Poultry Feedstuffs

Supply, Composition and Nutritive Value

Edited by J.M. McNab and K.N. Boorman

Poultry Science Symposium Series

Vol. 26

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Poultry Science Symposium Series Volume Twenty-six

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PREFACE

This volume contains the proceedings, revised and updated as appropriate, of the 26th Poultry Science Symposium held at the Peebles Hotel Hydro, in the Scottish Borders near Edinburgh, between 22 and 24 June 1999. This represented a continuation of the biennial series of Symposia, begun in 1964 by the British Egg Marketing Board, but organized since 1985 by the United Kingdom Branch of the World's Poultry Science Association. Each symposium is devoted to a specific topic within the field of poultry science and the lectures at the meeting are by invitation to those who are authorities in their own field. The symposia have traditionally been organized by an *ad hoc* national committee but the speakers are international, those at the 26th Symposium coming from nine different countries.

The objective of the organizing committee of the Symposium on *Poultry Feedstuffs, Supply, Composition and Nutritive Value* was to consider the present and future supply of feed ingredients, the chemistry of the major nutrient classes as it relates to feeding value, the methods available for assessing nutritive value, and the modification of feedstuffs by exogenous enzymes. The coverage is intended to range from basic science to application, emphasis being given to scientific excellence and commercial relevance, to extensive review and ultimately to the production of authoritative and high-quality papers.

Partly because of their economic importance in the performance of poultry, fleeting references to feedstuffs have appeared in several recent symposia. However, not since Symposium 19 held in 1985 has the topic been dealt with in any great detail. After the passage of 14 years it was, therefore, felt appropriate and timely to assess progress in an area which has seen vigorous research activity and which excites considerable commercial interest among companies concerned with the supply of feed ingredients, feed supplements and compounded feed.

The Symposium was divided into five sections, the opening session covering the current and future supply of feedstuffs, where political, agronomic and socio-economic factors were discussed. The second and third sessions, respectively, dealt with the qualitative and quantitative aspects of the nutritional components of feedstuffs, while the fourth considered some of the more important factors which are perceived to influence nutritive value. The fifth session was concerned with dietary enzymes, arguably the topic that made the greatest impact on the way poultry were fed during the last decade of the 20th century.

At the end of the first session and before dinner on the first evening of the meeting, a lively poster session was held, where 16 topics closely related to the

theme of the Symposium stimulated considerable interest among the 160+ delegates attending the meeting. Abstracts of these are included in this volume.

This book is designed to describe the thinking behind the way poultry are being fed at present and why. It is hoped that, like its predecessors, it will be widely used as a reference text and make a significant contribution to the development of international poultry science.

Organizing committee: J.M. McNab (Chairman); M. Dryburgh and E. Stewart (Secretaries); K.N. Boorman; C. Fisher; P.W. Garland; P.M. Hocking; K.J. McCracken; M.G. MacLeod; C.C. Whitehead.

PART I
Present and future supply of feedstuffs

CHAPTER 1
Agronomic and political factors influencing feedstuff use

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In agronomic terms and in the context of the global trading economy, it is appropriate to look at feedstuffs use for livestock production on a worldwide basis. Account must be taken of demographic factors such as population growth and urbanization. Incomes are also a significant determinant of livestock product consumption and, thus, the use of feedstuffs. Significant increases in demand for livestock products, and thus feedstuffs, are predicted in the early years of the next century, the bulk of which will occur in the developing world, most notably in China and South-East Asia. Pressures on land use will emphasize the role of increased crop yields in supplying requisite feed volumes. Declining rates of crop yield increase thus give cause for concern. The potential of biotechnology in squaring this equation is examined together with growing political opposition to such techniques. The importance of emphasizing the role of science in livestock production and feedstuffs usage is stressed[1].

INTRODUCTION AND OBJECTIVES

This chapter discusses some of the agronomic and political influences on feedstuffs use.

In agronomic terms and in the context of the global trading economy, it is increasingly appropriate to look at feedstuffs use for livestock production on a worldwide basis. In addition, there is a need to look at other factors that have entered the equation in more recent years. These factors apply with particular force in certain regions of the world.

Agronomic factors to be investigated include cropping area and productivity. Account must be taken of demographic factors such as population growth and urbanization. Incomes are also a significant determinant of livestock product consumption and, thus, the use of feedstuffs. Nor may cultural factors be ignored.

It will be shown that political factors are now becoming a significant input into livestock production systems, particularly in those parts of the western world rich enough to afford such sentiments.

[1]This paper was revised and updated in October 2001.

 Poultry Feedstuffs: Supply, Composition and Nutritive Value (eds J.M. McNab and K.N. Boorman)

No attempt is made to generate specific forecasts of feedstuffs usage. Instead, general indications are given, based on the predicted evolution of the main factors identified in the agronomic analysis.

FEEDSTUFFS – THE WORLD SCENE

It is virtually impossible to quantify all use of livestock feedstuffs on a world-wide basis.

In the first place, a significant proportion of livestock feeding takes place outside the purview of the formal economy in the non-commercialized sector. To some extent, data are not easily accessible even for those feedstuffs produced on an industrial basis.

In the 2000/01 cereal year, it is estimated that 683 million tonnes of wheat and coarse grains were used to feed livestock worldwide (USDA FAS, 2001). This volume represents approximately 47% of wheat and coarse grain usage for all purposes. Over the past 10 years, use of grain for livestock feed has increased at an average annual rate of 0.6%.

Use of coarse grains for feedstuffs accounted for 582 million tonnes in the 2000/01 season; approximately two-thirds of all consumption. Over the past 10 years, use of coarse grains in feed has increased by an average of approximately 0.9% per year. In contrast, just over 100 million tonnes of wheat was used to feed livestock last season, representing 17% of all wheat consumption.

Since the 19th century, the crushing of oilseeds for human consumption has yielded a valuable input for livestock production. In the 2000/01 season, 175 million tonnes of oilseed meals were consumed worldwide, of which two-thirds was soybean meal. Average growth in the consumption of oilseed meals during the past 7 years has been 3.5%. This highlights the increasingly industrial nature of feedstuffs production as the requirement for higher protein feed constituents has become apparent, notably in poultry and pig production.

THE EVOLVING PATTERN OF LIVESTOCK PRODUCT CONSUMPTION

The evolution of large-scale urbanized industrial societies in the west during the last quarter of the 19th century and the first 75 years of the present century was characterized by significant increases in the consumption of livestock products. This has been driven by increases in population and disposable income and by falls in the real relative price of livestock products.

In traditional societies, meat eating is reserved for ceremonial or festive occasions and the bulk of both energy and protein nutrition is derived from foodstuffs of vegetable origin. Such production of livestock as takes place occurs on a non-commercial basis. Consumption of livestock products is largely confined to intermediate products such as eggs or milk. The rural nature of such societies, the inadequacy of a distribution system and the frequent absence of electricity for essential refrigeration reinforce this.

In traditional societies, cultural and religious prohibitions also play an important role in determining patterns of livestock product consumption and feedstuffs usage. Such prohibitions might or might not be modified along with the changing social and economic nature of society. They will, however, play an important role in determining the early shape of such development as does take place. For example, it is difficult to envisage, certainly in the short to medium term, the evolution of a successful beef-based livestock product industry in India in those regions where the predominant religious tradition and practice is Hindu; similar considerations apply to pork in the case of Islam.

Significantly, no society displays obvious significant religious-cultural restraints on poultrymeat or fish consumption.

CHANGING CONSUMPTION PATTERNS

In recent years, there have been significant changes in the worldwide pattern of livestock product consumption.

In the developed world, income elasticities of demand for livestock products have fallen sharply. This will be discussed in more detail subsequently, but the significance of the income variable in the consumption function has diminished relative to other considerations.

In emerging economies, notably those of Asia and, in particular, China, demand for livestock products is increasing very rapidly, albeit this process has been interrupted by economic difficulties since 1996, less so in China than in Pacific Rim countries. This is a function of, in some but not all cases, expanding population, rising money incomes and increasing urbanization.

Population growth worldwide appears to have peaked in the late 1960s when it stood at 2.1% a year. This factor underpinned the Club of Rome's report in 1972 predicting a crisis of Malthusian proportions. This has, so far, not ensued because of falling rates of population growth and the effects of the Green Revolution in agricultural production. Longer-term projections from the United Nations suggest that, for the first 20 years of the next century, population growth will average 1.4% annually. This will be divided into 0.4% for the developed countries and 1.7% for the developing nations. Another source suggests that, from a total of 6 billion in 2000, world population is expected to grow to 7.5 billion in 2020, equivalent to an average annual rate of growth of 1.1%.

The role of urbanization, as people seek work in cities and towns, is an important element in determining the demand for livestock products and thus feedstuffs. In 1960, around 22% of the population of the developing countries resided in urban areas. By 1980, this figure had increased to 30% (Pinstrup-Anderson, 1992). One study suggested that, by 2000 and as a result of the extraordinary growth of industry in China and other Asian countries, urban dwelling in the developing countries would account for 44% of the population. In global terms, it has been noted that, during the 20th century, the world's population grew from 1.5 billion to 6 billion; the urban population grew from 200 million to around 3 billion, half the total.

A number of studies have suggested that urbanization exerts a significant effect on qualitative food demand. Dietary transitions noted include a move away from staple crops such as sorghum, maize and millet towards cereals requiring less preparation time and, significantly, towards livestock products and other processed foods. Also, significantly, such studies which have been carried out in Asia note not only a substantial increase in the demand for livestock products but also increased preference for wheat relative to rice (Bouis, 1994).

Urbanization is a by-product of economic growth and, in recent years, economic growth as measured by GDP has been significantly greater in the developing countries, notwithstanding economic disruption in Asia and the remarkable evolution of the US economy in the 1990s. One author, discussing long-term projections for Asian economic growth in the early 1990s, commented that 'the high growth rates in developing countries are projected to continue in future' (Rosegrant *et al*., 1995). This prediction was not borne out by events, but there remained little doubt that, once economic stabilization has been achieved, Asian and other developing countries would resume relatively high rates of economic growth. This optimism will be moderated by the US economic slowdown that has already had severe knock-on effects in South-East Asia, further compounded by the effects of the terrorist outrages in the USA.

The effects of such growth on the demand for livestock products and thus for feed are, nevertheless, difficult to quantify. Income effects will vary from country to country. In the developing countries, FAO studies indicate that income elasticities of demand can range from –0.4 in the case of some of the more traditional staple crops such as maize to +0.3 for high quality rice and, for a range of meats, from +0.2 to +0.9.

SATISFYING INCREASED DEMAND FOR LIVESTOCK PRODUCTS – THE INPUT REQUIREMENT

As livestock production becomes more commercialized in response to increased consumer demand, a major consideration will be the extent to which a particular country can satisfy increased demand for feedstuffs on its own account and the extent to which it will have to rely on imports.

Studies indicate that, in the developing countries, price elasticities of supply for most crops including those for livestock feed are, in general, fairly small (Huang *et al.*, 1995). This implies that increased agricultural production to feed-growing populations will depend on autonomous growth in areas cultivated and in the yield per hectare of cultivated land. A number of factors are relevant here, including public and private research and development, conventional plant breeding, wide-crossing and hybridization breeding, biotechnology and the development of supportive infrastructure such as agricultural extension, markets and the availability of irrigation.

There is widespread agreement that the production increases that characterized the 1960s through to the early 1980s cannot be regarded as typical. This partly reflects the exhaustion of the Green Revolution effects but it also reflects other factors, notably the reduced availability of cultivable land and some serious deficiencies in infrastructure investment, notably in irrigation.

The area planted worldwide to wheat and coarse grains for the 2000/01 harvest was 514 million hectares; 3.1% less than it was 30 years ago. This reflects diversion of land to other crops but it is also a by-product of the increasing urbanization of the planet, including the transfer of farm land to industrial activity and the abandonment of many small-scale farming enterprises; a process which has not, especially in many developing countries, been accompanied by sufficient investment in mechanized agriculture.

Final production in 2000/01 is projected at 1.44 billion tonnes; 49% greater than 30 years ago. This is due to increased yields per hectare, equivalent over the past three decades to almost 1.5% a year. While this may appear satisfactory, it is a matter for major concern that the rolling 5-year % increase in cereal yields, as shown in Fig. 1.1, has been falling since the mid-to-late 1980s. There are a number of reasons for this, which are specific to different regions of the world. In the developed world, the decline in the growth of cereal yields per hectare is primarily due to policy measures designed to draw down cereal stocks and to substitute direct payments to farmers for farm-price support programmes. In Eastern Europe and the former Soviet Union, economic collapse and subsequent economic reforms further depressed already low productivity. In developing countries, particularly in Asia, the slow-down in cereal productivity growth has been a function partly of growing water shortages and of inadequate public investment, notably in irrigation infrastructure. There is also, ominously, clear evidence of diminishing returns at work in that ever-increasing use of fertilizers, water and other inputs are needed to sustain yield gains.

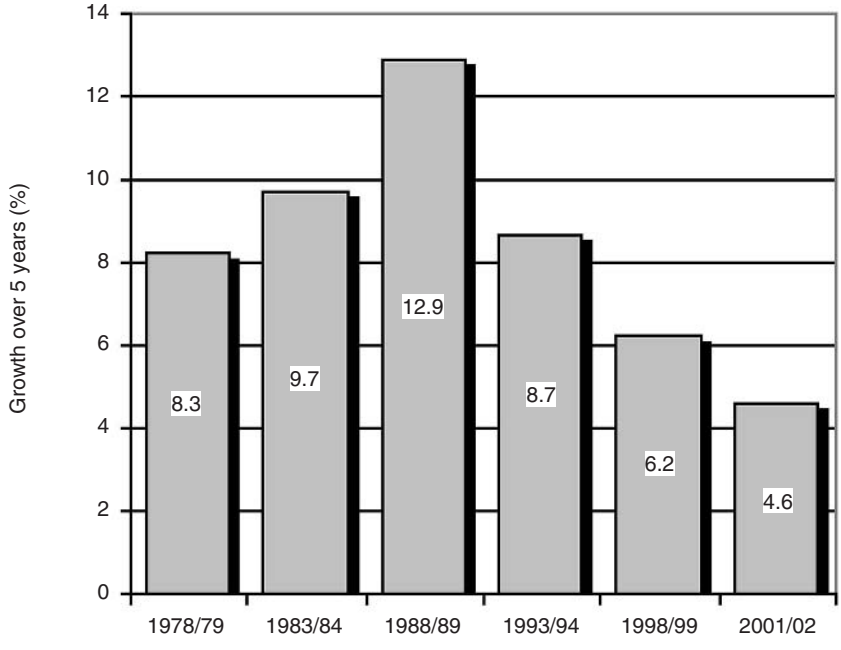

Fig. 1.1. Five-year rolling yield increase for wheat and coarse grains.

These factors are expected to slow growth in cereal yields worldwide from 1.6% a year in 1982–1997 to 1.0% a year in 1997–2020. This is a challenge that we are going to have to address in the next 20 years or so.

For 30 years, we have relied upon increased yields to provide the world with the wheat and other grains required to feed humans and livestock. It needs to be borne in mind, as one distinguished progenitor of the Green Revolution has pointed out, that observers have tended to focus overly on high-yielding wheat and rice varieties as if they alone can produce the yield improvements noted during the 1970s and 1980s. Certainly, modern plant varieties can uplift yield curves owing to their more efficient plant architecture and the incorporation of genetic sources of resistance to disease and insect infestation. However, they can only achieve significantly improved yields over traditional varieties if systematic changes in crop husbandry are made, such as in planting dates and rates, fertilizer application, water management, and weed and pest control. For example, higher soil fertility and greater moisture availability for growing food crops also raises the potential for the development of weeds, pests and disease. Complementary improvements in weed, disease and insect control are thus also required to achieve maximum benefit.

If the potential of the Green Revolution is becoming played out for whatever reasons, we shall have to look to other means to provide world food and feed requirements. The debate over transgenic crops is of clear relevance in this context.

EMERGING PATTERNS OF LIVESTOCK CONSUMPTION

The main engine driving the growth of demand for livestock feeds, and thus feedstuffs use, worldwide in recent years has been the long expansion, during the 1980s, of livestock product consumption in East Asia and, most notably, in China. This is not to exclude the effects of increased demand for livestock products in either the developed world or in Latin America and other emerging economies.

Between 1994 and 1999, the consumption of poultrymeat rose by 11 million tonnes or by over 25%. Over half the additional consumption between 1994 and 1999 took place in Asia where consumption has risen by almost 50%.

These data are remarkable in that they include the period in which the Asian economies were described, not without a degree of Western *schadenfreude*, as in a state of economic meltdown. China is, of course, the dominant economic entity of Asia. Consumption of poultrymeat in China grew by almost 5 million tonnes or over 70% in the 5-year period. If we look at growth in poultrymeat consumption over the period in question, it is evident that most of this growth occurred in 1994–1996 before the region's economic difficulties of 1997. For example, Chinese poultrymeat consumption in 1995 grew by 25%, and then fell progressively to 5% in 1998 and (forecast) 1999. Growth in the region as a whole in 1995 was 18%; this fell progressively to 3% in 1998 and was expected to be only 4% in 1999.

The role of economic growth in determining poultrymeat consumption is, of course, self-evident. In Brazil, consumption rose by almost a quarter in 1995; in 1998, growth was only 2.5%, reflecting the effects of Brazil's economic difficulties, but it bounced back in 1999.

Data subsequent to 1999 are not available on a basis that is consistent with that shown in Table 1.1. The former suggests that poultrymeat consumption worldwide rose by around 5.57 million tonnes or 10.5% between 1997 and 2000. China recorded an estimated increase of 1.71 million tonnes or almost 16%. Significantly, in the wake of the Asian economic meltdown in 1997–1998, it was Brazil and Mexico that recorded increases in poultrymeat consumption in excess of 30% between 1997 and 2000. Less dramatic but significant figures show Asia well in the lead in increasing pork consumption (USDA FAS, 1999). Out of the 6.2 million additional tonnes of pork consumed between 1994 and 1999, 95% was accounted for by Asian countries, largely China.

FUTURE AGRONOMIC INFLUENCES ON FEEDSTUFFS PRODUCTION – SUMMARY

In considering the future development of feedstuffs use worldwide, we need to look at a number of factors.

Primarily, the demand for feedstuffs will reflect demand for livestock products. This will be a function, *inter alia*, of population and income growth and is likely to be greatest in the developing countries, notably those of China and South-East Asia. It cannot be stressed too highly that the development of livestock product and thus feedstuffs demand is expected to show high rates of variation within the developing world. Most simulations suggest that growth in sub-Saharan Africa and in the Indian subcontinent will be relatively slow.

Table 1.1. Global poultrymeat consumption by region 1994–1999.

	1994	1995	1996	1997	1998	1999	Volume change 1994–99	% Change 1994–99
Asia	11,215	13,242	14,678	15,646	16,140	16,805	5,590	50
North America	14,259	14,385	14,848	15,145	15,574	16,502	2,243	16
South America	4,196	4,937	4,757	5,227	5,375	5,515	1,319	31
EU	6,829	6,993	7,406	7,412	7,588	7,754	925	14
Middle East	1,178	1,270	1,385	1,598	1,674	1,743	565	48
Africa	1,078	1,193	1,251	1,346	1,416	1,511	433	40
Eastern Europe	811	810	872	935	987	1,004	193	24
Oceania	489	490	493	522	569	586	97	20
Former Soviet Union	2,002	1,968	2,008	2,099	1,737	1,564	–438	–22
Total	42,057	45,288	47,698	49,930	51,060	52,984	10,927	26

Source: FAS post reports, official statistics, and inter-agency analysis.

The extent to which feedstuffs production and usage in the developing world increases will depend on competition for the available resources of land, labour and capital. It is, for example, suggested that where land is the main constraint, farmers may prefer to concentrate on high-value crops for fast-growing urban markets rather than on feedstuffs production. While production of livestock is expected to increase in the developing world, it is questionable whether they will produce requisite feedstuffs themselves or import them. Certainly, most recent studies suggest a substantial net increase in developing countries' net imports both of livestock products and feedstuffs during the first quarter of the 21st century.

The extent to which yield growth for most cereals has declined in recent years is of concern because of the pressures being exerted on cultivable land. Again, this stresses the combined effect of industrial encroachment and inadequate investment in infrastructure and irrigation.

CONSUMER POWER

In this section of the chapter, the background to consumption of feedstuffs is discussed with particular reference to the consumption of livestock products.

The Use of Statistical Method in the Analysis of Consumption

A very generalized form of analysis defined consumption of any product as follows:

Consumption = Income, Price, Underlying Demand, Dummy Variable, Error

$$C_{(1..n)} f\,(Y_{(1..n)}, P_{(1..n)}, Du_{(1..n)}, Vd_{(1..n)}, \epsilon_{(1..n)})$$

In a collection of papers marking the 50th anniversary of the National Food Survey, one contributor suggests that each of these variables has in turn dominated the consumption of livestock products since the end of the Second World War. Ritson and Hutchins (1991) suggested that post-war food consumption in Britain could be divided into five phases to which the present author would add a sixth. These are as follows, and are applicable in general terms to all developed European economies but not to the USA.

1951–1960 Return to normal diets

With the end of rationing in 1951 and the availability of more plentiful supplies of food, consumers were enabled to return to what would have been regarded as a more normal pattern of consumption, given the constraints of prevailing incomes and prices.

1960–1970 Income-driven demand

Increasing consumption of livestock products reflected rising real disposable consumer income. As people felt wealthier, they felt freer to trade up to more expensive products or to consume more of existing products. This period can be summed up in Harold Macmillan's immortal political slogan for the General

Election of 1959; 'You've Never Had It So Good'. Consumption of some more traditional food products such as canned meats and sausages declined, while that of fresh meat and poultry as well as cheese increased.

1970–1980 Price

The 1970s were a disturbed period in post-war UK history. The UK joined the EU in 1973 and this required a 5-year period of transition to higher EU farm prices, including those for livestock products. The oil-shock of 1973 caused a period of rapidly rising world commodity prices. In addition, this was a period of considerable social unrest, epitomized by the 3-day week following the start of the miners' strike and the two General Elections of 1974; the first fought on the basis of 'Who Governs Britain'.

1980–1990 Underlying demand and dummy variables

The Lawson boom of the late 1980s created new patterns of consumption; of livestock products no less than of Porsches and designer clothing. Part of this can be described as 'lifestyle', such as the abandonment of the family lunch on Sunday and the increased incidence of convenience food and takeaway eating. A spin-off of the lifestyle effect has been the increasing consumer concern with 'healthy eating'. This has impacted on milk and butter consumption while benefiting poultry consumption at the expense of the red meats. Dummy variables, used to represent discrete events such as the *Salmonella* crisis in 1988, the lead contamination problem that affected dairy feeds in 1989 and the ongoing BSE crisis that rumbled on through much of 1989–1990, finally breaking in full fury in 1996, assume much greater importance during this period.

Post 1990 The collapse of consumer confidence

There can be little doubt that, in the UK and in Western Europe in general, consumer confidence in the food they eat has been eroded by succeeding 'food scares' and a remarkable degree of ineptitude on the part of governments in the management of those scares.

Only if it is argued that governments are constitutionally incapable of learning from past mistakes, are the actions of the Belgian government over the dioxin scandal comprehensible. In the UK, the BSE crisis undermined more than consumer confidence in beef. The jury may still be said to be out on the question of who, if anyone, was to blame but, in recent comments about government trustworthiness over GM foods, the BSE saga is often quoted by the opponents of GM technology to illustrate the non-credibility of the government and thus, by extension, the credibility of the lobby groups.

It has been a long-standing principle on the part of the feedstuffs industry that science and scientific method should determine how feedstuffs are used to feed poultry as in any other form of livestock production. It cannot, however, be assumed that this principle will, in future, be accepted by consumers or, at least, by their self-appointed guardians in the consumer and environmental movements.

It must be noted, none the less, that two recent controversies, one non-food related and the other definitively so, have been sparked off by allegedly

scientific evidence which has been used by organizations to create alarm which has been enthusiastically taken up by the media. The Brent Spar oil platform controversy pursued by Greenpeace was based on quite erroneous evidence as to the amount of pollution that would ensue should the platform be sunk, as originally intended, in deep Atlantic waters. Greenpeace subsequently apologized to Shell Oil for their misrepresentation of the facts – a fact studiously ignored by most of the media on the grounds that it is the height of political incorrectness to attack Greenpeace – but the damage was done.

The debate over genetically modified organisms (GMOs) is, potentially, much more critical not just to the feedstuffs industry but to science in general.

The GM controversy – it cannot surely be called a debate – initiated by the publication of a letter supporting the cause of Dr Arpad Pusztai of the Rowett Research Institute, whose work on the effects of GM potatoes, genetically modified to express a lectin originating in the snowdrop, on the gut of rats has achieved a certain notoriety. Again, reputable bodies, including the Royal Society, have questioned the scientific basis of this work. No matter. This hare has been set running and the feed industry and the livestock industry must face the unpalatable fact that further factors have entered the Research and Development equation.

The first is the emergence into positions of prominence of organizations whose belief in the moral rightness of their cause – and it is a moral rightness because no scientific consideration is involved – is comparable with the self-righteousness of a 17th-century Witchfinder-General or the Dominican-inspired Inquisition. Governments are in the position, increasingly, of having to defer to such organizations. Brussels' immediate reaction, for example, to the dioxin scandal was to announce plans to review the list of permitted ingredients and the schedule of undesirable substances in feedstuffs. Since dioxins are not, as far as the author is aware, a permitted ingredient in feedstuffs, this response seems less than relevant. However, it leads to a much more significant general point.

Over the past decade, three controversies have afflicted the livestock industry. These references do not include sporadic outbreaks of *Salmonella*, *E. coli* 0157, *Campylobacter* and *Listeria*.

For more than a decade, a debate has raged over hormone-based growth promoters in beef production, largely as a result of a trade dispute with the USA that permits the use of such substances. Most scientific evidence tends to support the view that, properly used, such substances pose no danger to either beef cattle or to human consumers of beef.

Bovine somatotrophin therapy in milk production has been licensed in the USA. This is a biotechnology product that significantly increases milk production. Welfare issues have been raised about its effect on dairy cows; the role of IGF-1 on human health remains controversial.

The latest controversy over GM foods affects the feedstuffs industry in that two important raw materials used by the feed industry, maize and soybeans, are directly affected.

Setting aside, for the moment, the biotechnology-related aspects of bovine somatotrophin and GM crops, these three areas of dispute are linked by one common factor. The products are science-generated and the body of fact avail-

able to us supports the argument for their use at the present time. This does not mean that we should close the books on further evaluation. Increasingly complex scientific and technical solutions to the problems of the livestock and feedstuffs industry will require increasingly complex review procedures. What is also clear, however, is that a political input will also be required and that the feedstuffs industry, increasingly science-based, needs to improve the presentation of its case. Whether this will be successful is questionable. One aspect of the debate is, however, eminently arguable. It is said that '*We do not need these GM crops*'. Whether this is so or not, it requires an answer to the question '*how are we going to meet the demand for crops to satisfy both human food and livestock feed demand?*'. It would be much easier for everyone concerned if this were a purely agronomic question. Politics is, however, now firmly entrenched in the debate and that is something that the livestock industry and its feedstuffs suppliers will fail to address to its own profound disadvantage.

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CHAPTER 2
The assessment of the economic value of output traits genetically engineered into crops used in animal feed

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INTRODUCTION

Next to Darwin's *Origin of Species*, published in 1859, and the nuclear issue of the late 20th century, genetic engineering has probably stimulated one of the most controversial scientific debates known to man. The argument has ranged from, on the one extreme, meddling with the fundamentals of nature to, on the other, the panacea to future food shortage. The argument surrounding these issues is classical risk versus benefit. The object of this manuscript is to examine the issues related to the assessment of the economic value of the genetic traits, which, after all, will drive commercial development, with respect to the use in animal feed of crops containing genetically modified output traits.

One overriding factor has driven the substantial commercial support for the science of genetic engineering of crops. That factor is the perception of the considerable commercial advantage and profits that will be made from selling seeds which can be demonstrated to produce greater returns for the grower and which will thus capture a greater proportion of the market for the seed producer. Leahy (2001) reported that of the 2001 oilseed rape crop in Canada, 55% would be down to herbicide-tolerant varieties, citing an increase of 10% in yield, better weed control, less fuel use and less soil damage resulting from reduced tillage as the main factors for adoption of the crop. The choice of traits which will be engineered into animals and plants and which will be taken up commercially will be determined by the additional economic value that the traits can bring. However, there is a major challenge to capturing the economic value of the added trait.

Two aspects of the genetic manipulation of crops need to be defined. These relate to the definition of the type of trait in terms of either agronomic factors related to the growth and protection of the plant (input traits (IPTs)) or

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quality factors related to the composition of plants (output traits (OPTs) or quality traits). IPTs relate to the manipulation of the plant genome to influence the growth characteristics of the crop, which may impart herbicide resistance, insect tolerance, drought resistance or any factor that specifically relates to the germination and growth characteristics of the plant. OPTs relate to the yield-composition characteristics of the plant. It is certain that the development of OPTs in crops with respect to animal production, is of greatest interest to the nutritionist. Below are listed six categories of OPTs adapted from those proposed by Barre and Aumaitre (1998) which would be of interest with respect to improvements in nutritional value:

- reduction or suppression of anti-nutritional and toxic factors in plants (tannins, anti-trypsin factors, lectins, glucosinolates, α-galactosides);
- increase in the resistance of plants to cryptogamic diseases (*Fusarium*, *Giberella*, *Aspergillus*) which produce mycotoxins such as aflatoxin, ochratoxin and tricothecenes;
- reduction in plant components having low digestibility (modify the fibre fraction);
- increase in nutrient density, protein and/or energy content;
- increase the biological value of protein by improving the profile of amino acids to achieve a composition closer to the needs of the animal thus reducing nitrogen excretion;
- improve mineral availability in the plant (e.g. incorporate phytase which can aid phosphorus availability).

A wide range of potentially modifiable OPTs have already been identified, and a comprehensive list based on developments in progress, identified in patent applications is presented in Table 2.1. Genetically modified plants will offer no interest to animal production unless the new traits will in some way significantly reduce the cost of the feed formulation. Barre and Aumaitre (1998) stated that the cost–benefit must be attainable in all the countries involved, to avoid delocalization of systems of animal production.

The aim of this manuscript is to address the range of factors which need to be considered when evaluating the economic benefits of OPTs in crops with specific relevance to livestock production and the specific challenges to capturing this economic value.

INPUT TRAITS AND THE MAJOR BENEFITS FOR GROWERS

Reaction of Growers to Speciality Biotech Crops

There are three major criteria which farmers consider when deciding on a strategy for planting crops with new agronomic traits. The first is the opportunity to enhance profit on the farm. The second is the reduction in cost of inputs. When genetically engineered herbicide resistance is incorporated into crops, management is made easier and cheaper for growers, since the number of herbicide applications to the crop is reduced. The third is the ease of incorporation of the new technology into the existing farm operations.

Table 2.1. Output traits of potential value for feed and food identified from patent applications.

Crop	Use	Trait	Improvement
Barley	Food	Flavour/yield	Improved malting quality
Chickpea	Feed	Amino acid	Increased amino acids (meth & lys)
Clover	Feed	Amino acid	Increased amino acids (meth & lys)
Canola	Food/Industrial	Oil	High lauric acid
	Industrial	Oil	High myristate
	Food	Oil	High stearic acid
	Food	Oil	High medium chain fatty acids
	Industrial	Oil	Speciality lubricant (waxes) jojoba oil
	Food	Oil	High long chain PUFA
	Food	Oil	High medium chain fatty acids
	Feed/food	Oil	Low saturates/high mono/low PUFA
	Feed	Oil	High oil
Cotton seed	Food	Oil	High oleic acid
Lucerne	Feed	Amino acids	Increased amino acids (meth & cys)
	Feed	Lignin	Improved digestibility/low lignin
Lupin	Feed	Amino acids	Increased amino acids/Basta resist
Maize	Feed	Amino acid	High protein with balanced amino acids
	Feed	Mycotoxin	Fumosin detoxifying
	Feed	Oil	High oil content
		Oil/amino acids	High oil with increased digestibility
		Oil/phosphorus	High oil with increased P availability
Palm	Industrial/Food	Oil	
Peas	Feed	Amino acids	Increased amino acids (meth)
Potato	Food	Shelf life	No browning
Rape seed	Industrial	Oil	High erucic acid
Soybean	Food	Oil	High oleic lower saturated fat
	Food	Oil	High stearic acid
	Food	Oil	High palmitic acid
	Food	Oil	Low saturated fat
	Feed/Food	Protein levels	Increased levels of protein
	Feed	Anti-nut factor	Low stachyose
Sunflower	Food	Oil	High oleic acid
Sorghum	Feed	Carotenoid	High carotene
Tomato	Food	Shelf life	Increased shelf life

The rapid adoption of IPTs is in no small way due to the very rapid and quantifiable capture of the value of the trait by the farmer, apart from the value gained by the seed producer who can effectively ring-fence the use of branded insecticides and herbicides. The very rapid adoption of agronomic traits can be seen from Table 2.2, where the surface area and percentage of treatment of the main crops in North America is given separately for herbicide tolerance and insect resistance. Gene stacking will allow the combination of these two traits with subsequent major advantages for the grower and Table 2.3 gives combinations of likely stacked traits which were tested in 1998.

Table 2.2. Surface area in North America sown to genetically modified crops exhibiting modified agronomic traits.

	Surface area sown (1000 ha)					
	Canola	Maize	Cotton	Soy	Potatoes	Total
Herbicide tolerance						
1995	17					17
1996	172		20	410		602
1997	1,095	308	451	3,695		5,549
1998	1,845	2,970	1,886	10,320		17,021
% surface 1998	30	9	38	36		23
Insect tolerance						
1995					0.7	0.7
1996		205	750		4.5	960
1997		1,870	902		12	2,784
1998		6,070	1,025		22	7,117
% surface 1998		18	20			19

Table 2.3. Examples of potential gene stacking.

	Surface area sown (1000 ha)			
	Maize	Cotton	Soy	Total
Insect + herbicide	Bt / LL[1]	Bt / RR[5] : Bt / BXN[2]		
multiple herbicide	IMI[3] / LL[1]		STS[4] / RR[5]	
1996				
1997		25		25
1998	50	500		550
% surfaces 1998	0.2	10		1.5

Tolerance to [1]glufosinate, [2]bromoxynil, [3]imidazolinone, [4]sulfonylurea, [5]glyphosate.

Bt maize, containing the insecticide protein derived from *Bacillus thuringiensis,* was introduced in 1996, and was presented to the market in 1997, whereupon it achieved high market penetration and high grower satisfaction. The introduction of this trait not only addressed areas with known European corn borer (ECB) problems, but also suggested that ECB was causing more widespread yield losses than had previously been anticipated. All major seed companies marketed Bt maize in 1997, although the Bt trait came from different events and different sources. Approximately 5.5 million acres of Bt maize were planted in 1997 and it is estimated that supply could accommodate the planting of 20 million acres in 1998, with predictions that 60% of maize acreage will be planted to Bt maize in the year 2005.

All seed companies are pricing Bt hybrids at a premium over conventional hybrids. Bt hybrids were selling for a premium of approximately US$5–10 per

acre or US$15–30 per unit. In 1997, on-farm performance of Bt maize indicated that yield increases were in the range of 10–30 bushels per acre over conventional non-Bt hybrids. The benefits provided for the grower are closely tied to the value of the maize and are greatly diminished in years of either low ECB infestation or low maize prices. Compared with control using insecticides, the net economic profit from the Bt trait will range from at least US$8–15 per acre.

It is estimated that the increased yield achieved with Roundup Ready soybeans is of the order of 1–2 bushels per acre (Wheat, 1998). This, plus the savings on herbicides, amounts to an increase in income of 30–50 cents per bushel, or between $11–18 per metric tonne, even allowing for the premium payments on the genetically modified seed (Wheat, 1998). Roundup Ready seed is priced so that the farmer is likely to see several dollars in savings per acre.

To date, the genetically enhanced plants that are in the market place contain IPTs conferring insect resistance and herbicide tolerance. Clarke and Ipharraguerre (2001) reported that the results of 23 research trials with genetically enhanced maize and soybeans indicated that the genetically enhanced plants were substantially equivalent in composition, digestibility and feeding value to the non-genetically modified varieties.

Thus for seed producers and growers the success of IPTs is immediately evident. They offer a rapid, substantial economic return, the value of the technology can be calculated and to date there has been no recorded negative impact on yield. The technology is easily incorporated into existing practice and there has been high grower satisfaction. Recent new data suggest that insect-tolerant crops, which suffer less insect damage and are therefore less prone to fungal attack, are less likely to be contaminated with *Fusarium* mycotoxins (Cahagnier and Melcion, 2000; Pietri and Piva, 2000). Reduced mycotoxin contamination is of great significance to both animal and human health. The above examples indicate the level of return and potential benefits that can be achieved with IPTs and this forms the new base-line for the value of any alternative trait that would either be included instead of, or stacked in combination with nutritional OPTs. The advantage to the agronomic traits described is that there is no alteration in the composition of the grain which could influence returns, for example, to the soybean crusher and there is no need for segregation and identity preservation.

OUTPUT TRAITS AND THE CHALLENGE OF VALUE CAPTURE

Investment in Growing Output Traits

The reasons why growers may adopt output/quality traits are somewhat different to those that drive the adoption of IPTs. Again the first and foremost criterion is profit enhancement. Secondly, farmers are also keen to be part of any emerging technology, and the third factor is risk management with a desire not to be left behind. For most if not all of the quality trait crops, the need to identity-preserve them, coupled with the current tendency for these crops to experience some yield lag, has resulted in premiums being paid to producers to contract grow

them. Generally, there are three types of costs associated with quality traits: (i) grower premiums/incentives; (ii) identity preservation costs; and (iii) end-user costs of adoption. The road from the initial targeted trait, through expression in the seed, via the logistics to insure that the integrity of the trait is maintained into and through the grain handling system and is found and proven to be acceptable to the end user, is formidable.

To be sold in significant volume, a feed quality trait needs to be adopted by at least two end-user segments out of poultry, pig, beef, dairy or export. Without broad appeal, a quality trait cannot become a major percentage of production. For traits to have broad appeal in the USA, they must compete directly with the two major crops: standard maize, of which approximately 72% is used for feed production and soybeans, of which 80% is in some way used in feed production. Furthermore, maize and soybean are the primary targets for development of output traits in feed crops.

Producers have become accustomed to being paid premiums for anything new, novel or under contract. Assuming that growers will act rationally, they will require a premium or some other form of compensation for the increased risk of growing a quality trait. Typically these premiums have been at the level of \$0.15 bu^{-1} for waxy maize to a high of \$18.00 bu^{-1} for organic clear hylum soybeans. The majority of premiums fall in the \$0.20 to \$0.50 bu^{-1}. Typical premiums for speciality grains without any yield lag are expected to be in the region of \$0.15 bu^{-1} minimum. There are few speciality crops without some sort of yield lag that would need to be additionally recompensed. Furthermore, growers have no preference as to what trait is inside the crop, unless the trait causes detrimental environmental or food safety effects. At present, few growers are willing to adopt a monoculture approach with these new crops, and the genetically engineered crop tends to represent up to one third of the entire acreage.

Identity Preservation and Vertical Coordination

In order to capture the value of an output/quality trait when used as a major component in animal feed, changes will be required in the infrastructure of the feed business. Capture of value from OPTs is more complex compared with IPTs since there are more links in the value chain. For IPTs there are typically three links in the chain, the gene-engineer, the seed producer and the farmer who grows the crop. For OPTs there may be as many as four additional participants in the chain (grain storage, grain treatment, compound feed producer, livestock producer). In order to achieve a situation in which the nutritional benefit imparted into the crop is transferred to the livestock producer, it is essential that, at each stage of the movement and processing, each of the participants who contribute in the process can gain economic benefit. It is even more important that none suffer any financial loss or are in any way compromised by handling the crop. The value of the trait engineered into the crop must be capable of being captured and the value of the 'uplift' to the end-user must be significant. The situation differs dramatically according to the number of participants in the value chain and the type of crop concerned.

Identity preservation starts with segregation of the crop on the farm where it is grown. New storage capacity or modification of current capacity may be needed. Changes include the way the quality trait is delivered from the farm to the end-user and the way end-users make feed. Identity preservation places new responsibilities on the traditional commodity grain system. The costs associated with identity preservation include the following.

- Additional storage: reduced turnover, partially full bins, delivery over time rather than all at once. Separate harvesting together with additional separate storage space will be required on farm. On-farm storage with flexibility to handle smaller lots of speciality crops is usually limited, and lack of storage flexibility will severely limit the number of farms that can participate in the production of crops with specific agronomic traits. This will be the situation until farmers are willing to take on board converting to 100% adoption of the new crop.
- The logistics of handling speciality crops during the harvest poses an additional problem, since this is a period of major pressure with the high volume of grain that must be processed in a limited time. The number of grain reception areas, dryers and conveyors, and whether the system is continuous or batch flow, all determine whether or not an elevator may be a player.
- Handling/segregation: management and labour to ensure segregation. The requirement for separate storage facilities will severely limit the adoption of transgenic crops. In the United States, grain handling systems are set up to handle large volumes and the normal bin size in elevators is between 100,000 and 300,000 bushels. Identity preservation of grain tends to go against all the established operating procedures of volume and simplicity.
- Risk management: quality traits cannot be hedged exactly; contamination risks.
- Transportation: containment; transport identification. Transportation is via trainloads where the unit is 75+ cars.
- Analysis: testing and identification of the improvement in the quality trait.
- Marketing: identification of specific buyers, contracting, coordination of delivery.
- Consumer specifications: the reluctance of consumers to adopt GMO material in the feed chain will further add to identification costs.

An additional cost to elevators includes the hidden cost of blending. In the traditional system, high and low quality grain lots are often blended together in order to achieve an overall quality which just achieves the required standard with a maximum allowable level of foreign material. Typical costs for identity preservation of maize and soybean meal are shown in Table 2.4. These costs compensate the grower, the distribution system and the processor. However, the technology developer and/or seed supplier and the end-user have not been rewarded, the end-user has only paid for costs incurred. For maize this represents a minimum of an additional value of US\$10 t^{-1} in order to adequately compensate all the participants in the chain and for soybean, US\$28 t^{-1} to compensate for the additional participants in the chain.

Table 2.4. Costs to move quality traits through the system.

	Maize		Soybeans	
Description	Low end ($ bu^{-1})	High end ($ bu^{-1})	Low end ($ bu^{-1})	High end ($ bu^{-1})
Grower premium	0.10	0.40	0.25	1.00
Identity preservation	0.10	0.25	0.10	1.80
Cost of adoption for end-user	–	0.05	–	0.03
IP at processor	–	–	0.05	0.80
Total	0.20	0.70	0.40	3.63

Soybean production is a particular case where additional consideration must be given to the value chain. Oil and meal are two co-products from the harvesting of soybean meal (Fig. 2.1). The oil is used as a high value product in human nutrition, with just 5% being used for industrial purposes, while 97% of the meal is used for animal feed. However, the price of the oil and meal is significantly influenced by world markets of other commodities. The world price of food-grade oil is in competition with palm oil, and the meal competes with fishmeal and rapeseed. Soybean crushing and the extraction of oil from the bean are crucial steps in the processing of the bean and hence the profitability of the oil crushing process is critical. The crushing process not only yields the oil but also destroys the anti-trypsin factors in the meal. The oil processing step is therefore essential to the production of animal feed-grade meal. However, it is the oil

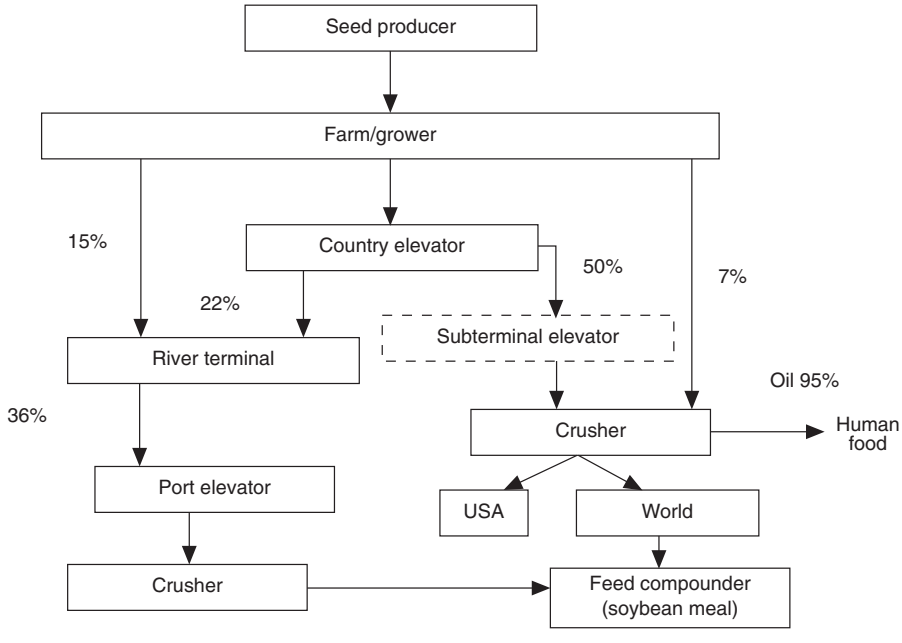

Fig. 2.1. Steps in the processing of soybean meal.

content of the bean that drives the price received by the grower who is penalized if the oil content falls below specific norms. The oil content of the bean therefore becomes a critical factor in defining the value of the crop and cannot be permitted to fall at the expenses of an added trait. Table 2.5 shows a number of the decision factors in the value chain of soybean meal.

There is one caveat to the above when the challenges to infrastructure do not apply and that is when the feed quality trait is to be fed directly on-farm. Such enterprises include cow/calf/suckler operations, a major proportion of dairy cow production and a major proportion of pig production. The barriers in terms of infrastructure may also cease to apply in situations where an integrated end-user can contract sufficient acres near a local feed mill, assuming that the product can be stored on the farm or at the mill.

One of the most important factors in limiting the numbers of growers who adopt either speciality or bioengineered grains will be the changing value of the crop itself. A premium of \$0.3 bu^{-1} for speciality grain when the commodity grain is valued at \$3.00 bu^{-1} represents a 10% increase in value. However, if the commodity crop is valued lower at \$1.75 bu^{-1} the same premium represents a 17% increase in return, which is obviously more attractive. It is predicted that from a low in 1998/99 the price of grain will steadily rise over the next 7 to 10 years. The premiums for nutritional traits are generally low and as the cost of grain increases they represent a diminishing return to the farmer.

To some extent, geography will dictate where contract grain production will occur. Sites close to the use of the grain, where the crop can be transported directly for processing, will result in nucleus regions of contract production.

HIGH OIL MAIZE, A MODEL FOR VALUE CAPTURE OF OUTPUT TRAITS

High oil maize (HOM) is a relatively recent development in crop production, which has been rapidly adopted by growers and feed compounders as a result

Table 2.5. Decision factors and consequences in the production chain of soybean meal.

Decision factor	Consequence
Grower	
Value of crop above production costs	Key decision on choosing crop for planting
Yield per hectare	Will not accept drop in yield: direct effect on profitability of crop
Trader (purchase based on three criteria)	
Oil content (18 ± 0.3% standard)	Low oil content incurs price penalty
Moisture content	Key aspect of grain quality
Fines	
Crusher	
Oil 18%	A 1% drop in oil content requires a 3% increase in value of meal to break even
Meal 77%	

of the benefits that can be realized in feed production. Although HOM was a trait achieved by traditional selection and not developed by genetic engineering, the capture of the additional economic value in the crop is an excellent example of the levels of value essential for adoption of the technology and the constraints which may be encountered.

In the first instance the problem of yield lag, which is an immediate constraint and disincentive for the grower, was overcome by using the Top Cross technique. This technology combines current high-yielding hybrids with high-oil genetic lines in which there is characteristically a yield lag. The female parent seed, which represents 90–92% of the maize in the field, which is a high yielding female hybrid, is male sterile. The high oil genes from the male plants that represent only 8% of the crop have an immediate impact (the Xenia effect) on the oil content of the resulting grain with absolute minimal yield lag.

In HOM a double benefit is claimed, the oil concentration rises from 4 to 7% and there is an additional 1% increase in protein (8.86 to 9.75%) with additional methionine and lysine. Although there is a reduction in starch content of the grain, the overall metabolizable energy content increases by 176 kcal kg^{-1} (equivalent to +4.5%) for poultry. The higher energy and protein content increases the energy and nutrient density of the grain, permitting an increase in overall nutrient density or alternatively the use of cheaper feed ingredients as a higher proportion of the ration. The improved protein quality with higher levels of lysine and methionine also allows a reduction in the supplementation with synthetic amino acids.

The primary determinants of the value of HOM as a feed ingredient are the price and nutritional value of normal maize (2-yellow dent maize) and the price of the alternative energy sources of which the most important is fat. Figure 2.2 shows the increase in value compared with 2-yellow dent maize when HOM is used in a typical broiler grower ration. There are two key points. It can be seen that there is a substantial increase in the value of the HOM, but the increase in value is dependent on the price of the grain. When there was an 85% increase

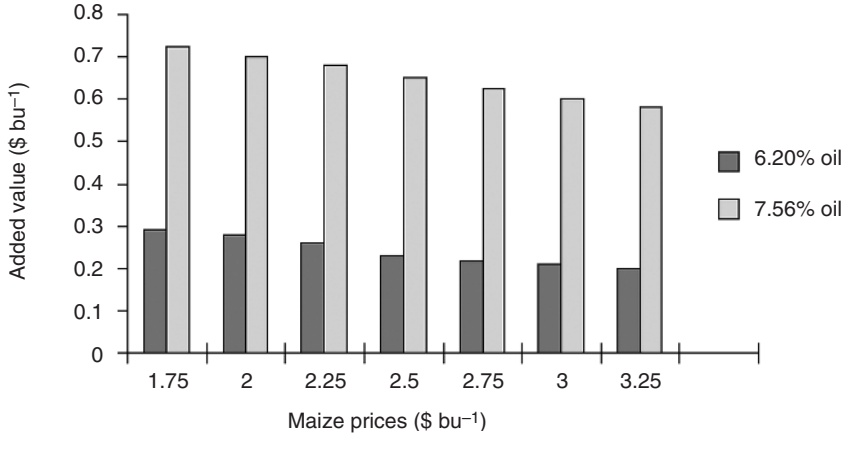

Fig. 2.2. The influence of the price of maize on the added value of high oil maize in broiler feed.

in the price of the grain, the added value of the HOM grain fell by 20–30%. This would at first appear illogical, however, as the price of the grain increases, it is the influence of the least cost formulation procedure that selects alternative energy sources and hence the value of the new trait is reduced. The situation is the reverse when the cost of alternative feed ingredients increases. The cost of supplementary fat has a major influence on the value of HOM (Fig. 2.3). Elevated prices for fat increase the value of the extra energy in HOM. Figure 2.4 indicates how the regional differences in the price of fat influence the potential markets for HOM. It is only in Europe and Asia, where the price of supplementary fat is higher than in the USA, that the value of the additional oil in the maize exceeds the minimum value of US$10 t^{-1}, which is the value indicated earlier as needed to recompense all the participants in the chain.

The importance of these examples is to demonstrate that there are several factors that have significant influence on the value of the added trait. It must be taken into account that feed formulations are derived by least cost formulation and that both the cost of the basic grain and cost of potential alternative feed ingredients will significantly influence the value of the trait.

Finally, the value of HOM is also influenced by the effect that the oil content has on manufacturing feed costs. With HOM there is, additionally, a potential 12% increase in grinding efficiency plus other factors such as dust

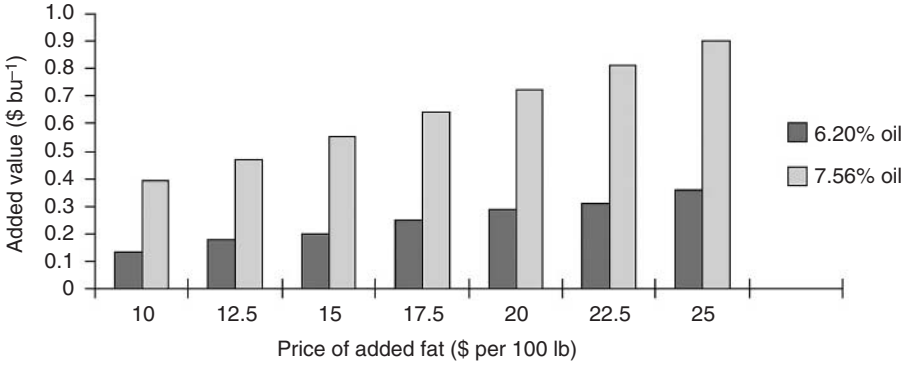

Fig. 2.3. The influence of the cost of supplementary fat on the value of high oil maize in broiler feed.

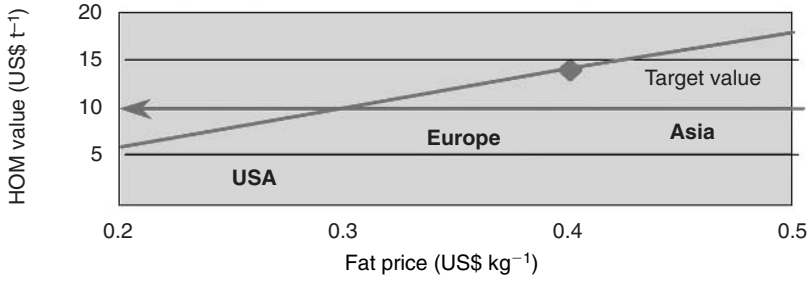

Fig. 2.4. The influence of the price of supplementary fat on the potential market for high oil maize.

reduction, mixing efficiency and pellet quality. All these factors need to be taken into consideration to determine the final value of the added trait. A summary of the key issues to be taken into consideration in evaluating HOM in feed formulae illustrates the complexity of the problem.

1. The animal species being fed (i.e. pigs, poultry, beef, dairy). The supplementation with HOM with a more nutrient-dense profile may offer an opportunity to incorporate less nutrient-dense ingredients with price advantages
2. Age of the animal and type of diet being fed (i.e. starter, grower, finisher). Two key factors, energy and protein level, will have the greatest impact on the formulas developed for different stages of the feeding programme. As the requirement for energy density increases, there is a greater premium for high energy-dense components. Increasing growth potential derived from poultry genetic programmes will accentuate the premium for HOC.
3. Tabulated metabolizable energy values of feed ingredients. The availability of current, relevant nutritional profiles and substantiated values for each feed ingredient used in the formulation will be critical.
4. Prices of primary protein sources and amino acid supplements. Since HOM has a higher level of total protein as well as higher levels of essential amino acids, well-substantiated profiles for both total and digestible amino acids will be critical in defining the final value.
5. Feed manufacturing efficiency and production goals. Feed pricing normally includes only the actual ingredients. The benefits that have already been explained for HOM in feed processing would be an additional factor to be taken into account.

In order for HOM to become widely adopted it must be attractive to both the integrated poultry and pig producers. Allied to this is the fact that the commodity fat market would be reluctant to allow significant penetration into these segments unless an alternative use is found for this commodity. Thus, with such a wide range of factors to take into consideration, as well as the dynamics of feed ingredient pricing, it is very difficult to provide a precise value for HOM on which to base an investment plan. However, this type of evaluation is essential before the value of a new OPT can be precisely quantified in animal nutrition.

DEVELOPMENT OF PROTEIN AND AMINO ACID TRAITS IN SOYBEANS

Research to genetically modify soybeans has targeted four broad areas: (i) improvement of protein concentration and/or essential amino acid composition; (ii) reduction and elimination of anti-nutritional factors; (iii) improvement in the profile and composition of soybean oil; and (iv) development of disease resistance, herbicide resistance and insect-tolerant lines. The high biological value and relatively high (approximately 44–48%) protein content of soybean meal makes it eminently suitable as a protein supplement in rations for pigs, poultry and ruminants. Furthermore, in least cost formulations soybean meal has a high value due to its ability to supply lysine which is often a first limiting

amino acid in any formulation. Increasing the methionine content of soybean meal would improve the biological value of the meal.

Three alternative approaches have been considered for increasing the methionine content of soybeans using genetic engineering. The advantages and disadvantages of each approach cannot be evaluated until samples of the meal are obtained and tested *in vivo*.

The three methods described are:

- Increase in the free methionine content.
- Insertion of a foreign protein with a high methionine content.
- Replacement of non-essential amino acids in an endogenous protein with the amino acid methionine.

There is a limit to the level of methionine that can be inserted into the plant since the high sulphur content of the amino acid results in high levels of methionine being toxic to the plant. The type of insertion may also influence the availability of the amino acid.

Increase in the Free Methionine Content of the Seed

In many seeds, storage proteins are formed from free amino acids. By increasing the free methionine content, i.e. the amino acid is not incorporated into a storage protein, the total methionine content of the seed can be increased. Results suggest that there is the potential to double the total methionine content of the seed by raising the level of free methionine, although the level is limited by the toxicity to the plant of the amino acid. To increase the free methionine content, the complete pathway of methionine production in the plant must be known and regulated. Usually there are negative feedback mechanisms that are initiated if any substance occurs in excess in the plant. These must be effectively turned off, if a specific amino acid is to be allowed to accumulate to very high levels. The exact site of the free methionine has not been identified, but it would be important for it not to interfere with the oil extraction process. In the free form, any mixing with the oil during extraction may hinder recovery of the amino acid in the meal.

Insertion of a Foreign Protein with a High Methionine Content

It is possible to express foreign proteins in the storage proteins of seeds. The amino acid composition of the foreign protein can be chosen to include a high level of a specific amino acid, thus increasing the level of the specific amino acid.

There are several difficulties with this approach. The chosen amino acid must represent a high proportion of the foreign protein in order to increase significantly the specific amino acid composition of the seed, and, furthermore, it is difficult to find such proteins that are completely innocuous. Alternatively, a large quantity of the foreign protein must be inserted into the plant. In this instance it is difficult to maintain the protein–energy balance in the plant. The

insertion of large quantities of the foreign protein may be at the expense of either the starch or oil content of the plant since the plant has a limited capacity to manufacture carbon skeletons. Raising the methionine content at the expense of reducing either the oil content or energy content of the seed would significantly reduce the economic value of the transgenic plant. Finally, the availability of the methionine in the foreign protein is unknown and this can only be identified by *in vivo* evaluation. It cannot be assumed that the digestibility of a foreign protein is equal to that of the parent proteins in the plant.

Replacement of Non-essential Amino Acids in an Endogenous Protein with the Amino Acid Methionine

A third approach is that the amino acid of choice (methionine) is used to replace non-essential amino acids in a specific endogenous protein of the plant. To date, 20–40 amino acid residues of the phaseolin protein that is found in kidney beans have been replaced by methionine. The phaseolin protein can then be returned to the parent plant. Such a development has the dual advantage of increasing a specific desirable amino acid and reducing the content of those amino acids that are not needed. This technique has been tested in kidney beans and rice. Since it is the endogenous protein of the plant that is altered, it would be expected that there would be no change in the digestibility of the protein and that the additional amino acid would be equal in digestibility to the amino acid in the parent protein. There appear to be few drawbacks to this technique, however, it is possible that the functional characteristics of the parent protein may be altered thereby reducing the viability/productivity of the plant. Secondly, a protein must be identified, which can be manipulated in the required manner. It was claimed that the methionine content of soybean meal could be increased by 80% using this technique.

Modification of the protein and amino acid composition of soybean meal is important because the value of the meal represents approximately 65% of the value of the bean. Chung and Pettigrew (1998) considered that high protein soybean meal had the greatest potential for technical feasibility, while the remaining alternatives still required significant research progress for their commercialization. High protein soybean meal has the potential to reduce the proportion of meal used in diets for both pigs and poultry. This compares with the situation when individual amino acids are the target. High lysine, with a 1.3% increase in lysine content in the meal, would result in a lower proportion of meal in the diet, a higher proportion of feed grains and no supplemental synthetic lysine. The meal would be of greatest benefit to pig producers and there would be little advantage in the poultry industry. Alternatively, the primary interest for high methionine soybean meal with a 0.32% increase in methionine content would be from poultry producers, who would be able to reduce reliance on supplemental synthetic methionine.

These examples of modification of amino acid content demonstrate that the market application of specific traits can be highly specific and that raw materials that presently have universal application can rapidly be restricted to

specialized markets. Storage and identity preservation of such materials for feed formulation in mills that produce feed for either pigs or poultry then becomes an additional consideration.

Chung and Pettigrew (1998) evaluated imputed prices (ξ level of specific nutrient × shadow price) of the new soybeans in a number of formulations to determine the price premiums which could be expected for a range of soybean meals with different OPTs. The estimated imputed prices are considered as the maximum amount of premium prices that livestock producers can pay for each alternative soybean meal. If livestock producers can gain extra benefit, the estimated prices are the premium prices in the market. However, in reality the premium prices would be determined somewhere between the price of conventional soybean meal and the imputed price of the new meals, depending on the efficiency of price transmission in the market system. As premium prices approach the imputed price, cost benefits for the livestock producers are expected to decrease. Therefore, feed cost savings estimated by comparing formulas with and without new high-quality soybean meals, given same ingredient prices, are the maximum estimates of premiums/costs savings for the livestock producers. Table 2.6 is adapted from the data presented by Chung and Pettigrew (1998); their analysis is based on industrial prices for supplementary amino acids and mean commodity prices based on the period January 1990 to December 1992. The objective of presenting this data is to compare the magnitude of the different premium estimates, rather than to consider the actual values that alter considerably, based on the price of raw materials.

Based on the weighted average values, the highest level of benefit is to be found for the high protein soybean meal (HPSM) product in feed for either

Table 2.6. Imputed prices for a range of different soybean meals.

Animal	Stage	Base model feed cost($ t^{-1})	Cost savings ($ t^{-1})		
			HPSM	HLSM	HMSM
Turkeys	0–4 weeks	148.16	7.97	2.89	4.08
	8–12 weeks	132.32	5.50	5.63	2.83
	16–20 weeks	115.21	3.44	0.07	0.74
	Weighted average	124.99	4.72	2.11	1.89
Broilers	0–3 weeks	148.11	6.44	0.48	3.30
	6–8 weeks	122.21	4.11	0.48	1.75
	Weighted average	129.77	4.84	0.48	2.28
Layers	0–6 weeks	115.63	1.63	0	0.66
	14–20 weeks	99.97	0.46	0.48	0.20
	Laying	105.84	0.99	1.03	1.23
	Weighted average	105.77	0.97	0.89	1.04
Swine	10–20 kg	127.95	5.28	0	NT
	50–80 kg	109.62	1.65	2.42	NT
	Weighted average	111.19	2.05	2.19	NT

HPSM: high protein soybean meal; HLSM: high lysine soybean meal; HMSM: high methionine soybean meal.

turkeys or broilers, with nearly equivalent levels of benefit. Although there were positive benefits for layers and swine, these were less than half the benefit for broilers and turkeys. Such a range in value that can be captured from the modified crop severely limits its market potential. It would appear, therefore, that these crops would need to be produced for particular species-specific, niche markets. In each instance, the highest level of benefit is found in the diet requiring the highest concentration of protein and highest amino acid specification, thus in the early growth phase. However, since this is the lowest volume of feed produced, compared with the later growth and finisher phases, the overall value is strongly weighted to that value which can be achieved in the late-growing animal. In broilers and turkeys, there is an approximate doubling of the added value when the high protein and high methionine traits are combined, compared with high methionine alone. High lysine has minimal additional value in broilers and layers and achieves greatest value in pigs, as would be expected.

Based on this analysis, the results indicate that the highest additional value that could be achieved for the HPSM diet was 11% above the conventional soybean price, thus any premium paid by livestock producers would not be likely to exceed 11% on the price of commodity grain. Again it must be emphasized that these evaluations are based on current market prices of synthetic amino acids. The key question is whether an 11% increase in the value of soybean meal would be sufficient for all the players in the chain leading to the production of the meal to gain sufficient benefit in order to invest in the identity preservation of the meal. As has already been emphasized, the answer to this question lies in the number of players in the chain and whether the number can be substantially reduced. Faced with such a challenge, there would undoubtedly be an adjustment in the price of synthetic amino acids, reducing the shadow price with a consequent decline in the imputed price. Chung and Pettigrew (1998) concluded that various price scenarios based on changes in the price of soybean meal had little effect on predicted savings and orders in cost benefits remained unchanged. However, changes in the availability of alternative protein sources such as meat and bone meal resulted in relatively large effects. This is exactly the same situation that was described for HOM, where the price of alternative energy sources such as fat had a major influence on the additional value of the oil in the grain. Since these alternative sources of energy and protein are by-products of the rendering industry, it must expected that the price of such materials could and would be adjusted downwards in the face of a challenge from alternative sources arising in plants. Figure 2.5 shows the estimated increase in the value of transgenic soybean meal, based on current market prices for lysine and methionine, containing increased levels of both lysine and methionine targeted to markets for either pigs or poultry. It can be seen that in neither case does the increase in value exceed US\$28 t^{-1} which was the minimum premium needed to compensate all the participants in the chain in order to identity-preserve the meal.

These calculations presume that the nutritional availability of the added nutrient is not different to the original endogenous nutrients in the parent plant. This may be a dangerous assumption to make given the known variability in digestibility of amino acids in a wide range of protein sources, as will be explained in a later section.

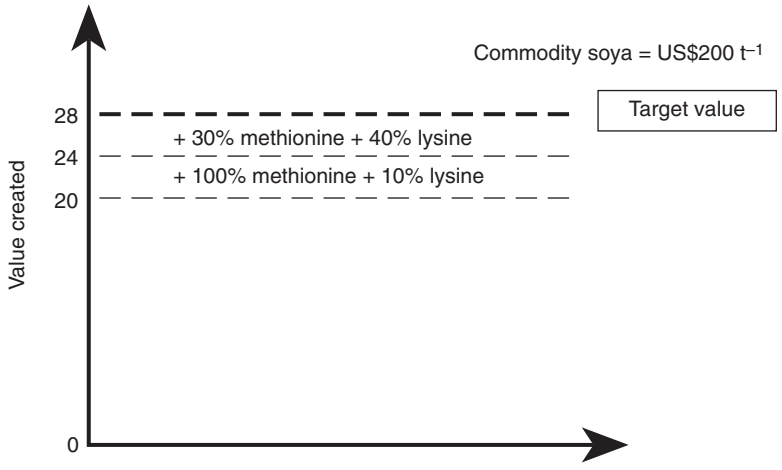

Fig. 2.5. Effects on increasing the levels of lysine and methionine in soybean meal on the additional value of the crop relative to the minimum value required to identity preserve the crop.

IMPROVEMENT OF PHOSPHORUS AVAILABILITY IN GRAIN

There is considerable interest in improving the phosphorus availability in grains, primarily as a means of reducing phosphorus pollution. Excretion of P into the environment results in it leaching into water courses, causing eutrophication of surface waters. Approximately half to two-thirds of the phosphorus in plant materials is present as phytic acid in a form of myo-inositol phosphate. Since monogastric animals do not naturally have a source of phytase in their gut, phosphorus in the form of phytic acid has very low digestibility. Phytic acid binds strongly to many other essential dietary minerals including calcium, zinc, magnesium, iron and copper, reducing their availability in the digestive tract. Major success has been achieved in reducing the problem by the addition of phytase enzyme to the feed of pigs and poultry. A phytase from *Aspergillus niger* has been developed for use in pig and poultry diets as the unique pH optima at pH 2.0 and pH 5.0 allow this enzyme to function well in the pH of the monogastric gut. The enzyme is not heat stable, therefore, to maintain the activity in feed, which may be processed and pelleted using heat, the phytase is sprayed onto the surface of the pellets shortly after they exit the pellet press.

Two alternatives have been suggested to replace the spray application of exogenous enzyme. In the first instance, the phytic acid content of the seed can be reduced, and in the second, phytase-enriched seeds may be produced in the plant. It is worthy to consider these two options, the potential economic value that can be added to the plant, and the risks and benefits that the two processes may bring.

Stilborn and Crum (1997) reported that a low phytic acid mutant maize showed an altered relationship between total phosphorus and phytic acid. The phosphorus released due to the reduction of phytic acid was present as inorganic phosphorus. This resulted in total phosphorus remaining the same, but with

approximately 35% reduction in phytate and 65% increase in available phosphorus. Stilborn and Crum calculated that the estimated additional value per bushel for high available phosphorus (HAP) maize was $0.036, $0.047 and $0.087 for broiler grower, turkey grower and peak lay layers, respectively. This calculation was based purely on the replacement of inorganic phosphorus in the diet, without additional value for the reduced excretion and management benefits. For maize costing $2.25 per bushel, this represents a range in additional value of 1.6 to 3.8% of the maize in diets for broilers and layers, respectively. Comparison of these increases in value with those obtained with HOM demonstrates the complexity of the problem in that it is only when HAP maize is used in diets for layers at peak lay that the added value begins to compete with the value of HOM. Obviously the value for HAP maize cannot compete with HOM unless the two traits can be stacked.

The alternative to reducing the phytic acid content of the grain is to synthesize phytase in the seed. The European scenario with diets based on wheat shows a similar minimal increase in economic value. Presently, phytase is marketed at a cost of £1.10 t^{-1} feed treated. The price of phytase is limited by the cost of the dicalcium phosphate which the phytase replaces and which is added to feed at a cost of £1.18 t^{-1} feed treated. Presently, without legislation to penalize phosphorus excretion, no premium can be added for the benefit of reduced phosphorus pollution. Based on a maximum and minimum price for feed wheat over the past 2 years of £110 and £75.00 t^{-1} respectively and 60% wheat inclusion in the diet, the value of supplying all the additional phytase via endogenous enzyme in the grain is small and only represents an additional value on the price of the grain of 1.6 and 2.4%, respectively. This value is obviously negligible and would not cover the costs of identity preservation of the grain. Such grains would only be of interest as the new commodity grain, and thus the value of the premium for the trait would soon be lost.

Furthermore, quality assurance of either low phytate grain or, in particular, high phytase grain would be a major problem. There are presently no routine assays for phytate and phytase and rapid nutritional evaluation of the crop in the field is required to set a market price. Consideration must also be given to the reliability with which the enzyme content of the grain can be predicted after processing. The present spray application of phytase onto pelleted feed avoids destruction of the enzyme during the application of heat when the feed is processed. There is a tendency for increased use of heat in the production of feed for broilers and pigs, as a means of sterilizing the feed, and temperatures in excess of 105°C are routinely used in expanders and extruders. It would be a prerequisite that the stability of the enzyme could be guaranteed at these high temperatures, in order to allow the routine processing of the feed without any restriction being placed on the feed processing system as a result of using the grain containing the phytase enzyme. Phytase enzymes are recognized to be particularly susceptible to destruction by heat. Under such circumstances the post-pelleting spray application of exogenous enzyme appears to offer the most versatile, cost effective and reliable alternative. These calculations strongly suggest that OPTs such as low phytate grain and the inclusion of phytase in the grain are interesting academic exercises, but have little commercial interest other than the opportunity to stack as part of multiple trait additions.

NUTRITIONAL EVALUATION OF OUTPUT TRAITS

One major concern with respect to commodity cereals is the ability to measure accurately and rapidly the nutritional composition of the grain being fed (Weigel, 1998). Weigel stated that, based on 15,000 samples of maize taken over the previous 10 years, the average protein content was 23.3% lower than literature values. In order to capture the value of a trait, it will be absolutely essential to know the value of the commodity crop and thus on what basis the uplift is being compared. This will bring new challenges to nutrient evaluation and the ability to rapidly and accurately determine specific nutrient values. Whether the added trait is energy, protein or amino acid based, it will be essential that the component can be measured with a degree of accuracy to guarantee a nutritional value different to the commodity product. This evaluation must be capable of being done cheaply and at an early stage in the grain handling process. The objective of the evaluation is somewhat different to classical feed evaluation, which is performed to define a nutrient value for feed formulation. In the new scenario, the evaluation must differentiate between loads of the crop which will be differentially priced before the final nutrient evaluation for feed formulation. Rapid evaluation using near infra-red reflectance spectroscopy (NIRS) may become key to estimating the nutrient value of genetically modified grain.

The question was posed earlier with respect to the amino acid composition of soybean meal, whether the amino acids inserted via genetic engineering were as available as those naturally occurring in the plant. Many factors influence amino acid availability in plants; coefficients of variability for true digestible lysine in poultry range from 9.5% in cottonseed meal to 1.9% in soybean meal (48% protein) (Rhône Poulenc, 1993). Williams (1999) recently reported on the use of NIRS for estimating the ileal digestible amino acids in a range of raw materials. Tests on maize with increased protein content, which was claimed to contain high levels of available lysine and methionine, suggested that whilst all the additional lysine was available, the availability of the additional methionine was not equal to that of the original methionine in the plant. Until the availability of the additional nutrients which are added via genetic engineering has been accurately estimated by the appropriate method, it cannot be assumed that their availability will be equal to that of the nutrient naturally occurring in the plant.

If the development of nutritional output traits is to grow and the full value of the traits is to be realized, the market requires the ability to know where the crop is, in what condition, at what volume and with what nutritional value, almost on an interactive basis. Unless the traits rapidly become commodities, grain analysis must become much more complex and this will be a cost that the traits must carry.

STACKING AND MULTIPLE TRAIT INSERTION

The analysis of agronomic IPTs at the start of this manuscript identified the major benefits they bring for the grower. Therefore, until a combination of IPTs plus OPTs can be inserted and stacked into plants, there will always be competition between the choice of the grower for immediate benefit brought

about by IPTs with the downstream benefits in nutritional value from OPTs which must be shared amongst the participants of the feed production chain. For growers, therefore, it is likely that IPTs will always be the traits of first choice.

Although techniques of gene insertion are now taken as a matter of course, the simultaneous transfer of several genes is less common since technically it is more difficult. However, recent developments suggest that simultaneous insertion of agronomic plus OPTs may soon be possible. Using ballistic bombardment with plasmids, each containing a transgene, Chen *et al.* (1998) succeeded in inserting 14 transgenes into a rice plant. Of the 125 plants grown from the tissues, 85% contained at least two new genes, and 17% contained more than nine insertions. The work demonstrated for the first time that in excess of 300 kb of foreign DNA could be inserted into the plant without affecting its morphology, growth or fertility. Furthermore, the fact that each of the 14 transgenes was inserted into the rice with the same probability demonstrates that the nature of the gene had no influence on its ability to insert into a strange genome. Inserting the gene is one thing, transmission to future generations is an additional challenge. The work of Chen *et al.* demonstrated that, as far as the third generation, there was no preference in the order of insertion on the stability of the 14 genes.

These results offer significant promise for the eventual introduction of combinations of traits. Nutritionally important OPTs, which could not compete alone in terms of value when the benefits from agronomic characteristics are an alternative, will become a more attractive choice when there is the option of stacking both OPTs and IPTs, compared with making a choice between one or the other. The opportunity to stack OPTs, and combine high oil with high essential amino acid content and thus significantly increase nutrient density, will increase the value of grain in least-cost formulations and thus be less sensitive to the changing cost of alternative raw materials. Gene stacking and high nutrient density will be the key to safeguarding the position of genetically modified grain.

CONCLUSIONS

The objective of this manuscript has been to review those factors that are important to the economic evaluation of genetically engineered traits in plants when used to supply nutrients in feed formulation, in particular for pigs and poultry. Least-cost ration formulation is the cornerstone on which feed formulation is based, and this system attributes simple economic values to specific quantities of available nutrients. Nutrients compete with one another on a cost basis. Many of the nutrients that are under consideration for transgenic insertion into plants are presently supplied as commodities with defined prices and nutritional values, prime examples are the synthetic amino acids, lysine and methionine. The presence of such readily available and defined commodities places severe limitations on the additional value that can be gained in plants from OPTs. Furthermore, for certain traits the value is highly sensitive to the price of the crop and to competing raw materials.

Any yield lag associated with speciality crops will be a major negative factor with respect to the adoption of the crops. There are technological limits to the development of high level OPTs and their economic evaluation is complex and influenced by many variables. Few OPTs are universally applicable, and rapid, simple, precise and low-cost methods of analysis are presently not available to quantify the trait on the farm. There are additional hidden costs relative to the handling of the crop that result in a required value over and above that based on the shadow price of the raw material. Contract production, which is not easily accepted by all growers, will be important for the production of crops with quality traits, until the new crop becomes the norm and the new commodity.

On face value, OPTs offer highly attractive new opportunities for nutritionists. The capture of the value is technologically and logistically challenging. If there is insufficient value within a single trait to share among the members of the feed production chain, it will be necessary for traits to be stacked and this will be a limit to the adoption of this new technology. However, all these problems apart, in the present environment it still remains to be seen whether the consumer will accept this new technology, because the controversy alluded to in the introduction has as yet not been settled. Unfortunately, the opposing debate that 'high yield conservation', exploiting high technology methods such as biotechnology in food and forestry, has the potential to protect the planet has not as yet convinced the most liberal of environmentalists (Avery, 2000).

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PART II
Nutritional components of feedstuffs: qualitative chemistry

CHAPTER 3
Carbohydrate chemistry of the feedstuffs used for poultry

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Carbohydrates represent a major part of poultry diets with contents ranging from 40% to 70%. The function of their digestible part is to supply energy to the host. The undigestible fractions of carbohydrates affect essentially the digestive tract, with effects which can be observed on its anatomy and histology, on the transit time, water losses, bacterial development, digestion efficiencies, etc. These functions and effects of carbohydrates vary according to their chemical and physical properties.

The basic units of carbohydrates are monosaccharides of cyclic form (furanose or pyranose), usually bearing six (hexose) or five (pentose) carbons. The unique features of monosaccharides compared to other organic compounds concern their hydrophilicity, due to the high number of hydroxyl groups on the molecule, and their ability to be linked to several compounds (up to four) through glycosidic ether or ester linkages, giving the possibility to form 'arborescent' structures dispersed in the three-dimensional space. These structures, combined with the possibilities of forming intermolecular bridges through covalent, hydrogen or ionic bonds, can result in 'network' superstructures.

The hydrophilicity of monosaccharides underlies the great water-solubility of many carbohydrates and explains the extended form of polysaccharides in water. This extended form explains why water-soluble polysaccharides of high molecular weight can strongly increase the viscosity of aqueous solutions. Thus, the major factor responsible for the viscosity supplied by water-soluble polysaccharides is the length of their molecules.

However, in many cases, because of strong intermolecular bonds, polysaccharides are not soluble in water. These water-insoluble structures, defined by intermolecular bonds, retain their hydrophilic properties and, hence, can attract a great quantity of water. As will be seen below, the occurrence of intermolecular bonds greatly depends on the chemical structures of carbohydrates.

The main chemical classification of carbohydrates is based on molecular weight: (i) the monosaccharides; (ii) the oligosaccharides (degrees of polymerization (DP) from 2 to 10); and (iii) the polysaccharides (DP>10).

 Poultry Feedstuffs: Supply, Composition and Nutritive Value (eds J.M. McNab and K.N. Boorman)

MONOSACCHARIDES

Glucose and fructose, the only monosaccharides occurring in feedstuffs for poultry, are usually very minor components (<1%). The linear configurations of glucose and fructose only differ in the carbonyl group position, which is located on C_2 in fructose and on C_1 in glucose. Thus, glucose and fructose are aldose and ketose, respectively. The reducing property of glucose, as in all aldoses, comes from this aldehyde function. In the alkaline condition, fructose also shows reducing properties because, in such a condition, fructose can be converted into aldehyde. In general, alkaline conditions lead to instability of monosaccharides, with breakage of the cyclic form, conversion of aldehyde and ketone functions into an enolic function and the subsequent risk of epimerization on C_2 with, for instance, the transformation of glucose into mannose.

The stability of monosaccharides is rather strong in neutral and mild acid conditions. In hot concentrated acid solutions, pentoses and hexoses undergo dehydration and transformation into furfural and hydroxymethylfurfural, respectively. These chemical products can react with various compounds such as orcinol or anthrone with the production of a coloured complex.

The reducing properties of monosaccharides and the ability of their dehydrated products to form a coloured complex can be used to measure their concentrations. However, these properties are not fully specific to monosaccharides and, often, do not allow stereoisomers to be distinguished.

Stereoisomers can be distinguished either by using specific enzymatic reactions or by means of gas or liquid chromatography. Gas chromatography requires the derivatization of monosaccharides in order to obtain volatile compounds. The most common derivatization procedures are acetylation of the reduced monosaccharides (Sawardeker *et al*., 1965), and trimethylsilylation (Sosulski *et al*., 1982). It should be noted that gas chromatography of trimethylsilyl derivatives of monosaccharides leads to the separation of their α and β anomers.

Glucose and fructose can be readily absorbed by enterocytes through two different specific carrier systems. Glucose and fructose enter the glycolysis pathway in different ways, which can induce different metabolic responses. However, in contrast to rats, fructose does not induce more fatty acid synthesis than glucose in chickens (Romsos and Leveille, 1975).

OLIGOSACCHARIDES

In general, linkage between two monosaccharides occurs through an ether link involving at least one carbon bearing the enol function of one of the monosaccharides, with one water molecule being eliminated. Thus, a molecule formed from several monosaccharides has no more than one monosaccharide with a reducing property (the reducing end group). Hence, to quantify total oligosaccharides (or polysaccharides) on the basis of reducing properties requires a previous hydrolysis.

Total monosaccharides and oligosaccharides can be separated from polysaccharides by extraction in hot ethanol:water (80:20) followed by decantation. Only a few polysaccharides (arabinans with low molecular weights) are soluble in the ethanolic extract, while all mono- and oligosaccharides are soluble. Decreasing the proportion of ethanol in the ethanol:water mixture results in an increase in the molecular weight of the extracted carbohydrates.

Individual oligosaccharides can be measured using gas, liquid or thin layer chromatography (Sosulski *et al.*, 1982; Quemener and Brillouet, 1983; Carré *et al.*, 1995b).

Disaccharides

The main disaccharide found in feedstuffs for poultry is sucrose (α-D-Glc*p*-(1–2)-β-D-Fru*f*). Its content in feedstuffs is usually lower than 10%. As the carbons bearing the enol functions of both glucose and fructose are involved in the ether linkage between glucose and fructose, the sucrose molecule has no reducing property.

The glycosidic linkage of sucrose can be hydrolysed by the invertase activity of the brush border membrane of enterocytes.

Maltose (α-D-Glc*p*-(1–4)-D-Glc*p*) and isomaltose (α-D-Glc*p*-(1–6)-D-Glc*p*) are essentially found as residues from starch hydrolysis in industrial products. Both maltose and isomaltose can be hydrolysed by the enzymes of the brush border membrane of enterocytes.

Lactose (β-D-Gal*p*-(1–4)-D-Glc*p*) is only found in milk products, where it can reach high concentrations (up to 75% of the dry matter). The digestive system of birds being devoid of lactase activity (Siddons and Coates, 1972), this sugar can only be degraded by bacteria. Such a degradation is probably slow, which probably explains why lactose can cause an increase in water losses (Carré *et al.*, 1995a), presumably due to its high osmotic power on account of its low molecular weight. High osmotic pressure can also be promoted by the organic acids produced by the fermentation of lactose (Carré *et al.*, 1995a).

α-Galacto-oligosaccharides

α-Galacto-oligosaccharides are linearly constituted with one, two or three α-(1–6) linked galactose units bound to the glucose unit of a sucrose moiety, corresponding to raffinose, stachyose and verbascose, respectively. As sucrose, these molecules have no reducing property. Raffinose is found in many plant materials at levels up to 2%. Stachyose and verbascose occur especially in the dicotyledonous seeds and their by-products, with contents that can reach 8 and 4%, respectively. The maximum content of total α-galacto-oligosaccharides that can be found in feedstuffs for poultry is about 12%. It can be estimated that their total content in poultry diets ranges between 0.5% and 4%, with soybean meal being a major source of variation.

As lactose, α-galacto-oligosaccharides cannot be degraded by the intestinal enzymes of chickens (Carré *et al*., 1994a). Thus, the nutritional consequences of α-galacto-oligosaccharides can be expected to be similar to those of lactose.

POLYSACCHARIDES

Polysaccharides in plant feedstuffs for poultry can be classified into non-cell wall components and cell wall components. This classification relies on the location of polysaccharides in plants: intracellular for non-cell wall components or extracellular for cell wall components.

The intracellular plant polysaccharides are essentially starch and fructans, whose main function in plants is to be storage components. Cell wall polysaccharides may have various functions. Many of them contribute to the rigidity, form and permeability variations of cell walls through the intermolecular bridges they can form. Others can also serve for storage with, in general, fewer or even no intermolecular bridges. In the latter case, the polysaccharides are not really bound to the cell wall matrix and are readily soluble in water.

Starch

Native starch occurs as granules with sizes ranging from 5 to 100 μm, depending on their origin. Starch molecules are formed with α-D-glucose units linked through (1–4) or (1–6) glycosidic bonds. α-(1–4) Bonds constitute the basis of backbones, while α-(1–6) bonds represent the branching points of lateral chains. Pancreatic α-amylase is able to hydrolyse most of the α-(1–4) bonds of starch, releasing maltose and branched oligosaccharides as end products. These end products are further hydrolysed by the maltase and isomaltase of enterocytes.

Two kinds of molecules are encountered in starch granules, namely amylopectins and amyloses. Amylopectins are arborescent molecules, while amyloses are mostly linear. DP are lower for amylose than for amylopectins. DP for amylopectins can be higher than 10^6.

Intermolecular hydrogen bonds between starch molecules determine the structure of starch granules. Inside the granules, a high density of hydrogen bonds with regular distribution defines crystalline zones. The variations in the frequency and location of crystalline zones and, more generally, the physical properties of starch granules partly depend on the proportion of amylose. It is generally believed that starch granules with high amylose contents (> 40%) tend to be more resistant than others.

The internal structure of starch granules makes them insoluble in water at 40°C. Thus, enzymatic hydrolysis may be limited due to their insufficient porosity arising from their physicochemical properties. Industrial treatments can alter the structure of starch granules, and, thus, can improve starch digestibility. The only risk in industrial treatments is the formation of retrograded starch. In the condition of a water excess, at 80–100°C, most of the starch granules are dispersed (gelatinization); then, with cooling down to 60–45°C, some intermolecular hydro-

gen bonds appear between the starch molecules, leading to a gel structure. As the temperature decreases further, the number of hydrogen bonds slowly increases, which may result in the formation of aggregates with crystalline structure, called retrograded starch, which is resistant to enzymatic hydrolysis. In the dry conditions used in most of the processes employed for poultry feeds, the temperature required for starch gelatinization is higher (at least 120°C). Thus, for pelleting, the most common process used in practice, only the outer part of the pellet may be affected by gelatinization. Moreover, it should be kept in mind that the proportion of gelatinized starch which is converted into retrograded resistant starch during storage is low (about 5%, Eerlingen *et al*., 1993). Thus, at least for pelleting, the risk of obtaining a significant amount of retrograded starch is negligible.

Several methods exist for starch determination. The polarimetric method, based on acid hydrolysis followed by determination of hydrolysis products by polarimetry, gives reliable data for cereals and slightly overestimated values for legume seeds. Methods based on glucose-releasing amyloglucosidase require a preliminary dispersion of starch granules. Such a dispersion can be made in boiling water (100°C), alkali or hot 95% dimethylsulphoxide (DMSO).

There is some risk of underestimation with boiling water (100°C), especially for retrograded starch and high amylose starch, due to some of the starch molecules remaining undispersed. The advantage in using the DMSO procedure is that it permits the amyloglucosidase step to take place at ambient temperature, as the diluted solution used at this stage (20% DMSO) impedes the formation of starch aggregates at ambient temperature.

Fructans

Fructans occurring in poultry diets are usually those from cereals. The main fructans found in cereals are levans formed with linear chains of β-D-Fru*f* linked by (2–6) bonds, having a terminal non-reducing end of sucrose. These linear chains can be more or less branched. It can be expected from these chemical structures that fructans will only be degraded by bacteria in the digestive tract of birds.

Molecular weights of fructans are not really high (<50,000) (Pollock and Chatterton, 1988) compared to many other polysaccharides. Some fructans can even be considered oligosaccharides. This probably explains in part the high water-solubility of fructans. Their rather low molecular weight means that their ability to produce high viscosity is limited.

Fructans are usually measured as the fructose released from acid hydrolysis of a water extract, corrected for fructose deriving from sucrose and α-galacto-oligosaccharides. The highest contents that can be expected in cereal grains for poultry are below 3% (Bach Knudsen, 1997).

Cell Wall Polysaccharides

Plant cell walls are extracellular semi-rigid structures surrounding each cell and connecting them together. Cell walls are formed by intermolecular associations

of various kinds of polymers including lignins, proteins and polysaccharides. Lignins (0–20%) and proteins (1–10% in cell walls) are usually minor components in cell walls.

The compositions of cell wall polysaccharides (CWP) vary widely inside cell walls, between types of cells and between plant species. CWP can be classified into cellulose, hemicelluloses, pectic substances and galactomannans.

Cellulose

Cellulose occurs in all cell walls where it represents 5% to 95% of whole polymers. Cellulose is constituted with very long chains of β-(1–4)-D-Glc*p* that may reach more than 10,000 units. This great regularity permits the formation of strong hydrogen bonds between cellulose molecules, resulting in the formation of water-insoluble crystalline microfibrils. Acid hydrolysis of this resistant structure requires the use of concentrated acids such as 72% sulphuric acid. Solubilization of cellulose is also very difficult, as even hot alkali treatments are not efficient. Hot *N*-methylmorpholine *N*-oxide, a solvent that can solubilize all cell wall polysaccharides (Joseleau *et al*., 1981), can be considered an efficient solubilizing agent for cellulose.

Hemicelluloses

The hemicelluloses which can be found in cell walls of feedstuffs for poultry include heteroxylans, (1–3, 1–4)-β-D-glucans, xyloglucans and mannans. When they have sufficient regular zones, these molecules are often linked with cellulose microfibrils through hydrogen bonds. They can also be bound to lignin by various covalent linkages, with a phenolic acid as an intermediate compound for example. The hemicelluloses involved in intermolecular bonds are water-insoluble at neutral or weakly acid pH ($3 < pH < 7$). Alkali treatments are able to split hydrogen bonds and the ester links responsible for some of the connections with lignin, which allows insoluble hemicelluloses to be solubilized.

When hemicellulose-related molecules have no intermolecular associations, they can be water-soluble without alkali treatment: this can be observed in some native arabinoxylans and (1–3, 1–4)-β-D-glucans of cereal grains.

Heteroxylans are formed with a β-(1–4)-D-Xyl*p* backbone bearing short side chains connected to C_2 or C_3 of xylose units. Heteroxylans are found both in dicotyledonous and monocotyledonous plants. The main heteroxylans in dicotyledonous plants are 4-*O*-methyl-glucuronoxylans or 4-*O*-methyl-glucuronoarabinoxylans; in these heteroxylans, the side chains are often constituted from single units of α-L-Ara*f* or (4-Me)-α-D-Glc*p* A. Concerning poultry feedstuffs, these heteroxylans can be found in the hulls from seeds and protein seed meals, and in lucerne and linseed meals.

α-L-Ara*f* units are practically always found in the heteroxylans of cereal grains. It may be noted that the bonds involving an α-L-Ara*f* unit are very sensitive to acid hydrolysis, as hydrolyses can be observed up to pH 3. Arabinoses often occur as single units in the lateral chains of these heteroxylans. A xylose or a galactose unit may also be connected to the lateral arabinosyl units; sometimes, a phenolic acid (ferulic or coumaric) can be bound to C_5 of arabinose through an ester link. Other side chains, including glucuronic acid, galactose or xylose, can also be found in the heteroxylans of cereals (Bacic *et al*., 1988).

Water-solubility of heteroxylans depends in part on the frequency of their side chains: a limited number of side chains favours hydrogen bonding either to cellulose or between xylan molecules and, hence, impedes water-solubility.

In cereals, water-solubility can also be impeded by covalent cross-links between arabinoxylan molecules, resulting from dimerization of the ferulic acids esterified on arabinose units. Ferulic acid (or coumaric acid) is also involved in the bonding between arabinoxylans and lignin in cereals, making these arabinoxylans insoluble in water. A great variability in the water-solubility of wheat arabinoxylans has been observed (0.36–0.83% water-soluble arabinoxylans in whole wheat dry matter; Saulnier *et al.*, 1995). It cannot be excluded that this variability comes from variations in cross-links between arabinoxylans, due to variations in the number of diferulic acid bridges. Cereal water-soluble arabinoxylans are not able to form a gel, except on addition of an oxidizing agent to the solution, which promotes the dimerization of ferulic acids from two different arabinoxylan molecules (Geissmann and Neukom, 1971).

In wheats, the variability in water-soluble arabinoxylan content is a major factor which explains the variation in potential applied viscosity (PAV) (R^2 = 0.92, n = 22) (Carré *et al.*, 2002) (for the definition of PAV, see below 'Chemical and physical analyses'). This is not surprising as the molecular weights of wheat water-soluble arabinoxylans do not show great variation between varieties (Cleemput *et al.*, 1993). It is notable that these molecular weights reached high values (>800,000) (Cleemput *et al.*, 1993). The PAV variability of wheats has been shown to depend essentially on the variety (Oury *et al.*, 1998).

In the dicotyledonous samples used for poultry feeding, heteroxylans are often weakly water-soluble, except in linseeds. Linseeds exhibit high levels (about 3%) of water-soluble heteroxylans (Cui *et al.*, 1994), which account for a major part of the viscosity measured for the linseed water extract (Cui *et al.*, 1994). The PAV value of whole linseeds was observed to be very high (18.5 ml g^{-1} DM; Bureau *et al.*, 1995) compared with wheat PAV values (1.6–5.7 ml g^{-1} DM; Oury *et al.*, 1998), which has to be related in part to water-soluble heteroxylan content which is much higher in linseeds than in wheat (see above). Linseeds show other peculiar features concerning viscosity: the real applied viscosity (RAV) is about the same as the PAV value (Bureau *et al.*, 1995), while RAV values of cereals are always lower than their PAV value (Carré *et al.*, 1994b). This means that, in contrast with cereals, linseeds do not contain enzymes able to hydrolyse their water-soluble heteroxylans. Moreover, a commercial enzyme preparation was observed to be much more active on water-soluble heteroxylans from wheat than on those from linseeds (Carré and Gomez, 1996, unpublished data). It cannot be excluded that this is a result of differences in the distribution of branching points on the xylose backbones, with wheat arabinoxylans perhaps having greater lengths of xylose segments with no branching point. Another explanation might be that the complexity of the lateral chains of linseeds heteroxylans impedes the activity of arabinose debranching enzymes. This comparison between enzyme sensitivities of heteroxylans from wheat and linseeds illustrates the importance of lateral chains, which may impede hydrolysis of polysaccharide backbones and, hence, the reduction of viscosity by enzymes.

(1–3, 1–4)-β-Glucans are linear polysaccharides that can display high molecular weight (1,000,000). These polysaccharides are for the most part only found in cereals. They have, in general, few units with repeated β-(1–4) linkages, which gives few possibilities for intermolecular linking by hydrogen bonds. Thus, some of them can be found as water-soluble components, for example in barley: the water-soluble β-glucan content of barley was found to be about 1% (Graham *et al*., 1988). The high PAV value of barley (19 ml g^{-1} DM) compared with wheat (3.4 ml g^{-1} DM) (Carré *et al*., 1994b) is probably accounted for by water-soluble β-glucans. The extended form of these polysaccharides, arising from their long linear irregular chains, accounts for a great part of their ability to create high viscosity. However, barley also contains water-soluble arabinoxylans (0.4%; Graham *et al*., 1988) which probably also contribute to the viscosity of the barley water-extract. It is noteworthy that viscosity is the main physical property of water-soluble β-glucans. Their ability to form a gel is generally considered to be rather low. The lack of branching points in β-glucans makes them very sensitive to hydrolysis by β-glucanases. Thus, in most cases, these enzymes should be efficient at reducing the viscosity induced by β-glucans.

Xyloglucans can be found as minor components in dicotyledonous seeds. They comprise (1–4)-β-D-Glc*p* backbones bearing, on C_6, short side chains consisting of α-D-Xyl*p*, β-D-Gal*p* and a-L-Fuc*p*. Xyloglucans are thought to be linked to cellulose by hydrogen bonds.

Mannans, constituted with (1–4)-β-D-Man*p* linear chains, are major components in the cell walls of palm-kernel and copra meals. Practically all mannans were found to be water-insoluble in copra meal (Brillouet *et al*., 1988). In other feedstuffs for poultry, these polysaccharides are often lacking, or occur as very minor components.

Pectic substances

Pectic substances are generally formed with backbones of (1–4) linked α-D-galacturonic acids interspersed by (1–2)-α-L-Rha*p*, bearing more or less extended side chains mainly consisting of several β-galactose or α-arabinose units. These side chains are often linked to the backbones through the C_4 of rhamnosyl units. Small amounts of free neutral polysaccharides, with structures similar to those of side chains, can also be found. Since they are not bound to the rhamnogalacturonan backbone, these free neutral polysaccharides are mostly water-soluble, as in rapeseed meals which contain noticeable amounts of water-soluble arabinosyl polymers (0.6%; Brillouet *et al.*, 1988).

The arabinose and galactose moieties of the side chains are organized either as more or less branched (1–4)-β-D-Gal*p* backbones or as highly branched (1–5)-α-L-Ara*f* backbones, the lateral chains on these backbones mainly consisting of one or several (1–5)-α-arabinosyl units.

Pectic substances are generally only found in cell walls from dicotyledonous plants. They do not occur in cereals. However, pectic substances were mentioned as being present in the cell walls of the starchy endosperm of rice (Shibuya and Iwasaki, 1978). Pectic substances are especially present in cotyledon cell walls, where they occur both as structural and as storage components utilized during seed germination. In cotyledon cell walls, most of them are

water-insoluble. This water-insolubility is due in part to intermolecular ionic bridges formed through Ca^{2+} cations interacting with the carboxylic groups of galacturonic acids. The existence of these calcium bridges requires incomplete methyl-esterification of galacturonic acids and a sufficient length for unbranched galacturonan segments.

When galacturonic acids are methyl-esterified, they become very sensitive to β-elimination, which can take place, in that case, at neutral or alkaline pH and boiling temperature (Albersheim *et al.*, 1960; Thibault, 1980). Such β-eliminations induce solubilization of pectic substances and a reduction in their molecular weights. Pectic substances can also be solubilized by a calcium chelating agent such as ethylenediaminetetraacetate (EDTA).

Using extraction conditions which minimize the risk of β-eliminations, the amount of water-soluble pectic substances is generally rather low in feedstuffs for poultry (<1%), except for rapeseed meal (1.4%), lucerne meal (1.5%) and lupin seeds (4.8%) (Brillouet *et al.*, 1988). For the latter three feedstuffs, PAV values might be expected to be in the range of that found for wheat. Comparing respective water-soluble polysaccharide contents and PAV values (Carré *et al.*, 1994b), it seems that the viscosity-inducing ability of water-soluble pectic substances of protein meals is somewhat lower than that of water-soluble arabinoxylans and β-glucans from cereals.

Galactomannans

Galactomannans consist of (1–4)-β-D-Man*p* backbones bearing single α-D-Gal*p* side chains attached to C_6 of mannose units. The number of branching points is often rather high, which makes them readily water-soluble. Galactomannans occur especially in legume seeds. However, in feedstuffs for poultry, they are usually very minor components. Noticeable amounts of water-soluble galactomannans were only found in soybean hulls (0.9%; Brillouet *et al.*, 1988). These polysaccharides probably explain the PAV value found for soybean shells (5.9 ml g^{-1}; Carré *et al.*, 1994b).

Chemical and physical analyses

Chemical and physical analyses of cell wall materials can be done at various levels of detail, depending on the aim and on the amount of information available.

Analyses of intra- and intermolecular bonds generally require complicated methods, such as selective chemical fractionations, gas chromatography coupled to mass spectrometry for analyses of methylated and acetylated monosaccharides, nuclear magnetic resonance studies, liquid chromatography of oligosaccharides released from enzyme hydrolysis, etc.

These studies are carried out in order to explain the cell wall organization, the mechanisms of plant cell growth, the physical properties of cell walls or isolated polysaccharides, or the sensitivity of polysaccharides or whole cell walls to degradation by enzymes or bacteria. In the field of nutrition science, the latter factor is a matter of consideration. However, concerning poultry, we have to keep in mind that bacterial degradation of cell walls targets essentially the water-soluble fraction (Carré *et al.*, 1995b). The study of sensitivity of cell walls to enzyme activities is another factor to consider in poultry nutrition as dietary

enzymes may be used to reduce the viscosity induced by water-soluble polysaccharides. One of the questions that can be raised concerning the use of enzymes is their ability to release cell wall materials into solution, which will depend on the chemical structure of the cell wall.

Other types of analyses quantify cell wall fractions. They are often used in poultry nutrition to estimate the dietary content of the cell wall undigestible fraction. In poultry, this fraction has been demonstrated to be close to water-insoluble cell walls (WICW) (Carré, 1993, for review; Carré *et al*., 1995b). However, many methods used in practice only supply values which are correlated to WICW. For instance, 'crude fibre' corresponds to cellulose added to a lignin fraction. 'Neutral detergent fibre' (NDF) is the measurement of WICW without the water-insoluble pectic substances, as EDTA is used to obtain the NDF residue. Thus, for cereals, NDF and WICW values are rather close, but for dicotyledonous plant materials, NDF values are lower than WICW (Carré and Brillouet, 1986).

Crude fibre and NDF methods are generally used because they are supposed to be quick. However, it is now possible to use a rapid practical method which supplies a direct measurement of WICW (Carré and Brillouet, 1989). The WICW methodology (Carré and Brillouet, 1989) aims to minimize the splitting of intra- and intermolecular bonds, especially in pectic substances which are very sensitive to pH and temperature (see above 'Pectic substances'): the amylolysis step at 100°C is as short as possible (10 min.) and is carried out at pH 5.6; the proteolysis step at pH 7.5 takes place at 40°C. Table 3.1 shows WICW contents for some feedstuffs used in poultry diets.

As WICWs are not degraded in the digestive tract of birds, they keep their physical properties throughout the digestion process. Thus, such physical properties may affect the properties of digestive contents and excreta: for instance, the water retention capacity of WICW (WRC_{WICW}) was observed to be correlated with the visual aspect of excreta (Carré *et al*., 1995c). A rapid method of WRC_{WICW} determination has been described (Carré *et al*., 1994b) and was observed to be additive (Carré *et al*., 1995c). However, WRC_{WICW} in diets also depends on the intensity of pelleting, the highest outlet temperatures resulting in a decrease in WRC_{WICW} (Carré *et al*., 1995c). This is probably due to a reduction in the space between cell wall molecules induced by pressure and water evaporation.

WRC_{WICW} varies greatly between feedstuffs (Table 3.1), according to the composition of cell walls. Lignin, a hydrophobic polyphenol polymer, induces a decrease in WRC_{WICW}. The effect of polysaccharides on WRC_{WICW} is very variable. Polysaccharides in WICW entrap water according to the space between them, which depends on the frequency of intermolecular bonds. It can be estimated that this frequency generally decreases in the order cellulose > hemicelluloses > pectic substances (see above). Thus, WRC_{WICW} varies also according to the relative amounts of the three types of polysaccharides. For instance, the high WRC_{WICW} value for soybean meal has to be related to low lignin and high pectic content of cell walls (Carré and Brillouet, 1986). On the other hand, the low WRC_{WICW} value for oats relates to a high lignin content and the absence of pectic substances in cell walls (Carré and Brillouet, 1986).

Table 3.1. WICW: water-insoluble cell wall content. WRC_{WICW}: water retention capacity of water-insoluble cell walls. PAV: potential applied viscosity. RAV: real applied viscosity. All data are expressed on a dry matter basis. Data are unique or mean values. Those in italics indicate minimum and maximum values and the number of samples within parentheses. WICW and WRC_{WICW} data are from Carré *et al.* (1995c), and PAV and RAV data are from Carré *et al.* (1994b), except for: [1](Oury *et al.*, 1998), [2](Svihus *et al.*, 2000), [3] (Bouguennec *et al.*, 1998), [4](Bastianelli, 1995), [5](Bureau *et al.*, 1995).

Ingredients	WICW (%)	WRC_{WICW} (g^{-1})	PAV (ml g^{-1})	RAV (ml g^{-1})
Maize	10.0	8.6	0.6	
Wheat	11.6[1]	8.6	3.2[1]	1.8[1]
	10.0–12.8 (121)[1]		*1.6–5.7 (121)*[1]	*1.0–3.4 (121)*[1]
Barley	18.0	7.6	19.7[2]	9.7[2]
			10.0–35.0 (20)[2]	*5.2–16.7 (20)*[2]
Triticale	13.3	9.5	4.1[3]	2.5[3]
			2.8–5.6 (30)[3]	*2.0–3.4 (30)*[3]
Sorghum (low tannin)	8.0	9.3	0.3	
Oat	31.0	4.3	20.7[2]	7.7[2]
			7.3–37.1 (20)[2]	*4.4–10.9 (20)*[2]
Rye	13.6	10.8	27.2	
Tapioca root	5.8	18.9	0.4	
Smooth peas (no tannin)	14.9[4]	12.7	0.7	
	12.9–16.6 (36)[4]		*0.3–1.5 (19)*	
Faba beans	16.5	10.3	1.5	
Whole soybean (toasted)	16.8	17.1	1.4	
Whole sunflower	27.1	7.3	0.6	
Whole rapeseed	20.9	10.4	0.7	
Whole linseed			18.5[5]	17.3[5]
Soybean meal (52.6% CP/DM)	18.9	16.0	2.0	
Sunflower meal	43.3	10.1	1.4	
Rapeseed meal	35.8	10.9	1.3	
Linseed meal			38.1[5]	36.9[5]
Maize gluten	1.5	3.5	0.3	
Wheat white shorts	18.5	10.1	4.5	
Maize gluten feed	36.5	5.9	1.3	
Maize distiller	32.4	6.9	3.7	
Lucerne meal (21% CP/DM)	38.6	17.8	2.3	
PX1	7.1	9.6	0.4	
Soybean husks	56.8	7.8	5.9	
Sunflower husks	81.6	5.3	0.6	
Rapeseed husks	66.0	5.7	0.7	

Another important property of cell walls is their possible effect on hardness of seeds and grains. Hardness affects the particle size after milling and, thus, may affect digestibility, especially of dicotyledonous seeds such as soybeans (Mitchell *et al.*, 1972), rapeseed (Shen *et al.*, 1983), peas (Carré *et al.*, 1991)

and faba beans (Lacassagne *et al*., 1991). For cereals, the effect of particle size on digestibility has only been identified for wheat bran (Saunders *et al*., 1969). This difference between dicotyledonous seeds and cereal grains might be related to differences in the cause of hardness: while cell walls probably contribute to the hardness of dicotyledonous seeds, their role is minor in the hardness of cereal grains such as wheat where the starch–protein association seems to be the most important factor (Barlow *et al*., 1973). However, there is no method at present for estimating the effect of cell walls on the hardness of dicotyledonous seeds.

Concerning poultry nutrition, the other point of interest in studying cell wall polysaccharides relates to their water-soluble fraction, as this may increase the viscosity of gut content, which may decrease nutrient digestibilities. Hence, from a nutritional point of view, the main interest about this fraction concerns its viscosity, not its amount or composition.

Thus, a method was specially developed to determine water-extract (pH 4.5) viscosity for any plant materials used in poultry diet formulations (Carré *et al*., 1994b). The pH (4.5) and temperature (19–23°C) used for the extraction were chosen in order to minimize chemical breaks of inter- and intra-molecular bonds. The extraction was done with or without pretreatment in hot ethanol:water (80:20). In the first case, plant enzymes are inactivated before the extraction and the measured viscosity is called 'potential viscosity'. In the second one, enzymes may be active (especially at pH 4.5, which is often rather close to the optimum pH of polysaccharide-hydrolysing enzymes) and the measured viscosity is called 'real viscosity'. The viscosity data are divided by the viscosity of the buffer, which gives relative viscosities (Vr), transformed into natural logarithms and then divided by the concentration (g ml^{-1}) of the plant material in the buffer extraction volume. The results '(Ln(Vr)) / (g ml^{-1})' expressed as ml g^{-1} are called 'applied viscosities', which leads to 'potential applied viscosities' (PAV) and 'real applied viscosities' (RAV). Inactivation of enzymes prior to water extraction has also been achieved by heat treatment in an autoclave (Svihus *et al*., 2000).

It should be mentioned that the viscosities measured in water-extracts of feedstuffs for poultry are rather low in many cases (< 2 mPa s), and hence require sensitive viscosimeters that can determine viscosities close to that of water (1 mPa s at 20°C). The mathematical transformation into 'applied' viscosity is done in order to obtain data that do not depend on the material concentration in the extraction volume, and which can be added together to enable diet formulation based on viscosity value (Fig. 3.1; Carré *et al*., 1994b). However, for RAV values, the additivity is not so certain because plant enzymes may have cross activities between feedstuffs.

When additivity calculation is used to estimate diet PAV values, a slight underestimation may occur if the diets are pelleted at a high temperature (about 100°C) in the die (Carré *et al*., 1994b), which is probably due to high shear and pressure stresses resulting in bonds being broken inside water-insoluble cell walls and subsequent release of polysaccharides into solution. High temperatures (> 90°C) in the die may also result in the inactivation of plant enzymes, and hence, increased RAV values (Carré *et al*., 1994b). On the basis

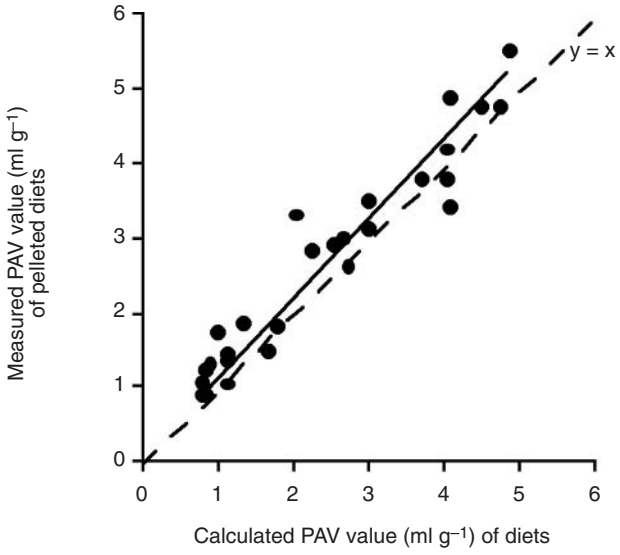

Fig. 3.1. Comparison between measured potential applied viscosities (PAV) of 27 pelleted diets and PAV values calculated on the basis of PAV values of ingredients, and use of the additivity principle. Figure after Carré *et al.* (1994b).

of a previous experiment (Carré *et al*., 1994b), diet RAV values can be predicted using the calculated diet PAV values (PAV_{cal}) and temperatures (T, °C) in the die, according to the following formula:

$RAV = 1.24 \times 10^{-5} \times (PAV_{cal} \times T)^2 + 0.012 \times T$; $R^2 = 0.78$; R.S.D. = 0.46; $n = 27$

With: $68 < T < 100$; $0.78 < PAV_{cal} < 4.84$

For cereals, RAV values are practically always lower than PAV values, especially for barley and oats (Table 3.1).

The positive correlations between turkey-poult water intake and *in vitro* viscosities were somewhat higher with RAV (r = 0.78) than with PAV (r = 0.68) (Carré *et al*., 1994b), which suggests plant enzymes remain active in the digestive tract of birds.

Cell wall-related analyses are sometimes done on digestive contents or excreta. In such cases, we have to keep in mind that gravimetric methods (crude fibre, NDF, WICW, etc.) were set for feeds, not for other materials. In excreta, uric acid may remain insoluble in the solutions used for solubilizing starch and proteins, which may lead to overestimated values. The other bias in using gravimetric methods may concern the comparison of data from excreta or digestive contents with those from feeds in order to calculate digestibilities. The gravimetric methods may lead to overestimated values in the feeds if feed grinding is not sufficient, because starch and proteins will remain in the residues. For instance, grinding on a 1 mm screen instead of 0.5 mm increased NDF contents by 4.0, 1.5, 7.5, 2.0 and 0%, for maize, wheat, peas, rapeseed meal and soybean meal, respectively (Carré *et al*., 1988). However, in

intestinal contents and excreta, levels of starch and protein are much lower than in feeds. Therefore, contamination by residual starch and protein may be much less than in feed residues, which may result in overestimation of the digestibility of cell wall components. In other words, gravimetric methods are not totally specific.

Thus, for digestibility measurements, other methods of high specificity may be preferred, such as determination of individual sugars released by acid hydrolysis (Carré and Leclercq, 1985; Longstaff and McNab, 1986; Slominski *et al*., 1994; Carré *et al*., 1995b). For individual neutral sugars, gas chromatography of acetylated reduced sugars (Sawardeker *et al*., 1965) is mostly used. Determination of total uronic acids may be carried out with colorimetric methods such as that described by Blumenkrantz and Asboe-Hansen (1973), which is of satisfactory specificity. The main difficulties in using methods based on individual sugar determinations lie in the possibilities of confusion with starch or oligosaccharides. Classically, starch and oligosaccharides are separated from non-starch polysaccharides (NSP) by enzyme hydrolysis of starch and subsequent fractionation by ethanol:water (80:20), in which oligosaccharides and starch hydrolysis products are supposed to be soluble, while total NSPs remain insoluble. However, some residual starch may remain in the ethanol:water insoluble fraction, which may require a correction of glucose data for contaminating starch.

There are two possibilities to quantify NSPs in water-insoluble and water-soluble fractions. A unique fractionation scheme may be used, or two fractionation schemes, with one devoted to the insoluble fraction and the other to the soluble fraction. In the unique scheme, the risk of relative error is low for total NSPs, a little bit higher for water-insoluble NSPs and very high for water-soluble NSPs (Graham *et al*., 1988), because of the degradative processes used to separate starch from NSPs. These processes may result in the release of NSPs from the water-insoluble fraction into the water-soluble one (Graham *et al*., 1988). The risk of relative error is much higher for water-soluble NSPs than for water-insoluble ones because, for most poultry feedstuffs, the proportion of water-soluble NSPs in total NSPs is low (< 20%; Brillouet *et al*., 1988; Graham *et al*., 1988). Considering the importance of water-soluble NSPs in poultry nutrition, the use of a unique scheme does not seem appropriate.

The interest in using two schemes lies in the fact that the fractionations can be specially adapted to the aim of the analyses. In a scheme adapted to water-soluble NSPs, the separation from starch can be done by aqueous extraction at ambient temperature, as the bulk of starch is not extracted. Hence, at ambient temperature and appropriate pH (4–6), the risks of cell wall degradation are limited and the risks of overestimation of water-soluble NSPs are decreased. Water-soluble NSPs can be separated from mono- and oligosaccharides using an ethanol:water (80:20) fractionation either before or after the aqueous extraction. It should be noted that treatment in boiling ethanol:water (80:20) does not induce a subsequent solubilization of starch at ambient temperature. Water-soluble NSPs can be quantified by gas chromatography of neutral individual sugars or by colorimetry or enzymatic determination (McCleary and Glennie-Holmes, 1985; Rouau and Surget, 1994). A correction for residual starch may prove necessary to avoid any confusion concerning glucose data from gas chromatography.

In a scheme specially adapted to water-insoluble cell wall components, it is not necessary to hydrolyse starch up to a complete transformation into small components soluble in ethanol:water (80:20): the fractionation only requires an aqueous solubilization of starch, which may readily be done using a short step at 100°C with the termamyl α-amylase. In addition to a time benefit, this short treatment reduces the risks of cell wall degradation, compared to the long-term treatments which are necessary for hydrolysing starch up to ethanol:water (80:20)-soluble components.

The amounts of water-soluble NSPs and WICW obtained from two separate schemes can be added in order to obtain the total 'NSP + lignin + cell wall proteins'. However, this total may be slightly underestimated. In comparison, a unique scheme of fractionation supplies more accurate data for the total. However, the knowledge of this total is of low nutritional interest compared to that of water-soluble NSP content on one hand, and that of WICW content on the other, as the nutritional implications of these two fractions are different in poultry. Thus, from a nutritional point of view, the greatest accuracy should be required for each of these two separate fractions, not for their sum, which would justify the choice of two specially adapted separate schemes.

CONCLUSION

The knowledge of chemical and physical features of diets is important in judging their nutritional consequences. However, in some cases, an animal response may correspond to a combination of so many dietary factors that it becomes difficult to distinguish them. In these cases, the prediction of animal responses based only on chemical and physical analyses of diets may be limited, either because some analyses are missing or because there are difficulties in building models that take account of interaction complexities. In such conditions, *in vitro* simulation systems could be useful. But the simulation approach presupposes that limiting physiological processes are known. Concerning poultry digestion, many points still require elucidation, such as first steps in the gizzard and the last ones involving enterocyte enzymes and carriers. More research in these areas would contribute to the development of efficient *in-vitro* systems for digestion simulation, which is needed when the physicochemical approach becomes too complex.

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CHAPTER 4
Nutritional components of feedstuffs: a qualitative chemical appraisal of protein

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ABSTRACT

Unlike plants, poultry and other animals cannot synthesize all 22 amino acids found in their tissue proteins. In addition, some amino acids cannot be produced *in vivo* at a rate fast enough to maximally support growth or other productive processes, such as egg production. Thus, 10–12 indispensable amino acids must be obtained from the diet. In addition, an adequate level of dietary non-specific nitrogen (dispensable amino acids) is also required for optimal growth and production. Since the scientific feeding of poultry involves the use of combinations of at least two or more feedstuffs (at minimum, one energy concentrate and one protein concentrate) in order to provide an adequate dietary level of both bioavailable indispensable amino acids and nonspecific nitrogen, a detailed knowledge of ingredient amino acid contents is requisite. This review will discuss, from a qualitative standpoint, the amino acid compositions of the most common poultry feedstuffs used worldwide. Where appropriate, relative deficiencies and/or excesses of indispensable amino acids will also be emphasized. Brief synopses on the prediction of feedstuff amino acid contents and the future role of biotechnology in improving plant protein quality are also presented.

INTRODUCTION

Derived from the Greek word *proteios*, meaning 'first' or 'of primary importance', the term *protein* is a collective one which encompasses an enormous group of closely related but physiologically distinct members. Proteins are polymers of amino acids and consist of one or more polypeptide chains. Although

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over 200 amino acids have been isolated from biological materials, only 20–22 of these are commonly found to be present in proteins (Maynard *et al*., 1979; McDonald *et al*., 1995). Moreover, each individual protein differs from all other proteins with regard to amino acid sequence (primary structure) and the manner in which the amino acid strands are connected to each other (secondary, tertiary, and quaternary structures). In many instances, specific proteins may exhibit unique secondary–tertiary structures that confer physicochemical properties which permit them to fulfill specific biological functions (Rodwell, 1996). Since a discussion of protein structure, function, and classification is beyond the scope of this review, the reader is referred to a general biochemistry text (e.g. Zubay, 1993; Stryer, 1995; Murray *et al*., 1996) for further information.

From a nutritional quality standpoint, amino acid content is the important distinguishing feature of a protein. However, individual plant and animal products are composed of many different proteins and, as a result, the overall amount of each of the approximately 20–22 different amino acids present in a feedstuff are dictated by the relative proportions of the various individual proteins present, which is genetically controlled.

AMINO ACID CONTENT OF POULTRY FEEDSTUFFS

Over 2000 different products (varietal differences excluded) have been characterized to some extent for animal feeds (Pond *et al*., 1995; Kellems and Church, 2002). In addition, the composition of a given feedstuff may vary widely due to differences in climate, soil conditions, maturity, cultivar, management and processing factors (Miller *et al*., 1964; Lilburn *et al*., 1991; Ravindran and Blair, 1991; Pond *et al*., 1995; Dale, 1996; Bath *et al*., 1998). Since detailed tabular information on the protein/amino acid contents of a multitude of nonruminant feedstuffs can be found in a variety of recent publications (Institut National de la Recherche Agronomique, 1989; Ministry of Agriculture Fisheries and Food Standing Committee on Tables of Feed Composition, 1990; Empresa Brasileira de Pesquisa Agropecuária, 1991; Ravindran and Blair, 1991, 1992; National Research Council, 1994, 1998; McDonald *et al*., 1995; Leeson and Summers, 1997; Bath *et al*., 1998; Centraal Veevoederbureau, 1998; Dale, 1998), this review will focus on the more common poultry feedstuffs and the data presented will consist of 'average' concentrations of amino acids most likely to be present, expressed on both an 'as-fed' basis and as a percentage of protein. Where appropriate, relative deficiencies and/or excesses of indispensable amino acids will also be emphasized. Moreover, in recognition of the increased availability and current widespread use of alternative feed ingredients by the poultry industry (e.g. bakery meal, wheat middlings, meat and bone meal, spent hen meal, poultry by-product meal, feather meal, blood meal, and various non-soybean plant protein sources), a special attempt was made to cite recent key references pertaining to these feedstuffs.

While it is recognized by the author that: (i) amino acid content does not necessarily equate with amino acid availability; and (ii) that a plethora of antinutritional factors that may affect amino acid availability and/or protein diges-

tion exist in many of the plant feedstuffs to be discussed, these two important topics are addressed elsewhere in this volume. Thus, their coverage in the present review will also be purposely limited. Lastly, readers interested in analytical methodologies for the determination of amino acids in feedstuffs are referred to three excellent publications: *Official Methods of Analysis* (Association of Official Analytical Chemists, 1990), Llames and Fontaine (1994) and Williams (1994).

ENERGY CONCENTRATES

In Europe and North America, energy concentrates (<20% CP (crude protein); Pond *et al*., 1995) used in poultry diets primarily consist of cereals and their by-products, while elsewhere roots and tubers, fruits and their by-products and other ingredients serve as cereal substitutes (Ravindran and Blair, 1991). With regard to cereals, maize is the most common energy feedstuff fed to poultry worldwide (Leeson and Summers, 1997), although substantial amounts of sorghum, wheat, barley, and rice/rice by-products are also used in poultry diets when price and supply allow for their inclusion. Other cereals fed on a much more limited basis include oats, rye, triticale and millet. Among the non-traditional (non-cereal/cereal by-product) energy feedstuffs, cassava root meal is particularly noteworthy because of its widespread use in poultry diets, particularly in Europe. For nutrient compositions of some lesser used by-products and unusual feedstuffs see Ravindran and Blair (1991) and Bath *et al*. (1998).

The CP content of feed grains is relatively low (8–12%; Table 4.1), although there are exceptions (e.g. wheat, which may sometimes be as high as 22%; McDonald *et al*., 1995; Pond *et al.*, 1995). However, because cereals and other energy supplements constitute ~60–70% of poultry diets, they typically supply a nutritionally important amount (~25–40%) of the total dietary protein (Lilburn *et al*., 1991; Ravindran and Blair, 1992). Thus, despite the fact that oilseeds might be more obvious candidates for genetically mediated increases in the relative richness of select indispensable amino acids (e.g. methionine in soybeans), analogous modifications in cereals would also be expected to have a significant impact on both the feed and poultry industries.

Of the nitrogenous compounds present in cereals, 85–90% are in the form of proteins (McDonald *et al*., 1995). In terms of dietary indispensable amino acid requirements of poultry, most cereal grains are moderately low to deficient in lysine, tryptophan, threonine and methionine (Scott *et al*., 1982; Ravindran and Blair, 1991; Pond *et al*., 1995). According to McDonald *et al*. (1995), the value of cereal proteins for promoting growth in young chicks is in the following order: oats>barley>maize=wheat. The relatively high ranking of oat protein for growth has been attributed to its slightly higher lysine content (Tables 4.1 and 4.2).

The amino acid composition of protein in a specific grain does not remain constant as protein concentration changes. For example, inverse relationships between protein content and the relative lysine-, arginine-, methionine- and cystine-richness of the protein, due to a shift among the major proteins within

Table 4.1. Crude protein (CP) and amino acid contents (%; as-fed basis) of selected energy concentrates.

Feedstuff[1]	CP	Arg	Gly	Ser	His	Ile	Leu	Lys	Met	Cys	Phe	Tyr	Thr	Trp	Val
Cereals and tubers															
Maize (3.8% oil)	8.5	0.38	0.33	0.37	0.23	0.29	1.00	0.26	0.18	0.18	0.38	0.30	0.29	0.06	0.40
Opaque-2 maize[2]	8.7	0.44	0.35	0.43	0.29	0.34	1.01	0.29	0.17	0.20	0.43	0.41	0.34	–	0.43
High oil maize (6% oil)[3]	9.5	0.44	–	0.49	0.25	0.26	1.14	0.29	0.24	0.25	0.45	–	0.33	–	0.36
Sorghum[4]	11.2	0.39	0.32	0.47	0.22	0.43	1.49	0.23	0.19	0.19	0.59	0.38	0.35	0.11	0.53
High-lysine sorghum[5]	12.2	0.64	0.48	0.57	0.29	0.49	1.25	0.45	0.17	0.25	0.55	0.40	0.43	–	0.66
Highly digestible sorghum[4]	8.9	0.40	0.33	0.41	0.20	0.28	1.03	0.25	0.17	0.19	0.42	0.29	0.31	0.10	0.40
Wheat, hard red	13.3	0.60	0.59	0.59	0.31	0.44	0.89	0.37	0.21	0.30	0.60	0.43	0.39	0.16	0.57
Wheat, soft white	10.2	0.40	0.49	0.55	0.20	0.42	0.59	0.31	0.15	0.22	0.45	0.39	0.32	0.12	0.44
Barley	11.0	0.52	0.44	0.46	0.27	0.37	0.76	0.40	0.18	0.24	0.56	0.35	0.37	0.14	0.52
High-lysine barley (Notch-1)[6]	13.8	0.74	0.57	0.46	0.29	0.49	0.90	0.55	0.21	0.26	0.67	0.46	0.42	0.15	0.70
Oats	11.4	0.79	0.50	0.40	0.24	0.52	0.89	0.50	0.18	0.22	0.59	0.53	0.43	0.16	0.68
Naked oats[7]	17.2	1.39	0.82	0.84	0.37	0.62	1.31	0.68	0.39	0.64	0.93	0.65	0.58	0.17	0.85
Rye	12.1	0.53	0.49	0.52	0.26	0.47	0.70	0.42	0.17	0.19	0.56	0.26	0.36	0.11	0.56
Triticale	11.8	0.57	0.48	0.52	0.26	0.39	0.76	0.39	0.26	0.26	0.49	0.32	0.36	0.14	0.51
Pearl millet	15.7	0.74	0.47	0.74	0.31	0.37	1.14	0.45	0.25	0.24	0.56	0.35	0.48	0.08	0.49
Rice grain, broken[8]	7.0	0.55	0.33	–	0.18	0.32	0.60	0.27	0.14	0.08	0.38	0.24	0.27	0.08	0.39
Cassava root meal[8]	2.5	0.23	0.17	–	0.03	0.05	0.06	0.03	0.02	0.02	0.05	0.06	0.05	0.01	0.07
By-products															
Bakery meal[9]	12.6	0.47	0.45	0.50	0.26	0.43	0.89	0.24	0.19	0.29	0.58	0.30	0.35	0.12	0.53
Maize hominy feed	10.0	0.47	0.40	0.50	0.20	0.40	0.84	0.40	0.13	0.13	0.35	0.49	0.40	0.10	0.49
Maize germ meal[10]	21.0	1.20	–	–	–	–	1.70	0.90	0.35	0.32	0.80	1.50	0.90	0.30	1.30
Rice bran	13.7	0.96	0.70	0.59	0.35	0.45	0.91	0.59	0.26	0.27	0.60	0.42	0.48	0.12	0.68
Wheat bran	15.4	1.02	0.81	0.67	0.46	0.47	0.96	0.61	0.23	0.32	0.61	0.46	0.50	0.23	0.70
Wheat red dog	15.3	0.96	0.74	0.75	0.41	0.55	1.06	0.59	0.23	0.37	0.66	0.46	0.50	0.10	0.72
Wheat middlings	16.0	1.15	0.63	0.75	0.37	0.58	1.07	0.69	0.21	0.32	0.64	0.45	0.49	0.20	0.71
Wheat shorts	16.5	1.18	0.96	0.77	0.45	0.58	1.09	0.79	0.27	0.36	0.67	0.47	0.60	0.21	0.83

[1]Unless indicated otherwise, data are from National Research Council (1994). [2]Data calculated from Mertz *et al.* (1964). [3]Data from Parsons *et al.* (1998). [4]Elkin *et al.* (unpublished). [5]Data from Featherston *et al.* (1975). [6]Data from Balaravi *et al.* (1976). [7]Data from Maurice *et al.* (1985). [8]Data (as % dry matter) calculated from Ravindran and Blair (1992). [9]Data from Ragland *et al.* (1999). [10]Data from Scott *et al.* (1982).

Table 4.2. Amino acid contents (g $100g^{-1}$ protein basis) of selected energy concentrates.

Feedstuff[1]	Arg	Gly	Ser	His	Ile	Leu	Lys	Met	Cys	Phe	Tyr	Thr	Trp	Val
Cereals and tubers														
Maize (3.8% oil)	4.47	3.88	4.35	2.71	3.41	11.76	3.06	2.12	2.12	4.47	3.53	3.41	0.71	4.70
Opaque-2 maize[2]	5.10	4.02	4.99	3.35	3.91	11.63	3.39	2.00	2.35	4.96	4.71	3.91	–	4.98
High oil maize (6% oil)[3]	4.63	–	5.16	2.63	2.74	12.00	3.05	2.53	2.63	4.74	–	3.47	–	3.79
Sorghum[4]	3.48	2.86	4.20	1.96	3.84	13.30	2.05	1.70	1.70	5.27	3.39	3.13	0.98	4.73
High-lysine sorghum[5]	5.27	3.90	4.67	2.39	4.01	10.27	3.67	1.39	2.08	4.54	3.32	3.52	–	5.41
Highly digestible sorghum[4]	4.51	3.72	4.63	2.26	3.16	11.63	2.82	1.92	2.14	4.74	3.27	3.50	1.13	4.51
Wheat, hard red	4.51	4.44	4.44	2.33	3.31	6.69	2.78	1.58	2.26	4.51	3.23	2.93	1.20	4.29
Wheat, soft white	3.92	4.80	5.39	1.96	4.12	5.78	3.04	1.47	2.16	4.41	3.82	3.14	1.18	4.31
Barley	4.73	4.00	4.18	2.45	3.36	6.91	3.64	1.64	2.18	5.09	3.18	3.36	1.27	4.73
High-lysine barley (Notch-1)[6]	5.41	4.13	3.32	2.15	3.57	6.54	4.00	1.56	1.89	4.87	3.37	3.06	1.07	5.12
Oats	6.93	4.39	3.51	2.11	4.56	7.81	4.39	1.58	1.93	5.18	4.65	3.77	1.40	5.96
Naked oats[7]	8.08	4.77	4.88	2.15	3.60	7.62	3.95	2.27	3.72	5.41	3.78	3.37	0.99	4.94
Rye	4.38	4.05	4.30	2.15	3.88	5.79	3.47	1.40	1.57	4.63	2.15	2.98	0.91	4.63
Triticale	4.83	4.07	4.41	2.20	3.31	6.44	3.31	2.20	2.20	4.15	2.71	3.05	1.19	4.32
Pearl millet	4.71	2.99	4.71	1.97	2.36	7.26	2.87	1.59	1.53	3.57	2.23	3.06	0.51	3.12
Rice grain, broken[8]	7.90	4.80	–	2.50	4.60	8.60	3.90	2.00	1.20	5.40	3.40	3.80	1.20	5.60
Cassava root meal[8]	9.00	6.90	–	1.00	2.00	2.50	2.80	0.70	0.60	2.00	2.50	2.00	0.40	2.80
By-products														
Bakery meal[9]	3.73	3.57	3.97	2.06	3.41	7.06	1.90	1.51	2.30	4.60	2.38	2.78	0.95	4.21
Maize hominy feed	4.70	4.00	5.00	2.00	4.00	8.40	4.00	1.30	1.30	3.50	4.90	4.00	1.00	4.90
Maize germ meal[10]	5.71	–	–	–	–	8.10	4.29	1.67	1.52	3.81	7.14	4.29	1.43	6.19
Rice bran	7.07	5.11	4.31	2.55	3.28	6.64	4.31	1.90	1.97	4.38	3.07	3.50	0.88	4.96
Wheat bran	6.62	5.26	4.35	2.99	3.05	6.23	3.96	1.49	2.08	3.96	2.99	3.25	1.49	4.55
Wheat red dog	6.27	4.84	4.90	2.68	3.59	6.93	3.86	1.50	2.42	4.31	3.01	3.27	0.65	4.71
Wheat middlings	7.19	3.94	4.69	2.31	3.63	6.69	4.31	1.31	2.00	4.00	2.81	3.06	1.25	4.44
Wheat shorts	7.15	5.82	4.67	2.73	3.52	6.61	4.79	1.64	2.18	4.06	2.85	3.64	1.27	5.03

[1]Unless indicated otherwise, data are calculated from National Research Council (1994). [2] Data from Mertz *et al.* (1964). [3]Data calculated from Parsons *et al.* (1998). [4]Elkin *et al.* (unpublished). [5]Data from Featherston *et al.* (1975). [6]Data from Balaravi *et al.* (1976). [7]Data calculated from Maurice *et al.* (1985). [8]Data from Ravindran and Blair (1992). [9]Data calculated from Ragland *et al.* (1999). [10]Data calculated from Scott *et al.* (1982).

wheat and sorghum, have been noted, while similar but lesser alterations have been reported in maize, barley, oats and rice (National Research Council, 1994). Thus, a traditional plant breeding strategy for improving the protein quality of a plant feedstuff would be to select against a nutritionally-poor protein in favour of others rich in an indispensable amino acid(s). This is essentially the situation that occurred over three decades ago during the discovery of the lysine-rich, *opaque-2* gene in maize (Mertz *et al*., 1964; see below).

Cereal Grains

Maize

The amino acid composition of maize (*Zea mays*) has been studied in detail by numerous investigators. Using a highly sensitive assay system based on ideal protein formulation, Fernandez *et al*. (1994a) recently reported that the limiting order of amino acids in maize was: (i) lysine; (ii) threonine; (iii) tryptophan; (iv) arginine, isoleucine, and valine; (v) methionine plus cystine; (vi) phenylalanine plus tyrosine; and (vii) histidine.

The endosperm contains approximately 90% of the protein in a maize kernel; thus, the proteins in this tissue dictate the nutritional quality of the grain. In most normal maize cultivars, the storage proteins (zeins) account for 60–70% of the endosperm protein (Hamaker *et al*., 1995). Since zeins are essentially devoid of lysine and tryptophan (Nelson, 1969; Shotwell and Larkins, 1989), they essentially dilute the contribution of these indispensable amino acids from non-zein endosperm proteins (Larkins *et al*., 1994). Thus, differences in the amount of lysine-poor zein and non-zein proteins determine the variation in lysine content among normal and *opaque-2* cultivars.

During the late 1960s it was assumed that *opaque-2* maize discovered by Mertz *et al*. (1964) would be widely cultivated because of its higher nutritional value. Unfortunately, it possessed a chalky soft kernel and exhibited reduced yields, lower resistance to fungi and insects in the field and in storage, and a longer drying time; because of these poor agronomic characteristics, most of the agricultural research centres and hybrid maize companies abandoned their research programmes on *opaque-2* maize (Mertz, 1994). However, by systematically introgressing *opaque-2* modifier genes into *opaque-2* genetic backgrounds, while simultaneously monitoring lysine content of the grain, plant breeders were able to develop a new type of *opaque-2* mutant, Quality Protein Maize, which resolved the long-standing deficiencies of the original *opaque-2* mutant and now offers the potential originally envisioned for high-lysine maize (Larkins *et al*., 1994).

Another genetically improved maize cultivar which has gained commercial feed ingredient status is high oil maize (HOM). Although it has been shown that the CP content often increases in proportion to the oil content (Watson and Freeman, 1975; Han *et al*., 1987; Bartov and Bar-Zur, 1995), others (Parsons *et al*., 1998) have reported no consistent relationship between ether extract and CP content of maize. However, Parsons *et al*. (1998) did observe that although most amino acids did not differ greatly

among conventional maize and three HOM varieties, the levels of lysine, methionine, cystine and aspartic acid generally increased (both on an absolute and a relative basis) as oil increased. They attributed the increased lysine content to an increase in germ protein, resulting from a preferential increase in the germ portion of the maize kernel.

Sorghum

Sorghum bicolor (L.) Moench, also known as milo, is one of the most drought-resistant cereal crops. Sorghum is also unique among cereals in that certain cultivars are able to produce relatively large amounts of polymeric, polyphenolic anti-nutritional compounds known as condensed tannins (Butler, 1989). However, the following discussion will be limited to low-tannin or tannin-free varieties, which are the ones predominantly grown around the world (Weaver *et al*., 1998).

In developed countries, sorghum is primarily used for poultry and livestock feeding, whereas in the developing countries the grain is mainly used as a human food. With the exception of high-lysine genotypes (see below), the indispensable amino acid profiles (as g 100 g^{-1} protein) of sorghum cultivars generally do not vary greatly and are comparable to that of normal maize (Table 4.2). However, it should be noted that, as a percentage of CP, methionine, lysine and threonine do not tend to increase directly in proportion to increases in CP content of the grain (Ward *et al*., 1988; Douglas *et al*., 1990; Elkin *et al*., 1996a).

High-lysine varieties of sorghum have also been developed and contain greater levels of this amino acid than 'normal' sorghum grains (Singh and Axtell, 1973; Featherston *et al*., 1975). In a manner analogous to *opaque-2* maize, the enhanced lysine content of select sorghum cultivars resulted primarily from an increase in the lysine-rich, non-kafirin proteins and a small decrease in the lysine-poor kafirin proteins (Weaver *et al*., 1998). Sorghum kafirins, like zeins in maize, are the prolamin storage proteins and are located within protein bodies (Hamaker *et al*., 1995).

Recently, sorghum cultivars possessing 10–25% higher protein digestibilities than normal sorghum were found within a high-lysine population (Weaver *et al*., 1998). Transmission electron micrographs revealed the presence of irregularly shaped protein bodies, which had numerous invaginations often reaching to their central areas (Oria *et al*., 2000). In contrast, normal sorghum genotypes, which have lower protein digestibilities, have spherical protein bodies which contain no invaginations. The resulting easy accessibility of digestive enzymes to α-kafirin, the major storage protein, combined with an increased surface area of the protein bodies of the highly digestible cultivars, appear to account for their high *in vitro* (Weaver *et al*., 1998) and *in vivo* (Elkin *et al*., 1996b) digestibilities. Although highly digestible, high-lysine sorghum cultivars hold great promise as value-added feed grains, their starch contents and availabilities are inferior to normal sorghums, thus offsetting their superior protein digestibilities and resulting in poorer broiler performance (Elkin *et al*., 1998). Studies are underway to improve the starch quantity and carbohydrate profile of the highly digestible lines.

Wheat

Once considered too expensive for inclusion in animal feeds (National Research Council, 1994), wheat (*Triticum* spp.) is now commonly used in many countries as the major energy source in poultry diets (Leeson and Summers, 1997). Of all the cereal grains, wheat is the most variable in protein (range of 10–18%) and amino acid content (Scott *et al*., 1982; Scott, 1987; Leeson and Summers, 1997) which, like those of maize and sorghum, are influenced by genetic and environmental factors. Most of the commonly grown varieties were developed with flour milling qualities in mind (National Research Council, 1994; Pond *et al*., 1995), although some breeding programmes were directed towards the development of cultivars with improved nutritive value for livestock (e.g. WS-3 wheat; Bowyer and Waldroup, 1987).

Wheats can be classified as winter or spring varieties, white or red, and hard or soft; thus, there is often confusion regarding the particular type of wheat being used. According to Leeson and Summers (1997), most winter wheats are white and soft, while spring wheats are red and hard, although advances in plant breeding have allowed for more variability as to where and when certain varieties may be planted. In general, hard wheats contain more protein (Pond *et al*., 1995) and are used primarily in bread making while the soft wheats are used only for the manufacture of biscuits and cakes (Leeson and Summers, 1997). In terms of poultry feeding, the use of higher-protein wheats is often economical because of the sparing effect that they exert upon the amount of soybean meal or other protein supplements needed (Scott, 1987).

Barley

Barley (*Hordeum* spp.) is widely grown in Europe and in the cooler climates of North America and Asia (Pond *et al*., 1995). Like sorghum grains, barley can often be grown in areas unsuitable for producing maize (Scott *et al*., 1982). Although a substantial amount is used in the brewing industry, a significant proportion of barley is used for animal feeding, where it is regarded as a medium energy-protein ingredient, falling between oats and wheat in most characteristics (Leeson and Summers, 1997). Moreover, with high grain costs in 1996 driving feed prices to historic levels, barley became a possible alternative grain to be used in broiler diets (Brake *et al*., 1997).

Like wheat, barley varies considerably in protein content (usually ~11–12% but can be as high as 14–16% (McDonald *et al*., 1995; Leeson and Summers, 1997)). In the USA, eastern barley averages ~12% protein while Pacific coast barley rarely exceeds 10% protein (Scott *et al*., 1982). Lysine is reported to be the first-limiting amino acid in barley, followed by threonine methionine and histidine (Fuller *et al*., 1979; McDonald *et al*., 1995). For normal cultivars containing between 10% and 14% CP, the lysine content has been described by the following equation: % lysine = 0.13 + 0.024% × CP (Leeson and Summers, 1997). Barley varieties containing ~25% more lysine than conventional barley have been shown to be superior in nutritive quality to their low-lysine isotypes (Balaravi *et al*., 1976; Eggum, 1978; Gabert *et al*., 1995) and hold promise as an energy source for non-ruminants. However, as is

the case of high-lysine maize and sorghum genotypes, many of the barley mutants exhibit inferior agronomic traits compared with their parent lines, and their starch contents may also be reduced (McDonald *et al*., 1995).

Oats

Oats (*Avena sativa*) represent less than 5% of the total world production of cereal grains, with most of the production being concentrated in the cooler climates of Europe and North America. Although protein content is relatively high and the amino acid profile is more favourable than that of maize, oats are not widely used for feeding swine or poultry because of their high fibre content (Pond *et al*., 1995; Leeson and Summers, 1997). As with other grains, protein and amino acid levels can vary due to cultivar and climatic effects.

Naked oats (*Avena nuda* L.) is a large hull-less oat species which is similar in texture to wheat and is grown primarily in China (Maurice *et al*., 1985). The protein content of naked oats (17–24%) exceeds that of other cereal grains (Table 4.1). Although Hsun and Maurice (1992) stated that the proportion of amino acids is not altered with the increase in protein content, data presented in Table 4.2 do not substantiate this claim, particularly for arginine, serine, isoleucine, methionine, cystine, tryptophan and valine.

Rye

Most of the existing research pertaining to the use of rye (*Secale cereale*) in poultry diets has concentrated on its growth-depressing properties in growing chicks rather than attempting to determine under what conditions rye might prove to be an acceptable energy feedstuff for poultry (Campbell *et al*., 1989). Rye grain is very similar to wheat in amino acid composition (McDonald *et al*., 1995), although rye protein is higher in lysine and slightly lower in both sulphur amino acids and tryptophan than wheat protein (Tables 4.1 and 4.2). Nevertheless, the feeding value of rye for poultry remains poor due to the presence of various anti-nutritional factors (Leeson and Summers, 1997). Moreover, rye is regarded as being the least palatable cereal grain (McDonald *et al*., 1995).

Triticale

Triticale is a hybrid cereal derived from a cross between wheat (*Triticum duriem*) and rye (*Secale cereale*). Grown commercially in central and northern Europe, North America and South America and used primarily in animal feeding (McDonald *et al*., 1995), triticale has a higher protein content than most wheats (Scott *et al*., 1982). In contrast to rye, triticale is well accepted by poultry and, because of its high protein content, allows for a reduction in the amount of soybean meal needed as a protein supplement (Scott, 1987).

Numerous cultivars have been developed with protein contents varying from 11% to 20%. Although deficient in tryptophan, triticale possesses an amino acid balance that is considered to be superior to that of wheat and rye, because it generally contains a higher proportion of lysine and sulphur amino acids (McDonald *et al*., 1995; Leeson and Summers, 1997). Flores *et al*. (1994) recently reported the chemical composition and amino acid and starch bioavail-

abilities of 18 Spanish triticale varieties. They observed a wide range in protein content (14.9–20.3%) with mean protein, lysine and threonine contents of 17.5%, 0.43% and 0.49%, respectively (all figures are on a dry matter basis).

Millet

Millets are small-seeded, annual cereal grasses that are grown primarily in the semiarid regions of Africa and Asia. In addition, they are the most drought-tolerant of all cereals (Ravindran and Blair, 1991). Pearl millet (*Pennisetum glaucum*), the most widely grown millet species, generally has a superior amino acid profile and a higher protein content as compared to maize (Adeola *et al*., 1994). Although pearl millet has been used to replace maize in duck diets (Adeola *et al*., 1994) and broiler diets (see Adeola *et al*., 1994, for brief review) without adversely affecting weight gains or feed conversion values, Ravindran and Blair (1991) consider the feeding value of pearl millet to be inferior to that of maize, but better or similar to that of sorghum, wheat and barley. More research is obviously needed to settle this important issue.

Rice

Rice (*Oryza sativa*) is the principal food cereal in tropical Asia. Rough rice (rice grains in their hulls) is often available during harvest season and, following grinding, up to 30% can be used in poultry diets with no adverse effects on growth or laying performance (Ravindran and Blair, 1991). Although lower in protein than all other cereals (Table 4.1), broken rice is comparatively rich in lysine (Table 4.2).

Cereal By-products

Maize by-products

Maize by-products result from both the dry and wet milling of maize. Scott *et al*. (1982) provide a complete description of the origin of these products, which has been briefly summarized below. Maize meal, hominy and maize grits are produced by dry milling; the resultant by-products are hominy feed, maize bran, maize germ cake and maize germ meal. *Hominy feed*, which contains ~10% CP, is a mixture of maize bran, maize germ, and part of the starchy portion of either white or yellow maize kernels as produced in the manufacture of pearl hominy. *Maize germ meal* (*dry milled*) is ground maize germ which consists of maize germ with other parts of the maize kernel from which part of the oil has been removed. *Wet-milled maize germ meal* consists of ground maize germ from which most of the solubles have been removed by steaming and most of the oil removed by hydraulic expeller or solvent extraction procedures. *Maize gluten feed* (~22% CP) is that part of the shelled maize (including bran) that remains after the larger portion of the starch, gluten and germ have been extracted in the wet milling manufacture of maize starch or syrup. *Maize gluten meal* is the dried residue remaining after the removal of most of the starch and germ and the separation of the bran by the processes employed in the wet milling manufacture of maize starch or syrup. Because of its high protein content (~60% CP), maize gluten meal is classified as a protein concentrate and will be discussed therein.

Wheat by-products

In the past, multiple names for the various by-products of the flour milling industry caused considerable confusion to feed manufacturers (for a historical perspective and potpourri of wheat by-product nomenclature, the reader is referred to Scott *et al*., 1982). Although their names still may vary in certain countries, the main wheat by-products available for animal feeding include wheat bran, wheat shorts, wheat mill-run and wheat screenings (Leeson and Summers, 1997). *Wheat bran* (~15% CP) is the coarse outer covering of the wheat kernel and is rich in fibre. Although its amino acid profile is comparable to that seen in whole wheat, it is little used in poultry nutrition, apart from specialized applications (Leeson and Summers, 1997). *Wheat shorts* is the product that remains after the extraction of the bran, flour and germ. The National Research Council (1994) actually places wheat shorts within a specific category of wheat flour by-products, which contain ~15–16% CP and are classified according to their fibre content: wheat middlings (7–9.5% fibre); wheat shorts (4–7% fibre) and wheat red dog (<4% fibre), which is the very finest particles of bran, endosperm and germ. *Wheat mill run* is a combination of wheat shorts and wheat bran. *Wheat screenings* are a by-product of the cleaning and grading of wheat that is destined for human consumption (Leeson and Summers, 1997). The composition of the screenings can be quite variable and although it consists of broken and cracked wheat kernels, wheat screenings can also contain other seeds, including wild oats, wild buckwheat and broken flax seed (see Scott *et al*., 1982 for the proximate analyses of 12 weed seeds commonly found in grain screenings).

Rice by-products

In addition to polished rice, several rice by-products are produced during the milling of rough rice and include broken rice, rice polishings, rice bran and rice hulls. Although broken rice has a nutritive value similar to maize and can be fed to all classes of poultry when prices are favourable, in many countries broken rice is usually mixed with whole rice and sold as a human food (Ravindran and Blair, 1991). Rice bran, the most important of the rice milling by-products, can vary widely in chemical composition due to the level of hull contamination. Good quality rice bran contains ~13% CP; however, its nutritional potential as a poultry feedstuff has not been adequately realized due to inconsistent chemical composition and rancidity problems associated with its lipid component (Ravindran and Blair, 1991). Nevertheless, in areas where maize is unavailable or very expensive, unadulterated rice bran, properly preserved, can be a most valuable ingredient, especially for ducks where the high fibre content is not a detriment (Scott and Dean, 1991).

Bakery By-products

Bakery meal

Bakery meal, otherwise known as *dried bakery product* (*DBP*), is a mixture of surplus and unsaleable materials collected from bakeries and other food

processors (Saleh *et al*., 1996). Although DBPs can differ considerably in nutrient content because of variation in raw ingredients and the use of non-nutritive fillers (Leeson and Summers, 1997), it is interesting to note that two different DBPs, which were fed in separate studies in different parts of the USA and reported 3 years apart, were remarkably similar in CP and indispensable amino acid contents (Saleh *et al*., 1996; Ragland *et al*., 1999). Because lysine digestibilities of DBPs may be reduced as a result of Maillard reaction products formed during cooking, Saleh *et al*. (1996) cautioned that the lysine value assigned to DBPs may need to be adjusted if they are included in diets which are marginal in lysine content.

Roots and Tubers

Cassava root meal

Cassava (*Manihot esculenta*), a tuber, is a staple food for over 500 million people in the tropics (Ravindran and Blair, 1991). However, cassava is also widely used in poultry feeds, particularly in Europe. Because cassava can contain high levels of cyanogenic glycosides (CG), it must be processed prior to use. In this regard, the introduction of low-CG cultivars, together with proper processing techniques, can yield a low-toxicity tuber meal (Garcia and Dale, 1999). *Cassava root meal* (CRM), the resultant product which is also known as tapioca, is primarily used as an energy source due to its high starch content (60–70%) and very low levels of fibre and CP (typically ~2.5%; Garcia and Dale, 1999). Ravindran and Blair (1991) pointed out that for the successful use of CRM as a substitute for maize, CRM-based diets must be supplemented with methionine, particularly when soybean meal serves as the main protein supplement. The reasons for this are twofold: (i) soybean meal is very deficient in sulphur amino acids (to be discussed below); and (ii) methionine apparently serves as a readily available source of labile sulphur for cyanide detoxification.

PROTEIN CONCENTRATES

The optimal use of protein concentrates (>20% CP) in poultry feeding programmes is essential for at least three reasons: (i) amino acids are critical nutrients for both rapidly growing meat-type birds and high-producing laying hens; (ii) protein concentrates are usually more expensive than energy feedstuffs; and (iii) the maximal use of dietary amino acids minimizes the production and excretion of nitrogenous waste products by the birds, thereby reducing the amount of nitrogen released into the environment. Although protein concentrates are available from an extremely wide variety of plant and animal sources (Scott *et al*., 1982; Ravindran and Blair, 1992; McDonald *et al*., 1995; Pond *et al*., 1995), this review will focus on the most common ones fed to poultry (Tables 4.3 and 4.4).

Table 4.3. Crude protein (CP) and amino acid contents (%; as-fed basis) of selected protein concentrates.

Feedstuff[1]	CP	Arg	Gly	Ser	His	Ile	Leu	Lys	Met	Cys	Phe	Tyr	Thr	Trp	Val
Plant sources															
Soybean meal	47.5	3.48	2.05	2.48	1.28	2.12	3.74	2.96	0.67	0.72	2.34	1.95	1.87	0.74	2.22
Canola meal	34.8	2.08	1.82	1.53	0.93	1.37	2.47	1.94	0.71	0.87	1.44	1.09	1.53	0.44	1.76
Cottonseed meal	41.4	4.66	1.69	1.78	1.10	1.33	2.41	1.76	0.51	0.62	2.23	1.14	1.34	0.52	1.82
Linseed meal[2]	34.0	3.33	1.94	–	0.75	1.53	2.04	1.29	0.65	0.68	1.63	0.99	1.36	0.61	1.84
Groundnut meal	49.0	5.33	2.67	2.25	1.07	1.55	2.97	1.54	0.54	0.64	2.41	1.80	1.24	0.48	1.87
Safflower meal	43.0	3.65	2.32	–	1.07	1.56	2.46	1.27	0.68	0.70	1.75	1.07	1.30	0.59	2.33
Sesame meal	41.0	4.68	2.04	1.72	0.99	1.51	2.68	0.91	1.22	0.72	1.93	1.48	1.40	0.62	1.91
Sunflower meal	36.8	2.85	2.03	1.49	0.87	1.43	2.22	1.24	0.80	0.64	1.66	0.91	1.29	0.41	1.74
Coconut meal	19.2	1.97	0.82	0.79	0.36	0.63	1.18	0.50	0.28	0.28	0.88	0.44	0.58	0.12	0.91
Palm kernel meal[2]	20.0	2.70	0.96	–	0.46	0.64	1.20	0.72	0.40	0.30	0.78	0.32	0.70	0.20	1.14
Faba bean (broad bean)[2]	23.0	1.98	0.87	–	0.55	1.01	1.47	1.43	0.18	0.30	0.99	0.78	0.85	0.23	0.85
Peas	23.8	2.23	1.00	1.08	0.59	0.97	1.65	1.68	0.24	0.33	1.10	0.73	0.84	0.18	1.10
Lupins, sweet[3]	33.3	3.53	1.30	1.70	0.77	1.00	2.40	1.50	0.20	0.40	1.20	1.20	1.33	–	1.10
Maize gluten meal	60.2	1.82	1.67	2.96	1.20	2.45	10.04	1.03	1.49	1.10	3.56	3.07	2.00	0.36	2.78
Animal and marine sources															
Meat meal	54.4	3.73	6.30	1.60	1.30	1.60	3.32	3.00	0.75	0.66	1.70	0.84	1.74	0.36	2.30
Meat and bone meal	51.6	3.28	6.65	2.20	0.96	1.54	3.28	2.61	0.69	0.69	1.81	1.20	1.74	0.27	2.36
Poultry by-product meal	59.5	3.94	6.17	2.71	1.07	2.16	3.99	3.10	0.99	0.98	2.29	1.68	2.17	0.37	2.87
Spent hen meal[4]	67.3	4.26	5.83	3.06	1.45	2.41	4.86	3.67	1.16	1.23	2.72	1.85	2.57	0.47	3.35
Feather meal	82.9	5.57	6.13	8.52	0.95	3.91	6.94	2.28	0.57	4.34	3.94	2.48	3.81	0.55	5.93
Blood meal, spray dried	88.9	3.62	3.95	4.25	5.33	0.98	11.32	7.88	1.09	1.03	5.85	2.63	3.92	1.35	7.53
Fish meal, menhaden	61.3	3.68	4.46	2.37	1.42	2.28	4.16	4.51	1.63	0.57	2.21	1.80	2.46	0.49	2.77
Tuna meal[5]	53.1	3.55	4.35	1.78	2.02	2.24	3.88	4.00	1.40	0.38	2.21	1.54	1.90	0.62	2.82

[1]Unless indicated otherwise, data are from National Research Council (1994). [2]Data (as % dry matter) calculated from Ravindran and Blair (1992). [3]Data calculated from Perez-Escamilla *et al.* (1988). [4]Mean values of three samples reported in Kersey *et al.* (1997). [5]Data from Zaviezo and Dale (1994).

Table 4.4. Amino acid contents (g 100 g^{-1} protein basis) of selected protein concentrates.

Feedstuff[1]	Arg	Gly	Ser	His	Ile	Leu	Lys	Met	Cys	Phe	Tyr	Thr	Trp	Val
Plant sources														
Soybean meal	7.33	4.32	5.22	2.69	4.46	7.87	6.23	1.41	1.52	4.93	4.11	3.94	1.56	4.67
Canola meal	5.98	5.23	4.40	2.67	3.94	7.10	5.57	2.04	2.50	4.14	3.13	4.40	1.26	5.06
Cottonseed meal	11.26	4.08	4.30	2.66	3.21	5.82	4.25	1.23	1.50	5.39	2.75	3.24	1.26	4.40
Linseed meal[2]	9.80	5.70	–	2.20	4.50	6.00	3.80	1.90	2.00	4.80	2.90	4.00	1.80	5.40
Groundnut meal	10.88	5.45	4.59	2.18	3.16	6.06	3.14	1.10	1.31	4.92	3.67	2.53	0.98	3.82
Safflower meal	8.49	5.40	–	2.49	3.63	5.72	2.95	1.58	1.63	4.07	2.49	3.02	1.37	5.42
Sesame meal	11.41	4.98	4.20	2.41	3.68	6.54	2.22	2.98	1.76	4.71	3.61	3.41	1.51	4.66
Sunflower meal	7.74	5.52	4.05	2.36	3.89	6.03	3.37	2.17	1.74	4.51	2.47	3.51	1.11	4.73
Coconut meal	10.26	4.27	4.11	1.88	3.28	6.15	2.60	1.46	1.46	4.58	2.29	3.02	0.63	4.74
Palm kernel meal[2]	13.50	4.80	–	2.30	3.20	6.00	3.60	2.00	1.50	3.90	1.60	3.50	1.00	5.70
Faba bean (broad bean)[2]	8.60	3.80	–	2.40	4.40	6.40	6.20	0.80	1.30	4.30	3.40	3.70	1.00	3.70
Peas	9.37	4.20	4.54	2.48	4.08	6.93	7.06	1.01	1.39	4.62	3.07	3.53	0.76	4.62
Lupins, sweet[3]	10.60	3.90	5.10	2.30	3.00	7.20	4.50	0.60	1.20	3.60	3.60	4.00	–	3.30
Maize gluten meal	3.02	2.77	4.92	1.99	4.07	16.68	1.71	2.48	1.83	5.91	5.10	3.32	0.60	4.62
Animal and marine sources														
Meat meal	6.86	11.58	2.94	2.39	2.94	6.10	5.51	1.38	1.21	3.13	1.54	3.20	0.66	4.23
Meat and bone meal	6.36	12.89	4.26	1.86	2.98	6.36	5.06	1.34	1.34	3.51	2.33	3.37	0.52	4.57
Poultry by-product meal	6.62	10.37	4.55	1.80	3.63	6.71	5.21	1.66	1.65	3.85	2.82	3.65	0.62	4.82
Spent hen meal[4]	6.33	8.66	4.55	2.16	3.57	7.22	5.45	1.72	1.83	4.04	2.75	3.82	0.70	4.98
Feather meal	6.72	7.39	10.28	1.15	4.72	8.37	2.75	0.69	5.24	4.75	2.99	4.60	0.66	7.15
Blood meal, spray dried	4.07	4.44	4.78	6.00	1.10	12.73	8.86	1.23	1.16	6.58	2.96	4.41	1.52	8.47
Fish meal, menhaden	6.00	7.28	3.87	2.32	3.72	6.79	7.36	2.66	0.93	3.61	2.94	4.01	0.80	4.52
Tuna meal[5]	6.69	8.19	3.35	3.80	4.22	7.31	7.53	2.64	0.72	4.16	2.90	3.58	1.17	5.31

[1]Unless indicated otherwise, data are calculated from National Research Council (1994). [2]Data from Ravindran and Blair (1992). [3]Data from Perez-Escamilla *et al.* (1988). [4]Mean values calculated from three samples reported in Kersey *et al.* (1997). [5]Data calculated from Zaviezo and Dale (1994).

Oilseed Meals

Oilseed meals are the protein-rich residues remaining after removal of most of the oil from oil-bearing seeds. Oilseeds have one particular characteristic in common in that they are subjected to manufacturing processes (Dale, 1996). As a result, such processes can increase the amount of nutrient variation already inherent in the raw product and can adversely affect the amino acid content/protein quality of the meal (Ravindran and Blair, 1992). The classic example of this is the formation of carbohydrate–amino acid reaction products (the so-called 'Maillard' or 'Browning' reaction products) when oilseed meals are overheated. Although such linkages are not hydrolysed by digestive enzymes, the amino acids may still be recovered from the protein following acid hydrolysis (Scott *et al*., 1982). Thus, an amino acid (especially lysine) may appear to be chemically present but is unavailable to the animal. Additional physical processing effects on the nutritive value of poultry diets are addressed elsewhere in this volume.

Soybean meal

Although the soybean (*Glycine max*) is an important legume crop grown for human consumption, particularly in Asia, soybean meal is by far the major plant protein concentrate used in poultry diets (Fernandez *et al*., 1994a; Dale, 1996). Thus, one could consider virtually all other lesser used plant, animal and marine protein concentrates as 'alternative' protein sources. However, in areas of the world where soybean meal is not readily available for animal feed use (e.g. Asia and the Pacific), a wide variety of protein-rich plant materials serve this purpose (Ravindran and Blair, 1992). Moreover, even in areas where soybean meal is plentiful, when its costs increase substantially (as in 1996), it becomes economically attractive to consider the use of alternative protein-rich ingredients (see below). Nevertheless, soybean meal remains the worldwide standard against which other protein sources are compared (Leeson and Summers, 1997).

The protein content of soybean meal is usually standardized at 44% or 48% CP by dilution with soybean hulls. Its amino acid profile is excellent with regard to feeding most types of poultry and, when combined with maize or sorghum, methionine is typically the only limiting amino acid (Leeson and Summers, 1997). This is because the soy proteins and the grain proteins complement each other, with the latter being deficient in lysine (e.g. ~3% of protein in yellow maize; Table 4.2) whereas soy is relatively rich in lysine (~6.5% of the total protein; Table 4.4) (Dale, 1996). Conversely, soy is deficient in sulphur amino acids (methionine and cystine), which comprise ~3% of the total protein (Table 4.4) as compared to ~4.5% of maize protein (Table 4.2) (Dale, 1996). Using the ideal protein concept to avoid dietary amino acid excesses, Fernandez *et al*. (1994a) reported the limiting order of amino acids in soybean meal as follows: (i) methionine plus cystine; (ii) threonine; (iii) lysine and valine; (iv) non-specific amino acid nitrogen; and (v) histidine. In a maize–soybean meal mixture, the first six limiting amino acids were found to be (in order) methionine, threonine, lysine, valine, arginine and tryptophan.

Like many of the common energy concentrates, soybeans and other protein-rich plant feedstuffs also contain anti-nutritional factors which can be destroyed by processing. Details on the subject of natural toxicants are beyond the scope of this review and the reader is referred to the excellent text of Cheeke (1998).

Canola meal

Rapeseed (*Brassica* spp.) is one of the world's major oilseed crops, exceeded in importance only by soybeans and oil palm as sources of edible oil. Although rapeseed meal can contain a plethora of toxicants and other deleterious factors (glucosinolates, erucic acid, sinapine, tannins, phytate, etc.), low glucosinolate/low erucic acid cultivars (so-called 'double zero' varieties) have been developed in Canada and the meal derived from these varieties can be used at much higher levels than typical rapeseed genotypes (Cheeke, 1998). Because the oil from these special cultivars was low in erucic acid, Canadian producers coined the term canola (for Canadian oil low acid) (Cheeke, 1998).

The protein content of canola meal (~35% CP) is lower than that of soybean meal (Table 4.3) and, although canola meal also contains less lysine per unit of dietary protein, it is relatively richer in methionine than soybean meal (~2% of total protein vs. ~1.4% in soybean meal (Table 4.4) (National Research Council, 1994; McDonald *et al*., 1995). Anderson-Hafermann *et al*. (1993) reported that overheating (autoclaving) canola meal reduced its analysed lysine content in a stepwise manner, from an initial value of 1.77% to 1.29% after 90 min. With the exception of arginine, which also decreased (from 2.08% to 1.89%, respectively), autoclaving had little or no effect on the concentration of the other amino acids in the canola meal. Although the authors attributed the lysine and arginine decreases to the formation of Maillard reaction products, the bound amino acids could not be recovered following acid hydrolysis, which contrasts with what is reported in Scott *et al*. (1982).

Cottonseed meal

Because of the growth habits of cotton (*Gossypium* spp.), cottonseed meal is available in many areas where soybeans do not grow (Pond *et al*., 1995). However, cottonseed meal is inferior in protein quality (lower in lysine and sulphur amino acids; Tables 4.3 and 4.4) as compared to the other major oilseed meals (Fernandez *et al.*, 1994b) and is not widely included in poultry diets, unless economic reasons dictate otherwise. Perhaps even more of a drawback to the use of cottonseed meal as a poultry feedstuff is that it contains several toxic substances, including gossypol and cyclopropenoid fatty acids (Cheeke, 1998). The development of glandless cotton varieties containing low levels of gossypol (but not totally devoid of cyclopropenoid fatty acids) has allowed for a more-widespread use of cottonseed meal in poultry diets. Ravindran and Blair (1992) claimed that cottonseed meal, in combination with lysine-rich supplements, may replace up to 40% of the protein from soybean meal in broiler diets without any ill effects. However, because of the effects of cyclopropenoid fatty acids on interior egg quality, cottonseed meal can only be used to a limited extent in layer diets.

Linseed meal

Linseed meal is obtained from flax (*Linum usitatissimum*). Although linseed meal has traditionally been used in ruminant feeds, recently there has been considerable interest in feeding full-fat flax seeds to poultry, because of its high content of linolenic acid (Leeson and Summers, 1997). The demonstrated cardiovascular health benefits of n-3 polyunsaturated fatty acids (PUFA), including linolenic acid, combined with the ease with which egg yolk fatty acids can be enriched with these compounds, has resulted in a plethora of research involving the feeding of flax to poultry in order to produce n-3 PUFA-rich products (for a recent review, see Van Elswyk, 1997).

From a protein quality standpoint, linseed meal is of poorer quality than soybean or cottonseed meals (McDonald *et al*., 1995), particularly with regard to its lower lysine content (Tables 4.3 and 4.4). In addition, the presence of linatine, an antipyridoxine factor, as well as moderate amounts of cyanogenic glycosides (primarily linamarin) and mucilage (Ravindran and Blair, 1992; McDonald *et al*., 1995; Cheeke, 1998), limit the use of linseed meal in poultry diets.

Groundnut meal

The groundnut (*Arachis hypogaea*) is an important oilseed crop in many developing countries. Unfortunately, groundnuts are very susceptible to contamination with aflatoxins, fungal metabolites produced by *Aspergillus flavus*. Aflatoxins, which are potent hepatotoxins, cause a wide variety of toxic effects in poultry (see Cheeke, 1998).

In terms of its CP content (44–47%), groundnut meal is comparable to soybean meal, although it is considerably lower in lysine and sulphur amino acids (Tables 4.3 and 4.4). Moreover, the protein quality of groundnut meal, like most oilseed meals, is reduced by excess heating during processing (Zhang and Parsons, 1996). Nevertheless, groundnut meal can be used successfully in broiler chicken diets if adequate levels of dietary lysine and methionine are provided (Zhang and Parsons, 1996). Taking advantage of its markedly lower methionine and cystine content, aflatoxin-free groundnut meal can serve as an excellent intact protein supplement for use in nutritional experiments examining dietary sulphur amino acid interrelationships (Featherston and Rogler, 1978) or methionine requirements of poultry (Elkin *et al*., 1986).

Safflower meal

The safflower (*Carthamus tinctorius*) is a warm-temperature crop grown in limited amounts for its oil (Pond *et al*., 1995). The undecorticated meal is high in fibre and low in protein. Removal of the hulls decreases the fibre content of the meal (~43% CP) from greater than 20% to 14% (Leeson and Summers, 1997). Because the protein quality of safflower meal is low due to a paucity of lysine, satisfactory performance of poultry will not result without blending safflower meal with other protein supplements and/or the use of synthetic amino acids (Scott *et al*., 1982; Ravindran and Blair, 1992). Analogous to the use of groundnut meal in sulphur amino acid studies, safflower meal is an excellent intact protein source that can be used experimentally to produce a lysine-deficient diet.

Sesame meal

Sesame (*Sesamum indicum*) is in great demand because of the excellent culinary properties of its oil (Ravindran and Blair, 1992). Although it contains ~41% CP, sesame meal is also very deficient in lysine and, as is the case with safflower meal, is sometimes used to advantage in formulating lysine-deficient diets for experimental purposes (Leeson and Summers, 1997). However, in contrast to safflower meal, sesame meal is an excellent source of methionine, cystine and tryptophan for both growing chicks and laying hens (Scott *et al*., 1982; Ravindran and Blair, 1992).

Sunflower meal

Sunflowers (*Helianthus annus*) are grown in cooler climates and can be a very useful ingredient as a protein supplement, especially in areas where soybeans are not produced (Scott and Dean, 1991). The worldwide production of sunflower meal ranks fourth behind soybean meal, cottonseed meal and canola (Zhang and Parsons, 1994). The CP and fibre contents of sunflower meal can be in the range 29–45% and 14–32%, respectively, depending on the dehulling and oil extraction processes employed (Villamide and San Juan, 1998). As compared to soybean meal, sunflower meal is relatively richer in sulphur amino acids but markedly lower in lysine (Table 4.4) and available threonine (Leeson and Summers, 1997). Despite these latter shortcomings, sunflower meal has been successfully used at levels up to 20% in broiler and layer diets (Zatari and Sell, 1990; Vieira *et al*., 1992).

Coconut meal

Coconuts from the coconut palm (*Cocos nucifera*) are dried to produce copra, which is processed into coconut oil and coconut meal, also known as copra meal (Ravindran and Blair, 1992). Despite being high in fibre (~14%), coconut meal is an economically important source of protein in many Asian and Pacific countries where other protein concentrates are not readily available. It contains 20–22% CP and is relatively low in indispensable amino acids, particularly lysine and histidine (Ravindran and Blair, 1992; McDonald *et al*., 1995). Although conflicting opinions exist regarding the suitability of coconut meal as a feedstuff for chickens (e.g. Ravindran and Blair, 1992 vs. McDonald *et al*., 1995), ducks can utilize aflatoxin-free copra meal advantageously, because they are better able to increase feed consumption and overcome diet dilution by fibre (Scott and Dean, 1991).

Palm kernel meal

Like coconut meal, meal prepared from the kernel of the oil palm (*Elaeis quineensis*) is high in fibre and contains ~20% CP. In contrast, palm kernel meal protein is relatively richer in most indispensable amino acids than coconut meal (Table 4.4) (Ravindran and Blair, 1992). Used primarily in Asia, where it is plentiful and low in cost, it may be sparingly used in maize–soybean meal diets, or as a replacement for coconut meal or groundnut meal (Ravindran and Blair, 1992).

Leguminous Seeds

Grain legumes, often referred to as pulses, include all the common peas and beans and are members of a large plant family with ~12,000 recognized species (Ravindran and Blair, 1992; McDonald *et al*., 1995). Although approximately 20 of these crops are cultivated primarily for human consumption in developing countries, significant quantities of seeds deemed unfit for human use are often made available for animal feeding (Ravindran and Blair, 1992). In general, legume seeds contain approximately 20–25% CP and are high in lysine and low in methionine. Although many leguminous plants are toxic to animals and man (Cheeke, 1998), cooking usually destroys most of the anti-nutrients (Ravindran and Blair, 1992; Brufau *et al*., 1998). However, prolonged cooking, like overheating of oilseed meals, will adversely affect protein quality.

Faba beans

Within the *Leguminosae* family, the chief member of the *Vicieae* tribe is *Vicia faba* L., commonly known as the faba bean, broad bean, horse bean and Windsor bean (McDonald *et al.*, 1995). Faba beans have been studied extensively during the past two decades as a protein-rich supplement for monogastric animals (for a recent review, see Brufau *et al*., 1998). As compared to soybean meal, the CP content of faba beans is lower and can be quite variable, depending upon the cultivar (range of 22–33% reported by Braufau *et al*., 1998). However, on a g 100 g^{-1} of protein basis, faba beans are fairly comparable to soybean meal in indispensable amino acid content (Table 4.4). Like sorghum, certain faba bean cultivars can contain condensed tannins, which can adversely affect animal performance by interfering with protein digestion.

Peas

Peas (*Pisum sativum* L.) are similar to beans in that they are regarded as a source of protein. Although peas have a comparatively better balance of amino acids than do beans, being higher in lysine and sulphur amino acids, methionine is still the first limiting amino acid (McDonald *et al*., 1995). Like many other grain legumes, peas contain a variety of anti-nutritive substances whose effects can be eliminated or minimized by processing (Igbasan and Guenter, 1996). Although peas generally cannot be included in broiler diets at levels greater than 20% because of adverse effects on growth rate and feed utilization, Igbasan and Guenter (1996) recently reported that chicks fed diets containing 40% micronized peas performed better than birds fed untreated peas or a wheat–soybean meal control diet. Micronization is a process in which grains are heated by infrared radiation, which reportedly causes rapid internal heating and starch gelatinization (McNab and Wilson, 1974).

Lupins

Low-alkaloid ('sweet') versions of lupins (*Lupinus* spp.) are being used increasingly as an alternative feedstuff for poultry in certain areas of the world (Leeson and Summers, 1997). As compared to soybean meal protein, lupin protein has a lower concentration of isoleucine, lysine, methionine, cystine, phenylalanine

and valine, but is richer in arginine (Table 4.4). Thus, despite a dietary amino acid pattern that is not considered to be well balanced (McDonald *et al*., 1995), Perez-Escamilla *et al*. (1988) reported that raw lupins could be incorporated into maize–soybean meal-based broiler and turkey diets at levels up to 30% without adversely affecting bird performance.

Other Plant Protein Concentrate Sources

Maize gluten meal

As discussed previously, maize gluten meal is the dried residue remaining after the removal of most of the starch and germ and the separation of the bran by the processes employed in the wet milling manufacture of maize starch or syrup. Maize gluten meal is very high in protein (~60%) and relatively rich in sulphur amino acids (Table 4.3). However, it is very deficient in lysine (Table 4.4), although with appropriate use of synthetic lysine sources, the product is a useful protein concentrate for poultry diets, particularly where high nutrient density is called for (Leeson and Summers, 1997).

Protein Concentrates of Animal Origin

Protein supplements derived from animal tissues originate primarily from inedible tissues from meat packing or rendering plants (Pond *et al*., 1995). They are widely used in poultry diets (Johnson and Parsons, 1997), but not as a source of protein *per se*; rather, they are included in small amounts to correct deficiencies of certain indispensable amino acids that result with the use of grain–oilseed meal mixtures (McDonald *et al*., 1995).

Animal protein supplements can vary widely in quality due to the nature of the raw materials and the processing methods employed (Parsons *et al*., 1997). Moreover, increasing awareness and public concern about microbial contamination of animal-based products may ultimately lead to additional requirements/regulations for their processing (e.g. pressure processing; Parsons (1999)) and thus, further variation in amino acid contents and digestibilities.

Tankage, meat meals, and meat and bone meal

Wet-processed meat meals, termed 'tankages', generally contain dried residue from mammalian tissues exclusive of hair, hoof, horn, manure, stomach contents and hide trimmings, especially prepared for feeding purposes by dry rendering (heated in steam-jacketed cookers) or by tanking under live steam (Scott and Dean, 1991). Meat meals are similar to tankage, with the exception that they are always dry rendered, and are usually blended with bone and tendonous tissues to produce meat and bone meal, which contains approximately 50% CP, 8% fat, 28% ash, 10% calcium and 5% phosphorus (Scott and Dean, 1991).

Meat and bone meal is typically composed of beef and pork materials, although lamb meal, which has become a common ingredient in pet foods, may find increased use in poultry diets in light of the recent ban on feeding ani-

mal meals containing ruminant tissues to ruminants (Johnson and Parsons, 1997). Variability in the nutritional quality of meat and bone meal is well documented, and the recent findings of Parsons *et al*. (1997) provide additional evidence in this regard. Proximate compositions and amino acid contents of 14 different meat and bone meal samples obtained from US and Canadian suppliers were reported. The mean CP, ether extract, ash, calcium and phosphorus contents (and ranges) were 51.6% (47.8–57.8), 12.3% (8.7–15.1), 22.8% (16.5–30.3), 10.0% (6.6–12.6) and 4.0% (2.6–5.7), respectively. The mean amino acid composition (and ranges) of the 14 samples, was as follows: arginine, 3.80% (3.54–4.30); cystine, 0.64% (0.34–1.38); histidine, 0.94% (0.74–1.26); isoleucine, 1.47% (1.17–2.11); leucine, 3.41% (2.93–4.20); lysine, 2.66%, (2.32–3.02); methionine, 0.72% (0.57–0.96); phenylalanine, 1.80% (1.54–2.15); threonine, 1.75% (1.53–2.18); and valine, 2.40% (2.06–3.21). Significant variability in amino acid digestibilities was also observed (Parsons *et al*., 1997). In a subsequent study from Parsons' laboratory, the proximate compositions and amino acid contents and digestibilities of 32 commercial meat and bone meal samples, varying in both raw material source and cooking systems, and processed at two different temperatures, were reported (Wang and Parsons, 1998a). The average CP, lysine, cystine and methionine contents (as-fed basis) were 50.4%, 2.57%, 0.62% and 0.71%, respectively. Although processing temperature influenced amino acid digestibilities, it had no consistent effect on amino acid or CP contents.

Based on both amino acid addition and deletion experiments, Wang *et al*. (1997) concluded that the order of amino acid limitation in meat and bone meal was: (i) tryptophan and sulphur amino acids (primary need for cystine); (ii) threonine; (iii) isoleucine and phenylalanine plus tyrosine; (iv) methionine; (v) lysine; and (vi) valine and histidine. The paucity of tryptophan is most likely a reflection of the high ash (bone) content of meat and bone meal, since bone protein is very deficient in this amino acid (Parsons, 1999).

Poultry by-product meal

Similar to meat meal, poultry by-product meal is produced essentially from waste generated during poultry meat processing. Since only one animal species is used, poultry by-product meal would be expected to be more homogeneous than meat meal and contain lower levels of calcium and phosphorus (Leeson and Summers, 1997). However, compositional variability of this product can arise from the inclusion of feathers, blood and viscera, with the former product necessitating higher cooking temperatures in order to hydrolyse the keratin proteins.

The presence of a significant amount of feathers can markedly influence the order of amino acid limitation in poultry by-product meals. For example, Main and Doghir (1981, 1982) found that methionine and lysine were the first- and second-limiting amino acids, respectively, in a product that contained 40% feathers, 40% blood and 20% viscera. In contrast, Wang and Parsons (1998b) used a more conventional sample, which was a blend of four poultry by-product meals obtained from commercial rendering plants in the USA and contained 70% CP, 0.89% cystine, 1.27% methionine, 3.79% lysine, 0.60% tryptophan and 2.54% threonine. They reported that the order of amino acid

limitation was: (i) sulphur amino acids (primary need for cystine); (ii) tryptophan; (iii) threonine and lysine; (iv) valine; and (v) isoleucine and histidine. A partial explanation for the different findings between the two laboratories is that the sample evaluated by Main and Doghir (1981, 1982) contained 3.73% cystine, which was indicative of a high proportion of feather protein. For chicks, feather meal is first- and second-limiting in methionine and lysine, respectively (Wang and Parsons, 1998b).

Spent hen meal

In order to provide table eggs for human consumption, approximately 250 million laying hens are produced annually in the USA (Kersey *et al*., 1997). Recent difficulties in disposal of birds at the conclusion of their laying cycle, combined with a reduced demand for Leghorn meat, has led to an increased interest in rendering these 'spent hens' into a by-product meal. Spent hen meal is thus a relatively new product which differs from poultry by-product meal in that the whole bird is rendered (Douglas *et al*., 1997; Kersey *et al*., 1997).

Spent hen meal can vary considerably in nutrient content. For example, Kersey *et al*. (1997) obtained products from three different renderers and found CP, fat, calcium and phosphorus levels to range from approximately 65 to 71%, 9 to 11%, 3.3 to 4.8%, and 1.9 to 2.3%, respectively. As would be expected, amino acid compositions as a percentage of the total sample varied widely, but when expressed as a percentage of CP, the values were fairly constant among samples. Douglas *et al*. (1997), who evaluated three additional spent hen meals, made similar observations to Kersey *et al*. (1997) but also concluded that spent hen meal has substantial nutritional value for poultry as a protein concentrate. Careful monitoring will be required in order to achieve a more uniform product composition.

Feather meal

Even with the best of processing conditions, feather meal still faces amino acid imbalance and digestibility problems (Lilburn, 1996). Because feather protein is extremely deficient for the chick in methionine, lysine, histidine and tryptophan, feather meal should only be used in limited quantities, and other protein supplements or free amino acids must be employed to correct the amino acid deficiencies (Scott *et al*., 1982; Eissler and Firman, 1996).

Lanthionine, a cross-linked sulphur amino acid which is formed during the heating of feathers, can be used both as an indicator of overprocessing or to monitor the presence of feathers in meat meal-type products (Leeson and Summers, 1997). Lanthionine is typically present in feather meal at a level of ~1.2% (Han and Parsons, 1991), which is ~25% of total cystine levels.

Blood meal

Ground dried blood is rich in CP (~80%) and lysine, arginine, methionine, cystine and leucine (Liu *et al*., 1989; McDonald *et al*., 1995) but has a poorly balanced amino acid pattern that limits its use in poultry diets (Scott, 1987). Blood meal is somewhat unusual in that it is deficient in isoleucine (Tables 4.3 and 4.4). Thus, analogous to the use of groundnut meal and safflower meal as

intact protein sources for the production of methionine- and lysine-deficient diets, respectively, blood meal has been used experimentally to produce an isoleucine deficiency (Scott *et al*., 1982).

Marine Protein Sources

Fish meal

Fish meal was once considered to be an indispensable ingredient for turkeys, although turkey feeds can now be formulated to contain all of the factors needed for optimal growth and reproduction without the use of this protein concentrate (Scott, 1987). Nevertheless, fish meal is still an excellent source of protein for poultry, because it contains adequate quantities of all indispensable amino acids and is especially rich in lysine and methionine (Scott *et al*., 1982). However, its use is generally limited in order to prevent a fishy taint to meat and eggs.

Menhaden and anchovy are the main fish species used for meal manufacture, with lesser quantities of herring meal produced in Europe (Leeson and Summers, 1997). Tuna meal, which is distinct from many other types of fish meal in that fillets are removed for human consumption prior to preparation, contains less protein than meals made from whole fish (Zaviezo and Dale, 1994). Not unexpectedly, most amino acids are proportionately lower in tuna meal vs. menhaden meal (Tables 4.3 and 4.4) or anchovy meal. The protein contents of fish meals may also vary considerably (range of 50–75% CP) and, as with other protein concentrates, overprocessing may significantly reduce the nutritional quality of the product (McDonald *et al*., 1995). In addition, gizzerosine, which is formed from histidine during the heating of fish meal and possesses histamine-type properties, can cause gizzard lesions due to an excessive stimulation of acid production by the proventriculus (Leeson and Summers, 1997).

PREDICTION OF FEEDSTUFF AMINO ACID CONTENTS

Use of Regression Analyses Based on Proximate Composition Data

Because of the expense and time delays associated with chemical analyses of feed ingredients, amino acid compositions are conventionally estimated using linear regression models (Cravener and Roush, 1999). Research has been conducted at several laboratories using regression analysis to estimate the amino acid composition of selected feed ingredients. Interested readers are referred to Tables 9–4 and 9–5 of the National Research Council (1994) publication, which present equations for predicting the amino acid contents of feedstuffs based on CP and from other proximate components, respectively. Although these equations are considered to be accurate, in situations where adverse growing conditions (e.g. drought) result in elevated CP levels, the prediction equations may significantly overestimate the indispensable amino acid content of feedstuffs such as maize (Lilburn *et al*., 1991).

Use of Artificial Neural Networks Based on Proximate Composition Data

The potential of artificial neural networks to predict the amino acid content of feedstuffs has been examined recently in three novel studies by Roush (Roush and Cravener, 1997; Cravener and Roush, 1999, 2001). As compared to regression analysis, artificial neural networks were found to more accurately predict the content of selected indispensable amino acids in maize, soybean meal, meat and bone meal, fish meal and wheat. Although the application of artificial neural networks in predicting the amino acid composition of feedstuffs is in its infancy, the improved accuracy of this methodology over conventional regression procedures may eventually translate into lower-cost poultry feed formulations, as well as reduced nitrogen pollution potential, through further minimization of the over-supplementation of indispensable amino acids (Cravener and Roush, 1999).

Use of Near-infrared Reflectance Spectroscopy

First developed in 1964 to measure moisture content, near-infrared reflectance spectroscopy (NIRS) technology is currently being utilized to determine the chemical composition of both individual ingredients and complete mixed feeds (Araba, 1997; Leeson and Summers, 1997; Fontaine *et al*., 2001), as well as their digestible amino acid contents (Van Kempen and Simmins, 1997). NIRS is actually a regression technique, with its predictions based on correlations between spectral information and reference data (Van Kempen and Simmins, 1997; Van Kempen, 2001).

The precision of NIRS technology, combined with its short sample analysis time, provides feed manufacturers with an efficient, economical quality control tool for the production of feeds that more closely match their intended nutrient profiles (Van Kempen and Simmins, 1997). Although NIRS has the potential to predict amino acids in a wide variety of feed ingredients and mixtures, its usefulness will depend entirely on the careful and conscientious calibration of the equipment (Leeson and Summers, 1997).

FUTURE ROLE OF BIOTECHNOLOGY IN IMPROVING PLANT PROTEIN QUALITY

In many fields of science today, the tools of molecular biology are being utilized in an attempt to produce plants and animals more efficiently while improving the quality and sustainability of the world's food supply. Thus, with due respect to the authors of the chapters dealing with the genetic manipulation of cereals and other crops, it would be remiss to not at least briefly acknowledge these exciting developments in the context of a discussion of the qualitative chemical appraisal of protein in poultry feedstuffs.

During the last two decades, there have been remarkable changes in the rate and range of potential modifications to the genetic composition of plants.

While herbicide-tolerant and insect-resistant product offerings dominate the $1.5 billion transgenic seed market today, the next-generation products of plant agricultural biotechnology are expected to focus on quality traits that improve crops as animal feed or human food (Thayer, 1999).

However, hurdles to market acceptance of genetically modified organisms (GMO) presently exist, particularly in Europe (Anonymous, 1999; Barling *et al*., 1999; Butler and Relchhardt, 1999; Hileman, 2001). In addition, even within nations that have readily accepted engineered crops, 'identity preservation' of GMO may be difficult and not cost effective (Thayer, 1999). Moreover, the production of an elite germplasm with improved quality traits may not necessarily guarantee the successful introduction of that transgenic crop into the marketplace. This was recently evidenced by the abandonment of plans to market sulphur amino acid-enriched canola and faba beans, because they were engineered with the methionine-rich, 2*S* albumin gene from the Brazil nut, whose protein product was subsequently shown to be highly allergenic in humans (Nordlee *et al*., 1996; Butler and Relchhardt, 1999).

Nevertheless, despite the above shortcomings, agricultural biotechnology is viewed by many as having great promise and, perhaps, as the only viable means by which we will be able to increase food supplies for an ever-expanding world population. From an animal agriculture perspective, the ability to markedly boost the sulphur amino acid content of legumes, or the lysine, threonine and tryptophan contents of cereals, is very exciting and significant in terms of economics, performance enhancement and environmental considerations (nitrogen excretion). Therefore, the successful development of high-yielding transgenic crops with enhanced protein quality would be expected to have a major impact on how we formulate feed for non-ruminant animals, and will certainly necessitate major revisions in both ingredient matrices and feedstuff composition tables.

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CHAPTER 5
An appraisal of fats and fatty acids

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The use of supplemental dietary fat in commercial poultry diets has been widespread since research in the 1960s demonstrated improved growth, feed efficiency, egg size and yield, and hatchability in poultry fed diets supplemented with fat (Fedde *et al.*, 1960; Renner and Hill, 1960, 1961; Young, 1961; Young and Garrett, 1963; Artman, 1964; Lewis and Payne, 1966). The characteristics of dietary fat sources needed for maximum utilization and energy value also have been the focus of considerable research (Garrett and Young, 1975; Wiseman, 1984; Sell *et al.*, 1986; Wiseman and Lessire, 1987; Hyughebaert *et al.*, 1988; Ketels and DeGroote, 1989; Wiseman and Salvador, 1991; Wiseman *et al.*, 1991). In addition to their recognized value as a dense source of energy, supplemental fats are excellent sources of essential fatty acids, enhance the absorption of fat-soluble vitamins, increase the palatability of the diet, reduce dustiness of the feed, and act as a lubricant to reduce wear of pellet mills (Bisplinghoff, 1992).

This paper will not address the issue of utilization, but will discuss sources of feed fats and the evaluation of their quality.

SOURCES OF FEED FATS

Two factors are of utmost importance with regard to the choice of feed fats, cost and metabolizable energy content, which is a function of digestibility and absorption. In early studies, investigators used tallow which is poorly utilized and has a relatively low energy value (Fedde *et al.*, 1960; Young, 1961). It was found subsequently that the saturated fatty acids in tallow were poorly absorbed and that by adding linoleic acid the utilization of tallow was improved to more than the predicted value of the mixture (Young and Garrett, 1963; Lewis and Payne, 1966). With the development of vegetable oil processing, acidulated soapstocks (mainly unesterified fatty acids removed during refining) became economical fat sources, either alone or blended with rendered fats (Bisplinghoff, 1992; Rouse and Petas, 1998). Research showed that unesterified saturated fatty acids were poorly absorbed, but that their utilization could be improved if they were combined with unsaturated fats (Young and Garrett, 1963). The apparent metabolizable energy (AME) of fats decreased linearly with increasing unesterified fatty acid content and this was particularly evident in young broiler chickens (1.5 weeks compared with 7.5 weeks) (Wiseman and Salvador, 1991).

 Poultry Feedstuffs: Supply, Composition and Nutritive Value (eds J.M. McNab and K.N. Boorman)

As the understanding of the requirements for maximum absorption has developed, the focus has been to use fats of the lowest possible cost, taking into consideration limitations for minimum concentrations of linoleic acid and maximum levels of unesterified fatty acids.

Today, many supplemental fats are by-products of the rendering industry (Lilburn, 1996) and fall into one of the categories shown in Table 5.1 (Bisplinghoff, 1992). Note that each category, with the exception of poultry oil, may contain fat from non-specified sources. Generally fats are categorized by 'titre', a measure of the solidification temperature of fat. Fats with a titre of 40 or above are considered to be tallow, whereas those with lower titre are classified as grease, regardless of the source. Examples of fatty acid profiles of these and other dietary fats are shown in Table 5.2.

Occasionally, unique fat sources will find a place in the industry. Ortiz *et al.* (1998) reported that fatty acids in de-hulled full-fat sunflower seed (incorporated up to 240 g kg^{-1} diet) were efficiently utilized (90% absorption by broilers). Rising *et al.* (1986) showed that fatty acids of calcium soaps of tallow were absorbed by laying hens 91% as well as the fatty acids of tallow alone. Calcium soaps are an excellent pellet binder as well as a source of energy and calcium for laying hens. Recently, much attention has been directed toward increasing the n-3 fatty acid content of poultry meat and eggs by feeding fat supplements with high n-3 fatty acid content such as linseed (Caston and Leeson, 1990; Cherian and Sim, 1992; Scheideler and Froning, 1996; Leeson *et al.*, 1998). Of particular interest is the increased content of eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3) found in fish oil (Hammershøj, 1995) or in an algae extract (Herber-McNeill and van Elswyk, 1998). Dietary fish oil can cause off-flavours in meat and López-Ferrer *et al.* (1999) found that replacing fish oil with linseed oil or rapeseed oil (Canola) for the final 1 or 2 weeks of feeding, or for the entire feeding period progressively decreased the content of fish oil fatty acids and improved the sensory quality of broiler meat.

Table 5.1. Common types of feed fats produced by renderers[a].

Category	Description
Feed grade animal fat	Primarily rendered beef and pork material; could include other animal sources
Poultry fat	Rendered solely from poultry, often used within an integrated industry
Choice white grease	Primarily from rendered pork products, but may contain beef and poultry fat if specifications are met
Tallow	Primarily from rendered beef or other ruminant tissue
Yellow grease	Primarily recycled restaurant grease (hydrogenated vegetable fats) but may contain other fats of dark colour or high unesterified fatty acid content
Blended animal and vegetable fat	May contain blends of all types of animal fat, vegetable oil, acidulated soapstock, restaurant grease

[a]Bisplinghoff (1992).

Table 5.2. Typical fatty acid profile of feed fats.

	Fatty acid (g kg^{-1} of total fatty acid)													
Category	14:0	16:0	16:1 n-7	17:0	18:0	18:1 n-9	18:2 n-6	18:3 n-3	20:0	20:1 n-9	20:5 n-3	22:1 n-9	22:6 n-3	IV
Tallow[a]	30	250	25	15	215	420	30							50
Choice white grease (lard)[a]	15	270	30	5	135	434	105	5						65
Poultry[a]	15	210	65		110	400	180	10						84
Yellow grease[a]	19	150	15	10	90	480	200	30						89
Soybean[a]		115			40	245	530	70						125
Maize[a]		163			25	290	550	65						125
Rape-high erucic acid[a]		33	2		15	214	142	70	7	123		389		144
Rape-low erucic acid (Canola)[b]		43	3		17	591	228	82	5	20		9		132
Sunflower[c]		60			64	284	581	1	6	3				118
Flax (linseed)[d]		59	2		34	162	167	575						200
Fish (menhaden)[e]	105	215	142		34	103				12	151	1	65	126

[a]Rouse and Petas (1998); [b]Leeson (1984); [c]Ortiz *et al.* (1998); [d]Kuksis (1978); [e]Opstvedt (1985).
IV = iodine value.

MEASURE OF FAT QUALITY

Fatty Acid Profile

The first measure of fat quality is to determine the profile of fatty acids (known as 'fat structure' in the industry) in order to characterize the extent of saturation and unsaturation. In today's technology, this is accomplished easily by direct extraction/transesterification to form the methyl esters that can then be analysed by gas–liquid chromatography (Sukhija and Palmquist, 1988). This procedure simultaneously determines the amount of fatty acids in the sample.

The fatty acid profile of restaurant grease has changed considerably in recent years. Public concern about the relationship between saturated fats and health resulted in pressure on fast-food chains to move away from tallow, the fat of highest quality for deep-fat frying. Table 5.3 shows that restaurant grease has become more unsaturated since 1987, and decreasing standard errors of means for individual fatty acids suggest that fats used by different chains are becoming more similar. The fats used most extensively now are hydrogenated vegetable oils, and this is reflected in the decreasing proportions of 16:0 and increasing proportions of *trans* fatty acids. Formation of *trans* isomers is also greater when the more-unsaturated fats are heated. It appears that processors, fat users, or both, have learned about quality control, judging from lower values for both initial peroxide values and peroxide values after oxygen treatment (AOM) in 1998 compared with 1994. Some adjustment in profiles is indicated also by the slightly lower iodine value in 1998, suggesting that the move to unsaturated frying oils had gone too far in 1994.

Table 5.3. Changes in fatty acid composition of restaurant grease from four leading fast-food chains in the USA (based on Rouse and Petas, 1998).

	Fatty acid (g kg^{-1})		
Parameter	1987	1994	1998
14:0	21.0 ± 12.6	6.5 ± 6.1	5.7 ± 3.9
16:0	213 ± 37.7	135 ± 19.5	138 ± 14.7
16:1	29.5 ± 9.0	11.0 ± 6.7	12.2 ± 6.6
18:0	123 ± 34.7	98.0 ± 31.0	99.2 ± 7.2
*c*18:1	450 ± 59.5[a]	410 ± 30.2	389 ± 24.1
*t*18:1	–	165 ± 35.5	170 ± 43.4
*c*18:2	108 ± 58.5	110 ± 84.5	105 ± 41.1
*t*18:2	–	35.0 ± 15.1	35.0 ± 12.0
18:3	14.5 ± 5.4	5.5 ± 4.7	1.0 ± 0
IV	63.1 ± 11.6	79.2 ± 14.0	75.7 ± 5.3
IPV	–	2.0 ± 1.3	0.9 ± 0.2
AOM	–	47.9 ± 5.3	23.0 ± 3.8

[a] *Trans* isomers not separated.
IV, iodine value; IPV, initial peroxide value, meq kg^{-1}; AOM, peroxide value, meq kg^{-1}, after bubbling oxygen for 20 h.

Unesterified Fatty Acids

Known in the industry as 'free fatty acids', the proportion of unesterified fatty acids can influence absorbability, as noted above. In higher-quality fats (tallow, choice white grease), the concentration of unesterified fatty acids is a measure of the history of the fat; a high proportion of unesterified fatty acids suggests that the fat has been abused (i.e. excess water content, heat or long storage). The content of unesterified fatty acids is not a measure of abuse in blended fats, which contain varying amounts of acidulated soapstock.

Moisture, Impurities and Unsaponifiables (MIU)

The measure 'MIU' should not exceed 2% in high quality fats. Not only does this non-fat material dilute the energy value, it also may be detrimental in specific ways. Moisture (M) contributes to fat hydrolysis, increasing the unesterified fatty acid content with the subsequent formation of sludge in holding tanks. Insoluble impurities (I) include any material not soluble in petroleum ether. These can cause clogging of screens and nozzles during transport. Insoluble impurities may include hair, hide, bone, plastic ear tags, etc. The unsaponifiable (U) matter includes ether-soluble, non-fatty acid material, such as sterols, hydrocarbons, fatty alcohols and pigments (Bisplinghoff, 1992). Potentially serious unsaponifiable materials are the polymers caused by oxidative degradation (see below), and in future, olestra, an indigestible sucrose polyester of vegetable oil which could become a problem if its use in frying oils becomes more prevalent.

Iodine Value

The iodine value (IV) is a simple and rapid measure of the total unsaturation of a fat. It is defined as the grams of iodine that will be absorbed by 100 g of fat (one unsaturated bond absorbs one mole of iodine). Oleic acid, for example, has an IV of 90. The IV is used often by industry to indicate the fatty acid profile (Rouse and Petas, 1998). However, IV provides no information about the fatty acid profile of the fat.

Other Considerations

Any feed fat should be certified free of pesticide and PCB residues, and stabilized with the addition of an approved feed or food-grade antioxidant (Rouse and Petas, 1998). Suggested industry quality specifications for blended feed fats are shown in Table 5.4. Fats should be stabilized sufficiently to pass the AOM stability test. The latter refers to 20 hours of bubbling oxygen under defined conditions. To be acceptable, a fat should emerge with no greater than 20 meq peroxide kg^{-1}, having had an initial peroxide content of less than 5 meq peroxide kg^{-1} fat. The 20 h AOM test is a measure of stability for storage, whereas the initial peroxide test indicates prior abuse and present quality of the

Table 5.4. Suggested quality specifications for feed fats (Rouse and Petas, 1998).

	Blended fat categories (%)				
	Animal	Poultry	Feed grade animal	Animal/ vegetable	Vegetable soapstock
Total fatty acids, minimum	90	90	90	90	90
Free fatty acids, maximum	15	15	15	15[a]	50
Moisture, maximum	1	1	1	1	1.5
Impurities, maximum	0.5	0.5	0.5	0.5	1
Unsaponifiable, maximum	1	1	1	3.5	4
Total MIU, maximum	2	2	2	5	6

[a]When blended feed fats contain acidulated soapstock this specification can be adjusted to allow for higher unesterified fatty acid found in this fat.

fat. There is debate in the industry as to whether the AOM test is a measure of quality (Bisplinghoff, 1992). Fats intended for use in poultry diets should contain no cottonseed soapstock or other cottonseed by-products and should be certified negative for chick oedema factor as determined by the modified Lieberman-Burchard test (Rouse and Petas, 1998). Fats should contain no more than trace amounts of nickel, a common catalyst for hydrogenation of vegetable oils, or other heavy metals.

QUALITY CONTROL – LABORATORY ASPECTS

Quality control of blended feed fats, with a special view of UK practices, was described competently by Edmunds (1990). He showed that the MIU of blended fats generally increased as unsaturation of the oil increased (Table 5.5), and could be variable, thus causing the energy value of the fat to vary. He described the low repeatability of measuring the unsaponifiable fraction, which contains the oxidized and polymerized fatty acids (Table 5.6). He concluded that the MIU, plus oxidized and polymerized fatty acids (non-elutable material, NEM) are useful indicators of fat quality, but consitute four non-specific and highly-variable assays. He proposed that NEM should be determined indirectly, as the difference between total mass and total fatty acids. Values he reported for NEM are shown in Table 5.7. A simple and rapid procedure for determining both the fatty acid content and profile by gas–liquid chromatography has been used in our laboratory for many years (Sukhija and Palmquist, 1988). Edmunds (1990) cautioned that correcting for NEM would undervalue the energy of fats according to the amount of glycerol in the fat. In pure triacylglycerol, this amounts to 10% of the weight (5% of the energy), whereas unesterified fats contain no glycerol. Thus, he advocated an additional measure to determine glycerol content.

Table 5.5. Unsaponifiable matter found in common feed fat raw materials (Edmunds, 1990).

	Unsaponifiable matter (g kg^{-1})	
Material	mean ± SD	*n*
Crude soybean oil	5 ± 2	6
Recovered vegetable oil	7 ± 4	36
Palm acid oil	15 ± 4	6
Tallow	2 ± 8	20
Fish acid oil	21 ± 5	4
Palm fatty acid distillate	23 ± 11	5
Mixed soft acid oil	3 ± 14	21
Soy/sunflower acid oil	64 ± 27	25

SD, standard deviation; *n*, number of samples.

Table 5.6. Repeatability of slip point (SP), free fatty acid (FFA), unsaponifiable matter (US), oxidized fatty acids (OFA) and moisture determinations on a sample of blended feed fat within a laboratory (Edmunds, 1990).

	SP(°C)	FFA (g kg^{-1})	US (g kg^{-1})	OFA (g kg^{-1})	Moisture (g kg^{-1})
n	10	10	10	10	5
Mean	268	364	19.7	8.6	6.2
SD	5.9	2.5	2.4	1.5	6
CV (%)	22	7	122	174	95

Table 5.7. Non-elutable material (NEM) values found in feed fat raw materials. Samples were assayed using 17:0 (free acid) as an internal marker. All data are corrected for any background 17:0 (Edmunds, 1990).

	NEM (g kg^{-1})	
Material	mean ± SD	*n*
Palm acid oil	40 ± 12	9
Palm fatty acid distillate	50 ± 14	5
Crude soybean oil	52 ± 1	3
Tallow	70 ± 13	59
Recovered vegetable oil	86 ± 29	91
Fish acid oil	102 ± 41	6
Mixed soft acid oil	105 ± 32	37
Soy/sunflower acid oil	124 ± 28	22

SD = standard deviation; *n* = number of samples.

COMMERCIAL STANDARDS FOR FAT QUALITY

A quality control chart, developed as a guideline for determining the types and frequencies of analyses for commercial fat use, is shown in Table 5.8. These analyses are used in addition to individual requirements that may be specified by individual users. Typical specifications by three large commercial users of blended fats in the USA are shown in Table 5.9.

SUMMARY

Blended fats are an important and economical source of energy and essential fatty acids for poultry. Information is available to determine the optimal combinations of saturated and unsaturated fatty acids and maximum unesterified fatty acids. When these are established, fat users must insist on adequate and regular quality control to ensure that a consistent and safe product is supplied.

Table 5.8. A quality control chart to guide number and frequency of analyses for commercial fats (Rouse and Petas, 1998).

Type of fat	Frequency code
1. Animal fat – tallow/grease	A – Every sample
2. Poultry fat	B – 1 of 2
3. Blended feed grade animal fat	C – 1 of 5
4. Blended animal and vegetable fats	D – Every sample – composite of 3
5. Vegetable soapstocks	E – Check each supplier periodically

		Type of fat				
Analysis	AOCS method[a]	1	2	3	4	5
Moisture	Ca 2A-45	A	A	A	A	A
Impurities	Ca 3-46	A	A	A	A	A
Unsaponifiable	TKLa-64T	C	C	B	B	A
Total fatty acid	G3-53	C	C	B	B	A
Free fatty acid	CA 52-40	B	B	A	A	A
PCBs – pesticides		D	D	D	D	D
Stability						
Initial peroxide	Cd 8-53	A	A	A	A	A
AOM test	Cd 12-57	A	A	A	A	A
Gossypol[b]	Halphen test	E	E	E	E	E
Iodine value	Cd 1-25	A	A	A	A	A
Fat structure	Ce 1-62	E	E	E	E	E

[a]AOCS (1997).
[b]Poultry use only.

Table 5.9. Typical purchase specifications by major users in the US fat industry (Rouse and Petas, 1998).

User 1		
Ingredient requirements	Minimum	Maximum
Moisture, %		0.5
Impurities, %		0.5
Unsaponifiable, %		1.0
Total MIU, %		**2.0**
Total fatty acids, %	90.0	
Free fatty acids, %		15.0
Iodine value	75.0	80.0
AOM stability, meq kg^{-1}		20.0
Fatty acid composition		
Linoleic acid, %	15.0	20.0
Oleic acid, %		50.0
Colour and special characteristics		
Colour	Tan to brown	
Odour	Typical, not rancid	
Special considerations		
Certified free of pesticide residues and PCB		
All fats stabilized with feed or food grade antioxidant		
User 2		
Specifications	Acceptance	Desired
Unloading temperature,°F	165	140
FFA, %	16	5
Total fatty acids, %	92	93
Moisture, %	1	0
Unsaponifiable, %	1	0
MIU, %	2	0
Linoleic acid, %	16	15
Chick oedema factor	Neg.	Neg.
Odour	Free from rancid or putrid odour	
Contamination	Free from visual contamination	
User 3		
Total fatty acid, %	90 minimum	
Free fatty acid, %	30 maximum	
Moisture, %	1.0 maximum	
Impurities, %	0.5 maximum	
Unsaponifiable, %	2.5 maximum	
Iodine value	85 ± 3	
Linoleic acid, %	23 minimum	
Stearic acid, %	12 maximum	
Gossypol	Trace	
Inorganic elements	Trace	
AOM stability	20 h	
Chick oedema factor	Negative	
PCB/pesticides	Below FDA tolerances	

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CHAPTER 6
An appraisal of trace elements: inorganic and organic

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INTRODUCTION

Trace elements fulfil more or less specific physiological and biochemical functions in the body, as summarized by Underwood and Suttle (1999). They predominantly act as catalysts in many enzyme and hormone systems. Deficiency symptoms include disturbances of many metabolic processes, resulting in reduced production performance, loss of appetite, reproductive disorders, impaired fat and carbohydrate metabolism and immune response.

Much research has been done to determine the bioavailability of inorganic trace element compounds. Most data are expressed relative to a standard source such as mineral sulphates or oxides. Recently, trace minerals have received new attention because:

1. It has been recognized that trace mineral contents in animal manure are far in excess of nitrogen. Mohanna and Nys (1998) calculated that Zn, Cu and Mn in poultry manure are far in excess of crop requirements, causing accumulation in the soil, especially in regions of intensive livestock production. This phenomenon is even more important in pig manure as in some pig diets high Cu and Zn concentrations are used because of growth promoting effects (Meijer and Kröger, 1973; Hahn and Baker, 1993). To avoid further accumulation of these trace elements in the soil, trace mineral compounds with higher bioavailabilities should be used, and/or safety margins lowered.
2. Additional positive effects of specific trace element compounds have been described on immune competence (e.g. Zn-methionine) and on fat and carbohydrate metabolism (e.g. Mn and Cu). Cr sources are also of interest because of their effects on fat and carbohydrate metabolism.

The use of organic sources can potentially improve intestinal absorption of trace elements as they reduce interference from agents that form insoluble complexes with the ionic trace elements. Moreover, positive responses have been described for several organic trace element compounds in various physiological processes. In this chapter, the bioavailabilities of both inorganic and organic sources of trace minerals and the factors affecting them are discussed. In addition, the potentials of different organic compounds are summarized.

 Poultry Feedstuffs: Supply, Composition and Nutritive Value (eds J.M. McNab and K.N. Boorman)

TRACE MINERAL RETENTION IN BODY TISSUES

Body retention of most trace elements is very low. Recently, Mohanna and Nys (1998) calculated the retention of Zn, Fe, Mn and Cu in broiler chickens during a complete production period using feed and carcass analysis. They concluded that the retentions (as percentage intake) of Zn, Fe, Mn and Cu were only 6, 10, 0.2 and 6, respectively. These retention values agreed fairly well with calculations done by van der Klis (1991), who concluded that 22, 15, 0.6 and 5%, respectively were retained by broiler chickens during a 6 week growth period. The considerably higher retention value for Zn was primarily caused by the higher Zn concentrations in the French diets. The low retention of trace elements is mainly ascribed to the relatively high dietary intakes, as the body contents of trace elements are rather stable in the second half of the production period (Mohanna and Nys, 1998). Dietary contents of trace elements are far in excess of the animals' requirements, as published by the NRC (1994), for the following reasons:

1. In general, the physiological requirements as published by the NRC (1994) are determined at low dietary concentrations of potential interacting agents. However, commercial diets contain large amounts of agents such as fibre, phytate and phosphate that may decrease trace element absorption. For this reason, large safety margins are used to avoid deficiencies. Moreover, knowledge of the bioavailability of trace elements in feed ingrediants is lacking and therefore trace elements from this source are neglected in feed formulation. Furthermore, physicochemical conditions in the intestinal tract can adversely affect trace mineral absorption (Mohanna *et al*., 1999). Finally, mutual interactions between minerals and trace elements for transport proteins in enterocytes and in intermediary metabolism, and the nature of organic molecules in the gastro-intestinal lumen, can affect the efficiency of absorption and utilization.
2. As trace elements play essential roles in many physiological processes, adequate dietary concentrations are crucial. Moreover, positive effects of dietary trace element concentration in excess of the physiological requirements on production performances of the animal have been reported.

METHODS OF EVALUATION OF THE BIOAVAILABILITY OF TRACE ELEMENT COMPOUNDS

The bioavailability of an inorganic nutrient was defined by Fairweather-Tait (1997) as the proportion in the diet that is utilized for normal metabolic functions. The results of bioavailability tests are not only affected by the characteristics of the mineral source, but also by the specific test conditions. Test conditions might affect intestinal absorption and the utilization in the body, such as the composition of the basal diet (practical or synthetic), the age and physiological state of the animals, and the response parameters used.

Before trace elements can be absorbed from the intestinal lumen, they should be in a soluble form, i.e. as ions or bound to low molecular weight lig-

ands. Once absorbed they are generally bound to transport proteins and transported to target organs where they fulfil their metabolic functions.

Many studies to determine the bioavailability of trace elements are based on dose–response curves relating supplementation rate of a trace element to the content of that trace element in specific body tissues or to the production performance of an animal. The experimental diets are formulated to contain adequate levels of all nutrients except the trace mineral to be studied. If necessary, chickens will be depleted during the first week of life by feeding a purified diet deficient in that trace element. The depletion period is followed by a repletion period in which the standard and test diets, containing increasing concentrations of the standard trace element source and the test source to be evaluated, respectively, are fed. The relative biological value (RBV) of the test product is calculated from the ratio of the slopes of the two response curves: 'slope of the test response' divided by 'slope of the standard response'. The RBV of the standard product is set at 100% (Fig. 6.1). Using this slope-ratio technique, a linear response relationship is a prerequisite. It was shown that this assumption is true for a wide range of intakes of Mn (e.g. Black *et al*., 1985) and Cu (e.g. Miles *et al*., 1998), using tibia Mn and liver Cu as a response criterion, respectively. Unlike Zn, the estimated bioavailabilities for Mn and Cu were similar at high (up to 1000 mg Mn kg^{-1}) and low supplementation rates (up to 100 mg Mn kg^{-1}) as described by Wedekind and Baker (1990b). For Zn, linearity was only valid up to intakes of about 30 mg kg^{-1}, using tibia Zn as a response parameter (Wedekind and Baker, 1990a). Wedekind *et al*. (1992) concluded that bioavailability assays involving higher Zn intakes, i.e. where there was a diminishing response per unit of supplementation, are invalid as they would overestimate the RBV of trace element sources with lower availabilities.

A complicating factor in using depletion/repletion techniques for the evaluation of Zn sources is the stimulatory effect of available Zn intake on feed intake in Zn-depleted birds. Under these conditions, Zn intake from the basal diet (mostly with an unknown availability) will confound the assessment of the

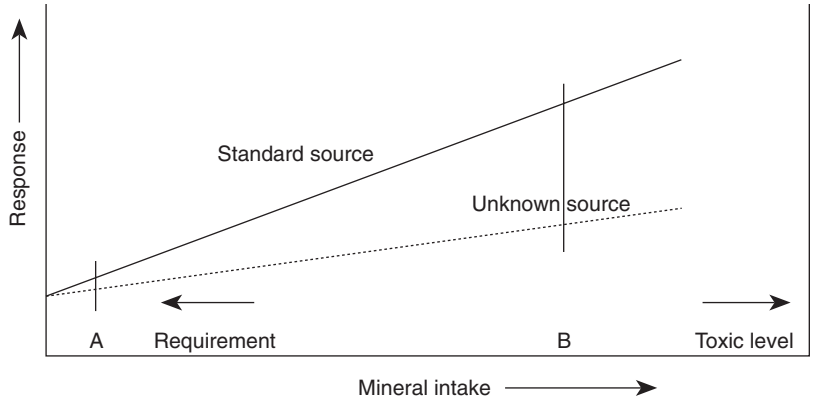

Fig. 6.1. Predicted tissue mineral response to dietary addition of a mineral. The biological value of the test product (with an unknown biological value) is expressed relative to the standard source (reference = 100) (from Henry *et al*., 1986).

Zn bioavailability of the test source. The accumulation of Zn in the tibia is therefore not only the result of differences in Zn availability, but also of differences in basal diet Zn intake (Wedekind *et al*., 1992). These problems can be overcome by using either purified basal diets containing no Zn or by feeding a basal diet, which meets the Zn requirements of the animal. However, in the latter case it should be ensured that dose rates do not exceed the linear range.

Black *et al*. (1985) conducted an experiment to determine which response criterion would be the most discriminative for the evaluation of Mn bioavailability. Based on the highest slope/SD ratio using kidney Mn and tibia Mn contents in 3-week-old chickens as response criteria, they concluded that these were better criteria than plasma, pancreas plus liver, and muscle Mn concentrations. Among others, Henry *et al.* (1986) have shown that estimation of the RBV of MnO (reference $MnSO_4.H_2O$) using Mn contents in bone, kidney and liver as response parameters resulted in values of 79%, 58% and 64%, respectively.

Nutritional balance methods are not very discriminative for estimating the relative availability of trace element sources, as the proportion of most trace elements absorbed in the intestine is rather low. Moreover, homoeostasis of elements such as Cu, Mn and Zn is regulated via endogenous secretions. This implies that only small differences between intake and excretion of trace elements must be measured. Although the limitations of balance methods can be overcome by the use of isotopes to label either the body pool or the trace elements in the feed, in order to enable calculation of the true absorbability, reports of balance methods on trace elements are as scarce as those of RBV tests. Furthermore, these techniques are only valid if it is assumed that the marker isotopes behave in a similar manner to the normal isotopes of the trace elements (Sandström, 1997). Although this assumption might be correct for inorganic trace element sources, it most likely does not hold for organically bound trace elements.

BIOAVAILABILITY OF INORGANIC AND ORGANIC TRACE MINERAL COMPOUNDS USED IN POULTRY FEEDING

Animal feed is usually supplemented with trace elements in the form of mineral oxides and sulphates via mineral premixes. Apart from these inorganic sources, organic sources are available. Ammerman *et al*. (1998) have summarized the biological values of different trace element sources for poultry (Table 6.1).

Since trace elements are absorbed from the intestinal tract as ions or as soluble low molecular weight ligands (Scott *et al*., 1976), the solubility of a source would directly affect its bioavailability. This was confirmed by Ledoux *et al.* (1991) who determined the *in vitro* solubility of four different copper sources in water, neutral ammonium citrate, 0.4% HCl and 2% citric acid and showed that sulphate and acetate had similar solubilities, oxide a low solubility, while carbonate solubility was intermediate. They showed the same ranking in RBV *in vivo* using 3-week-old chickens. Ammerman *et al*. (1998) concluded in their review that the soluble trace mineral compounds, such as sulphates and chlorides, are well utilized by the animal, while less soluble compounds, such as carbonates and oxides, have lower relative biological availabilities.

Table 6.1. The relative biological value (RBV) of different Cu, Mn and Zn sources in poultry as reviewed by Ammerman *et al.* (1998).

Copper			Manganese			Zinc		
Compound	RBV	*n*	Compound	RBV	*n*	Compound	RBV	*n*
$CuSO_4.5H_2O$	100		$MnSO_4.2H_2O$	100		$ZnCl_2$	100	
Cu-lysine	105	2	$MnCO_3$	55	2	$ZnSO_4$	100	
Cu-methionine	90	2	MnO_2	30	3	$ZnCO_3$	105	2
Cu acetate	100	3	Mn-methionine	120	1	Zn methionine	125	3
$Cu(HCO_3)_2$	115	1	MnO	75	8	Zn lysine	110	1
$CuCO_3$	65	2	Mn-proteinate	110	2	ZnO	55	2
$CuCl_2$	110	2	MnCl	100	1	Zn-proteinate	100	1
$Cu_2(OH)_3Cl$	105	2						
CuO	0	4						
Cu_2O	100	1						

n is the number of observations; all values are rounded to the nearest '5'.

In general, basal diets with low concentrations of potential interacting agents are used to determine differences in bioavailabilities of trace element sources, although the dietary content of potential chelating agents can affect the outcome of studies to determine RBV. Fly *et al*. (1989) observed that the RBV of Mn-methionine was 130% in comparison to MnO when a semi-synthetic diet was used, while the difference between Mn sources was much more pronounced in a practical maize–soy diet (RBV of Mn-met was 174%). These results were confirmed by Wedekind *et al*. (1992) using a purified basal diet, a basal diet with a soy-isolate and a practical maize–soy diet, for estimation of Zn-methionine. They found RBV estimates of 117%, 177% and 206% for Zn-methionine using $ZnSO_4.H_2O$ as a reference. These results illustrate the higher potential of organic trace element sources when fed in commercial diets rather than in purified experimental diets. Aoyagi and Baker (1993) suggested that differences in RBV of Zn in Zn-lysine and Zn-methionine, found using a purified diet with isolated soy protein, were possibly due to a higher fraction of Zn-lysine being dissociated in the upper small intestine compared with Zn-methionine, causing the former to be less protected from potent chelating agents in the gut.

POTENTIAL CHELATING AGENTS

As indicated, the outcome of availability studies is affected by the composition of the basal diet, because of the presence of trace minerals with an unknown availability in the feedstuffs used, and because dietary food components such as phytate and fibre bind trace minerals, inhibiting absorption. Especially in the complex diets used in practical animal feeding, organic trace mineral components might have a higher positive value than established in experiments using

semi-synthetic diets. Previously mentioned differences in RBV of organic Mn (Fly *et al*., 1989) and Zn sources (Wedekind *et al*., 1992) suggest that, with increasing concentrations of potential interacting agents, the use of trace mineral chelates will be more beneficial than under experimental conditions when semi-synthetic diets are used.

Trace elements are absorbed as ions or soluble compounds, as illustrated for Zn in Fig. 6.2. In the case of practical diets containing high concentrations of potential chelating agents, the use of organically bound trace elements can have benefits, as a higher proportion of the dietary supplement can become available for absorption in the host animal. Therefore, organic trace element compounds should be soluble, with a relatively high stability constant to effect absorption before the element is bound in the intestinal lumen to feed residues that are not absorbed. On the other hand, the stability of such organic complexes should not be too high, as in that case it will prohibit bonding to the endogenous transport proteins in the animal.

The adverse effects of dietary phytic acid, one of the most widespread mineral chelators, on the availability of (trace) minerals have been recognized for decades. It was demonstrated *in vitro* that phytic acid binds polyvalent cations, dependent upon the pH and the ratio between phytic acid and the minerals (Nolan *et al*., 1987). The adverse effects of phytate on trace mineral absorption have been studied by supplementing diets with phytases. Several experiments

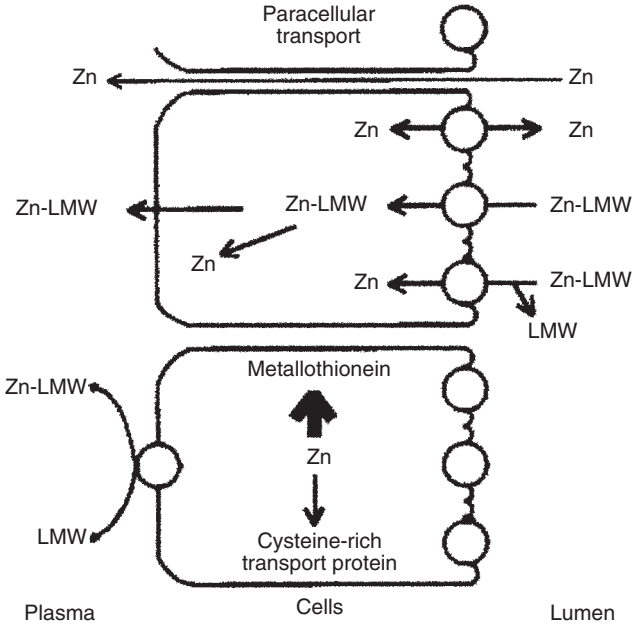

Fig. 6.2. A model for zinc absorption. Zinc absorption can be physiologically divided into two processes: uptake of Zn from the GI lumen into the enterocyte (top) and transport of Zn from the enterocyte into the circulatory system (bottom) (reprinted from Swinkels *et al.*, 1994). LMW, low-molecular-weight ligand.

have demonstrated a positive effect of dietary phytase supplementation on the bioavailability of Zn (Yi *et al*., 1996; Mohanna and Nys, 1999). However, the results of these experiments were dependent on the dietary trace mineral concentrations used and on the response parameter chosen. Mohanna and Nys (1999) observed a significant improvement in bone tissue Zn deposition in broiler chickens during an experiment from 5 to 21 days of age. They used a maize/soybean meal diet that was supplemented with 800 units phytase kg^{-1} with a dietary Zn content close to the requirement (40 mg kg^{-1}). At higher Zn contents (up to 70 mg kg^{-1}) any increase in tibia Zn was not significant. Also, Yi *et al*. (1996) showed a linear improvement in bone tissue Zn concentration with dietary phytase supplementation (inclusion rate of 600 IU kg^{-1} of a maize/soybean meal diet). Others showed inconsistent effects of phytase on Zn absorption in broiler chickens (Roberson and Edwards, 1994; Sebastian *et al*., 1996) or failed to show a positive effect of phytase on availability of trace minerals such as Cu (Aoyagi and Baker, 1995) and Mn (Mohanna and Nys, 1999). The lack of effect of dietary phytase supplementation on Cu availability has been ascribed to the improved bioavailability of antagonizing elements in Cu absorption. This is supported by the fact that dietary supplementation with 1,25-dihydroxycholecalciferol, which stimulates Ca absorption from the intestinal tract, also stimulated Cu absorption (Aoyagi and Baker, 1995).

BENEFICIAL EFFECTS OF TRACE MINERAL COMPOUNDS IN EXCESS REQUIREMENTS

Growth promoting effects of 'pharmacological' doses of dietary Cu are well recognized in piglets, and such positive effects have also been demonstrated in poultry (e.g. Pesti and Bakalli, 1996; Ewing *et al*., 1998; Miles *et al*., 1998). Dietary Cu concentrations of 125–250 mg Cu kg^{-1} diet increased growth and decreased feed conversion ratio. Cupric citrate was more efficacious for promoting growth than $CuSO_4.5H_2O$ (Pesti and Bakalli, 1996). Ewing *et al.* (1998) also showed that Cu-citrate at an inclusion rate of 63 g Cu kg^{-1} diet resulted in a larger improvement in weight gain during a complete production period than 125 g Cu kg^{-1} diet from $CuSO_4.5H_2O$ or Cu oxychloride. They added these Cu sources to a maize/soybean-meal basal diet containing 10 mg Cu kg^{-1} from vegetable sources. High concentrations of copper were shown to reduce total plasma and breast muscle cholesterol and plasma triglycerides (Konjufca *et al*., 1997). However, Cu concentrations greater than 350 mg kg^{-1} were shown to be toxic, i.e. resulting in poorer performance.

PERSPECTIVES OF CHROMIUM

Chromium (III) is involved in carbohydrate and fat metabolism (Nielsen, 1994), stimulating insulin action (Anderson, 1987). Under physiological and neutral pH conditions, levels of the free ionic form are extremely low, due to formation of complexes predominately with phytate and phosphate. Therefore, Cr must

bind with endogenous or dietary ligands to facilitate absorption, e.g. with some amino acids, such as histidine, and oligopeptides that chelate Cr during its passage through the small intestine. Nicotinic acid, oxalic acid and ascorbic acid may also stimulate Cr absorption (Ducros, 1992). However, it is not known whether the effect of these compounds derives from keeping Cr in solution or whether they are part of co-transport systems (Aggett, 1985). Cr absorption takes place in the proximal part of the small intestine. Absorption from inorganic Cr sources is low and varies from 0% to 3% (Anderson, 1987), while its absorption from Cr picolinate is somewhat higher (1% to 5%, Gargas *et al.,* 1994).

Supplementing laying hen diets with 400 mg kg^{-1} Cr from Cr picolinate stimulated energy and protein utilization and decreased serum cholesterol concentrations in 36-week-old laying hens (Kim *et al*., 1997). The serum cholesterol results agreed with those of Yih and Hsu (1997), who supplemented layer diets with 200 mg kg^{-1} Cr from Cr nicotinate, Cr picolinate and Cr yeast and observed decreased serum and egg cholesterol concentrations. Steele and Rosebrough (1981) observed an improved daily gain and feed conversion ratio in turkeys with supplements of 20–80 mg kg^{-1} $CrCl_3.6H_2O$. Part of these effects of Cr were also described for 'pharmacological' inclusion rates of Cu (e.g. Bakalli *et al.*, 1995). However, to the present time, Cr supplementation of pig and poultry diets is not allowed in the European Union.

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PART III
Feedstuff quality: quantitative assessment

CHAPTER 7
Digestive processes in poultry from a physiological viewpoint

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ABSTRACT

Gastrointestinal (GI) contractions (i.e. motility) are required to: (i) move digesta through the GI tract; (ii) help in mechanically reducing the size of swallowed food items (especially in the gizzard); and (iii) mix mechanically reduced digesta with digestive secretions (e.g. enzymes, acids). The bulk and density of the digesta regulate the rate at which foods passes through the tract, i.e. food items such as complex carbohydrates, which are more difficult to digest, pass more slowly than easily digested items. This regulation is accomplished by hormones secreted from the gut and pancreas, and also by intrinsic nerves within the gut and extrinsic nerves arising from the brain and spinal column.

INTRODUCTION

In the absence of ingesta there is minimal gastrointestinal (GI) motility. Eating, or even the anticipation of eating, increases motility, with the type of nutrient ingested determining the amount and type of motility that occurs.

MATERIALS AND METHODS

GI motility (contractions of the gut) is commonly detected by strain-gauge transductors (SGT) surgically sutured directly on to the surface of the tract. SGT can be surgically implanted on the serosal surface of any part of the GI tract. Wire leads from each SGT are passed through the surgical opening in the abdomen and sutured to the skin on the back of the bird. The leads are inserted into small three-pin plugs. To obtain information about GI motility, each plug is attached to a transducer on a physiological recorder. This emits a very small current, which flows through the SGT. When the SGT are distorted by a GI contraction, the amount of current flowing through the SGT changes. The change detected by the transducer is recorded on a moving chart as an upward deflection.

 Poultry Feedstuffs: Supply, Composition and Nutritive Value (eds J. McNab and N. Boorman)

RESULTS AND DISCUSSION

The gross anatomy of the GI tract of poultry must be understood in order to appreciate the significance of the contractile activity (Duke, 1984, 1986a,b, 1989). The anatomy of the gastric area of poultry is unique (Fig. 7.1). The proximal portion of the stomach is called the proventriculus or glandular stomach, and it is richly supplied with glands through which are secreted mucus, hydrochloric acid and pepsin. The latter two play a major role in the digestion of protein. The distal portion is known as the muscular stomach or gizzard, the main function of which is to grind the grains eaten by poultry. It is also the 'chamber' wherein the chemical digestion of proteins is initiated. Small stones (i.e. 'grit') aid this process. The muscular stomach consists of two pairs of opposing muscles, namely the thin and thick muscle pairs (Fig. 7.1). Our earliest studies established that every contractile cycle began with the simultaneous contraction of both thin muscles, followed by a contraction that started in the proximal duodenum near the pylorus and spread throughout the entire duodenum. The thick muscles of the muscular stomach then contracted and the cycle ended with a contraction of the glandular stomach. The contraction of the thick muscles provided the grinding force to comminute the ingested grains.

Several factors affect the frequency of this 'gastroduodenal' contraction cycle. During the day, when the birds are feeding, gastric contractions occur about three times per minute and the duodenum has three contractions for each gastric cycle. Frequencies during the night drop to less than one per minute among birds that have been allowed to feed, and to nearly zero in fasted birds. Therefore, both darkness and fasting depress GI contractile activity.

A unique type of duodenal contractile activity was first observed during studies in our laboratory on the gastroduodenal contraction sequence, namely the duodenal reflux. About three times each hour, gastric activity ceased when two very large duodenal contractions were recorded. Using simultaneous radiography and recordings from implanted SGT, we were able to determine that this activity was a reflux of nearly all of the duodenal contents back into the muscular stomach. It is presumed that these reflexes permit the increased digestion of the contents of the duodenum by allowing them to pass through the duodenum more than once. We have found that refluxes could be stimulated by infusion of nutrients into the duodenum, e.g. infusion of an amino acid solution caused a duodenal reflux and then slowed duodenal contraction frequency for about 20 min. Contractile amplitude was also decreased. Infusion of a hydrochloric acid solution caused a reflux and then slowed duodenal contractions for about 12 min. The solution was used to simulate gastric contents high in hydrochloric acid. An infusion of a hypertonic solution of sodium chloride caused many duodenal refluxes for more than 20 min. Lastly, an infusion of maize oil also caused multiple refluxes and contractile frequency was depressed for more than 35 min. The response to the maize oil, however, did not occur immediately upon infusion of the oil, but took about 6 min to develop. Based on similar research in mammals, we believe that the infusion of the oil caused the release of the GI hormone cholecystokinin. The concentration increased in the blood stream over several minutes; when a critical concentration was

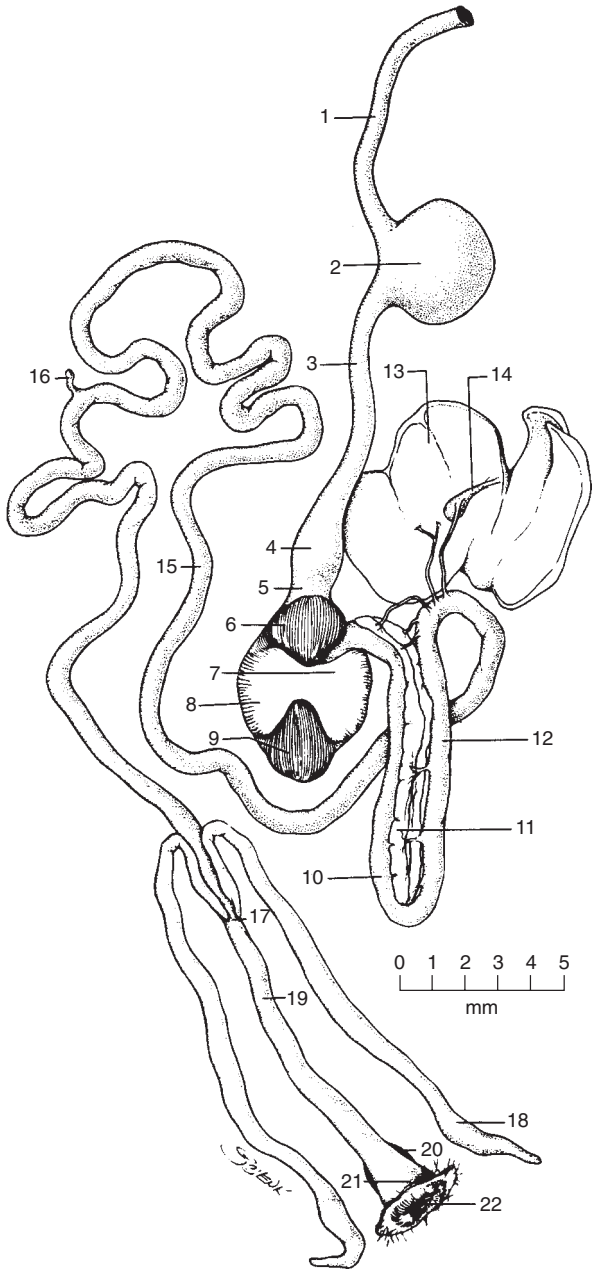

Fig. 7.1. Digestive tract of a 12-week-old turkey. 1, Precrop oesophagus; 2, crop; 3, postcrop oesophagus; 4, proventriculus; 5, isthmus; 6, thin craniodorsal muscle; 7, thick cranioventral muscle; 8, thick caudodorsal muscle; 9, thin caudoventral muscle (6–9 gizzard); 10, proximal duodenum; 11, pancreas; 12, distal duodenum; 13, liver; 14, gallbladder; 15, ileum; 16, Meckel's diverticulum; 17, ilececocolic junction; 18, caeca; 19, rectum; 20, bursa of Fabricius; 21, cloaca; 22, vent. Reprinted from *Dukes' Physiology of Domestic Animals*, 10th edn, Melvin J. Swenson (ed.). Copyright ©1984 Cornell University Press. Used by permission of the publisher, Cornell University Press.

reached, gastric and duodenal contractile frequency was depressed. Apparently, amino acids, hydrochloric acid and hypertonic solutions initiate a neurally mediated reflex, which depresses contractile activity almost instantaneously.

CONCLUSION

It can, therefore, be concluded that individual nutrients may have major effects on GI motility, either by slowing contractile frequency and, thereby, passage rate through the intestine, or by stimulating duodenal refluxes, which also slow passage through the intestine and additionally re-expose the intestinal contents to the gastric digestive enzymes. Fasting or darkness also result in increased digestion. Increasing digestion would be likely to benefit a fasting bird or a bird in the dark and unable to find its food.

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CHAPTER 8
Digestibility and bioavailability of protein and amino acids

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INTRODUCTION

The optimum use of feedstuffs to supply amino acids (AA) required by animals is of great importance for accurate and efficient formulation of animal diets. To accomplish the latter, the bioavailability or digestibility of the AA in feed ingredients must be known. It is well established that in most feedstuffs the amounts of digestible AA are substantially less than the total amounts. Many studies have been conducted to determine bioavailability or digestibility of AA in feedstuffs and many excellent reviews have been published on the topic, including Rerat (1971), Meade (1972), McNab (1979), Sibbald (1987) and Ravindran and Bryden (1999). Interest in AA bioavailability in poultry feedstuffs has increased during the last two decades, partly due to the development and increased use of more-rapid digestibility assays that have permitted more research to be conducted. For example, the National Research Council (NRC) first published a table of feedstuff AA digestibility coefficients for poultry in 1994. In addition, several other publications have extensive tables on AA digestibility coefficients for poultry feedstuffs (Sibbald, 1986; Parsons, 1991; Rhone-Poulenc, 1993, 1995; Heartland Lysine, 1996; Ravindran *et al.*, 1998).

The primary purpose of the current chapter is to review several of the various methods or assays proposed to determine AA bioavailability in poultry feedstuffs and to discuss their usefulness for practical evaluation of feed ingredient samples. The emphasis will be on the most commonly used animal (*in vivo*) assays. Terminology, factors affecting AA bioavailability and use of AA bioavailability values in feed formulation will also be discussed.

TERMINOLOGY

Many different methods have been used to estimate AA bioavailability. These procedures can be broadly classified into two groups, *in vitro* (laboratory) and *in vivo* (animal) assays. The terms used to refer to results of *in vitro* assays are usually straightforward since each assay has a specific set of laboratory procedures. Several different *in vivo* assays have been used to assess AA

 Poultry Feedstuffs: Supply, Composition and Nutritive Value (eds J.M. McNab and K.N. Boorman)

bioavailability, and the terminology associated with these is sometimes confusing. The results of the various *in vivo* assays do not all mean the same thing in terms of AA bioavailability. Bioavailability is a term that includes all of the processes of digestion, absorption and metabolism or utilization. Bioavailability is often defined as the amount of AA that is absorbed in a form suitable for utilization. It is usually measured in growth assays where bioavailability is based on the utilization of AA by growing animals.

'Digestibility' is a term that is associated with bioavailability, with which it is sometimes used synonymously. Digestibility of AA is determined in some types of nutritional balance assay and is classically defined as the difference between the amount of AA consumed and that excreted in the faeces, divided by the amount consumed. Digestibility is usually determined by one of three methods. In addition to the classical faecal collection method, AA digestibility can be based on collection of ileal contents from surgically modified or killed animals. Further, when poultry are used, the total excreta (faeces + urine) are usually collected. The latter assay actually measures 'metabolizable AA' by classical definition since urinary AA are included in the calculation. However, the term 'digestibility' is commonly used and will be used in this review when referring to excreta collection assays, since the term 'metabolizable AA' is uncommon and the amount of AA excreted in the urine has been estimated to be small (Sibbald, 1987). Digestibility assays only measure digestion and absorption of AA and do not measure utilization. Thus, digestibility is not always synonymous with bioavailability. In this review, the term bioavailability will be used when referring to results of growth assays which measure AA utilization based on animal performance, and the term digestibility will be used for nutritional balance assays in general.

The terminology becomes more confusing when digestibility values are corrected in some manner for endogenous AA losses or excretion. Classically, the corrected values have been referred to as 'true' digestibility values although they are now sometimes referred to as 'standardized' digestibility values (Stein, 1998; Rademacher, 1999). The latter term is being adopted because of the many different methods that are used to estimate or correct for endogenous losses and because of the ambiguity of the term 'true'. In addition, the term 'real' AA digestibility was proposed by de Lange *et al.* (1990) for AA digestibilities determined with an N-isotope dilution technique.

FACTORS AFFECTING BIOAVAILABILITY OF AMINO ACIDS

Many factors can influence bioavailability of AA in feedstuffs. Some of the most common factors are processing conditions, presence of anti-nutritional compounds, physical and chemical composition of the protein, and dietary fibre (Sauer and Ozimek, 1985; Sibbald, 1987). From a practical standpoint, the effects of processing are probably the most important because most ingredients used in poultry diets, particularly high-protein ones, are heat-processed. Either under- or over-processing can reduce AA bioavailability; however, over-

processing or excessive heat processing and/or pressure is probably a more frequent problem. Examples of over-processing are shown for several oilseed meals in Table 8.1 and meat and bone meals in Table 8.2.

Commercial samples of several oilseed meals were overprocessed to different degrees by autoclaving at 105 kPa and approximately 121°C for increasing amounts of time. The effects of over-processing on AA digestibility varied greatly among AA, with effects being greatest for lysine, intermediate for cystine, and much less for methionine. In addition to the reduced digestibility of lysine caused by autoclaving, the concentration of lysine in the oilseed meals decreased considerably. Some decrease in cystine concentration was also observed for soybean meal but not much for the other meals. Autoclaving generally had no effect on methionine concentration. The decrease in lysine concentration indicated that lysine destruction, probably due to formation of advance Maillard reaction products, was occurring with excess heating. My laboratory has observed the same types of effects in numerous industrial overprocessed soybean meals. Due to the lysine destruction, the concentration of lysine as a proportion of the crude protein is usually decreased in moderately to severely overprocessed oilseed meals and this can be a useful first indicator of over-processing.

Processing conditions can have considerable effects on AA digestibility of animal protein meals such as meat and bone meal (MBM; Table 8.2). The data from Wang and Parsons (1998a) are selected results from the evaluation of 32 MBMs processed in several different commercial rendering systems at two (low or high) processing temperatures. It is apparent that System B produced MBM with much higher AA digestibilities that System A. Also, processing temperature greatly affected AA digestibility, particularly for System A, where the lower processing temperature yielded higher AA digestibility. Interestingly, the effects of processing temperature on AA digestibility were greatest for cystine and least for methionine, with lysine being intermediate. At least part of the effect of the processing system was probably associated with processing temperature, because the processing temperatures used in System A were higher than those used in System B. In addition to the type of system and temperature, processing pressure had large effects on AA digestibility of MBM (Table 8.2). As processing pressure increased from 0 to 210 and 415 kPa, digestibility of all AA decreased, with the largest and most remarkable effects being observed for cystine. The effects of pressure processing were studied due to concerns about BSE, in which context it is being recommended that MBM be cooked under pressure in an attempt to reduce the incidence of BSE in cattle and the risk of Creutzfeldt–Jakob disease (CJD) in humans. The combined results of the studies summarized in Table 8.2 clearly indicate that processing conditions greatly affect AA digestibility of MBM and that the effects are greatest for cystine. Moreover, the results show that MBM with very high AA digestibility (e.g. System B with low temperature) can be produced with good processing conditions.

Table 8.1. Effect of over-processing on measured concentrations (g kg^{-1}) and digestibility coefficients (%) of lysine, cystine and methionine in several oilseed meals.[1]

	Lysine		Cystine		Methionine	
Treatment	Concentration	Digestibility	Concentration	Digestibility	Concentration	Digestibility
Normal SBM	32.7	91	7.0	82	7.1	86
Moderately overprocessed	29.5	78	6.6	69	7.1	86
Severely overprocessed	27.6	69	6.3	62	7.0	83
Normal SFM	14.3	86	5.6	81	5.7	85
Moderately overprocessed	10.4	54	5.5	77	5.4	79
Severely overprocessed	8.4	43	5.0	73	5.2	78
Normal CM	17.7	80	8.7	73	8.2	81
Moderately overprocessed	15.4	65	8.3	64	7.4	70
Severely overprocessed	12.9	37	8.4	54	8.1	72
Normal GM	16.6	87	6.9	75	4.7	81
Moderately overprocessed	14.9	72	6.7	67	4.6	76
Severely overprocessed	12.6	57	6.9	60	4.7	78
Normal CSM	19	65	7.0	72	6.0	72
Moderately overprocessed	–	52	–	48	–	75
Severely overprocessed	–	46	–	35	–	71

SBM, soybean meal (Parsons *et al*., 1992); SFM, sunflower meal (Zhang and Parsons, 1994); CM, canola meal (Anderson-Haferman *et al*., 1993); GM, groundnut meal (Zhang and Parsons, 1996); CSM, cottonseed meal (Fernandez *et al*., 1994).

Table 8.2. Effect of processing system, temperature and pressure on digestibility coefficients (%) of cystine, lysine and methionine in meat and bone meals.

System and product[a]	Temperature (°C)	Pressure (kPa) applied	Digestibility (%)		
			Cystine	Lysine	Methionine
A (2L)	132	–	39	85	88
A (2H)	152	–	20	78	87
A (3L)	132	–	50	81	90
A (3H)	152	–	31	71	83
B (7L)	110	–	71	92	91
B (7H)	140	–	62	90	91
B (8L)	110	–	59	91	91
B (8H)	140	–	51	87	86
1		0	65	75	79
2		210	44	64	75
3		415	14	45	58

[a]Values for the first eight products indicate processing system and product from Wang and Parsons (1998a). Values for the last three products are from Shirley and Parsons (2000).

METHODS FOR DETERMINING AMINO ACID BIOAVAILABILITY *IN VIVO*

The two primary types of *in vivo* assays are usually referred to as direct and indirect assays. The indirect assays include microbiological assays, insect assays and plasma AA assays (Sibbald, 1987). The two main types of direct assays are the growth assays and the balance assays. Actually, within this classification, the growth assay could be considered a direct assay whereas the balance assay could be considered indirect. As discussed earlier, the growth assay measures the utilization of dietary AA for growth, and thus includes all of the processes of digestion, absorption and utilization for protein synthesis. Conversely, the balance or digestibility assay estimates bioavailability indirectly by measuring the amounts of AA not digested and absorbed. The balance assays are used much more extensively than growth assays because of savings in time and expense. The emphasis of this section will be on reviewing the growth assay, the precision-fed caecectomized excreta digestibility assay and the ileal digestibility assay, because these are by far the most commonly used methods.

Growth Assays

The growth assay is usually considered the absolute method for determining AA bioavailability. This assay is often called the slope-ratio assay, although standard curve methodology can also be used (Sasse and Baker, 1973). The growth assay measures the ability of a protein or feedstuff to replace a specific limiting or deficient AA. A basal diet deficient in the test AA is supplemented with increasing amounts of the crystalline test AA or increasing amounts of the

test feedstuff(s) to produce linear growth response curves. Bioavailability is then calculated by regression analysis and from the ratio of the slopes of the growth response lines for the test AA and test feedstuff.

The specific procedures used in the growth assays vary considerably among laboratories. Some investigators use empty body-weight gain, feed efficiency ratio or carcass nitrogen or AA as the response parameter rather than body-weight gain. Further, some researchers regress the response parameter on dose rate or concentration of AA in the diet, whereas others regress it on the amount of AA consumed by the animals. Unless feed intake is controlled, it is better to use AA intake as the independent variable in the regressions since animals respond to the amount of AA consumed, not the concentration in the diet. Some laboratories use basal diets composed of intact protein in their growth assays, whereas others use crystalline AA basal diets. At the University of Illinois, crystalline AA basal diets are routinely used and chick weight gain is the response parameter, which is regressed on the amount of supplemental test AA or test ingredient consumed under an *ad libitum* feed regimen.

Although the growth assay is generally considered the absolute standard, the results of the assay can be influenced by many factors other than the limiting test AA. One factor of particular concern is the effect of the AA profile of the test ingredient on the response parameter being measured. Many studies have shown that the AA profile of the test ingredient may negatively affect growth (Table 8.3). In the studies summarized in Table 8.3, the bioavailability of the crystalline test AA was compared when increasing amounts were added alone to the deficient basal diet (bioavailability assumed as 100%) or when increasing amounts were added as part of an AA mixture that simulated the measured total or digestible AA profiles of various test ingredients. In both situations, exactly the same limiting AA entity was being added; thus, the bioavailability should have been the same, i.e. 100%. However, in almost all studies, the bioavailability of the test AA was substantially less than 100% when it was being added with all the other AA of the test ingredient. These results indicate that the AA profile or the excess AA being supplied by the test ingredient have a negative effect on growth, either by reducing the bioavailability of the test AA or by some other means. Consequently, it has been recommended that bioavailability values for ingredients in slope-ratio assays should be estimated by comparing the slope of the test ingredient regression line with that of the regression line generated from the AA-simulated mixture, rather than the slope of the line generated by feeding the test AA alone. For example, in the Hirakawa and Baker (1986) study cited in Table 8.3, when the slope from a mixture of maize gluten meal/sesame meal/meat and bone meal was compared with the slope of an AA-simulated mixture rather than with the limiting AA fed alone, the bioavailability of lysine apparently increased from 56% to 93%.

Another factor that can influence growth assay results is the effect of the test ingredient on feed intake, most importantly the intake of the basal AA-deficient diet. Studies at the University of Illinois since the 1970s (Netke and Scott, 1970; Fernandez and Parsons, 1996a) have suggested that weight gains of

Table 8.3. Influence of amino acid profile of test ingredients on estimates of amino acid bioavailability by slope-ratio growth assay[a].

Feedstuff, amino acid, study	Bioavailability of crystalline amino acid fed alone	Bioavailability of crystalline amino acid fed as feedstuff-simulated amino acid mixture
Soybean meal, sulphur amino acids, Robel and Frobish (1977)	100	87
Soybean meal, lysine, Baker (1978)	100	80
Ingredient mix, lysine, Hirakawa and Baker (1986)	100	60
Feather meal, lysine, Han and Parsons (1991)	100	68
Feather meal, sulphur amino acids, Han and Parsons (1991)	100	92
Soybean meal, lysine, Fernandez and Parsons (1996a)	100	91
Cottonseed meal, lysine, Fernandez and Parsons (1996a)	100	72
Soybean meal, valine, Fernandez and Parsons (1996a)	100	71
Cottonseed meal, valine, Fernandez and Parsons (1996a)	100	72
Meat and bone meal, lysine, Wang and Parsons (1998b)	100	99
Meat and bone meal, methionine, Wang and Parsons (1998b)	100	85

[a]Bioavailability values based on total body weight gain (not partitioned) during the assays. Value for amino acid fed alone expressed as 100 and that for amino acid fed in amino acid mixture expressed relative to 100.

chicks fed *ad libitum* in growth assays should be partitioned to reflect only that growth attributable to the supplemental test AA or test ingredient consumed. The partitioning procedures were outlined by Netke and Scott (1970). Under *ad libitum* feeding conditions, feed intakes of chicks vary among dietary treatments, and chicks fed diets supplemented with the test AA usually consume more feed than those fed diets supplemented with the test ingredient. Consequently, the former chicks also consume more of the basal diet AA. Since growth is a function of the total amount of AA consumed (basal + supplemental), differences in feed intake among treatments may bias the results. The partitioning of growth to reflect only that due to supplemental test AA or ingredient consumption represents an effort to adjust for differences in feed intake. Studies have generally shown that this partitioning procedure yields higher bioavailability estimates. The partitioning procedure is quite simple if crystalline AA diets are used, since the bioavailability of the AA in the basal diet is 100%. Unfortunately, the procedure is not as straightforward when intact protein basal diets containing AA of unknown bioavailability are used.

Two of the general primary disadvantages or limitations of growth assays are the time and expense required. In addition, the bioavailability values obtained in growth assays are often variable and lack precision. Using a crystalline AA basal diet increases the sensitivity and precision of the assay but also the expense. The design of a growth assay is strengthened if at least three supplemental levels of each test ingredient are fed so that good tests for linearity can be conducted. The time and expense of a growth assay can be decreased greatly by using the standard curve method rather than the slope-ratio method. In the standard curve assay, only one level of test ingredient is fed (Han and Parsons, 1991). Thus, the number of treatments is reduced and the number of ingredients that can be evaluated is increased. However, the standard curve method does not permit tests for linearity and provides little or no information concerning any adverse effects of the test ingredient on growth.

It is obvious that many factors can influence the AA bioavailability values obtained with growth assays. Thus, the results of these assays should not be accepted without question. On the other hand, the growth assay is still the *only* method that actually measures AA bioavailability *per se*.

Precision-fed Caecectomized Cockerel Excreta Assay

Most AA digestibility determinations with poultry have been made with the total excreta collection method. Only a few studies have used the faecal method, because it requires surgical modification to separate the urine and faeces. A very rapid and simple excreta collection assay was first described by Likuski and Dorrell (1978) and Sibbald (1979). The general assay involves fasting mature cockerels for 24–48 h, administering 30 g of feedstuff via crop intubation and collecting excreta quantitatively for 48 h after feeding. Excreta are also collected from roosters that are fasted throughout the trial to measure endogenous AA excretion. This assay is usually referred to as the 'precision-fed cockerel assay'.

The use of faecal or excreta collection methods with poultry is subject to criticisms concerning the effects of hind-gut bacteria on AA excretion. To reduce the effects of the hind-gut bacteria, some investigators have used ileal collection methods, which will be discussed in the next section. The use of caecectomized birds in digestibility assays is an alternative to using ileal collection. Since the caeca comprise the major portion of the hind-gut area in poultry, removal of the caeca should eliminate most of the effects of the hind-gut bacteria on AA excretion. Caecectomy is much simpler and more rapid than other surgical procedures such as ileal cannulation and the animals are much easier to maintain after surgery. Thus, many laboratories now use caecectomized cockerels in the precision-feeding assay (for example, Parsons, C.M., University of Illinois; Rhône Poulenc, Antony, France; Firman, J., University of Missouri) hence, the assay is now often called the 'precision-fed caecectomized cockerel assay'. Although several groups are now using caecectomized cockerels, it is the opinion of this author that caecectomy, or any other technique to reduce microbial interference with AA

excretion, is not absolutely required for poultry. The size or capacity of the hind-gut in poultry is much less than that of most mammals. General AA digestibility values for ingredients determined in conventional birds are often not greatly different from those determined in caecectomized birds. Han and Parsons (1991) and Parsons *et al.* (1997) reported that although AA digestibility values for feather meals and meat and bone meals were generally lower in caecectomized roosters than in conventional cockerels, the values among samples were highly correlated and the correlation between both types and values and growth assay values were similar. Thus, although using caecectomized cockerels is generally preferred to using conventional cockerels, it seems that the latter still provide useful AA digestibility data for poultry.

The precision-fed cockerel assay has become probably the most frequently used assay for poultry. Reasons for this include reduced time, reduced expense, the need for only a small amount of feed sample, no need for a digesta marker and the ability to obtain AA digestibility values for any specific ingredient regardless of palatability. Thus, the assays work well for routine evaluation of large numbers of feed samples. Another particular advantage of the precision-fed cockerel assay, or balance assays in general, over the growth assay is the ability to obtain a cystine digestibility value. The latter is very difficult to obtain in growth assays, because two separate assays need to be conducted, one for methionine plus cystine and another for methionine alone and then the cystine bioavailability can be calculated by difference (Parsons, 1986).

The primary criticism of the precision-fed cockerel assay is that it is an abnormal feeding or consumption situation and thus the results obtained may not apply to normal *ad libitum* feeding. There have been little or no data to support the latter concern. Another concern probably of greater importance is that the ingredients are fed to adult birds. This is probably not a substantial problem for most ingredients; however, for ingredients such as wheat and barley, the assay may overestimate AA bioavailability for young birds. Another weakness of the cockerel assay is that AA digestibility values can be quite variable for low protein ingredients such as grains, which result in very low AA intakes (only 30 g of ingredient is fed). However, low AA intakes are a problem with most AA bioavailability/digestibility assays.

Ileal Amino Acid Digestibility Assays

The two basic methods of determining ileal digestibility are to insert a cannula into the terminal ileum or to kill the birds and remove the ileal contents. Techniques for ileal cannulation have been described by Raharjo and Farrell (1984) and Gurnsey and James (1985). The usefulness of ileal cannulation is limited by the surgical expertise required, variation in flow of digesta through the cannula (e.g. blockage) and rejection of the cannula.

Payne *et al.* (1968) first proposed the method of collection and analysis of ileal contents after killing the birds, and this method has been used by many

groups since. The general procedure used mostly involves feeding diets to chickens for approximately 2 weeks, killing the chickens (cervical dislocation, CO_2 gas, anaesthesia), and collecting the intestinal contents from the vitelline diverticulum to the ileal–caecal junction. Kadim and Moughan (1997) suggested that collecting material from the last 15–20cm is preferred. The procedure requires use of a digesta marker, with chromic oxide or acid insoluble ash being used most frequently. Acid insoluble ash is becoming more popular as it is easily measured and is very consistent. However, it requires much more sample than chromic oxide.

The ileal digestibility assay (using killing) is becoming more frequently used. One reason is the increased interest in the effects of enzymes on AA digestibility in feed ingredients. The ileal assay is probably preferred over the precision-fed cockerel assay for enzyme evaluation (Zanella *et al.*, 1999). The main limitations of the ileal assay are time and expense in that a 2-week feeding trial is required for the use of a digesta marker. In addition, the assay is not well-suited for determining AA digestibility values for individual feed ingredients. The best method of killing the chicks and best area for collection of intestinal contents are also still being disputed.

Correcting Digestibility or Balance Assay Values for Endogenous Amino Acid Losses

There have been many studies of correction for endogenous losses of amino acids over the last 30 years. An excellent review of the subject was recently published by Ravindran and Bryden (1999). There is still disagreement over the proper or best method for estimating endogenous AA losses and the terminology for corrected and uncorrected values. Classically, values which have not been corrected are called apparent digestibility, and those that have been corrected are called true digestibility. However, other terms, such as standardized and real, are also used. More discussion will be presented on terminology later in the section. The main criticism of using apparent digestibility values is that they are influenced by level of feed or AA intake, with values being underestimated when AA intakes are low.

There are many procedures for estimating endogenous AA losses. These include fasting, feeding an N-free diet, regression analysis based on several intakes of the diet or ingredient and extrapolation to AA excretion at zero AA intake, isotope markers such as ^{15}N, a peptide alimentation ultrafiltration technique that involves feeding peptides from enzymatically hydrolysed casein, and the guanidination or homoarginine technique. These procedures were reviewed by Ravindran and Bryden (1999). The most commonly used methods have been those using N-free diet and fasted animals, mainly because they are much easier than the other methods. There is no question that the estimated amount of endogenous AA losses varies greatly among procedures. An example is shown in Table 8.4 where endogenous AA losses estimated using guanidinated casein in the homoarginine method were much higher than those for the N-free diet or regression analysis methods

Table 8.4. Endogenous amino acid losses (g kg^{-1} dry matter intake) in ileal digesta of broiler chickens estimated using different methods (from Siriwan *et al.,* 1994).

Amino acid	N-free diet	Regression analysis	Guanidinated casein	SEM
Aspartic acid	0.61	0.81	1.69	0.08
Threonine	0.54	0.61	1.48	0.10
Serine	0.51	0.97	2.11	0.15
Glycine	0.74	1.78	3.08	0.24
Glutamic acid	0.29	0.31	0.72	0.05
Alanine	0.27	0.27	0.69	0.04
Valine	0.42	0.50	1.02	0.07
Methionine	0.07	0.08	0.11	0.01
Isoleucine	0.20	0.52	0.91	0.06
Leucine	0.47	0.48	1.04	0.08
Tyrosine	0.32	0.15	0.49	0.04
Phenylalanine	0.29	0.21	0.51	0.04
Histidine	0.14	0.13	0.31	0.03
Lysine	0.24	0.19	0.56	0.05
Arginine	0.25	0.18	0.54	0.05
Total	5.36	7.19	15.26	0.55

SEM, standard error of the mean.

(Siriwan *et al.*, 1994). When comparing endogenous AA estimates among assay methods and studies, the fasted animals and N-free diet methods give the lowest values, the regression method gives intermediate values and the isotope, peptide alimentation and homoarginine methods give substantially higher values.

The fact that many different methods are used to estimate endogenous AA losses has contributed to the development of different terms being used for what has historically been known as true digestibility. Two such terms are real digestibility and standardized digestibility. Real digestibility, because it includes a correction for endogenous losses that are specific to individual diets or ingredients, is usually used for isotope marker techniques and sometimes for the peptide alimentation methods. As mentioned earlier, standardized is sometimes used as a less ambiguous alternative to 'true' and because the endogenous correction is based on a summary of several previously published studies. Although different procedures are being used, it seems that all the different methods discussed are measuring what has classically or historically been known as true digestibility. Thus, the justification for using terms such as real or standardized seems questionable. When true digestibility is being estimated, it seems that the author can simply describe the method being used to estimate endogenous AA losses in that particular study.

The question that then arises is whether digestibility values should be stated on an apparent or true basis. If AA intake is high in the assay, the question is mostly academic because the two values are similar. However, if AA intake is low, such as for grains or in the precision-fed cockerel assay, then the values generally need to be corrected for endogenous AA losses. If

values are then expressed on a true digestibility basis, which procedure for measuring endogenous AA losses should be used? In the precision-fed cockerel assay, fasted birds are commonly used. It can be argued that endogenous AA losses are being underestimated by the latter method. However, the use of fasted birds seems to yield a good baseline endogenous AA value that results in reasonable and repeatable digestibility coefficients and removes most of the effect of different AA intakes. If higher endogenous values from the other methods discussed are used to correct precision-fed cockerel digestibility values, the digestibility coefficients for grains are consistently above 100%, often as high as 150%. Moreover, if a feed ingredient causes increased endogenous AA losses (e.g. due to high fibre or an anti-nutritional factor such as a protease inhibitor), it seems that the increased loss should be counted against the ingredient. This statement is further strengthened by the fact that digestibility coefficients for individual ingredients have been reported to be additive (Fuller *et al.*, 1994; Fernandez *et al.*, 1995; Angkanaporn *et al.*, 1996). Thus, using fasted birds for the endogenous AA correction in the precision-fed cockerel assay may underestimate endogenous losses for birds fed some ingredients; however, it seems to be appropriate. Furthermore, it can be argued that it is better to underestimate endogenous AA losses slightly than to overestimate them.

Comparison of Values Among Methods

When comparing AA bioavailability and digestibility values among methods, there is good agreement in some cases and not such good agreement in others. In general, AA bioavailability values determined with the slope-ratio growth assay are often lower than those determined by the precision-fed caecectomized cockerel assay (Table 8.5). The difference is sometimes small but can also be very large (e.g. lysine in feather meals 1 and 2, Table 8.5). A large part of that difference is due to the very low lysine bioavailability values obtained for these two poor quality feather meals. The low content of bioavailable lysine combined with the poor AA balance of feather meal protein results in very little growth response from the feather meals, hence a very low and imprecise bioavailability value. This situation can be a problem with growth assays. For example, my laboratory has not been able to obtain a methionine bioavailability value for feather meal (very deficient in methionine) by growth assay because no growth response has been obtained from adding feather meal to a methionine-deficient basal diet. As discussed earlier, another contributing factor to some of the large differences between growth assay and cockerel digestibility values in Table 8.5 is the negative effect of the ingredient AA profile on growth. For example, the latter accounted for all of the difference between slope-ratio lysine bioavailability and lysine digestibility determined with cockerels for a mixture of maize–gluten meal/meat and bone meal/sesame meal observed by Hirakawa and Baker (1986) in Table 8.5.

It is also possible that some of the differences between growth assay bioavailability values and cockerel digestibility values are due to the fact that not

Table 8.5. Comparison of slope-ratio amino acid bioavailability values (%) with precision-fed caecectomized cockerel digestibility values (%).

Study	Feedstuff[a]	Amino acid	Slope-ratio bioavailability[b]	Caecectomized cockerel digestibility
Parsons (1986)	MBM	Lys	70	79
	MBM	Met	75	75
Hirakawa and Baker (1986)	Mixture of ingredients	Lys	60	89
Han and Parsons (1991)	FM 1	Lys	–6	58
	FM 2	Lys	8	67
	FM 6	Lys	54	73
	FM 7	Lys	63	73
Fernandez and Parsons (1996a)	SBM	Lys	90	87
	CSM	Lys	54	61
	SBM	Val	83	92
	CSM	Val	75	86
Fernandez and Parsons (1996b)	SBM	Lys	88	88
	SBM DEX	Lys	35	63
Wang and Parsons (1998b)	MBM 1	Lys	92	81
	MBM 2	Lys	71	71
Wang and Parsons (1998b)	MBM 1	Met	91	85
	MBM 2	Met	83	73

[a]MBM, meat and bone meal; FM, feather meal; SBM, soybean meal; CSM, cottonseed meal; SBM DEX, soybean meal autoclaved with dextrose for 30 min.

[b]Estimates based on total body weight gain (no partitioning) compared with response to increasing amounts of the test amino acid fed alone, not as part of an amino acid mixture simulating the profile of the test ingredient.

all of the digestible or absorbed AA are available for protein synthesis. Batterham (1992) reported the results of several experiments with pigs in which growth assay values were lower than ileal digestibility values. He subsequently concluded that substantial amounts of dietary AA were being absorbed in forms that were unsuitable for protein synthesis. In general, we have not observed the large differences between growth assay and digestibility assay values in poultry that Batterham (1992) reported in pigs. For example, Batterham reported that the difference between lysine digestibility and bioavailability in cottonseed meal for pigs was approximately 40 percentage units, whereas we found only 7 percentage units in poultry (Fernandez and Parsons, 1996a). In summary, when considering all of the factors that can affect slope-ratio growth assay values, the agreement between the latter values and caecectomized cockerel digestibility values is reasonable in most cases.

A comparison of AA digestibility coefficients determined with the precision-fed cockerel excreta assay (NRC, 1994) and those determined with the chick ileal digestibility assay (Ravindran *et al.*, 1998) for selected ingredients and AA is presented in Table 8.6. Values for lysine and methionine found by the two methods are in general agreement except for meat meal. Part of the latter difference

Table 8.6. Comparison of precision-fed cockerel excreta amino acid digestibility coefficients (%) with chick ileal digestibility coefficients (%).

Ingredient	Amino acid	Rooster excreta true digestibility[a]	Chick ileal apparent digestibility[b]
Maize	Lys	81	81
	Met	91	90
	Thr	84	69
Sorghum	Lys	78	74
	Met	89	84
	Thr	82	66
Soybean meal	Lys	91	85
	Met	92	90
	Thr	88	76
Canola meal	Lys	80	76
	Met	90	90
	Thr	78	64
Meat meal	Lys	59	67
	Met	85	72
	Thr	79	57

[a]NRC (1994).
[b]Ravindran *et al.* (1998).

may be due to differences in samples evaluated. In contrast to lysine and methionine, ileal digestibility coefficients for threonine are consistently lower than the excreta digestibility values. Part of this difference may be due to the fact that the ileal values are on an apparent digestibility basis whereas the excreta digestibility values are on a true digestibility basis. The concentration of threonine in endogenous secretions has been reported to be high.

Summary

The precision-fed caecectomized cockerel assay continues to offer many advantages as the best method for routine evaluation of feed ingredient samples. It is far less expensive and faster than any of the other methods and requires only a small amount (less than 500 g) of sample. The caecectomy surgical procedure is relatively easy, and, once completed, the birds can be used repeatedly (once per month) in assays for at least 2 years. Comparisons of the digestibility coefficients with those of growth assay and ileal digestibility assays indicate that the values are reasonably accurate. The chick ileal digestibility assay is also useful and will probably become more widely used because it is probably the preferred assay for enzyme evaluation. It is questionable whether the precision-fed cockerel assay is appropriate for enzymes because only one small dose of feed is given to fasted birds. The growth assays, isotope, peptide alimentation and homoarginine methods are too complicated, laborious and expensive for routine use. However, further work

is worthwhile to compare the latter assays, the precision-fed cockerel assay and the chick ileal assay, on the same samples to elucidate any substantial errors or corrections/modifications needed in the digestibility values. Most laboratories using the precision-fed cockerel assay would be very willing to cooperate with laboratories using the other procedures for comparisons between methods.

PRACTICAL APPLICATION OF AMINO ACID DIGESTIBILITY VALUES

Formulation on a Digestible/Bioavailable Basis Compared with a Total Amino Acid Basis

Numerous studies have shown that there are advantages to formulating diets on a digestible/bioavailable AA basis compared with a total AA basis (Table 8.7). Formulating diets containing substantial concentrations of sunflower meal, rapeseed meal, cottonseed meal, meat and bone meal or spent hen meal on a total AA basis resulted in reduced growth performance compared with a maize–soybean meal diet. Formulating the former diets on a digestible AA basis improved growth performance in all cases and often resulted in performance equivalent to that obtained from the maize–soybean meal control diet. However, it is important to note that diets containing high concentrates of some ingredients, such as rapeseed meal, cottonseed meal, low-quality meat and bone meal or spent hen meal did not yield optimum performance even when formulated on an equal digestible AA basis. Further work with the latter three ingredients showed that additional AA supplementation had no positive effect, indicating that the adverse effects of these ingredients was not AA related.

Digestible Amino Acid Requirements

A potential problem with using digestible AA values in routine feed formulation is that almost all published AA requirements are based on total AA values. Dietary treatments in requirement studies usually consist of a basal diet deficient in the AA under study and the basal diet supplemented with several concentrations of the deficient AA in crystalline form. Although it is not possible to determine digestibility of AA in the basal diet feed ingredients used in previously published studies on AA requirements, total AA digestibility coefficients, such as those of the NRC (1994), can be used to estimate digestible AA contents of the ingredients used in those studies. The digestible AA contents of the natural feed ingredients in the basal diet can then be summed and added to the amount of supplemental crystalline AA required (bioavailability = 100%) to

Table 8.7. Growth performance of chicks fed on diets based on total or bioavailable/digestible amino acid (AA) concentrations.

Dietary treatment	Weight gain (g)	Gain/feed ratio
Smith (1968)		
1. Complete crystalline AA reference diet	89	0.72
2. As 1 + 7% feather meal – equal total AA basis[a]	60	0.60
3. As 1 + 7% feather meal – equal bioavailable AA basis[a]	84	0.69
Green (1987)		
1. Maize/soybean meal diet – equal total AA basis	837	0.459
2. Maize/sunflower meal diet – equal total AA basis	761	0.435
3. Maize/rapeseed meal diet – equal total AA basis	602	0.373
4. Maize/soybean meal diet – equal digestible AA basis[b]	863	0.474
5. Maize/sunflower meal diet – equal digestible AA basis[b]	871	0.500
6. Maize/rapeseed meal diet – equal digestible AA basis[b]	769	0.444
Rostagno *et al.* (1995)		
1. Maize/soybean meal reference diet	2333	0.560
2. As 1 + by-products – equal total AA	2241	0.541
3. As 1 + by-products – equal digest AA	2330	0.559
Fernandez *et al.* (1995)		
1. Maize/soybean meal reference diet	271	0.656
2. As 1 + 20% cottonseed meal – equal total AA	252	0.615
3. As 1 + 20% cottonseed meal – equal digestible AA	272	0.655
4. As 1 + 40% cottonseed meal – equal digestible AA	236	0.617
5. As 4 + amino acids	229	0.626
Wang and Parsons (1998c)		
1. Maize/soybean meal reference diet	326	0.690
2. As 1 + 20% low-quality MBM – equal total AA[c]	288	0.595
3. As 1 + 20% low-quality MBM – equal digestible AA[c]	304	0.641
Douglas and Parsons (1999)		
1. Maize/soybean meal reference diet	325	0.648
2. As 1 + 15% SHM A – equal total AA[c]	287	0.541
3. As 1 + 15% SHM A – equal digestible AA[c]	310	0.606
4. As 1 + 15% SHM B – equal total AA[c]	292	0.552
5. As 1 + 15% SHM B – equal digestible AA[c]	328	0.633

[a]Total or bioavailable AA equal to the reference diet. Bioavailability determined by chick growth assay.
[b]Diets were supplemented with lysine.
[c]MBM, meat and bone meal; SHM, spent hen meal.

calculate a requirement based on digestible AA. Parsons (1991) reviewed 28 published studies on the lysine and total sulphur AA requirements of broiler chickens, turkeys and laying hens and concluded that the digestible AA requirements were 8% to 10% lower than the total requirement when averaged over studies. The modest difference between total and digestible requirements can be attributed to two primary factors. First, the digestibility of lysine and sulphur AA in most of the dietary ingredients used in the basal diets is high (e.g. soybean meal, maize gluten meal). Second, a large proportion of the test AA in the

diets that meet the requirements is often provided by the crystalline AA (digestibility = 100%). Indeed, crystalline lysine or methionine frequently accounted for 25% or more of the total lysine or methionine in the diets defining requirements in the studies reviewed by Parsons (1991). The results of these comparisons indicate that digestible AA requirements are approximately 8%–10% lower than published values for total requirements.

Another method of estimating digestible AA requirements is to calculate the digestible AA concentrations in a few simple feed formulae that the nutritionist is already using and has confidence. These calculated digestible AA concentrations are then used as the digestible AA requirements.

Using Digestible Amino Acids Values in Feed Formulation

The application of AA digestibility values to routine feed formulation can be accomplished by several methods. The procedures used differ mainly in the degree to which the feed ingredient/requirement matrix is modified. The most comprehensive method is to convert all feed ingredient values to digestible AA values, and also to convert all requirements to digestible AA requirement values. Another less extensive approach is to allow the values for maize and soybean meal and the AA requirements to remain as total concentrations and to modify the total concentration values for other grains and high protein ingredients relative to maize and soybean meal, respectively, based on relative digestibilities. Thus, maize and soybean meal serve as reference points since they comprise the majority of most diets. The main advantage of this method is that fewer changes need to be made in the feed ingredient matrix. For example, the digestibility coefficients for several of the grains such as wheat and sorghum are similar to that of maize and would not need to be changed. This same relationship holds true for some high-protein ingredients relative to soybean meal. The digestibility coefficients of AA in fish meal and maize gluten meal are similar to those of soybean meal and probably would not need adjustment. However, the digestibility coefficients of AA in ingredients such as meat meal, poultry-by-product meal, canola meal and feather meal are lower than those for soybean meal, indicating that values for the total concentration of AA should be decreased. Two main disadvantages of this method are: (i) it does not fully maximize the concept of AA digestibility; and (ii) it may underestimate the value of crystalline AA supplements. For example, the bioavailability or digestibility of crystalline lysine is 111% that of soybean meal.

There are other modifications of these two methods for incorporating AA digestibility values into practical feed formulation. Most nutritionists are already applying the concept of AA digestibility in some manner.

IN VITRO ASSAYS

This topic will be discussed only briefly here. Extensive reviews of *in vitro* assays have been published by Sibbald (1987), Fuller (1991) and Ravindran

and Bryden (1999). My laboratory has had substantial success with only two assays, namely the KOH protein solubility assay and the pepsin digestibility assay. The KOH assay measures protein solubility by stirring ingredient samples in KOH (2 g l^{-1} for 20 min) (Araba and Dale, 1990). This assay generally works well for oilseed meals (see references in Table 1 footnote) to detect overprocessing or excess heating. Further recent work suggests that the protein dispersibility index method may also be useful for monitoring the quality of soybean meal (Batal *et al.*, 2000). The pepsin digestibility assay (AOAC, 1984) works reasonably well at detecting poor quality animal protein meals if the concentration of pepsin is reduced from 0.2% to 0.01 or 0.002%. Both the KOH and pepsin digestibility assays have limitations and are not as accurate or as sensitive as one would like. There continues to be a great need for the development of good *in vitro* assays that are easy and fast enough to be used in commercial feed ingredient quality control programmes. A number of groups are currently evaluating the use of near infrared reflective spectroscopy (NIR). The latter method has great potential but it is still too early to know if it will be accurate enough for AA digestibility assessment.

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CHAPTER 9
The quantitative contribution of fat to metabolizable energy

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INTRODUCTION

Fats and oils have approximately twice the dietary energy-yielding potential of digestible carbohydrates, they may contain essential fatty acids and fat soluble vitamins, their physical texture reduces dust in feed mills and they promote palatability of diets. These materials, accordingly, have assumed considerable importance as ingredients in compound poultry feeds in recent years. The dietary energy value of any raw material is governed primarily by: (i) its chemical composition; and (ii) the degree to which it is digested by the bird in the supply of energy-yielding substrates. Fats and oils vary considerably in both these factors such that the range of dietary energy values with this group of raw materials is probably greater than for any other. The chemical composition of fats and oils is considered elsewhere in this volume (see Palmquist, Chapter 5). Accepting that the fats and oils of greatest importance in this context are neutral triacylglycerides rather than phospholipids, the chemical variables of greatest importance in the context of energy-yielding potential of fats and oils are the degree of saturation and the chain length of constituent fatty acids. As soapstocks and acid oils are also perfectly acceptable components of dietary fats and oils, the proportion of free fatty acids is also of importance.

There have been numerous reviews on the physiological bases for fat and oil digestion in poultry (e.g. Freeman, 1976, 1984; Krogdahl, 1985). The major site of fat digestion in poultry is the duodenum. Basically, the process consists of emulsification of dietary fat by conjugated bile salts, followed by hydrolysis of triacylglycerides by pancreatic lipase into mixtures consisting essentially of 2-mono-acylglycerides and free fatty acids. The subsequent absorbability of these products is dependent upon their solubility in bile salt micelles. Polar solutes are more readily incorporated into micelles, which explains the relatively higher absorbabilities of unsaturated fatty acids, compared with saturated fatty acids, and the well-established observation that unsaturated fatty acids have higher digestibilities than those that are saturated (e.g. Renner and Hill, 1961). Accordingly, oils, which are relatively unsaturated, have a higher dietary energy value than the more-saturated fats – this also explains why hydrogenation of

 Poultry Feedstuffs: Supply, Composition and Nutritive Value (eds J.M. McNab and K.N. Boorman)

oils (even partial) is associated with a reduction in dietary energy value. The relative superiority of an intact triacylglyceride compared with hydrolysed fat in terms of dietary energy value is also well known (e.g. Young, 1961; Sklan, 1979), attributable presumably to the importance of mono-acylglycerides in the overall absorptive process.

However, implications for the quantitative assessment of dietary energy value (which in the context of the current chapter will be expressed as apparent metabolizable energy; AME) have only been addressed comparatively recently. There still remains a persistence among applied nutritionists to refer to this class of raw materials in terms of origin alone rather than chemical composition. Whilst sourcing of raw materials is assuming greater importance, as the food chain seeks to become increasingly proscriptive as to what may or may not be permissible as a raw material in poultry diets, it still remains the case that it is chemical structure and not name or origin that determines AME. The importance of this point is reinforced by the observation that fats and oils are invariably included into diets as mixtures of individual commodities (referred to as 'blends'). It is the objective of this chapter to examine the quantitative contribution of variability in chemical structure of fats and oils to their AME.

ROLE OF CHEMICAL COMPOSITION OF FATS AND OILS ON AME

Degree of Saturation

There have been many terms employed to describe degree of saturation of fatty acids. Estimation of the number of double bonds through, for example, iodine number is used widely. However, the value of the term in the context of this chapter is limited. It would appear that the key difference between fatty acids is the presence (unsaturated) or absence (saturated) of double bonds, not the number of double bonds. Thus an oil based predominantly on oleic acid (C18:1, e.g. rape seed oil) would in all probability have a similar AME value to one based essentially on linoleic acid (C18:2, e.g. soybean oil) even though the iodine number of the latter would be considerably higher. This explains the lack of precision of equations based on iodine number in attempting to predict the AME of fat and oil blends.

Accordingly, a more effective approach is to establish the ratio of unsaturated to saturated fatty acids (U/S). From knowledge of the physiology of fat digestion and absorption, the probable response of AME to U/S is presented in Fig. 9.1. In a comprehensive series of poultry metabolism trials (Wiseman and Lessire, 1987; Wiseman and Salvador, 1991; Wiseman *et al.*, 1991) examining the AME of a number of fats and oils (together with their blends), it was possible to quantify this response (see Fig. 9.2) – use of the descriptors 'tallow' and 'soybean oil' is merely a guide as to the approximate relative values of two commodities with these 'names'; the presence of two responses attributable to age of bird (young referring to 1.5 and old to 7.5 weeks of age, with the latter extending to adults) is based on the well-known improvement in dietary utilization of fats in older birds (e.g. Fedde *et al.*, 1960; Carew *et al.*, 1972; Wiseman and Salvador, 1989).

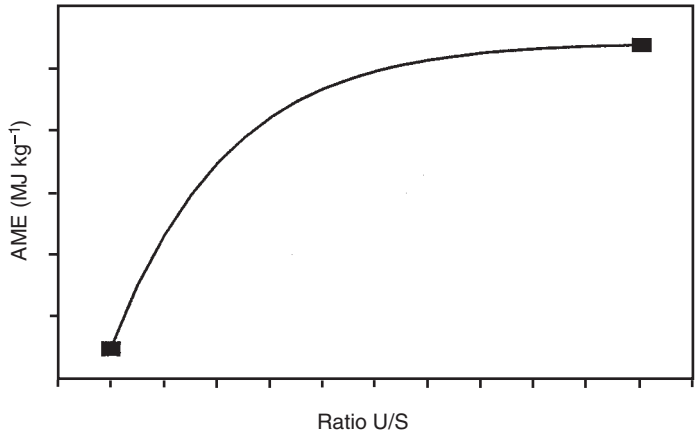

Fig. 9.1. Predicted relationship between the degree of saturation of a fat (expressed as the ratio of unsaturated – U – to saturated – S – fatty acids) and its apparent metabolizable energy (AME) value.

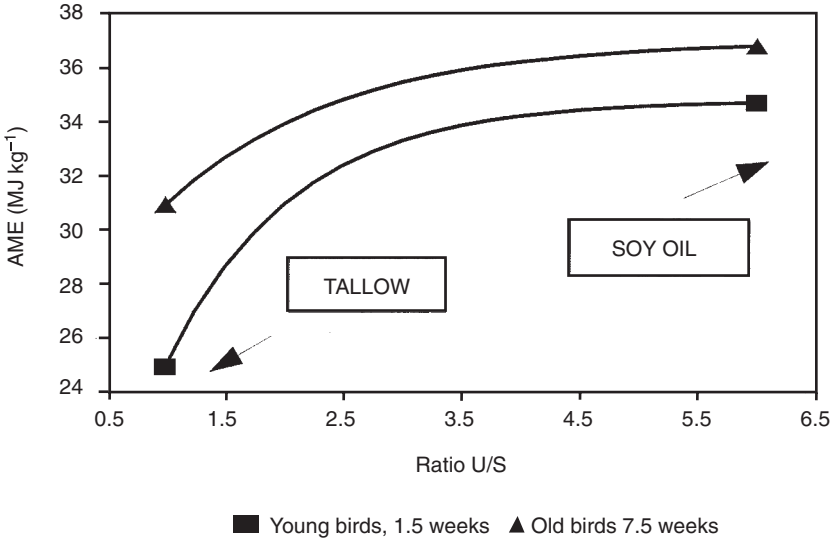

Fig. 9.2. Modelled relationship between the degree of saturation of a fat (expressed as the ratio of unsaturated – U – to saturated – S – fatty acids) and its apparent metabolizable energy (AME) value. Young and old refer to birds aged 1.5 and 7.5 weeks, respectively.

It has been thought for some considerable time that the effects of degree of saturation cannot be considered in isolation from the phenomenon of 'synergism', when the AME of a relatively saturated fat is improved if blended with a more unsaturated oil. The physiological basis for this has been established when considering individual fatty acids. Thus the absorbability of stearic acid (C18:0) is improved if blended with linoleic acid (e.g. Renner and Hill, 1961). However, extending this principle to a consideration of fats and

oils is not straightforward. Thus the established improvement in the AME with increasing unsaturation (higher U/S) is reliant not only on the greater utilization of the increasingly important 'U' fraction but also of the 'S' fraction (which, whilst declining in concentration, is still present). Therefore, 'synergism' between individual classes of fatty acid has already been included in the non-linear responses described in Figs 9.1 and 9.2. Thus 'synergism' is not a numerical improvement in the AME of a blend of two sources over and above that which would be predicted from their individual AME values. A 50:50 mixture of two sources with respective AME values of 35 and 27 MJ kg^{-1} would yield an AME of 31 MJ kg^{-1}, not higher. The basis for the calculation of AME is presented in Fig. 9.3; the response to U/S is curvilinear whereas the response to the relative proportion of the two sources is linear. It is crucial to appreciate that the U/S of a 50:50 mixture of two sources is not the numerical mean of their individual U/S.

Inclusion of Free Fatty Acid Content

Although degree of saturation of fats and oils is a major chemical variable, it is by no means the only factor of relevance to AME values. Whilst hydrolysed fats and oils (i.e. those with high concentrations of free fatty acids – FFA) have lower AME values than their corresponding intact triacylglycerides, it is crucial that the response of AME to FFA is assessed to allow the conse-

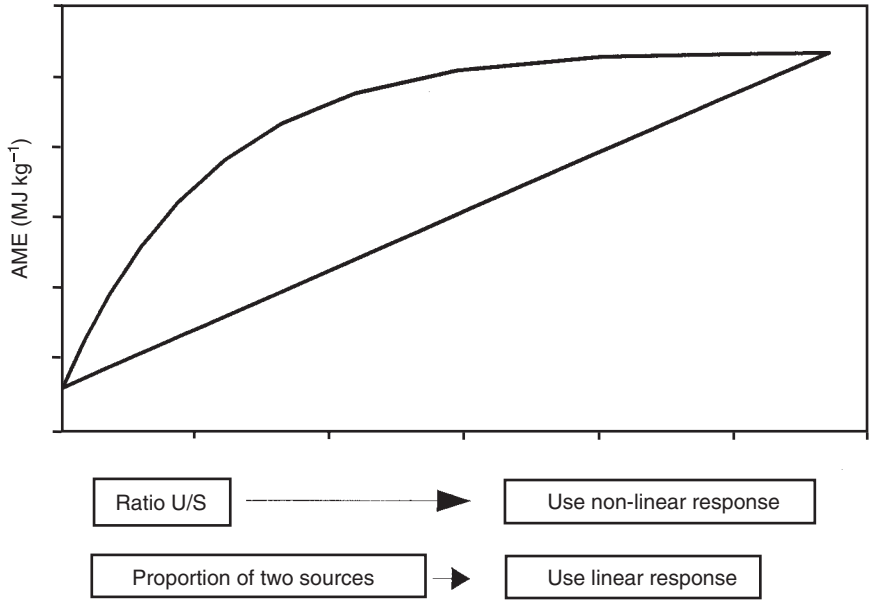

Fig. 9.3. Calculation of apparent metabolizable energy (AME) value of a mixture of two fats based either on ratio of unsaturated – U – to saturated – S – fatty acids or proportion of the two sources in the mixture.

quences of an incremental increase in FFA to be quantified. Representative data emerging from the series of poultry metabolism trials described above (Wiseman and Salvador, 1991) are presented in Fig. 9.4. These trials, in addition to evaluating the two extreme sources, also determined the AME of mixtures with intermediary FFA content in order to define the response of variable FFA content. It is evident that the reduction in AME with increasing FFA is linear in all cases. This is not to say that FFA levels should be minimized but rather to argue that knowledge of FFA is a fundamentally important step in establishing AME values of fats and oils.

Thus U/S (non-linear response on AME) and FFA (linear response on AME) can now be combined into one predictive model (it having been established that U/S and FFA do not interact) which is presented in Table 9.1. Solutions for these functions over varying U/S, at two FFA concentrations and for two ages are presented in Fig. 9.5. The range in values is of the order of 12 MJ kg^{-1}. Although this is in itself of major importance to the efficiency of utilization (and, indeed, economics of purchase) of this group of raw materials, there are other consequences emerging. Thus if a fat blend has a coefficient of digestibility of say 0.9 then, for every 10 g of the blend consumed, 1 g will be voided; with a coefficient of 0.7, the corresponding output will be 3 g. Problems of greasy and capped litter, with their consequent link to carcass quality, may well be associated with high concentrations of fat in excreta.

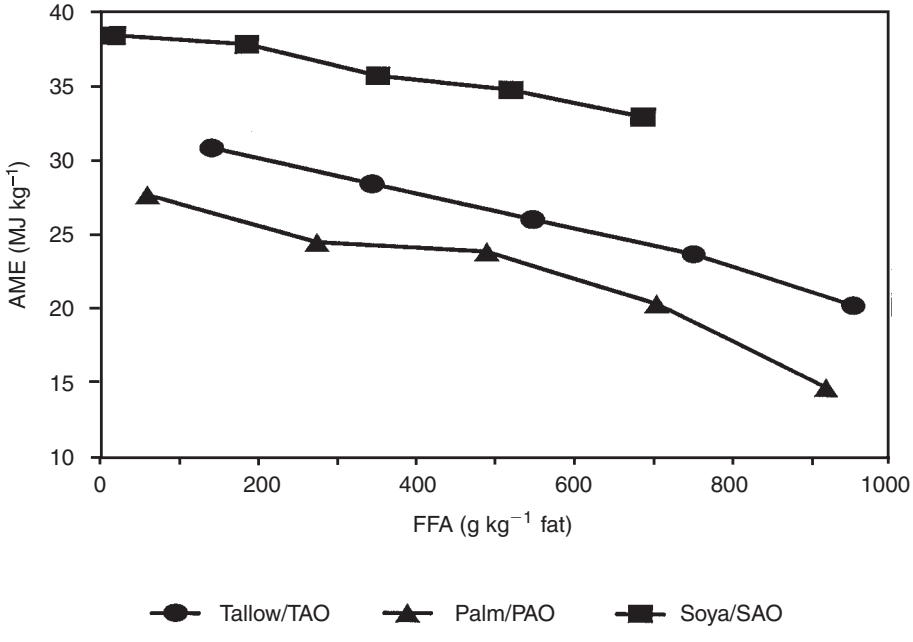

Fig. 9.4. Relationship between free fatty acid (FFA) content of three materials and their respective acid oils (AO), together with mixtures thereof, on apparent metabolizable energy (AME) value.

Table 9.1. Functions employed to predict the dietary energy value of fats.

Function:	Dietary energy (MJ kg^{-1} fat) = $A + B \times FFA + C \times e^{(D \times U/S)}$		
Where:	U/S = ratio of unsaturated to saturated fatty acids in fat		
	FFA = free fatty acid content (g kg^{-1} fat)		
Value of constants:			
		Young[a]	Old[b]
	Constant		
	A	38.112	39.025
		±1.418	±0.557
	B	–0.009	–0.006
		±0.002	±0.001
	C	–15.337	–8.505
		±2.636	±0.746
	D	–0.506	–0.403
		±1.186	0.088
	PV[c]	0.816	0.925

[a]1.5 weeks of age.
[b]7.5 weeks of age.
[c]Proportion of variance accounted for by function.

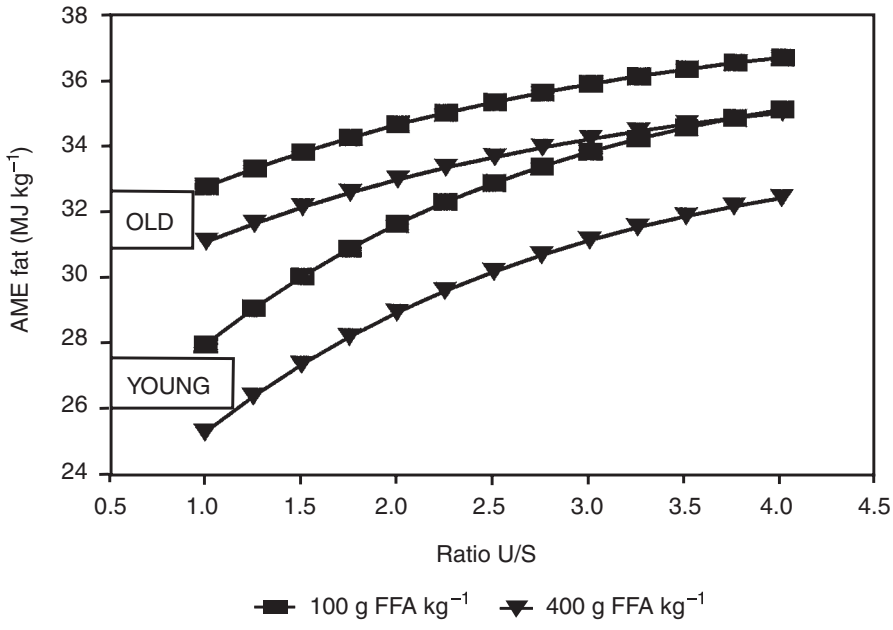

Fig. 9.5. Calculation of apparent metabolizable energy (AME) value of fats based on ratio of unsaturated – U – to saturated – S – fatty acids and free fatty acid (FFA content). Young and old refer, respectively, to birds aged 1.5 and 7.5 weeks. Functions are presented in Table 9.1.

Chain Length

Most fats and oils employed in the manufacture of blends for compound poultry diets consist of fatty acids with chain lengths ranging over the comparatively modest C16–C20. Those with chain lengths of more than C20 are unlikely to be of major importance, as the source of these is invariably fish oils which, because of their oxidative instability, are associated with off-odours. However, it is not impossible that fatty acids with chain lengths of less than C16 (or, rather, sources containing such fatty acids) might be used. Two sources which have high concentrations of short chain fatty acids are coconut and palm kernel oil (not to be confused with palm oil), and a mixture of the two together with the respective 'acid oil' of high FFA content (Table 9.2) and blends of intermediary FFA content were evaluated by Wiseman and Blanch (1994). Figure 9.6 and Table 9.2 present data derived from the trial and also the predicted responses as calculated from the model presented in Table 9.1 with three means of expression of U/S. It is clear from the responses that, in the calculation of U/S, the saturated fraction should be based on stearic (C18:0), palmitic (C16:0) and myristic (C14:0) acids but that lauric acid (C12:0) should appear in the unsaturated fraction.

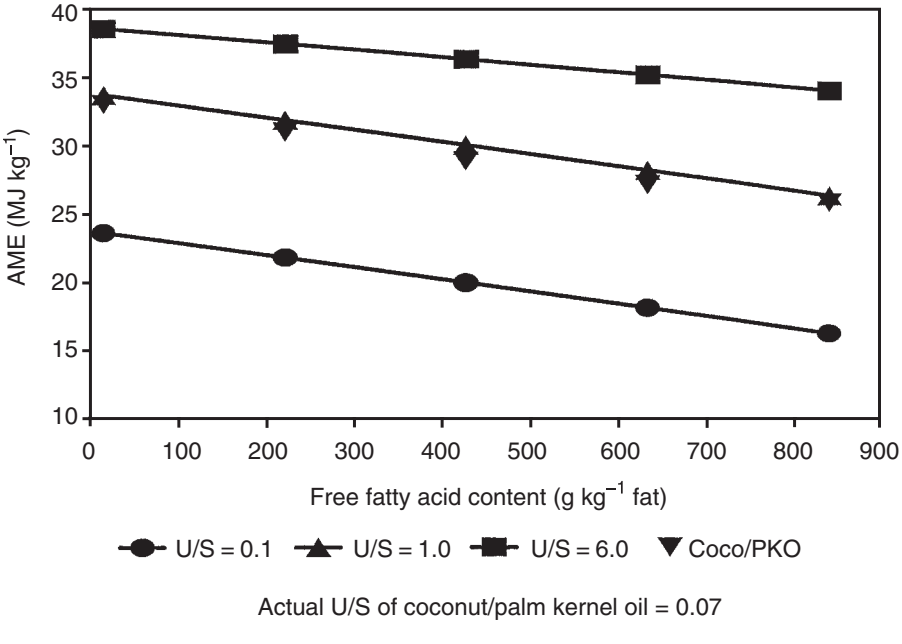

Fig. 9.6. Calculation of apparent metabolizable energy (AME) value of fats based on ratio of unsaturated – U – to saturated – S – fatty acids and free fatty acid (FFA content) compared with determined data for a mixture of coconut/palm kernel oil, the respective acid oil and mixtures thereof.

Table 9.2. Mean chemical analyses of coconut/palm kernel oil blend (CP) and its acid oil (CPAO) – all data expressed as g kg^{-1} oil.

Fatty acid profile[a]	CP	CPAO
8:0	95	42
10:0	71	41
12:0	494	462
14:0	171	170
16:0	78	101
18:0	24	26
16:1	0	0
18:1	53	129
18:2	12	21
18:3	0	0
20+	1	7
U/S[b]	0.07[c]	0.19
	2.66[d]	2.36
	8.78[e]	6.86
Free fatty acids (g kg^{-1} oil)	13.8	839.0

[a]Notation indicates length of carbon chain followed by number of double bonds.
[b]Ratio of unsaturated to saturated fatty acids:
[c]All saturated fatty acids included in 'saturated' fraction;
[d]Only C14:0, C16:0 and C18:0 appearing in 'saturated' fraction;
[e]Only C16:0 and C18:0 appearing in 'saturated' fraction.

Changes Occurring During Processing

Fats and oils will also have probably been subjected to a range of processing conditions during manufacture and subsequent use, such as refining, cracking, rendering and heating (e.g. recovered vegetable oils) which may be responsible for further changes to chemical structure (e.g. Artman, 1969; Wiseman, 1986). As these raw materials are generally relatively unstable (the more so the greater the degree of unsaturation), they are therefore prone to some form of degradation under a variety of conditions. A large number of modifications to the chemical structure of fats and oils following heating have been identified, ranging from simple oxidation products through to dimerization and polymerization (linear and cyclic) of both fatty acids and triacylglycerides depending upon the substrate in question and the conditions operating. This has prompted numerous studies on the chemical commodities produced during heating and the nutritional implications.

The biological effects of feeding these modified structures are also extremely varied both in terms of the actual response in the animal and its severity. It should be noted that even minor adverse biological consequences would have serious repercussions for output from poultry production. Initially, digestibility and hence dietary energy value will be reduced. The consequences will be that animals will not perform to expectations and also that an increasing

amount of dietary fat will pass through the gastrointestinal tract and be excreted. It is also possible that the presence of modified fat structures within the gastrointestinal tract may interfere with the overall digestive process so that general nutrient uptake is impaired. Furthermore, an actively oxidizing fat or oil will destroy other nutrients present, including some vitamins.

Perhaps more concern has been expressed over whether any toxic products are generated following fat and oil heating/oxidation. It does appear, however, that the majority of these products are only sparingly absorbed and thus would not present a threat. However, in the case of oxidized fats and oils, defence mechanisms in the gut mucosae to prevent absorption could be stretched so that overall nutrient absorption would be reduced. It is certainly the case that death in laboratory animals fed heated fats and oils has been recorded (Andrews *et al.*, 1960), but these are extreme cases related in all probability to the physical properties of the materials evaluated rather than to their chemical toxicity.

Because of the potential adverse effects of feeding heat-damaged/oxidized fats and oils, there has been considerable interest in developing chemical methods for the detection of such damage. It is important to note that any method adopted has to be one that measures all products collectively if it is to have any practical application. Peroxide value (PV) has been employed widely for this purpose, but it is an unsound method. In tracing the change in PV over time (Poling *et al.*, 1962) an increase followed by a decrease was observed. Thus a low PV value may indicate, on the one hand, a commodity that had not undergone any degradation but, on the other hand, one that had been seriously denatured. PV measured over time might be an improvement, but the rate of production of peroxides may equal their subsequent degradation so that, overall, PV remains constant although the material is deteriorating. Measurement of 'oxidized fat' has been employed, but the evaluation is solvent dependent and would not measure those complexes which would not be soluble in polar solvents.

Free fatty acid content has been employed frequently to assess damage to oils used in the human food industry, but is inappropriate for fats and oils for incorporation into compound diets for poultry. This is because materials of high FFA content are perfectly acceptable ingredients for blends (whilst being of lower energy value than the original triacylglyceride). This also explains why assessments of molecular weights or sizes are inappropriate, because a fatty acid trimer (of no dietary energy-yielding value) would generate similar data to a triacylglyceride (of high value).

A technique which has found favour is one based upon estimating the total non-elutable material (NEM) of a fat or oil using quantitative gas–liquid chromatography (Waltking *et al.*, 1975; Edmunds, 1990) and incorporating a glycerol correction (glycerol, the backbone of triacylglycerides, is present following hydrolysis and derivatization of fatty acids; as such it would appear in the NEM fraction although it is not associated with 'damaged' structures). Whilst this method merely measures collectively most degraded structures within fat or oil, it does at least provide guidance as to whether the commodity (for example some recovered vegetable oils from frying operations) may have been excessively heated.

The possible reduction in dietary energy value likely to result from damage was studied by Wiseman *et al.* (1992) employing a refined sunflower oil which was, subsequently, extensively heat damaged (Table 9.3). Chemical analysis revealed an increase in FFA and NEM content following such treatment but little difference in the proportion of individual fatty acids. The two commodities (together with mixtures of the two) were evaluated for AME and data generated were compared with values predicted from U/S and FFA. It was evident that the dietary energy value of the NEM fraction in this material was of the order of zero. This indicates the problems identified with heat-damaged fats, although no account was taken of other issues associated with the presence of the NEM fraction (e.g. reduction in general nutrient uptake).

The consequences of failure to record NEM are presented in Fig. 9.7. The conventional means of quality control of fats and oils (other than U/S and FFA) are based on moisture plus unsaponifiable matter plus impurities (MUI). However, in the example given, two sources with identical MUI and assumed equal FFA and U/S would not be of the same AME because source B would have a higher NEM content.

INTERACTIONS BETWEEN FATS/OILS AND DIETARY 'FIBRE'

Although the model described above has allowed the estimation of the AME of a fat from knowledge of its free fatty acid content together with chain length and degree of saturation of constituent fatty acids (and including non-elutable content and age of bird), there have been recent studies of the utilization of

Table 9.3. Mean chemical analyses of refined sunflower oil (RS) and sunflower acid oil (SAO). All data expressed as g kg^{-1} oil.

Fatty acid profile	RS	SAO
14:0	0.7	1.4
16:0	69.3	82.4
17:0	2.0	2.7
18:0	44.7	47.9
16:1	1.1	2.0
18:1	196.1	190.5
18:2	651.1	619.6
18:3	1.3	2.0
20+	16.4	25.3
Other	17.3	26.2
U/S	7.32	6.25
Non-elutable material	41.0	136.6
Free fatty acids	0.0	388.0
Water	2.0	8.2
Unsaponifiable material	0.6	82.0
Oxidized fatty acids	0.4	14.8
Impurities	0.01	0.1

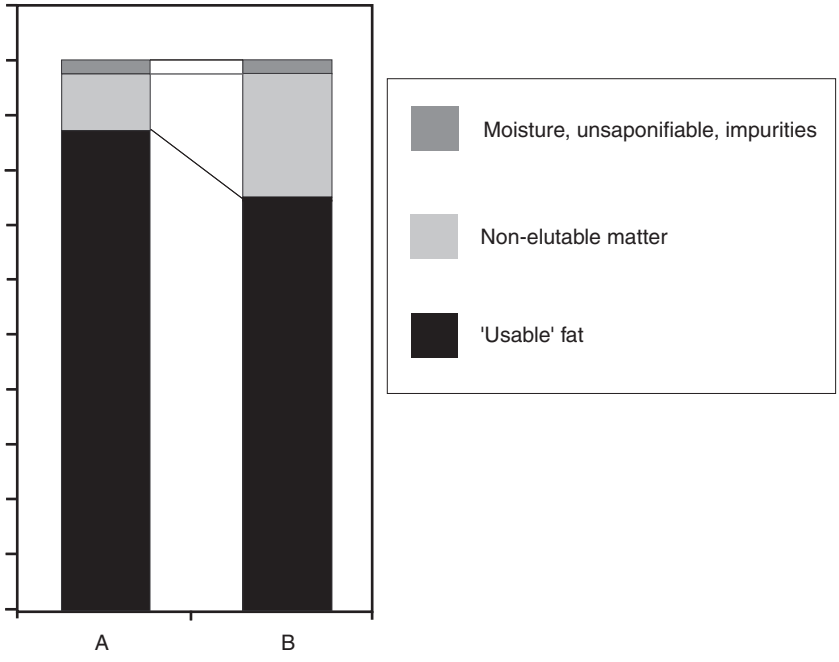

Fig. 9.7. Demonstration of importance of estimating non-elutable matter within a fat blend on nutritional value.

dietary fats and oils in poultry based on digestive efficiency as influenced by other dietary constituents. One major area is the role of 'dietary fibre'. This has been examined in human nutrition in the context of reducing the postprandial hyperlipidaemic response in subjects prone to obesity. Interestingly, the objectives of these programmes are to reduce lipid uptake through reducing the efficiency of overall lipid digestion, whereas in poultry nutrition the objective is the opposite. Thus, routes whereby fat digestion and absorption are compromised in humans are of interest in poultry in order to avoid such possibilities.

Emulsification is a key stage in fat digestion, and the size together with the physical properties of the subsequent droplets is an important determinant of the degree of binding of pancreatic lipase – the greater the size, the smaller the surface area per unit of mass of fat presented and, hence, the lower the efficiency of lipolysis. In a recent *in vitro* study, Pasquier *et al.* (1996) examined the role of different soluble dietary 'fibres' of varying intrinsic viscosity on the degree of emulsification and lipolysis of triacylglycerides. The data generated indicated that there was a relationship between viscosity and droplet size (Fig. 9.8) which in turn was associated with the degree of triacylglyceride lipolysis.

These studies have now been extended into poultry, with the additional factor of exogenous enzyme addition, in an attempt to overcome the negative features, including, principally, viscosity-promoting potentials of soluble non-starch polysaccharides (NSP, e.g. Danicke *et al.*, 1997a,b; Langhout *et al.*, 1997). The subject is covered in detail in this volume (see Bedford, Chapter 17), but the

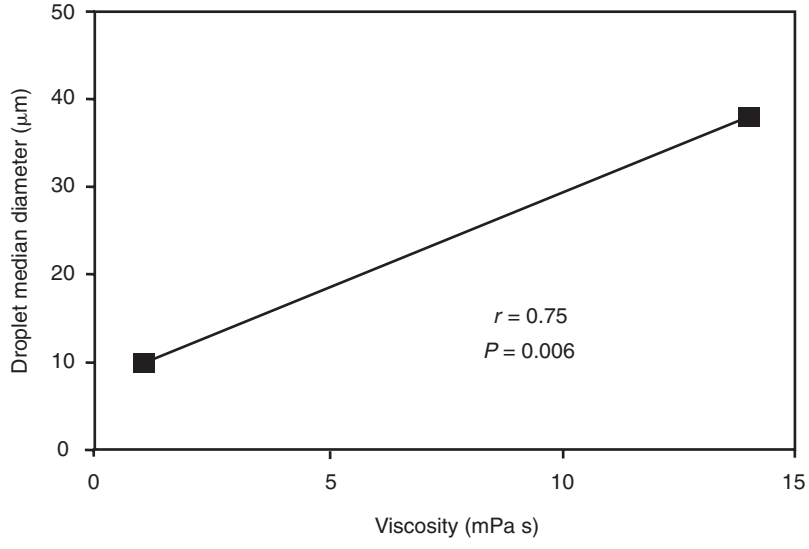

Fig. 9.8. Influence of viscosity on degree of emulsification of an oil as determined through droplet size (from Pasquier *et al*., 1996).

general conclusions from these and other studies are that the presence of NSP within the intestinal milieu will compromise digestion and absorption of dietary fats. The mechanisms are associated with lowered degree of emulsification, reduced activity of pancreatic lipase and decreased micelle formation. The problems are, accordingly, more evident the greater the degree of saturation of dietary fat. The reasons for these effects are in all probability correlated with increased viscosity *in vivo*, which will also interfere with nutrient diffusion, which is in itself linked to molecular weight and size of the NSP fraction. More indirect effects may be associated with motility of digesta and frequency of peristaltic contractions (see Duke, Chapter 7 this volume). Finally, the role of gastro-intestinal microflora (which may be encouraged by increasing NSP concentration) in bile salt deconjugation may be important.

GENERAL CONCLUSIONS

Although interactions with other dietary ingredients have been established, they remain to be subsequently quantified. It should be borne in mind that the studies by both Danicke *et al.* (1997a,b) and Langhout *et al.* (1997), which have established that NSP are linked to reduced fat digestibility (the more so the greater the degree of saturation), employed diets that were unusual (high levels of rye and/or high levels of saturated fats) in order to demonstrate effects. What remains to be determined are the quantitative effects these responses might have on the model that has been derived to predict the AME of fats under conditions in which they are likely to be utilized. Initial observations tend to suggest that these effects are unlikely to be of major consequence.

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CHAPTER 10
The availability of calcium and phosphorus in feedstuffs

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In a review describing different calcium and phosphorus sources utilized for feed formulations, Waldroup (1996) reported that although 12 minerals are considered essential minerals for poultry and pigs, meeting the calcium and phosphorus needs of these animals is of the greatest concern to nutritionists and producers. This concern is warranted due to the relative quantities needed and due to the adverse effects on the animals that occur when inadequate dietary levels are fed. Producers need to make informed decisions based on animal health, production and economics as to how they will meet the needs of the animals, especially in the case of phosphorus, which is relatively expensive compared with calcium. The quality of calcium and phosphorus sources is important in that the availability of the sources used will determine the quantity that is required in the diet and the amount that will be in the manure of the animals. This latter point is becoming of greater importance as evidence indicates that phosphorus from manure has a negative effect on water quality. Because of the relatively high cost of adding phosphorus to diets, there has been a large amount of research dedicated to determining the necessary dietary levels and quality of phosphorus sources in poultry diets. In contrast, calcium is typically inexpensive compared with phosphorus and at present poses no ecological concern, consequently there has been less emphasis placed on calcium requirement (with the exception of shell quality of laying hens) and source quality research.

Calcium and phosphorus are the two most abundant minerals found in the body, due mainly to their major involvement in bone formation. Calcium and phosphorus are also necessary for efficient feed utilization and weight gain. Calcium is essential to the formation of eggshell and is required for the formation of blood clots, muscle contraction and transmission of nerve impulses. Calcium is also involved in the regulation of heartbeat, can act as an activator or stabilizer of enzymes and is involved in hormone secretion. Phosphorus is needed for normal muscle growth and egg formation, is a component of the nucleic acids of the genetic code, and of phospholipids, and is also a component or activator of a large number of enzyme systems. Phosphorus aids in maintaining osmotic and acid-base balance, is a factor in energy metabolism (ATP), amino acid metabolism and protein

 Poultry Feedstuffs: Supply, Composition and Nutritive Value (eds J.M. McNab and K.N. Boorman)

production. Lack of adequate dietary calcium and phosphorus in growing poultry leads to abnormal calcification of bones, known as rickets. Visible symptoms of rickets are swollen joints, enlargement of end bones and rubbery beaks and may be the result of inadequate calcium, phosphorus or vitamin D in the diet of the bird. Older birds subjected to inadequate dietary calcium and phosphorus develop brittle and weak bones (osteomalacia). In laying hens, calcium deficiency first manifests itself as thin and weak eggshells. A prolonged and severe lack of dietary calcium can lead to a complete cessation of egg production.

Calcium and phosphorus can be provided in poultry diets by a large number of sources. A portion of diet calcium and phosphorus content is provided by the plant feedstuffs used to provide dietary energy and protein. Table 10.1 shows a list of plant feedstuffs and their calcium and phosphorus contents. Typically, a large proportion of plant feedstuffs' phosphorus is in the form of phytate phosphorus, which is not highly available to the bird. Since plant feedstuff components of poultry diets generally do not provide enough of these minerals to meet the needs of maintenance, growth and production, diets are typically supplemented with concentrated calcium and phosphorus sources. Sources of calcium and phosphorus are listed in Table 10.2. Limestone and oyster shell are the more commonly used sources of calcium for poultry diets, as they are relatively inexpensive. A larger variety of phosphorus sources exist. Natural, unprocessed phosphorus sources tend to have lower and more variable phosphorus contents than the higher priced, chemically processed phosphates. Selection of calcium and phosphorus sources to use to supplement diets is based on local availability and price.

ABSORPTION AND EXCRETION OF CALCIUM AND PHOSPHORUS

Van der Klis (1993) has provided an extensive review of calcium and phosphorus absorption and excretion by poultry. Table 10.3 lists the sites of absorption and excretion for both calcium and phosphorus in poultry. Hurwitz and Bar (1969, 1970) observed net secretion in the duodenum of broilers but net absorption in the duodenum of laying hens. Most calcium absorption has been found to occur in the duodenum and jejunum in broilers and layers (Hurwitz and Bar, 1970, 1971; van der Klis *et al.*, 1990). In laying hens, absorption has also been observed in the lower gastrointestinal tract. The secretion and absorption of calcium by different intestinal segments in laying hens has been found to be dependent on the stage of eggshell formation (Hurwitz and Bar, 1965; Nys and Mongin, 1980; Waddington *et al.*, 1989). Calcium is transported across the intestinal membranes by a saturable, active (transcellular) process and a non-saturable (paracellular) process. The saturable (active) process can be affected by the nutritional and physiological status of the bird (van der Klis, 1993). During calcium restriction, active transport is significantly increased (Hurwitz and Bar, 1969; Hurwitz, 1989). Excretion of calcium from the cell has been described as an active process (Wasserman *et al.*, 1992).

Table 10.1. Calcium and phosphorus content of common plant feedstuffs (National Research Council, 1994).

Feedstuffs	Calcium content (%)	Total phosphorus content (%)	Non-phytate phosphorus content (%)	Non-phytate phosphorus (% of total phosphorus)
Lucerne meal, 17% CP	1.44	0.22	0.22	100.0
Barley	0.03	0.36	0.17	47.2
Buckwheat	0.09	0.32	0.12	37.5
Canola meal, 38% CP	0.68	1.17	0.30	25.6
Maize gluten meal, 60% CP	–	0.50	0.14	28.0
Maize, grain	0.02	0.28	0.08	28.5
Cottonseed meal, 41% CP	0.15	0.97	0.22	22.6
Distillers dried grains	0.10	0.40	0.39	97.5
Distillers dried solubles	0.35	1.27	1.17	92.1
Oats, grain	0.06	0.27	0.05	18.5
Groundnut meal	0.20	0.63	0.13	20.6
Pearl millet	0.05	0.32	0.12	37.5
Rice bran	0.07	1.50	0.22	14.7
Rice polishings	0.05	1.31	0.14	10.7
Rye, grain	0.06	0.32	0.06	18.8
Safflower meal, 43% CP	0.35	1.29	0.39	30.2
Sesame meal, 43% CP	1.99	1.37	0.34	24.8
Soybean meal, 44% CP	0.29	0.65	0.27	41.5
Soybean meal, 48% CP	0.27	0.62	0.22	35.4
Soy protein concentrate	0.02	0.80	0.32	40.0
Sunflower meal, 45% CP	0.37	1.00	0.16	16.0
Wheat bran	0.14	1.15	0.20	17.4
Wheat middlings	0.12	0.85	0.30	35.3
Wheat, hard winter	0.05	0.37	0.13	32.0

The absorption of phosphorus by the gastrointestinal tract has been found to be similar to, but not dependent on the absorption of calcium (Wasserman, 1981). Absorption of phosphorus in broilers was determined to be most efficient from the duodenum to the upper jejunum (Hurwitz and Bar, 1970), with no net absorption occurring in the lower gastrointestinal tract. Layers have been shown to absorb phosphorus throughout the whole intestine, but the rate of absorption declines in the lower tract. Large amounts of endogenous phosphorus have been shown to be secreted into the duodenum of laying hens (Hurwitz and Bar, 1965). As is the case with calcium, laying hens show differences in phosphorus absorption and excretion based on stage of eggshell formation. Wasserman and Taylor (1973) suggested that the absorption of phosphorus is a saturable, active process. Favus (1992) described a cotransport system for sodium and phosphorus to effect the movement of phosphorus into the cell and identified facilitated diffusion as a means of phosphorus leaving the cell.

Table 10.2. Common sources of calcium and phosphorus (Waldroup, 1996).

Source		% Ca	% P
Limestone		38	–
Oyster shell		38	–
I. Calcium phosphates			
	A. Natural or unprocessed		
	Low fluorine rock phosphate	32–35	12–15
	Curacao phosphate (guano)	36	13–15
	Colloidal phosphate (soft phosphate)	18–20	9–10
	Bone meal, steamed	23–26	8–18
	B. Chemically processed		
	1. Dicalcium phosphates		
	Di/mono calcium phosphates	15–23	18–23
	Mono/dicalcium phosphates	15–18	20–21
	Precipitated dicalcium phosphates	24–26	18–22
	2. Defluorinated phosphates	30–36	14–18
II. Sodium phosphates			
	Monosodium phosphate	–	25
	Disodium phosphate	–	21
	Sodium tripolyphosphate	–	25
III. Ammonium phosphates			
	Monoammonium phosphate	–	24
	Diammonium phosphate	–	20
IV. Phosphoric acid		–	23–24
Fish meals		2–14	2–7
Meat and bone meals		4–14	2–10
Poultry by-product meals		2–10	2–8

Table 10.3. Sites of calcium and phosphorus absorption or secretion in broilers (van der Klis, 1993).

Site	Calcium	Phosphorus
Duodenum	Secretion	Secretion
Upper jejunum	Absorption	Absorption
Lower jejunum	Absorption	Absorption
Upper ileum	Absorption	No change
Lower ileum	No change	No change

Factors which may affect gastrointestinal absorption of calcium and phosphorus include dietary concentration and physical and chemical forms of these minerals, passage rate of feed and viscosity of digesta, chelating agents and mineral interactions, gastrointestinal tract pH, and interactions with dietary protein, fat and carbohydrate (van der Klis, 1993).

URINARY EXCRETION OF CALCIUM AND PHOSPHORUS

The reason retention of minerals has more meaning than digestibility or availability of minerals is because the kidney plays an important role in the amount of mineral that will be retained or found in the excreta. In order to determine the digestibility or availability of minerals for a feedstuff, the separation of the urinary minerals from the faecal minerals is necessary.

Calcium and phosphorus content of poultry urine is determined by the rates of kidney secretion and reabsorption of the minerals. The quantity of calcium and phosphorus excreted by the urine is thus dependent on glomerular filtration rates, tubular reabsorption rates and tubular secretion rates. Factors affecting kidney excretion of calcium and phosphorus are listed in Table 10.4. Wideman (1984) noted that blood or plasma inorganic phosphorus concentrations had only an indirect influence on the amount of phosphorus secreted by the kidney and that only a small fraction of secreted urinary phosphorus was composed of organic phosphates. Wideman (1987) suggested that calcium availability for eggshell formation was the controlling parameter for laying hen urinary calcium and phosphorus excretion patterns. The author noted that urinary phosphorus excretion increased and urinary calcium excretion decreased when bone minerals are mobilized for eggshell formation. Another major determinant of urinary phosphorus excretion is parathyroid hormone, which inhibits tubular reabsorption of inorganic phosphorus. Both high dietary calcium and low dietary phosphorus have been shown to depress urinary phosphorus excretion. A high calcium, low phosphorus diet resulted in very high urinary calcium excretion (Wideman, 1987). Increased dietary phosphorus leads to increased kidney phosphorus excretion, whereas low dietary phosphorus stimulates phosphorus reabsorption by the kidneys (Wideman, 1989).

Total excreta calcium and phosphorus content then is the summation of unabsorbed gastrointestinal ingested and secreted calcium and phosphorus from the gastrointestinal tract plus urinary calcium and phosphorus. The dietary and physiological factors affecting the digestibility, absorption and secretion of minerals in the gastrointestinal tract, along with factors affecting the excretion of calcium and phosphorus in the urine, will need to be taken into consideration.

Table 10.4. Factors that affect avian urinary calcium and phosphorus (Wideman, 1987).

Parathyroid hormone
Dietary calcium and phosphorus levels
Vitamin D
Stage of eggshell formation
Calcitonin – ?

EVALUATION OF CALCIUM AND PHOSPHORUS AVAILABILITY IN FEED INGREDIENTS

Traditional Biological Value Assays

Sullivan and Douglas (1990) published an excellent review of phosphorus bioassays performed from 1945 to 1990, wherein they traced the development of relative biological value bioassays and discussed the primary variable which can affect the data obtained from such studies. Dietary phosphate sources have been primarily evaluated for poultry with 2–3 week feeding trials. The biological value of a phosphorus source is determined by feeding chicks or poults different amounts of the test phosphates in a phosphorus-deficient diet for a 2–3 week period and comparing the weight gain, feed conversion, proportion of tibia ash, or other performance parameter with a phosphorus source designated to be a standard phosphate (usually a food reagent grade phosphorus source). The standard phosphate used is assigned a biological value of 100 (for 100% available), and the feedstuff phosphates assigned relative biological values compared with the biological value of the standard phosphorus source. Numerous reference standards have been employed, including potassium phosphates, sodium phosphate, and mono-, di- and tri-calcium phosphates. Both conventional and purified diets have been used in these types of assays. Though useful in qualitatively comparing different phosphorus sources, these studies are limited in that they provide only values relative to the standard chosen and other factors (Table 10.5), such as selection of response criteria, also may play a role in determining the value assigned to a phosphorus source. These bioassays provide phosphorus bioavailability data and response criteria specific to a chosen standard but lack the ability to provide actual data needed to determine available, digestible or retainable phosphorus in a feedstuff for poultry.

Compared with the large amount of research conducted on biological values of phosphorus sources, little research has been done in this manner to compare calcium sources. Dilworth *et al.* (1964) determined relative availabilities of four feed-grade calcium sources for starter period chicks, utilizing a USP grade calcium carbonate as the standard. The authors determined that defluorinated phosphates resulted in high relative calcium availability based on weight gain and tibia ash compared with a low fluorine rock phosphate and a soft phosphate (Table 10.6). Spandorf and Leong (1965) found the biological

Table 10.5. Factors affecting phosphorus relative bioavailability bioassays (Sullivan and Douglas, 1990).

1. Selection of response criteria
2. Reference of standard phosphate selected
3. Diet composition – purified or practical
4. Ca:P ratio
5. Species and type of fowl
6. Bioassay length

Table 10.6. Calcium availability of feed-grade phosphates (Dilworth *et al.*, 1964).

Calcium source	Relative availability (%)
$CaCO_3$ – USP	100
Low fluorine rock phosphate	90
Defluorinated phosphate A	95
Defluorinated phosphate B	92
Soft phosphate	68

availability of calcium in 12 menhaden fish meals to be similar to that of calcium in limestone and dicalcium phosphate. Reid and Weber (1976) reported that bioavailability of feed-grade calcium sources ranged from 73.3% to 109.4% compared with a reagent-grade calcium carbonate. Calcium availability can be dependent on the particle size of the source, especially at marginal dietary levels. Hillman *et al.* (1976) reported that small particle-size limestone resulted in increased weight gain, feed efficiency and calcium availability to the turkey poult at low dietary calcium levels. This effect of small particle size has also been shown with male broiler chicks (Guinotte and Nys, 1991a). These same authors and others (Cheng and Coon, 1990; Rao and Roland, 1990; Guinotte and Nys, 1991b) have noted that the converse is true in laying hens; large particle size calcium sources result in improved bone ossification, eggshell quality and performance. It has been suggested that the increased availability for laying hens of larger particle size sources is the result of a slower rate of passage through the digestive tract of the hen, allowing absorption when needed for eggshell formation (Zhang and Coon, 1997). The particle size needs a minimum diameter of 0.9–1.0 mm to be retained in the gizzard for more efficient utilization during the time of eggshell formation. The availability and performance differences, due to particle size of calcium sources, have been most evident when the hens' daily intake of calcium is marginal to deficient. Anderson *et al.* (1984) observed that chicks can pass excess calcium of medium particle size through the digestive tract more rapidly than small particle size calcium sources. The faster passage rate of the medium size particles for broilers may limit overall calcium utilization. McNaughton (1981) reported that particle size of the calcium source could also affect phosphorus utilization in the chick.

RETAINABLE CALCIUM AND PHOSPHORUS

The nutritionist today needs an actual biological retention value for key minerals to assess the true impact of dietary formulations on animal performance and on the elements remaining in animal waste, which will potentially affect the ecological system. The relative biological availability assays for calcium and phosphorus provide a valuable tool for comparing the feeding value of different sources of calcium and phosphorus. The information obtained from a

relative biological availability assay has limited value for a nutritionist formulating diets. The relative biological value is dependent on a standard which is assumed to be of higher availability than the other sources being evaluated and is dependent on a performance response criterion rather than on actual mineral retained and excreted. The calcium or phosphorus source used as a standard may not be 100% available, so the availability obtained for the mineral for a test ingredient has limited value. Retention bioassays measure both ingested and excreted calcium and phosphorus and would provide the means to calculate actual mineral retained. The ability to detect changes in calcium and phosphorus retention will provide important information needed to assess the economic value of increasing dietary concentrations of these key minerals and also to evaluate the effect of adjusting other nutrients on calcium and phosphorus utilization. Retention is defined herein as the amount of ingested mineral (calcium or phosphorus) that is not excreted. In the case of phosphorus, retainable phosphorus can include that from both non-phytate and phytate sources assessed with a balance method using a Celite marker as reported by Gueguen (1996). Thus:

Total phosphorus retained = non-phytate phosphorus retained + phytate phosphorus retained

Phosphorus retention (%) = (total phosphorus ingested – total phosphorus excreted)/total phosphorus ingested) × 100

Excreted mineral as a proportion of ingested mineral can be determined either by the total collection technique or by the use of an indigestible marker. The above equation can also be used to determine retention of calcium. Although many authors use the terms available or digestible to refer to the above calculation, here we will use the term retainable in order to differentiate from the relative biological availability studies. Also, non-phytate phosphorus and available phosphorus are often inaccurately used interchangeably, although studies have shown that non-phytate phosphorus is not 100% 'available' and phytate phosphorus is not 100% 'unavailable'. The term digestibility, often used to describe the utilization of amino acids, is also less descriptive than would be desired, as it is a term for describing the disappearance of a nutrient from the gastrointestinal tract, but does not account for the excreta calcium and phosphorus from the urine. For the sake of clarity in the following discussion, data from authors calculated according to the equations above will be referred to as retained, rather than available or digestible as in the original publication.

Retention bioassays can provide requirement information based on the actual amount of calcium and phosphorus retained, rather than the amount fed. This allows for a determination of a requirement irrespective of source quality and provides a tool for predicting the amount of a mineral that will be present in the excreta. Retention bioassays also can provide information on the availability of calcium and phosphorus in plant feedstuffs, including phytate phosphorus. Determination of requirements based on retention of minerals is based on the concept put forth by Sibbald (1982) and illustrated in Fig. 10.1.

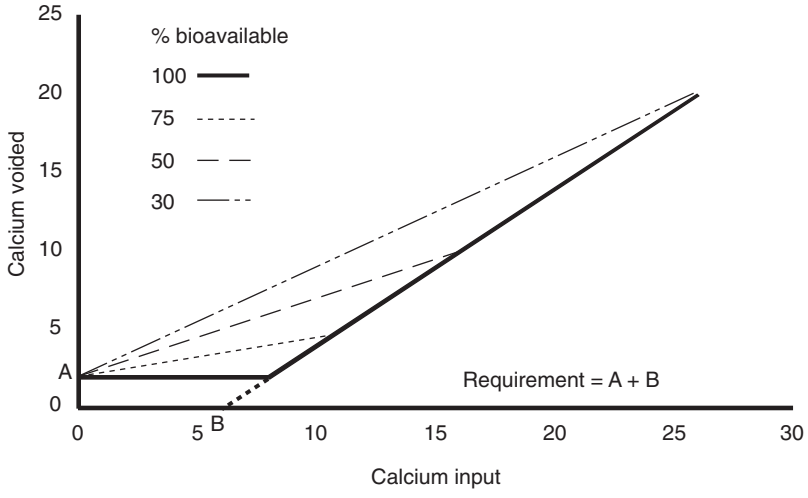

Fig. 10.1. Theoretical response of excreted calcium at different intakes and source quality (Sibbald, 1982).

Retention Assays

Commercial egg layers and pullets

Hurwitz and Griminger (1962) determined calcium retention of laying hens (at production rates of 0.7 eggs per bird day or more) using synthetic diets with differing rates of calcium supplementation and total excreta collection. The authors determined calcium retention to be approximately 50–55% when the birds were in calcium balance and optimum shell thickness was obtained (3 g day^{-1}). At higher daily intakes of calcium, percentage retention of calcium decreased. Rao and Brahmakshatriya (1976) fed pullets at three calcium concentrations and determined calcium retention at 18, 21 and 24 weeks of age using a total collection method. They observed that at 18 weeks of age, calcium retention was increased for the higher calcium diets (Table 10.7). However, at 21 and 24 weeks of age, although net retention increased, percentage calcium retention decreased with increasing dietary calcium. Keshavarz (1986) reported similar results based on total collection with laying hens fed on a basal diet containing maize and soybean meal. The author noted that as calcium concentration increased, net calcium retained increased although percentage retention of calcium and phosphorus decreased (Table 10.8). Phosphorus retention decreased as phosphorus intake increased. Dietary phosphorus had no impact on calcium retention. Scott and Balnave (1991) used acid-insoluble ash as a marker and observed that calcium retention for sexually maturing pullets was affected by age, temperature and metabolizable energy content of the diet. Nahashon *et al.* (1994), using a chromic oxide as a marker, determined the calcium and phosphorus retentions from maize and soybean meal diet for laying pullets to be 58.9% and 24.4%, respectively. They noted that an addition of fat (10 g kg^{-1}) increased calcium and phosphorus retention from the diet, but more fat addition (30 g kg^{-1}) produced no further benefits.

Table 10.7. Calcium retention in pullets (Rao and Brahmakshatriya, 1976).

Diet calcium (%)	% calcium retention		
	18 weeks	21 weeks	24 weeks
1.05	33.33	32.91	40.90
2.20	48.46	26.99	37.69
3.30	39.90	21.57	37.56
% Total P = 0.72%			

Table 10.8. Retention of calcium and phosphorus by laying hens at 69 weeks of age (Keshavarz, 1986).

Diet calcium (%)	Calcium intake (g day^{-1})	Calcium retention (%)	Phosphorus intake (g day^{-1})	Phosphorus retention (%)
3.50	3.48^{c}	49.8^{a}	0.74^{a}	31.3^{a}
4.50	4.50^{b}	44.9^{ab}	0.75^{a}	25.4^{b}
5.50	5.42^{a}	39.1^{b}	0.68^{b}	20.2^{b}
Diet non-phytate P %				
0.24	4.25^{b}	46.2^{a}	0.54^{c}	30.5^{a}
0.44	4.64^{a}	45.8^{a}	0.75^{b}	25.3^{ab}
0.64	4.51^{a}	41.8^{a}	0.88^{a}	21.2^{b}
Ca × P interaction	$P < 0.05$	NS	$P < 0.05$	$P < 0.05$

^{a,b,c}Means followed by different letters in each column under calcium and phosphorus levels are significantly different ($P < 0.05$).

Broilers

A large portion of the data on calcium and phosphorus retention by broilers has been in conjunction with research on the utilization of phytate phosphorus and the effects of an added phytase enzyme. Two excellent reviews on phytate and the use of microbial phytase in poultry diets have been provided by Ravindran *et al.* (1995) and Sebastian *et al.* (1998). Factors affecting retention of phytate phosphorus are similar to those affecting retention of phosphorus in general. Sebastian *et al.* (1998) listed the following as factors affecting phytate phosphorus utilization: dietary calcium and phosphorus concentration, dietary vitamin D_3 concentration, age of bird, phytase activity of dietary ingredients, fibre and genotype. Qian *et al.* (1997) used a total collection method to determine the calcium and phosphorus retention from maize and soybean meal diets by 21-day-old male broilers. The authors determined calcium retention to range between 42% and 67%, depending on the calcium : total phosphorus ratio, the addition of 66 or 666 μg kg^{-1} diet vitamin D_3, and the addition of 0–900 phytase units kg^{-1} diet. Phosphorus retention from the diets ranged from 51 to 68%, depending on the same factors. The addition of vitamin D_3 increased phosphorus and calcium retentions, as did the addition of phytase. This agrees with earlier findings of

Mitchell and Edwards (1996). Calcium and phosphorus retentions were both increased as the calcium : total phosphorus ratio was reduced from 2.0 : 1 to 1.1 : 1. Simons *et al.* (1992) have estimated that the addition of phytase to a maize and soybean meal diet can lower the need for monocalcium phosphate supplementation of broiler diets by 1 g kg^{-1}. Mitchell and Edwards (1996) found that the addition of 5 μg of 1,25-$(OH)_2$ vitamin D_3 and 600 units phytase kg^{-1} broiler diet can reduce the amount of supplemental phosphorus needed by 2 g kg^{-1}.

Feedstuffs

Little research has been reported on the calcium and phosphorus retention from individual feedstuffs. Van der Klis and Versteegh (1999) measured the retention of phosphorus by 3-week-old male broilers from a large number of feedstuffs (Table 10.9). They utilized a total collection method and a synthetic diet in which the test feedstuff provided most of the phosphorus. Diets were standardized

Table 10.9. Phosphorus content and retention of feedstuffs (van der Klis and Versteegh, 1999).

	Total P content (%)	Retainable phosphorus (% of total phosphorus)
Plant feedstuffs		
Beans	0.49	52
Lupin	0.30	72
Maize	0.30	29
Maize gluten feed	0.90	52
Maize feed meal	0.51	50
Peas	0.41	41
Rape seed	1.09	33
Rice bran	1.72	16
Soybean (heat treated)	0.55	54
Soybean meal (solvent extracted)	0.71	61
Sunflower seed (solvent extracted)	1.19	38
Tapioca	0.09	66
Wheat	0.34	48
Wheat middlings	1.08	36
Animal feedstuffs		
Bone meal	7.6	59
Fish meal	2.2	74
Meat meal	29	65
Meat and bone meal	60	66
Feed phosphates		
Calcium sodium phosphate	18.0	59
Dicalcium phosphate (anhydrous)	19.7	55
Dicalcium phosphate (hydrous)	18.1	77
Monocalcium phosphate	22.6	84
Mono-dicalcium phosphate (hydrous)	21.3	79
Monosodium phosphate	22.4	92

with the test ingredient to contain 1–8 g calculated kg^{-1} retainable phosphorus and all diets contained 5 g kg^{-1} calcium. Leske and Coon (1999) determined the calcium and phosphorus retentions from various plant feedstuffs with broilers and laying hens. They used synthetic diets in which the test feedstuff was the only source of phosphorus. Diets were offered to the birds with or without added phytase. The data (Tables 10.10 and 10.11) indicated that the dietary ingredients used influenced phytate phosphorus hydrolysis and total phosphorus retention for both broilers and layers. The effect of phytase supplementation on phosphorus retention was also dependent on ingredient. The addition of phytase increased calcium retention from maize and soybean meal in broilers.

Five day bioassay

Leske and Coon (2001) conducted a retention bioassay to determine the total phosphorus retention of a reagent grade monocalcium phosphate (MCP; J.T. Baker, Phillipsburg, NJ 08865, USA, product 426-05) and three commercially available feedgrade mono- and dicalcium phosphates (M/DCP). Male broiler chicks were offered a standard starter diet until 10 days of age (240 g mean body weight). Eighty-eight chicks of mean body weight ± 13 g were then placed in individual cages and offered 20 semi-synthetic test diets (four chicks per diet), in which the majority of the phosphorus came from the test source. Phosphorus concentration was adjusted by replacing cellulose with eight concentrations of the MCP, and four concentrations each of the commercially available phosphorus sources. Celite™ (Celite Corp., Lompoc,

Table 10.10. Hydrolysis of inositol hexaphosphate and calcium and total phosphorus retention by 3-week-old broilers of seven feed ingredients with or without 600 FTU phytase as determined with a 2-day excreta collection (Leske and Coon, 1999).

Feed ingredient	Phytase	*n*	Calcium retention	Hydrolysis of IP6	Total P retention
			%	%	%
Soybean meal	–	9	69.8^{fgh}	$34.9^{b,wx}$	$27.0^{b,y}$
Soybean meal	+	8	87.2^{a}	$72.4^{a,h}$	$58.0^{a,h}$
Maize	–	9	64.0^{ghi}	$30.8^{b,wx}$	$34.8^{b,wx}$
Maize	+	10	72.8^{def}	$59.0^{a,i}$	$40.9^{a,i}$
Rice bran	–	10	33.3^{j}	$33.2^{b,wx}$	$15.5^{b,z}$
Rice bran	+	9	62.0^{hi}	48.0^{ajk}	$26.5^{a,k}$
Canola meal	–	10	70.5^{defg}	$36.7^{b,w}$	$39.4^{a,w}$
Canola meal	+	10	73.4^{def}	$55.8^{a,ij}$	$45.7^{a,i}$
Barley	–	8	78.6^{bcd}	$32.2^{b,wx}$	$40.3^{b,w}$
Barley	+	8	85.6^{ab}	$71.3^{a,h}$	$55.5^{a,h}$
Wheat middlings	–	8	74.8^{cdef}	$29.1^{b,x}$	$31.9^{b,xy}$
Wheat middlings	+	8	78.3^{bcde}	$52.2^{a,ijk}$	$43.4^{a,i}$

[a,b]Hydrolysis of IP6 and total P retention, means within a specific feed ingredient treatment and column with differing superscripts are significantly different ($P \leq 0.05$).

[h,i,j,k]Hydrolysis of IP6 and total P retention, means within the added phytase (+) treatment and same column with differing superscripts are significantly different ($P \leq 0.05$).

[w,x,y,z]Means within the no added phytase (–) treatment and same column with differing superscripts are significantly different ($P \leq 0.05$).

Table 10.11. Hydrolysis of inositol hexaphosphate and retention of total phosphorus by laying hens of three feed ingredients with or without 300 FTU phytase as determined with a 3-day excreta collection (Leske and Coon, 1999).

Diet	*n*	Food intake (g day^{-1})	SD	Hydrolysis of IP6	SD	Total P retention	SD
				%			%
Soybean meal	7	65.9^{abc}	23.3	25.7^{b,y}	4.7	36.8^{b,x}	8.4
Soybean meal and phytase	7	61.6^{bc}	21.5	62.4^{a,c}	9.5	53.4^{a,c}	9.5
Maize	7	48.0^{c}	18.2	23.0^{b,y}	11.0	28.6^{b,y}	4.4
Maize and phytase	8	46.3^{c}	23.1	52.0^{a,d}	6.3	44.7^{a,d}	3.0
Rice bran	8	79.1^{ab}	23.6	36.1^{b,x}	8.5	35.9^{b,x}	3.5
Rice bran and phytase	8	82.3^{a}	21.7	50.9^{a,d}	5.5	43.0^{a,d}	6.1

^{a,b,c}Means within column with differing superscripts are significantly different ($P \leq 0.05$).
^{a,b}Means within a specific feed ingredient treatment and column with differing superscripts are significantly different ($P \leq 0.05$).
^{c,d}Means within the added phytase treatment and same column with differing superscripts are significantly different ($P \leq 0.05$).
^{x,y}Means within the no added phytase treatment and same column with differing superscripts are significantly different ($P \leq 0.05$).

CA 93436, USA) was added to the diets as an acid-insoluble ash marker. Broilers were acclimatized to cages and test diets for 3 days prior to initiation of experiment. Individual stainless steel trays were placed under each cage for excreta collection. Excreta were collected for 48 h.

In conjunction with the 5 day bioassay, a second experiment was conducted using 200 10-day-old male broiler chicks (average weight = 248 g) from the same flock placed into cages, ten chicks per cage. Each cage was assigned one of the previously described diets. The chicks were offered unlimited access to both water and experimental diets for a 2-week period. At 24 days of age, feed consumption was recorded, all chicks were weighed, killed by CO_2 asphyxiation and the tibiae were collected, cleaned and frozen for analysis. Phosphorus sources, diets and excreta samples were analysed for acid-insoluble ash using the dry ash and hydrochloric acid digestion technique of Scott and Balnave (1991). Diet and excreta phytate phosphorus were measured as IP6 (inositol hexa-phosphate) using ion-exchange chromatography (Bos *et al.*, 1991). Total phosphorus and calcium were measured by an inductively coupled plasma (ICP) emission spectroscopic method (AOAC, 1990).

Phosphorus retention from the basal diet was determined as 43.2% (Table 10.12). It was determined that the non-phytate phosphorus portion of the maize and soybean basal was only 65.5% retainable, not 100% as is widely assumed when formulating diets. However, the 32.3% retention of the phytate phosphorus masked the incomplete retention of the non-phytate phosphorus. Although true for the maize and soybean diet, this may not be the case when other ingredients are used. The data indicated that retentions of total phosphorus

Table 10.12. Phosphorus retention from the basal diet (Leske and Coon, 2002).

Diet	Phytate phosphorus retention (%)	Non-phytate phosphorus retention (%)	Total phosphorus retention (%)
C	32.3	65.5	43.2

Based on retention of individual feed ingredients.

and phosphorus from calcium phosphates supplements were dependent on the dietary concentration of phosphorus (Table 10.13). When feeding extremely low levels of a test calcium phosphate source (<10% of non-phytate phosphorus) it was impossible to separate the retention from phosphorus source from that of the basal due to variability. Maximum total phosphorus retention occurred at or below an intake of 197.3 mg total phosphorus day^{-1} (Fig. 10.2). With the bioassay diets used, this equates to a retainable phosphorus content of 2.4 mg g^{-1} (Fig. 10.3). Retainable phosphorus levels in the diet greater than 2.4 mg g^{-1} resulted in a linear increase in daily excretion of phosphorus. The MCP source decreased from 94% retention when the non-phytate phosphorus level of the diet was 0.21% to a retention of 58.5% when the non-phytate phosphorus level was equal to the NRC suggested level of 0.45%. Similarly, calcium retention declined as calcium dietary concentrations exceeded 7.45 mg g^{-1} (Fig. 10.4, Table 10.14).

Using this type of bioassay, it is possible to determine a 'requirement' based on a chosen performance parameter. Chick performance and bone development results from the accompanying 2-week feeding trial are given in Table 10.15 and Figs 10.5 and 10.6. Notice that retainable phosphorus content did not increase at the same rate as the total or non-phytate phosphorus content of the diet, due to the reduction in retention at higher dietary phosphorus concentrations. The amount of retainable phosphorus required to maximize bone strength was 4.4 mg g^{-1} diet or 205 mg day^{-1}, respectively. A retainable phosphorus requirement expressed in this manner would be irrespective of the source.

Seong and Coon (unpublished) recently conducted a phosphorus retention experiment using three different phosphorus sources, with the objective of determining if phosphorus retention is best expressed with a general broken line or two-line segmented model, linear (straight line) model or a polynomial model. The researchers also compared the relative bioavailability of P (RBP) of the phosphorus sources with their respective retention values. Male broiler chicks were offered a standard starter diet until 10 days of age (approx. 240±15 g mean body weight). The 10-day-old male chicks (n = 252) were then placed in separate cages and offered semi-synthetic test diets consisting of maize, soy, and ten levels of the three phosphorus sources. The three sources tested were two commercially available defluorinated phosphate samples and a reagent-grade dibasic, dihydrate, calcium phosphate from Fisher Scientific (C129-12) utilized as a standard for comparison. The

Table 10.13. Retention of phosphorus from various sources at different inclusion rates (Leske and Coon, 2002).

Diet	Source	Diet total P (mg g^{-1})	Diet NPP (mg g^{-1})	Source NPP (mg g^{-1})	*n*	Total P retention (%)	SD	Total NPP retention (%)	SD	Source P retention (%)	SD
1	MCP	3.234	1.109	0.071	4	53.2^{fg}	3.8	69.3^{ef}	2.3	X	
2	MCP	3.281	1.156	0.118	3	54.5^{defg}	2.9	70.2^{de}	4.3	X	
3	MCP	3.748	1.627	0.592	3	67.6^{a}	4.3	80.2^{a}	2.2	98.0^{a}	2.7
4	MCP	4.215	2.098	1.065	4	62.5^{abc}	4.5	76.4^{abc}	2.9	94.0^{ab}	3.3
5	MCP	6.667	4.573	3.551	4	52.0^{g}	3.7	60.1^{g}	3.4	58.5^{f}	4.4
6	MCP	7.835	5.751	4.734	4	52.7^{g}	0.7	60.0^{g}	2.1	58.9^{f}	2.6
7	MCP	9.002	6.930	5.918	3	39.8^{h}	2.0	48.3^{h}	2.6	45.4^{g}	3.1
8	MCP	11.338	9.286	8.285	4	45.0^{h}	4.0	49.5^{h}	4.9	47.6^{fg}	5.5
9	M/DCP-1	3.264	1.139	0.102	3	60.7^{bcde}	1.8	76.7^{abc}	0.8	X	
10	M/DCP-1	3.464	1.341	0.305	4	59.8^{cdef}	1.9	70.6^{de}	1.8	87.9^{abcd}	7.9
11	M/DCP-1	3.764	1.644	0.609	3	66.0^{abc}	3.0	76.2^{abc}	1.1	94.4^{a}	3.1
12	M/DCP-1	3.963	1.846	0.812	4	61.6^{abc}	3.9	70.4^{de}	2.5	76.6^{de}	5.7
13	M/DCP-2	3.262	1.138	0.100	3	54.4^{defg}	7.8	63.0^{g}	5.5	X	
14	M/DCP-2	3.459	1.337	0.300	4	53.8^{efg}	7.9	64.2^{fg}	7.6	72.9^{e}	25.6
15	M/DCP-2	3.755	1.635	0.600	4	59.7^{cdef}	6.3	71.5^{cde}	2.5	82.0^{abcde}	6.7
16	M/DCP-2	3.952	1.834	0.800	4	60.5^{bcde}	7.0	72.0^{bcde}	3.0	80.3^{cde}	6.9
17	M/DCP-3	3.270	1.145	0.108	4	63.8^{abc}	4.3	74.1^{bcde}	2.5	X	
18	M/DCP-3	3.483	1.360	0.324	4	66.8^{ab}	4.2	75.2^{abcd}	5.0	88.3^{abcd}	10.9
19	M/DCP-3	3.802	1.683	0.648	4	63.7^{abc}	8.3	77.3^{ab}	6.2	90.5^{abc}	14.1
20	M/DCP-3	4.015	1.897	0.864	4	61.7^{abcd}	2.9	72.7^{bcde}	5.1	81.2^{bcde}	11.3

MCP, monocalcium phosphate; M/DCP, feed grade mono/dicalcium phosphates; NPP, non-phytate P.
[1]Source P retention calculated assuming that phosphorus retention of the basal diet was constant for all inclusion rates of added phosphorus sources. X=Source phosphorus retention values calculated to be over 100%, removed from analysis.

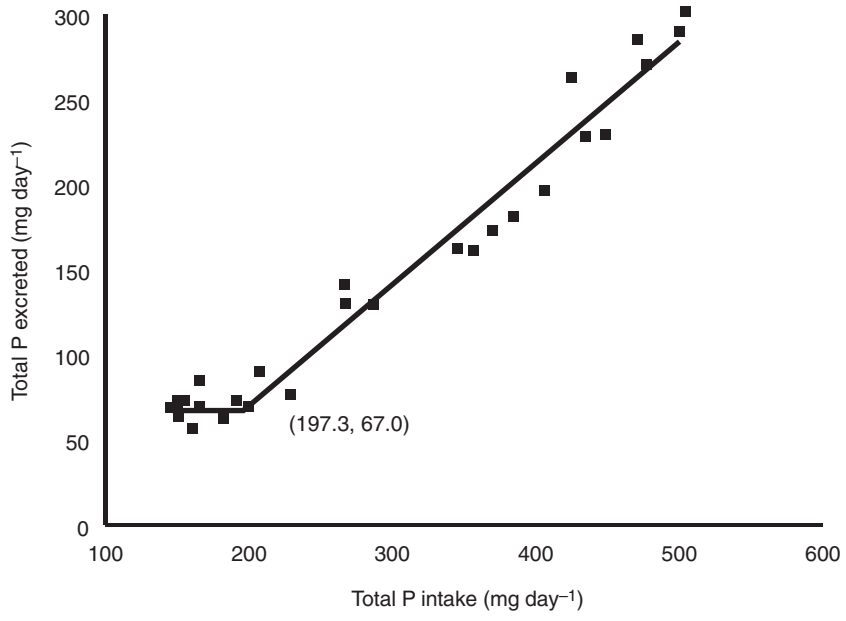

Fig. 10.2. Effect of total phosphorus intake on total phosphorus excretion in chickens (10–15 days old). Point of inflexion found by segmented line analysis (Leske and Coon, 1999).

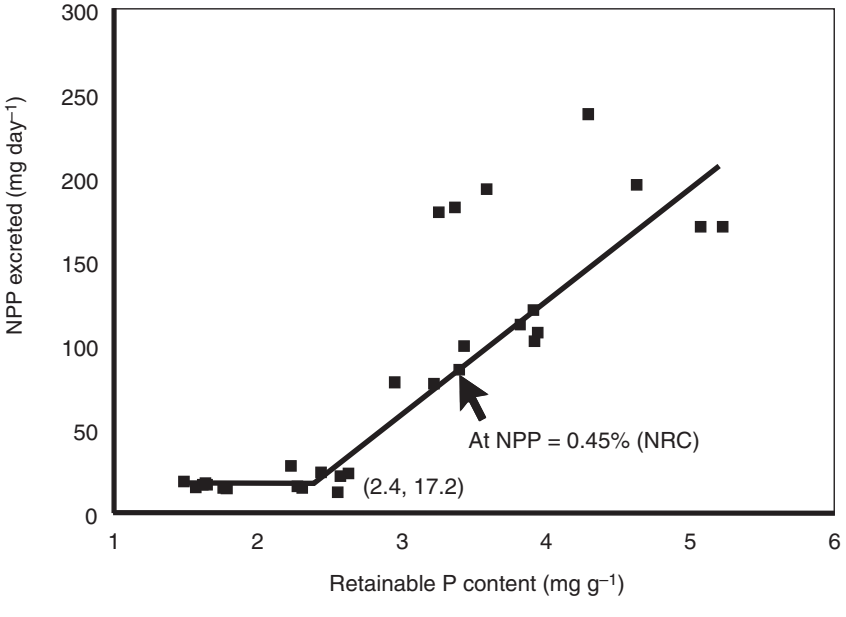

Fig. 10.3. Effect of retainable phosphorus content on non-phytate phosphorus (NPP) excretion in chickens (10–15 days old). Point of inflexion found by segmented line analysis (Leske and Coon, 1999).

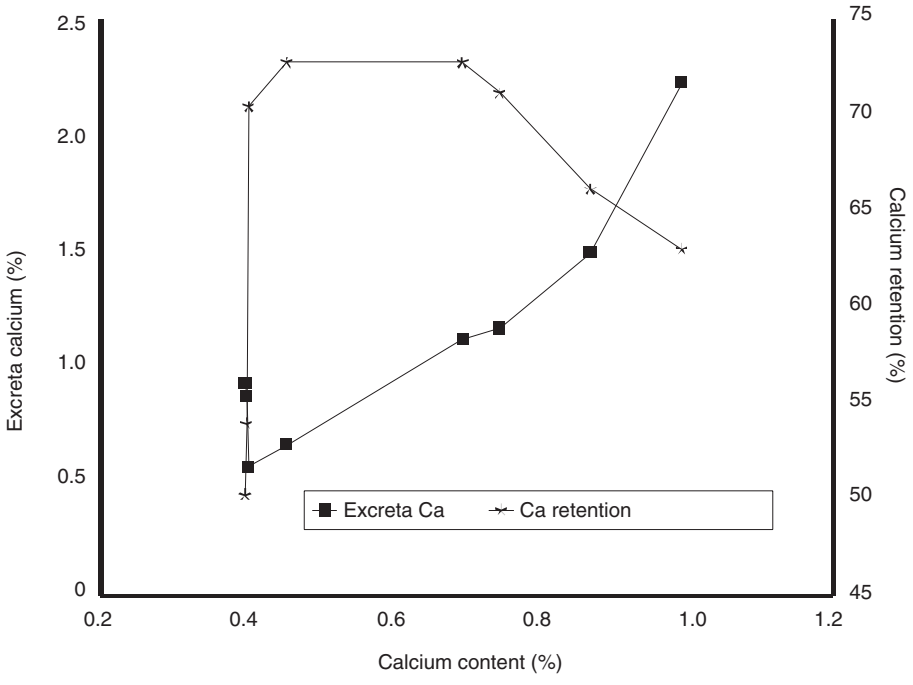

Fig. 10.4. Effect of dietary calcium content on calcium excretion and retention in chickens (10–15 days old; Leske and Coon, 1999).

levels of dietary non-phytate phosphorus in the test diets ranged from 0.1% to 1.0%. The phosphorus levels that were fed provided retention and performance data both above and below the National Research Council (1994) recommended level of 0.45% non-phytate phosphorus. Twelve birds were offered the semi-synthetic basal diet with no added phosphorus supplementation in order to determine phosphorus retention for the basal. Eight individual chicks per phosphorus source level were utilized for the two defluorinated phosphorus sources. Acid-insoluble ash (Celite) was added to the test feed and used as a marker. Chicks were acclimatized to the cages and diets for 3 days prior to a 48-hour excreta collection on day 5. At 21 days of age, the experiment was terminated, all birds were euthanized and the right tibia was collected. The tibias were cleaned and tested for strength by the shear force measurement described by Wilson (1991) using an Instron Universal Testing Machine. The bones were then extracted with ether prior to ashing. Diets and excreta were analysed for total phosphorus by ICP and acid-insoluble ash (AOAC, 1990). Phytate phosphorus was analysed by the modified HPLC method of Bos *et al.* (1991).

The data of bone strength, percentage bone ash and bone ash weight were analysed by a general broken line regression method as seen in Figs 10.7–10.9. The bone characteristics were improved by phosphorus supplementation. These improved responses to varying phosphorus levels were

Table 10.14. Efffect of increasing inclusion rates of calcium phosphate sources on retention of diet calcium (Leske and Coon, 2001, unpublished data).

Diet	Source	Diet Ca (mg g^{-1} DM)	Total P level (mg g^{-1} DM)	NPP level (mg g^{-1} DM)	Retainable P level (mg g^{-1} DM)	*n*	Excreta Ca (mg g^{-1} DM)	SD	Ca retention (%)	SD
1	MCP	3.968	3.234	1.109	1.720	4	9.211^{cd}	0.493	50.15^{g}	2.55
2	MCP	3.994	3.281	1.156	1.788	3	8.608^{de}	0.174	53.91^{efg}	3.39
3	MCP	4.019	3.748	1.627	2.534	4	5.547^{f}	2.314	70.40^{abc}	15.00
4	MCP	4.540	4.215	2.098	2.634	4	6.533^{ef}	0.933	72.65^{ab}	2.96
5	MCP	6.936	6.667	4.573	3.467	4	11.095^{c}	1.254	72.67^{ab}	3.17
6	MCP	7.451	7.835	5.751	4.129	4	11.562^{c}	1.476	71.13^{a}	1.89
7	MCP	8.685	9.002	6.930	3.583	4	14.808^{b}	2.646	66.07^{abcd}	4.28
8	MCP	9.944	11.338	9.286	5.102	4	22.222^{a}	3.461	63.04^{bcde}	6.54
9	M/DCP-1	3.769	3.264	1.139	1.981	4	7.789^{def}	1.835	61.14^{cde}	8.48
10	M/DCP-1	4.058	3.464	1.341	2.071	4	7.533^{def}	1.822	63.07^{bcde}	6.94
11	M/DCP-1	4.240	3.764	1.644	2.484	4	7.768^{def}	1.896	65.33^{abcd}	8.67
12	M/DCP-1	4.571	3.963	1.846	2.441	4	7.807^{def}	1.229	68.22^{abcd}	4.86
13	M/DCP-2	3.853	3.262	1.138	1.775	3	9.350^{cd}	1.699	50.70^{fg}	12.31
14	M/DCP-2	3.962	3.459	1.337	1.861	4	9.263^{cd}	1.430	53.05^{efg}	10.04
15	M/DCP-2	4.600	3.755	1.635	2.242	4	6.744^{ef}	1.459	69.66^{abcd}	6.18
16	M/DCP-2	4.558	3.952	1.834	2.391	4	6.215^{ef}	1.608	72.27^{ab}	7.22
17	M/DCP-3	3.913	3.270	1.145	2.086	4	8.067^{de}	1.007	60.29^{def}	7.32
18	M/DCP-3	4.176	3.483	1.360	2.327	4	7.117^{def}	1.357	68.12^{abcd}	4.48
19	M/DCP-3	4.764	3.802	1.683	2.422	4	7.508^{def}	1.840	70.33^{abcd}	7.18
20	M/DCP-3	5.010	4.015	1.897	2.477	4	7.594^{def}	1.063	71.50^{ab}	4.69

DM, Dry matter; MCP, M/DCP see Table 10.13.

Table 10.15. Bone data from 2-week feeding trial accompanying 52-day phosphorus retention bioassay (Leske and Coon, 2002).

Diet	Source	Total P level (mg g^{-1})	NPP level (mg g^{-1})	Retainable P level (mg g^{-1})	*n*	Breaking strength (kg)	SD	Bone ash (%)	SD	Bone ash (g per tibia)	SD
1	MCP	3.234	1.109	1.720	10	6.8^{hi}	2.3	41.1^{h}	2.8	0.69^{ghi}	0.09
2	MCP	3.281	1.156	1.788	10	6.1^{i}	1.6	41.9^{gh}	2.5	0.64^{I}	0.07
3	MCP	3.748	1.627	2.534	10	9.4^{def}	1.9	45.0^{cde}	2.8	0.78^{ef}	0.07
4	MCP	4.215	2.098	2.634	9	13.3^{c}	2.8	47.3^{b}	1.2	0.90^{d}	0.08
5	MCP	6.667	4.573	3.467	9	15.7^{b}	2.6	50.2^{a}	1.0	1.08^{c}	0.07
6	MCP	7.835	5.751	4.129	9	17.2^{ab}	3.6	52.0^{a}	1.8	1.18^{ab}	0.13
7	MCP	9.002	6.930	3.583	9	16.7^{ab}	2.3	50.4^{a}	1.8	1.12^{bc}	0.10
8	MCP	11.338	9.286	5.102	10	18.1^{a}	1.4	51.8^{a}	1.2	1.22^{a}	0.13
9	M/DCP-1	3.264	1.139	1.981	9	7.0^{hi}	1.8	42.9^{fgh}	2.5	0.70^{ghi}	0.08
10	M/DCP-1	3.464	1.341	2.071	10	8.4^{efgh}	2.3	42.4^{fgh}	2.3	0.76^{efg}	0.08
11	M/DCP-1	3.764	1.644	2.484	9	9.0^{defg}	2.1	44.1^{def}	2.2	0.76^{efg}	0.10
12	M/DCP-1	3.963	1.846	2.441	10	9.3^{defg}	1.7	46.3^{bc}	2.0	0.83^{de}	0.10
13	M/DCP-2	3.262	1.138	1.775	9	7.5^{ghi}	1.8	42.0^{gh}	2.4	0.69^{ghi}	0.07
14	M/DCP-2	3.459	1.337	1.861	9	7.9^{efghi}	1.5	43.6^{efg}	2.7	0.73^{fgh}	0.06
15	M/DCP-2	3.755	1.635	2.242	10	9.3^{defg}	1.4	46.1^{bcd}	1.9	0.79^{ef}	0.08
16	M/DCP-2	3.952	1.834	2.391	10	9.8^{de}	1.9	46.0^{bcd}	2.6	0.81^{e}	0.07
17	M/DCP-3	3.270	1.145	2.086	10	7.6^{fghi}	2.3	41.7^{gh}	3.2	0.67^{hi}	0.09
18	M/DCP-3	3.483	1.360	2.327	10	8.0^{efghi}	2.2	43.3^{efg}	2.8	0.72^{fgh}	0.05
19	M/DCP-3	3.802	1.683	2.422	10	10.4^{d}	1.9	47.1^{b}	1.3	0.81^{e}	0.08
20	M/DCP-3	4.015	1.897	2.477	10	10.9^{d}	2.7	46.6^{bc}	1.6	0.80^{ef}	0.13

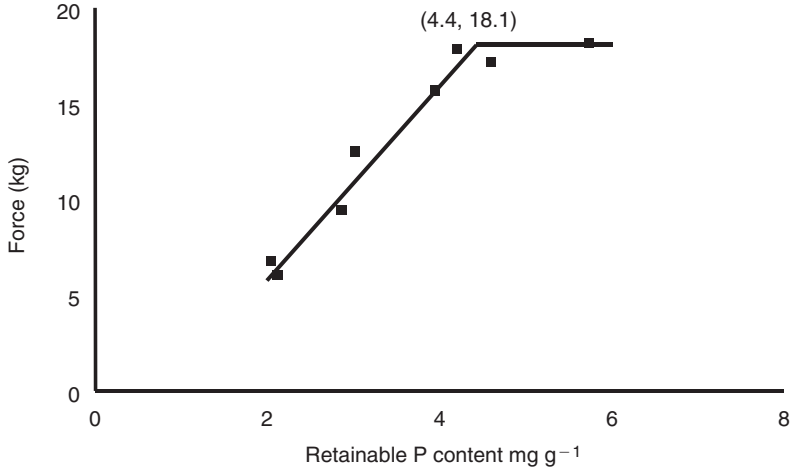

Fig. 10.5. Effect of dietary retainable phosphorus on bone breaking strength in broilers (10–24 days old).

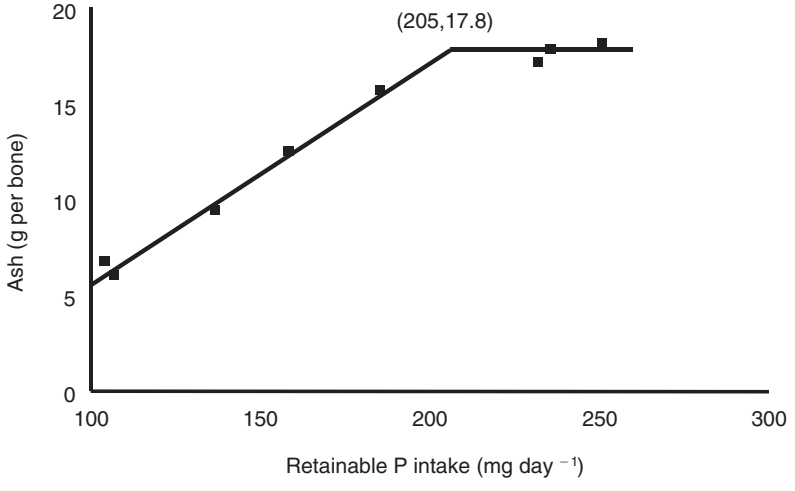

Fig. 10.6. Effect of daily retainable phosphorus on bone breaking strength in broilers (10–24 days old). Point of inflexion found by segmented line analysis (Leske and Coon, 1999).

linear to breakpoints. The slopes and breakpoints of the three sources were obtained and summarized in Table 10.16. The relative biological availability of phosphorus with bone data for tested sources was determined by the slope ratio procedure reported by Fernandes *et al.* (1999) and presented in Table 10.17. A dibasic, dihydrate, calcium phosphate from Fisher Scientific was used as a standard to determine the relative biological availability of test phosphorus diet sources: defluorinated phosphate 1 and defluorinated phos-

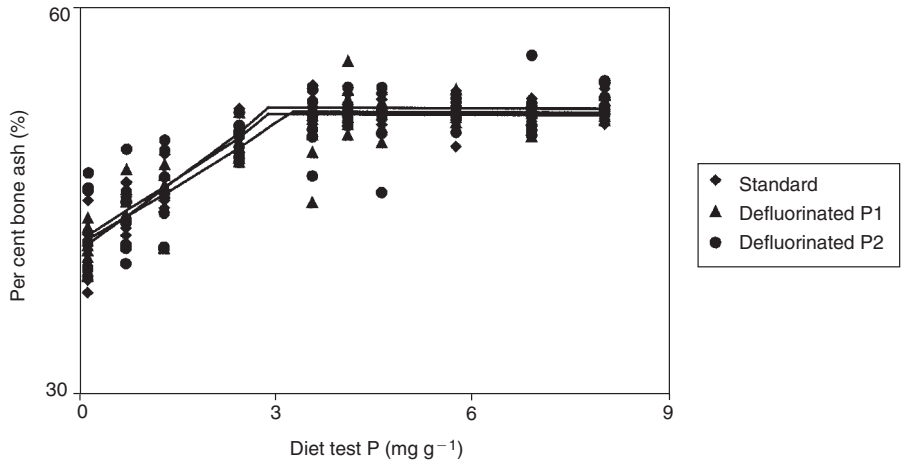

Fig. 10.7. Tibia per cent bone ash from 21-day-old broilers fed three different test P sources.

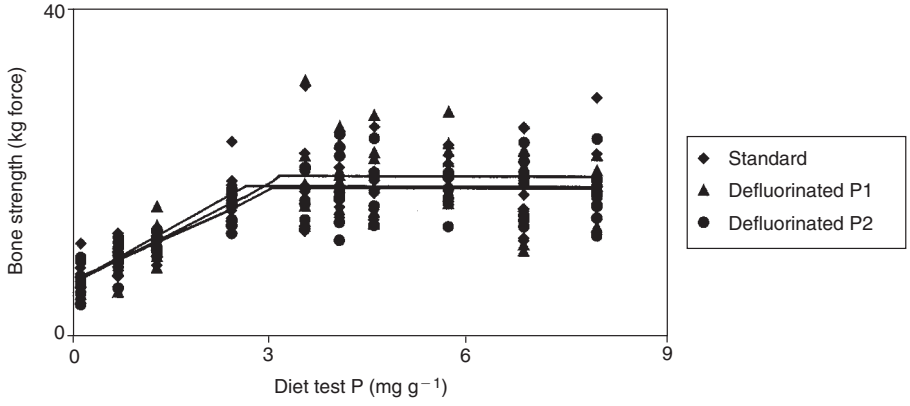

Fig. 10.8. Tibia bone strength from 21-day-old broilers fed three different test P sources.

phate 2. The non-phytate phosphorus (NPP) 0.45% in the test diets from natural ingredients and the test phosphorus sources corresponded to 3.6 mg g^{-1} of added test phosphorus. The phosphorus requirement for this age of broiler as determined only from bone parameters was almost identical to the NRC (1994) requirement of 0.45% as seen in Table 10.16. The breakpoints shown in Table 10.16 are based only on the test phosphorus added to the basal diet, but the basal diet also contained 0.1458% NPP and 0.3314% total P from the natural ingredients. The amount of NPP or available phosphorus necessary to produce the requirement or breakpoint for percentage fat free tibia bone ash ranged between 0.43 to 0.47% (Table 10.16).

The data of total phosphorus in excreta in relation to dietary test phosphorus levels were subjected to general broken, two lines, straight line and polynomial regression analysis methods (Robbins, 1979; Cody, 1997). The general broken line equation for regression analysis was $Y=L–U\ (R–X_{LR})$, where L was

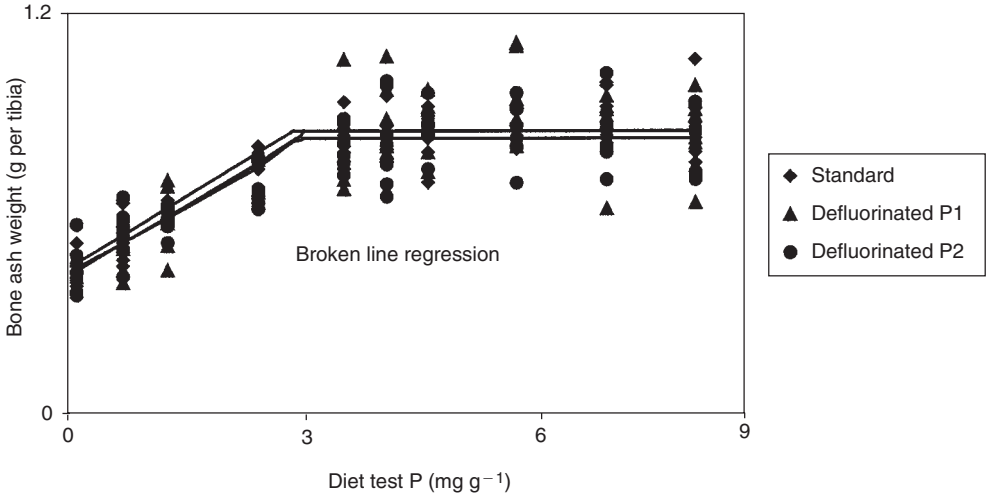

Fig. 10.9. Tibia bone ash weight from 21-day-old broilers fed three different test P sources.

Table 10.16. The comparisons of parameters from bone strength, per cent bone ash, and bone ash weight using broken line regression analysis for three different phosphorus sources (Seo and Coon, 2002).

	Breakpoint and slope for bone ash weight	Breakpoint and slope for per cent bone ash	Breakpoint and slope for bone strength
Standard Defluorinated Phosphate 1	2.88:0.87, 0.14	2.92:52.3, 3.86	2.63:18.9, 4.57
Defluorinated Phosphate 2	3.01:0.88, 0.15	2.86:51.9, 3.51	3.13:20.1, 4.27
	2.96:0.85, 0.14	3.24:52.0, 3.22	3.04:18.7, 3.74

The general equation of the broken line regression is $Y = L + U\ (X_{XR} – R)$, where L is the ordinate and R is the abscissa of the breakpoint, X_{XR} means X less than R, and U is the slope of the line for X_{XR}. The standard errors for breakpoint of bone ash weight (L, mg g^{-1}) are 1.15, 1.70 and 1.00, respectively for three P sources and 7.94, 11.64 and 7.42 for breakpoint (R, mg g^{-1}). The standard errors for slope are 0.016, 0.024, and 0.018, respectively. The standard errors for breakpoint of per cent bone ash (L, %) are 20.9, 17.6, and 37.1, respectively for three P sources and 10.8, 10.1, and 23.1 for breakpoint (R, mg g^{-1}). The standard errors for slope are 0.3, 0.4 and 0.6, respectively. The standard errors for breakpoint of bone strength (L, kg) are 5.6, 5.3 and 42.7, respectively for three P sources and 2.47, 2.53, and 22.8 for breakpoint (R, mg g^{-1}). The standard errors for slope are 0.87, 0.88, and 0.64, respectively. The breakpoint P can be modified to dietary NPP by adding 1.46 (mg g^{-1}) to the abscissa values. The NPP and total P for the basal diet was 0.146% and 0.331%, respectively, on an air-dried basis.

Table 10.17. Relative bioavailability (%) of P for the two test phosphorus sources from bone data.

	Bone ash weight	Per cent bone ash	Bone strength
Standard	100	100	100
Defluorinated phosphate 1	107	91	93
Defluorinated phosphate 2	100	83	82

Seo and Coon (2002).

the ordinate and R was the abscissa of the breakpoint (this means the estimated requirement for U was the slope of the line for X_{LR} which means $X<R$ and X_{LR}–R is zero as $X>R$). The equation for the two line regression was $Y = \alpha + (\beta_1 * X) + \beta_2 * (X-\gamma)$, where $Y = \alpha + (\beta_1 * X)$ for $X<\gamma$, $Y= \alpha$ is the ordinate for $X=0$ and γ is the value of the breakpoint for intersection of the two lines, and β_1 and β_2 are the slopes of the two lines. The breakpoints were obtained by the two line and broken line analysis method. The total and test phosphorus retention at breakpoint of three P sources was determined by a balance method using Celite as a marker as reported by Gueguen (1996). The P in the excreta from the three sources as determined by the two line method is presented in Fig. 10.10. The retention percentage was also determined at 0.45% of NPP for added test phosphorus for the three phosphorus sources. The break-points and retention of phosphorus for the two line and broken line regression models for the three phosphorus sources are shown in Tables 10.18 and 10.19.

The mean square errors and coefficients of determination (R-square) of predicted phosphorus levels in the excreta using the four different models are shown in Table 10.20. The mean square error of the straight line model was much larger than the mean square error from other analysis models. The coefficient of determination (R-square) was also determined to measure the best fit of data. The R-square of the polynomial method was slightly higher and closer to unity than the R-square value for the straight line model. The differences between measured excreta phosphorus from predicted values for four different

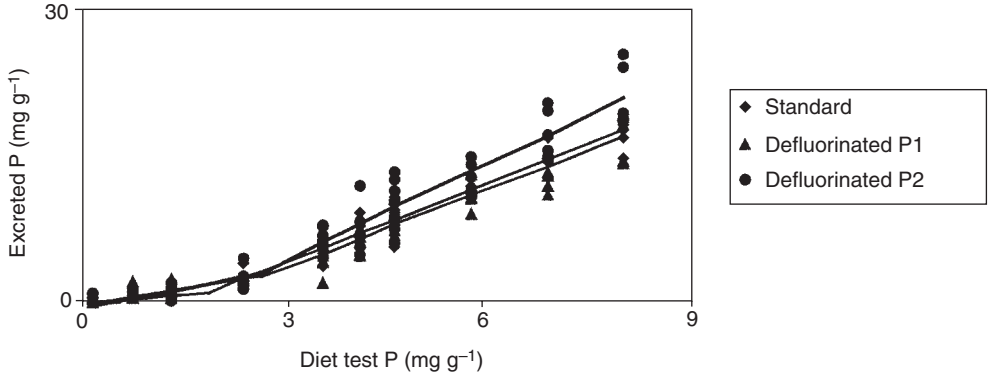

Fig. 10.10. Phosphorus in excreta from 10–15 day-old chicks fed three different test P sources.

Table 10.18. The comparison of phosphorus excretion and retention of chicks fed three different phosphorus sources using best fitted two-line regression analysis.

	Alpha, gamma and slopes for total P	Alpha, gamma and slopes for test P	Retention (%) at gamma of total and test P	Retention (%) for test P at NPP (0.45%)
Standard	2.69, 4.74, 0.62, 2.72	0.06, 1.84, 0.56, 2.73	67.11, 82.78	60.27
Defluorinated phosphate 1	1.22, 5.51, 1.09, 2.71	–0.11, 2.63, 1.09, 2.71	65.15, 77.59	63.75
Defluorinated phosphate 2	0.27, 5.28, 1.33, 3.33	–0.40, 2.40, 1.32, 3.33	59.83, 70.74	60.27

The general equation of the broken line regression is $Y = \alpha + (X <= \gamma)*(\beta_1 * X) + (X > \gamma)*(\beta_1 * \gamma + \beta_2 *) (X - \gamma)$, where α is the ordinate and γ is the abscissa of the breakpoint, and β_1 and β_2 are the slopes of the line. The standard errors for alpha (mg g^{-1}) for total P are 1.91, 1.39 and 2.1 respectively for the three P sources and 0.42, 0.45, and 0.64 for test P. The standard errors for gamma (mg g^{-1}) for total P are 0.34, 0.48 and 0.56 respectively and 0.32, 0.48 and 0.56 for test P. The standard error range for slopes were 0.1–0.6.
Seo and Coon (2002).

Table 10.19. The comparison of phosphorus excretion and retention of chicks fed three different phosphorus sources using best fitted broken line regression analysis.

	Breakpoint and slope for total P	Breakpoint and slope for test P	Retention (%) for total P at break	Retention (%) for test P at break	Retention (%) for test P at NPP (0.45%)
Standard	4.48:4.94, 2.72	1.61:0.46, 2.72	68.9	84.4	64.7
Defluorinated phosphate 1	4.72:5.17, 2.71	1.84:0.63, 2.71	69.4	86.3	71.5
Defluorinated phosphate 2	4.56:4.96, 3.33	1.70:0.47, 3.33	65.5	76.2	60.80

The general equation of the broken line regression is $Y = L + U (X_{XR} - R)$, where L is the ordinate and R is the abscissa of the breakpoint, X_{XR} means X less than R, and U is the slope of the line for X_{XR}. The standard errors for ordinates (L, mg g^{-1}) are 2.27, 3.05 and 4.67 respectively for the three phosphorus sources and 0.14, 0.17 and 0.25 for breakpoints (R, mg g^{-1}). The standard errors for slope are 0.08, 0.12 and 0.25, respectively. The breakpoint for test P retention can be converted to NPP retention by adding 1.46 (mg g^{-1}) to the abscissa values. The dietary NPP in the basal was 0.146% on an air-dried basis.
Seo and Coon (2002).

models are plotted in Fig. 10.11. Regression is a parametric statistical method in which the residuals are normally distributed with constant variance. The trend lines of residuals for the four different models are also shown in Fig. 10.11. The residuals of simple linear regression (plotted with a dashed line) did not distribute with constant variance, whereas the residuals from the other models (plotted with solid lines) were distributed with a constant variance. The residuals of three models (broken, two and polynomial line) were very similar

Table 10.20. The comparison of phosphorus excretion for chicks fed reagent-grade dicalcium phosphate (standard) using four different regression analysis models.

	Linear line	Polynomial	Broken line	Two line
Mean square error	1.79382	1.18116	1.1540	1.1499
R-square	0.9494	0.9686		

Seo and Coon (2002).

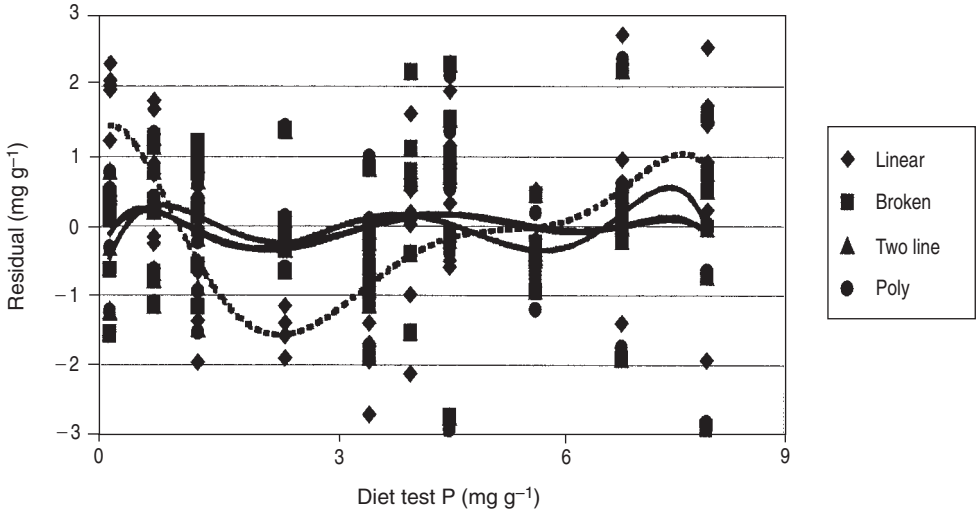

Fig. 10.11. Residual plot for measured excreta P and predicted excreta P from four different models for 10–15-day-old chicks fed standard dicalcium phosphate.

and close to the zero line for all the dietary levels of added test phosphorus. The research indicates that the straight line analysis method for predicting the total phosphorus in the excreta in relation to dietary phosphorus does not fit as well as the other regression models.

The relative bioavailability of P from defluorinated phosphate 1 from bone data was 7–11% higher than from defluorinated phosphate 2 (Table 10.17). The total phosphorus retention of diets containing defluorinated phosphate 1 and defluorinated phosphate 2 at breakpoint from two line regression analysis was 65.15% and 59.83%, respectively (Table 10.18). The percentage retention of added test P from defluorinated phosphate 1 and defluorinated phosphate 2 at breakpoint was 77.6% and 70.1%, respectively (Table 10.18). The retention of P from defluorinated phosphate 1 at the 0.45% NPP level was 3.5% higher than that of defluorinated phosphate 2. The percentage retention of dietary total phosphorus from diets containing defluorinated phosphate 1 was 69.4% and was 3.9% higher than total phosphorus from diets containing defluorinated phosphate 2 by the broken line regression method (Table 10.19). The retention at breakpoints for added test P was 86.3% and 76.2% for defluorinated

phosphate 1 and defluorinated phosphate 2, respectively. The retention of test P using the broken line regression model at the 0.45% NPP level was 71.5% and 60.8% for defluorinated phosphate 1 and defluorinated phosphate 2. The breakpoints and retention for total P and added test P from the two line analysis can be compared to the parameters from broken line model in Tables 10.18 and 10.19. The data from the broken line model were higher than data from two line model because the slope of the broken line model was forced to zero.

In summary, Seo and Coon (2002) showed the relative bioavailability of phosphorus for two defluorinated phosphorus samples compared to a dicalcium phosphate standard, and ranked the two test P samples the same as the total phosphorus retention and test P retention determined with the 5-day bioassay balance method for the test P sources. The bioavailability of phosphorus values determined with bone parameters is much higher than test P retention values, because the values are compared to a standard whereas the retention values are not relative to a standard. The bioavailability of phosphorus from bone, or performance data based on comparing test phosphorus to a standard, does not provide information about dietary phosphorus that is not retained and is lost in the faeces, which make the values less meaningful when formulating diets for digestible phosphorus. The relative bioavailability of P and total P retention of defluorinated phosphate 1 was slightly better than the relative bioavailability of P and total P retention of defluorinated phosphate 2 in this study. The defluorinated phosphorus 1 test sample produced relative bioavailability of P values for tibia ash weight slightly better than the standard, whereas the standard provided more available P for the per cent tibia ash and bone strength assays (Table 10.17). The retention of test P from defluorinated P1 at the breakpoint and at 0.45% NPP was 86.3% and 71.5%, respectively, when using the broken line regression model, compared to 84.4% and 64.7% for same P levels for the reagent-grade dicalcium phosphate standard. The excretion of phosphorus in relation to dietary phosphorus is best expressed by regression analysis other than straight line linear models. The retention of phosphorus was approximately 10–15% higher for test phosphorus sources when measured at the breakpoint determined with a two line or segmented line model compared to the phosphorus retention measured at the 0.45% NPP level. The breakpoint for maximum P retention based on the segmented line regression was approximately 0.20% dietary NPP (Table 10.19), which is approximately half of the 0.45% dietary NPP needed for optimum bone development (Table 10.16). The breakpoint for maximum P retention was determined to be approximately 0.24% retainable P from research conducted by Leske and Coon (2002), with 0.39% retainable P being determined as the breakpoint (requirement) for bone parameters. Leske and Coon (2002) also showed that dietary phosphorus levels needed for maximum broiler weight gain and bone development in floor pen studies were substantially higher than dietary phosphorus levels needed for maximum phosphorus retention.

It has become increasingly clear that retainable P values for individual feedstuffs are needed in order to allow poultry meat and egg producers to make feed formulation decisions about feed phosphorus and commercial phytase based on the economical value of products compared to the economic disadvantages of phosphorus in poultry waste.

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CHAPTER 11
Vitamins in feedstuffs

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ABSTRACT

Information on natural vitamin contents of feedstuffs is published in various tables. However, much of the information may be out of date, with information on modern varieties of a number of feedstuffs largely lacking. It is assumed that most of the vitamins in feedstuffs are available to chickens. Exceptions are biotin, for which there are good bioavailability data, nicotinic acid, for which data are limited, and folic acid. The natural vitamin contents of diets can vary considerably depending upon ingredient composition. Dietary contents of individual vitamins rarely meet minimum requirements for broilers, but may meet requirements for laying hens in several cases. Diets are normally supplemented with multivitamin mixtures. These vitamins are manufactured by different chemical and fermentation techniques and incorporated into products to try to maximize stability and distribution during mixing. Vitamins are highly stable in premixes in the absence of choline or trace minerals. The main losses occur during feed processing, particularly during extrusion, expansion and pelleting. The likely losses of individual vitamins during all stages of feed preparation and storage must be taken into account when setting dietary inclusion specifications.

INTRODUCTION

Vitamins in poultry feeds have two origins; they are natural components of the ingredients used to make the diet, and they can be added in concentrated form as a supplement. Both sources are important in practical nutrition. The natural feed vitamins can make an important contribution towards meeting birds' needs and it is useful for the nutritionist to be aware of likely feed contents. Tables of feedstuff vitamin contents are widely reproduced in various publications (e.g. NRC, 1994), though the origins of the data and the relevance to modern feedstuff varieties may be uncertain. There may also be uncertainties over the bioavailability to birds of chemically or microbiologically determined amounts. In practice, natural contents rarely provide the intakes thought to be needed by birds to meet their normal requirements and also to provide a margin of safety to meet extra metabolic demands imposed by stresses and other factors. Supplemental vitamins are then needed to make up the difference. Vitamin profiles vary considerably between feedstuffs, so the natural vitamin content of diets

 Poultry Feedstuffs: Supply, Composition and Nutritive Value (eds J.M. McNab and K.N. Boorman)

can vary considerably, depending upon the individual ingredient composition. Thus optimum vitamin supplement compositions for poultry can vary widely, depending upon the main dietary ingredients used in different parts of the world.

Amounts of supplemental vitamins added to diets need to take into account possible losses prior to consumption by the bird. Synthetic vitamins are usually highly stable, but the aggressive processing conditions used largely to curb microbial contamination during feed preparation can have an important impact on vitamin stability. A knowledge of likely processing losses is thus important in determining the initial amount of a supplemental vitamin needing to be added to a diet to ensure an adequate final intake by the bird. These topics are discussed in more detail in the remainder of the chapter.

TABLES OF FEEDSTUFF VITAMIN CONTENT

Vitamin contents of feedstuffs can be determined by a variety of techniques. There are chemical techniques based upon spectroscopy, chromatography or immunoassay for many vitamins. Biological assays based on the growth of specific microorganisms have been widely used to measure some B-vitamins in particular. The results of various assays have been compiled into tables which have been widely reproduced and repeated, generally in such a way that the original source of the information is not identifiable. There do not appear to have been any recent experimental reassessments of vitamin contents of feedstuffs, so there are a number of uncertainties relating to tabular values. These include age of the data and relevance to modern feedstuff sources or types, and the nature and reliability of the assay method. Natural variation in the vitamin content of a particular feedstuff with region, season, and perhaps processing conditions, is also likely to occur. It may therefore be difficult to assess the reliance that can be placed upon a single value in a feedstuff vitamin composition table. The values in Table 11.1 (taken from Whitehead and Portsmouth, 1989) have been biased towards the lower end of ranges of values reported from a variety of sources. This means that calculations should give a reasonable indication of the likely minimum natural vitamin content of poultry diets.

BIOAVAILABILITY

The values in Table 11.1 assume that, for most vitamins, the analytically determined values are nutritionally available to poultry, but this is not true for all vitamins. There are several vitamins where incomplete availability has been proven or suspected. The determination of vitamin bioavailability is a somewhat uncertain procedure. The simple approach is to use a chick growth response bioassay, in which the growth of chicks fed a diet containing a high proportion of the feedstuff under test is compared with growth on diets containing graded supplements of pure vitamin with the feedstuff under test being replaced by an ingredient not containing the vitamin. It is then assumed that differences in growth rate are related to changes in vitamin content, rather than

Table 11.1. Vitamin contents of feed ingredients (per kg)[a]. From Whitehead and Portsmouth (1989).

	Vitamin E (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)		Pantothenic acid (mg)	Pyridoxine (mg)	Biotin (μg)		Folic acid (mg)	Cobalamin (μg)	Choline (mg)
				A	B			A	B			
Barley	6	4	1	42	14	5	2	130	20	0.2	0	900
Maize	7	4	1	15	5	4	6	65	65	0.2	0	435
Maize gluten	20	2	2	15	5	13	8	160	160	0.5	0	250
Oats	7	6	1	12	4	10	1	220	65	0.2	0	800
Sorghum	5	4	1	40	13	8	3	250	65	0.2	0	600
Wheat	7	4	1	36	12	8	0.8	80	4	0.2	0	900
Wheatfeed	35	15	2	90	30	13	10	300	15	0.8	0	950
Cassava	0	1	0.5	10		15	1	50	1		0	450
Peas	0	1.8	0.8	17		7	1.5	140	?	0.3	0	650
Cottonseed meal	15	6	4	35		9	5	230	?	0.8	0	2600
Rapeseed meal	15	2	3	160		9	6	900	500	2	0	6500
Soybean meal	2	6	2	18	18	12	6	235	235	2	0	2500
Sunflower seed meal	8	2	3	176		30	10	1000	280	1.5	0	2600
Fish meal	4	2	5	33	33	7	3	100	100	0.2	88	3500
Herring meal	5	0.4	5	105	105	15	3	130	130	0.2	88	3500
Meat and bone meal	1	0.5	4	44	44	3	2	75	75	1	40	1600
Lucerne	100	3.4	13	20		25	6	540	400	4.2	0	250

[a]Values represent conservative estimates for feed formulation purposes (i.e. are biased towards the lower end of reported ranges).
For niacin and biotin, the 'A' values are total contents and 'B' values are estimated bioavailable contents. For other vitamins, bioavailability is assumed to be high.

to other changes in diet composition. Statistical tests can be applied to confirm the validity of this assumption, but doubts may remain (Southern and Baker, 1981). Assay methods measuring more specific metabolic criteria of vitamin status overcome this problem, but are rare.

Biotin

Biotin bioavailability varies widely among feedstuffs. Bioavailability is generally low in a range of cereals, especially wheat in which bioavailability is only about 5%. The low available biotin content of wheat-based diets accounted for the occurrence of fatty liver and kidney syndrome in different parts of the world and led to biotin becoming a routine supplement for broiler diets. In contrast, biotin is fully available in maize and also has high availability or content in a range of oilseed meals. Biotin in animal proteins is also fully available, though total contents are relatively low. Bioavailability has been established in quite a wide range of feedstuffs by different technical approaches involving responses in growth (Frigg, 1976, 1984) and in the biotin-dependent enzyme pyruvate carboxylase (Whitehead *et al.*, 1982), so overall information on bioavailable biotin contents, as indicated in Table 11.1, can be considered to be good.

Niacin (Nicotinic Acid)

Nicotinic acid is another vitamin that is not wholly available in all feedstuffs, but information is not nearly so detailed as for biotin. Nicotinic acid availability is thought to be only about 30% in a number of cereals, but it is fully available in oilseed meals (Manoukas *et al.*, 1968; Carter and Carpenter, 1980). More comprehensive data on the bioavailability of this vitamin would be helpful.

Folic Acid

This vitamin presents some different problems. It is present in feeds in a range of chemical structures. The fundamental active form is pteroylmonoglutamic acid, which is readily extracted from feedstuffs and is fully available. Folic acid is almost wholly present in this form in animal proteins. However, in feedstuffs of vegetable origin, folic acid also occurs in more-complex forms linked to chains of up to nine glutamate moieties. The polyglutamates are less soluble and must be first hydrolysed by conjugase enzymes to liberate the monoglutamate before the vitamin can be absorbed. The digestive system of poultry contains conjugase enzymes, but there is doubt about the extent of degradation of polyglutamate chains during digestion. Measurements of extractable 'free' folic acid will thus give an underestimate of the folic acid nutritive value of a feedstuff, but measurements of total folate released after

hydrolysis will give an overestimate. Since there can be up to a sixfold difference between free and total folate in some feedstuffs (e.g. soybean meal), the measure of uncertainty over the available folate contents of diets is considerable. Bioassays have been described (Keagy and Oace, 1982; Hoppner and Lampi, 1991) but do not seem to be sensitive and do not appear to have been applied to poultry.

Choline

There were doubts about the availability of choline in some feedstuffs following the report by Molitoris and Baker (1976) that choline appeared to be only about 60–75% available in soybean products in an assay that involved the replacement of maize starch with the test materials. However, more recently Menten *et al.* (1997) confirmed full availability of choline in soybean meal using an assay in which the meal was compared with meal that had been washed to remove choline. It was concluded that the results of Molitoris and Baker (1976) had been distorted by growth responses to changes in the diet contents of other nutrients, such as methionine, that are known to interact with choline. Other studies have shown that choline in lecithin, a major choline-containing component in soybean meal, is fully available.

VITAMIN CONTENTS OF DIETS

The likely available vitamin contents of broiler diets of different compositions are given in Table 11.2 and can be compared with minimum requirements listed in Table 11.3. Total contents for some vitamins remain quite constant over a wide range of diet compositions. This is especially true for thiamin (at concentrations above the requirement) and riboflavin (at concentrations below the requirement). Other vitamins are more variable, with complete absences of vitamin A and vitamin B_{12} in some diets and two- to threefold variations in the contents of other vitamins. Biotin contents of diets vary considerably depending upon whether wheat or maize is the main cereal source. With the exception of thiamin and (occasionally) pyridoxine and choline, natural vitamin contents of diets are less than minimum requirements. Hence supplementation is necessary.

SUPPLEMENTAL VITAMINS

Typical supplementation rates for UK diets are given in Table 11.4. The ranges are relatively wide for some vitamins and can be accounted for by variations in diet composition, margins of safety thought desirable and uses of vitamins for specific purposes (e.g. additional vitamin E to promote immunological enhancement or meat quality). For laying hens, it is apparent that it may not be necessary to provide supplements containing all vitamins.

Table 11.2. Natural available vitamin contents of broiler starter diets (per kg). From Whitehead and Portsmouth (1989).

	Wheat-based		Maize-based				Mixed			
Main ingredients (g kg^{-1})										
Maize			500	490	460	600	420	300	480	100
Wheat	500	600					120	70	150	100
Cassava								100		250
Peas							50			50
Rapeseed meal				50						
Soybean meal	400	150	400	300	300	150	260	220	230	300
Sunflower seed meal				50	50			40		
Fish meal		100		60	50	100	20	40	40	50
Meat and bone meal		50				50	60	50		50
Vitamin contents (per kg)										
Vitamin A (IU)	0	0	1250	1220	1150	1500	1050	750	1200	250
Vitamin E (mg)	4	5	4	4	5	5	4	3	5	2
Thiamin (mg)	4	3	4	4	4	3.2	3.6	3	3.7	3
Riboflavin (mg)	1.5	1.7	1.5	1.5	1.5	1.7	1.6	1.5	1.4	1.3
Niacin (mg)	13	14	10	18	26	11	12	17	9	12
Pantothenic acid (mg)	9	7	7	7	7	5	6	7	6	11
Pyridoxine (mg)	3	2	5.5	5.4	5.5	4.5	4.5	4	4	3
Biotin (μg)	96	52	130	122	139	90	103	90	90	93
Cyanocobalamin (μg)	0	11	0	5	4	11	4	5	3	5.7
Folic acid (mg)	0.9	0.4	0.9	0.5	0.6	0.4	0.7	0.7	0.6	0.7
Choline (mg)	1450	1350	1210	1300	1580	1070	1139	1115	1060	1280

Table 11.3. Vitamin requirements of chickens (per kg diet).

	Broiler		Chick		Hen	
	Starter	Finisher	Starter	Grower	Laying	Breeding
Vitamin A (IU)	1400	1400	1300	1300	3600	3600
Vitamin D_3 (IU)	800	800	200	200	400	400
Vitamin E (mg)	10	10	10	5	10	10
Vitamin K (mg MSB)	0.4	0.4	0.4	0.4	0.7	0.7
Thiamin (mg)	1.5	1.5	1.2	0.5	0.7	0.7
Riboflavin (mg)	5	4	3	2	2.5	4
Niacin (mg)	70	45	30	20	8	10
Pantothenic acid (mg)	12	10	10	6	7	10
Pyridoxine (mg)	4	3	3	2	2	4
Biotin (μg)	180	120	120	30	30	100
Cyanocobalamin (μg)	12	10	12	5	12	15
Folic acid (mg)	2.5	2	0.5	0.3	0.3	0.5
Choline (mg)	1300	1000	1000	300	300	300

MSB, menadione sodium bisulphite.
IU vitamin A = 0.3 μg retinol.
IU vitamin D_3 = 0.025 μg cholecalciferol.

Table 11.4. Typical vitamin supplementation rates for UK poultry diets (per kg diet).

	Broiler			Pullet	
	Starter/grower	Finisher	Breeding	Rearing	Laying
Vitamin A (kIU)	10–15	8–12	12–15	8–12	7
Vitamin D_3 (kIU)	4–5	4–5	3–3.5	3	2.5–3.5
Vitamin E (mg)	30–180	25–180	30–100	20	7.5–20
Vitamin K (mg)	2–3	2–3	3	1	1
Thiamin (mg)	1.5–2.5	1–2	3	0	0–1.5
Riboflavin (mg)	6–8	6–8	10–18	4	2.5
Niacin (nicotinic acid) (mg)	35–60	25–35	40	20	5–10
Pantothenic acid (mg)	12–18	8–12	18	8	4–8
Pyridoxine (mg)	4	1.5–3.5	4	2	0–1
Biotin (μg)	180–250	100–150	150–350	100	0–?
Vitamin B_{12} (μg)	10–16	10–14	20	8	5–10
Folic acid (mg)	1.5–2.5	1–2.5	3	1	0–1
Choline (mg)	250–350	175–300	300	300	0–200

kIU vitamin A = 0.3 mg retinol.
kIU vitamin D_3 = 0.025 mg cholecalciferol.

Supplemental vitamins are produced either by chemical synthesis or fermentation. Both methods usually result in the final preparation of crystalline, chemically similar products. Vitamin B_{12} is an exception. Chemical synthesis results in the cobalamin cation, which can be crystallized as cyano- or hydroxy-cobalamins. Fermentation, however, results in the organic forms methyl- and adenosyl-cobalamin. Further chemical treatment of the fermentation products may be used to convert these forms to cyanocobalamin but some vitamin B_{12} products may contain some or all of the cobalamin in the form of the methyl or adenosyl derivatives. The availability of these different forms is probably high (Brandt *et al.*, 1979), but there is little published information relevant to poultry.

Other synthetic vitamins can be fed in different forms, but these usually have high potency. For instance, nicotinic acid and nicotinamide have equal potency for chickens. Recent research has confirmed that thiamin mononitrate has the same potency as thiamin chloride-hydrochloride in chickens (Geyer *et al.*, 2000) though benfotiamin, a lipid-soluble thiamin derivative used in human nutrition, has higher potency.

VITAMIN STABILITY

The vitamins mostly have complex chemical structures that are susceptible to modification and degradation under a range of conditions. Hence stability of vitamins is an important issue. The menadione structure of vitamin K_3 is especially labile, particularly when heated, and is usually manufactured as a complex (e.g. with bisulphite or nicotinamide) to improve its stability. Other vitamins are manufactured in beadlets encased in gelatin or dextrin to improve stability and also to improve dispersion during feed mixing.

The main causes of vitamin instability are chemical reactions induced by heat, moisture, changes in pH, pressure and friction. Vitamins generally do not interact with each other, with the exception of choline chloride which acts aggressively towards other vitamins. For this reason choline chloride is usually not included in vitamin premixes, but is added to diets as a separate supplement. The inclusion of trace minerals in a vitamin supplement can increase vitamin losses through initiation of redox reactions. The stability of different vitamins in premixes is shown in Table 11.5 (BASF, 1994a). Vitamins in premixes not containing choline chloride or trace minerals have good stability when stored under cool conditions. Losses can start with diet mixing, when friction and heating in the presence of the great variety of chemical substances found in feeds can start degenerative changes. Electrostatic forces that build up in machinery may also attract vitamins such as riboflavin and folic acid and affect their distribution in diets.

The main vitamin losses in diets occur as a result of high temperatures and pressures experienced during diet processing. These are associated with extrusion, expansion and pelleting procedures. The more aggressive conditions used in recent years to combat *Salmonella* and other microbiological contamination of feeds has had a particularly destructive effect on vitamin contents of diets. The extent of the destruction depends upon the temperature used and the length of the exposure. Stabilities under a range of temperatures and dwell times during expansion are given in Table 11.6 (BASF, 1994b). Losses can also occur during storage of processed feed, and these losses may be greater where the processing conditions have disrupted protective treatments. The overall effect of the whole range of storage and processing factors under one set of expanding and pelleting conditions on overall vitamin stability is given in Table 11.7. Losses under the conditions specified were generally about 25–30% for most vitamins, though vitamin K losses were much higher (65%). Such losses must be taken into account in setting the initial dietary addition rates for particular vitamins. Losses from these heating processes could be substantially overcome if vitamins were added

Table 11.5. Vitamin stability (%) over time in premixes containing choline chloride and trace minerals. From BASF (1994a).

	Retention		Average loss per month
	1 month	6 month	
Vitamin A beadlet	85	58	8
Vitamin D_3 beadlet	91	65	6
Vitamin E acetate	95	82	3
Vitamin K (MSBC)	64	0	38
Thiamin HCl	70	27	17
Riboflavin	95	56	9
Pyridoxine	92	56	9
Ca-pantothenate	95	58	8
Niacin (nicotinic acid)	95	58	8
Biotin	93	57	9
Vitamin B_{12}	98	89	2
Folic acid	85	43	12
Choline Cl	99	91	2

MSBC, menadione sodium bisulphite complex.

Table 11.6. Stability (%) of vitamins under different expanding conditions during feed preparation. From BASF (1994b).

	Temperature (°C) × time (s)		
	93 × 30 110 × 5	115 × 30 132 × 5	149 × 30 165 × 5
Vitamin A beadlet	98	94	79
Vitamin D_3 beadlet	99	96	88
Vitamin E acetate	99	95	87
Vitamin K (MSBC)	80	60	30
Thiamin HCl	94	85	58
Riboflavin	95	88	75
Pyridoxine	96	90	80
Ca-pantothenate	97	91	81
Niacin (nicotinic acid)	95	89	70
Biotin	96	90	68
Vitamin B_{12}	99	96	90
Folic acid	96	90	69
Choline Cl	100	98	96

MSBC, menadione sodium bisulphite complex.

in liquid form to diets after processing. However, problems might arise in trying to keep all vitamins in solution. Liquid vitamins would also lack the specific protections used for solid products and so might be less stable during storage. Finally, it must be stressed that the stability values quoted in Tables 11.5, 11.6 and 11.7 are meant to be illustrative only. Different manufacturers produce vitamins and vitamin products by different methods and to different specifications. It is therefore unlikely that all products for a particular vitamin will have the same stability, but comparison of specific products is beyond the scope of this chapter.

Table 11.7. Stability (%) of vitamins during various stages of feed preparation and storage. From BASF (1994b).

	Premix (1 month)	Expanding[a] 120°C × 10 s	Pelleting[b] 93°C × 30 s	Storage (2 weeks)	Overall retention
Vitamin A beadlet	99	95	90	92	78
Vitamin D_3 beadlet	99	97	93	93	83
Vitamin E acetate	99	96	93	93	87
Vitamin K (MSBC)	98	65	65	85	35
Thiamin	99	94	89	98	81
Riboflavin	99	90	89	97	77
Pyridoxine	99	92	87	95	75
Ca-pantothenate	99	93	89	98	80
Niacin (nicotinic acid)	99	91	90	93	76
Biotin	99	92	89	95	77
Vitamin B_{12}	100	97	96	98	91
Folic acid	99	92	89	98	79

MSBC, menadione sodium bisulphite complex.
[a]Temperature and retention time in expander.
[b]Pelleting temperature and conditioning time.

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CHAPTER 12
Energy utilization: measurement and prediction

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INTRODUCTION

Evaluation of energy yield from the diet is of crucial importance in practical and research nutrition. One of the major reasons for its importance is that energy content has a key role in the control of food intake. This means that the intake of individual substances is strongly influenced by the nutrient:energy ratio. Reliable estimates of the bird's energy requirement and the availability of energy from the food are therefore essential foundations for the accurate formulation of a diet which provides biologically, economically or environmentally optimal intakes of specific nutrients. Energy is not a chemical entity; it is a summation of the biologically available energy of the chemical constituents of the food. There is a good argument for describing a diet in terms of nutrient chemical contents (e.g. amino acids, carbohydrates, fats), but this should be done in the knowledge that some of the bird's physiological control mechanisms may perceive these substrates as contributors to energy supply rather than as specific chemicals. It is therefore necessary to describe a feedstuff or ingredient in terms of both its biological energy value and its chemical composition.

A survey of feed evaluation in Europe (de Boer and Bickel, 1988) listed the wide range of energy evaluation systems in use. The section on poultry was conspicuous for its comparatively high degree of consensus, with almost all countries using a metabolizable energy system (metabolizable energy, ME = gross energy – [faecal + urinary energy]). ME is a reliable index of what is available to the bird for maintenance and production, but not a predictor of how efficiently the bird then uses what is available. Its low variability (Hill and Anderson, 1958) is a consequence of ignoring many of the bird's metabolic responses to its food. ME is assumed to be linearly additive, which is practically convenient but not exhaustively tested (MacLeod *et al.*, 1996). There have been many authoritative reviews on ME (e.g. Miller, 1974; Sibbald, 1982; Fisher, 1989; Fisher and McNab, 1989). This review will, therefore, attempt to introduce or reiterate some of the key ideas on ME but will not attempt to repeat comprehensive coverage. It will also try to introduce some alternative approaches to energy evaluation. As long as requirements for energy and provision of energy by diet ingredients are expressed in the same form, accurate relativity between energy values may be more critical than absolute accuracy in allowing substitution of ingredients

 Poultry Feedstuffs: Supply, Composition and Nutritive Value (eds J.M. McNab and K.N. Boorman)

during diet formulation; internal consistency is therefore essential in any feed evaluation system, although a systematic bias in both response estimates (or 'requirements') and 'bioavailability' may be tolerable. The yardstick of any feed evaluation system is how well it satisfies these requirements.

ENERGY PARTITION

Any discussion on energy evaluation must be informed by a knowledge of how energy is partitioned among different functions. A classical representation is shown in Fig. 12.1, which is followed by a list of terms used in energy evaluation (Table 12.1).

ME MEASUREMENT AND PREDICTION

Three general types of energy balance experiment for measuring ME were identified by Fisher and McNab (1989) and are quoted below:

1. Traditional assays which involve preliminary feeding periods to establish 'equilibrium' conditions. Differences in carryover in the digestive tract between the beginning and end of the assay period ('end-effects') are controlled by trying to ensure that they are the same. Complete diets must be used in most cases and substitution methods used for ingredients.
2. Rapid assays, using starvation before and after giving a known aliquot of test feed to control 'end-effects', but which permit the birds free access to the feed. Again, complete diets and substitution methods must be used in most cases.
3. Rapid assays, as above, but using tube-feeding (or force-feeding or precision feeding) to place the feed directly in the bird's crop. These methods usually avoid the need to substitute feed ingredients into a basal diet.

Examples of all three types will be briefly described below and can easily be ascribed to a category.

Apparent Metabolizable Energy

The word 'apparent' refers to the fact that the droppings collected for AME measurement contain matter endogenous to the bird (e.g. gut secretions, gut epithelial cells) as well as undigested food material and urine. It should be noted that only the urine is correctly termed 'excreta' in a physiological sense. The combined faeces and excreta of the bird should strictly (if unexcitingly) be called 'droppings', although 'excreta' has entered common useage, presumably because it sounds more scientific. A typical protocol for AME measurement (The European Reference Method, Bourdillon *et al.*, 1990a) is shown in Fig. 12.2. This is one of many variations on the same basic technique. If an ingredient cannot be fed alone, for practical reasons or because of unpalatibility, AME is usually determined by substitution of the test ingredient for part of a basal

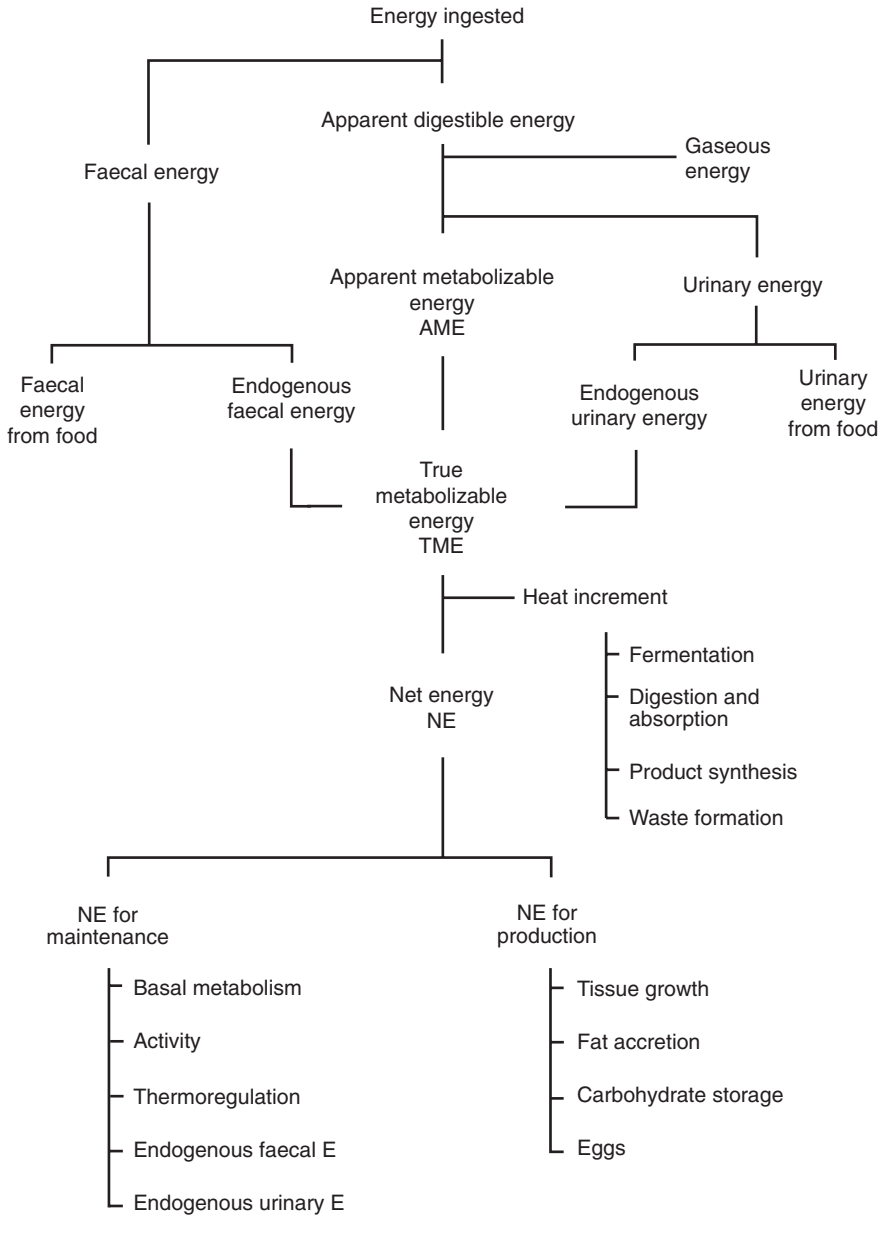

Fig. 12.1. Partition of dietary energy intake by the domestic fowl (after Sibbald, 1982).

diet. The assumption is then made that the AME of the basal diet is a constant and that any change in the AME of the mixture is attributable to the test ingredient. The inherent defects of this approach are that all statistical error and the effects of any interactions between the basal diet and the test ingredient are inevitably attributed to the test ingredient.

Table 12.1. A glossary of equations, symbols and abbreviations used in food energy evaluation (based on EAAP Energy Metabolism Symposium, 1976 (EAAP, 1976); suggested conventions)

Apparent metabolizable energy (AME) intake:	I_{AME}	=	$I_E - (F_E + U_E)$
True metabolizable energy (TME) intake:	I_{TME}	=	$I_E - (F_E + U_E) + (F_{E,b} + U_{E,b})$
Energy retention:	R_E	=	$I_E - (F_E + U_E) - H$
Net availability of TME:	k	=	dR_E/dI_{TME}
Net energy per g of food:	NE	=	kI_{TME}/I

Symbols and abbreviations:

I	=	rate of food intake (by weight if no subscript)
R	=	rate of retention
F	=	rate of faeces production
U	=	rate of urine production
k	=	net availability of ME
d	=	difference or change in a measurement (delta)
AME	=	apparent metabolizable energy
TME	=	true metabolizable energy
E	=	gross energy
b	=	fasted (b from basal)
m	=	maintenance
o	=	egg production
f	=	fat deposition
p	=	protein deposition

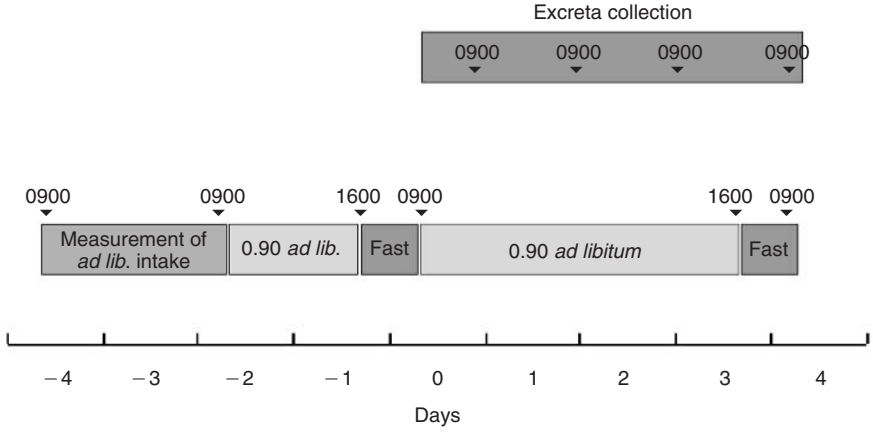

Fig. 12.2. The 'European Reference Method' for determination of apparent metabolizable energy (AME) (Bourdillon *et al.*, 1990a).

Rapid methods of AME measurement

Farrell (1978) developed a rapid method for measurement of AME, which was later modified by Farrell *et al.* (1991). In this method, adult cockerels were trained to consume their daily food allowance in 1 h. The birds were then fasted for 36 h before being given a fixed quantity of pelleted test food. Droppings were collected for the next 42 h. There have been other variations on this technique (e.g. du Preez *et al.*, 1986).

Marker methods

Metabolizability of energy, like the digestibility of other nutrients, can be measured by marker ratio methods. Markers used include chromic oxide, titanium dioxide (Peddie *et al.*, 1982) and endogenous or added acid-insoluble ash (Scott and Hall, 1998).

Nitrogen correction

Correction to zero nitrogen retention makes the simplification that the feedstuff evaluated is used entirely as an energy source. The justification for this correction is that ME is purely an energy evaluation system, so materials should be assessed only for their energy value. The correction involves calculating (by the following equation) the additional energy which would appear in the excreta if any nitrogen retained were instead to be catabolized and excreted.

$$([\text{faecal+urinary}]\ \text{energy})_N = ([\text{faecal+urinary}]\ \text{energy}) + 34.4\ (\text{N intake} - [\text{faecal+urinary}]\ \text{N})$$

where 34.4 kJ g^{-1} is the mean gross energy of the nitrogenous excretory products in the bird.

The correction to excreta nitrogen is negative in many assays because of low nitrogen intake and N-corrected ME is therefore higher than the uncorrected value. However, in cases where there is sufficient protein intake to give a positive nitrogen retention, N-corrected ME is lower than the uncorrected value. This has sometimes prompted the criticism that high-protein raw materials are 'penalised' by correction to zero nitrogen balance. However, it is to be expected that high-protein materials are being included in the diet as sources of amino acids and that their inclusion will be determined on this basis, rather than their energy value. Nitrogen correction is also applied to endogenous energy losses (EEL) in measuring TME; since nitrogenous matter makes up a large proportion of endogenous losses, EEL_N is much lower than EEL (McNab and Blair, 1988).

True Metabolizable Energy

True metabolizable energy is ME corrected for endogenous energy losses (Table 12.2). It should not be taken as inextricably linked with tube-feeding techniques, as it often is. A correction for endogenous losses can be applied to measurements made by any ME technique. However, TME came into vogue at the same time as Sibbald's (1976) method, because the limited food intake consequent on this technique meant that endogenous losses became a more significant factor in the accuracy of the measurement. The relationship between AME, TME and endogenous energy loss is shown graphically in Fig. 12.3, which also contains the equation for the relationship. This relationship was explored thoroughly by Jonsson and McNab (1983).

Table 12.2. Roslin true metabolizable energy (TME) protocol (McNab and Blair, 1988).

Time (h)	Tube-fed birds	Fasted birds (for EEL)
0	Food withdrawn	Food withdrawn
24	50 ml glucose solution	50 ml glucose solution
48	50 g test material	50 g glucose (granulated)
72	50 ml water Palpate crop	50 ml water
96	Droppings collection Period may be extended by 24 h	Droppings collection

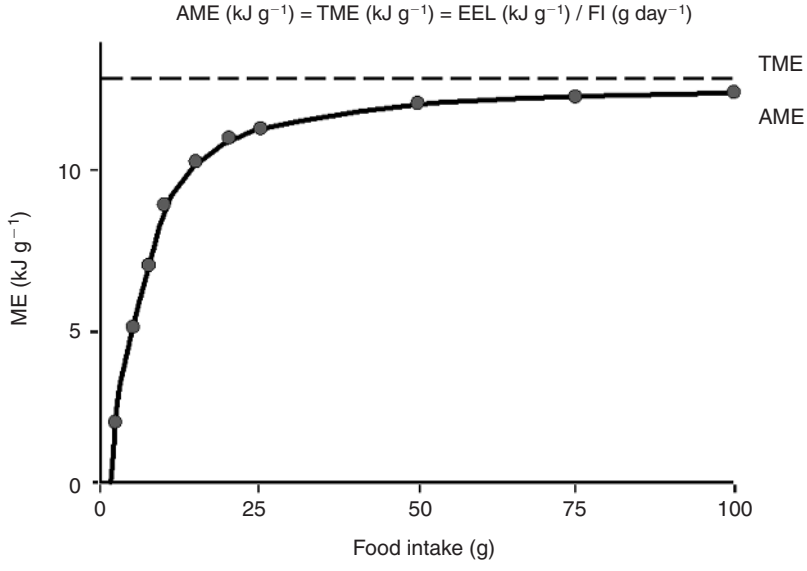

Fig. 12.3. The relationships between apparent metabolizable energy (AME), true metabolizable energy (TME) and food intake. EEL is endogenous energy losses. The curve was drawn using a realistic estimate of EEL. The figure demonstrates how a correction for EEL becomes larger and more necessary as food input decreases.

Feeding Methods

Tube-feeding has the advantages of precision in the measurement and timing of intake and of allowing the feeding of substances which (although nutritious) may be unpalatable at high concentrations (Table 12.3). There have been assertions that tube-feeding may not be as suitable as longer-term 'self-feeding' for assessing the efficacy of feed enzymes. If there is a scientific basis for these doubts, it would probably be that the gut microflora need time to adapt to the changes in substrate composition produced by the action of the exogenous (feed) enzymes. I have been unable to find published evidence for this effect. In any case, the effect of enzymes

Table 12.3. Comparison of tube-feeding and 'self-feeding' for evaluation of raw materials and diets; the greater the number of asterisks, the greater the advantages of the technique.

	Tube-feeding	Self-feeding
'End effects' (carry-over etc.)	***	**
Accuracy in measuring food intake	***	**
Accuracy in timing of feeding	***	*
Accuracy in timing of collection	***	***
Feeding of unpalatable raw materials	***	*
Errors due to substitution	***	*
Tests for action of exogenous enzymes	**	***

on metabolizability and digestibility is often not large enough to allow the detection of a superimposed treatment (such as mode of feeding) with statistical confidence.

The advantages of tube-feeding would also be applicable to the measurement of the net energy of raw materials or whole diets. On the other hand, tube-feeding clearly circumvents feeding activity; the absence of upper alimentary tract and central stimuli of feeding may also affect centrally mediated general locomotory activity or other energy-using processes. MacLeod (1991) used rapid-response indirect respiration calorimetry (Lundy *et al.*, 1978; MacLeod *et al.*, 1985), with simultaneous Doppler-radar activity measurement (MacLeod *et al.*, 1982) to measure the effect of tube-feeding on energy metabolism and physical activity (Table 12.4). The thermogenic effect of feeding was about 30% less with tube-feeding, over-estimating net energy by 4%. This difference was entirely accounted for by the reduction in feeding-related activity. The theoretical relationship between true and apparent ME can be confirmed when birds are given the same amount of food by tube or by self-feeding and there is no difference due to feeding method (MacLeod, 1991). Both AME and TME were the same in tube-fed and self-fed birds. Variance of all measurements was lower in tube-fed birds, probably because of the removal of variation in food intake.

Table 12.4. A comparison of results obtained by tube-feeding and self-feeding the same quantities of a complete diet (MacLeod, 1991).

	Self-fed		Tube-fed	
	Mean	SEM	Mean	SEM
Food intake (g)	46.4	3.95	50.0	0.01
Gross energy intake (kJ day^{-1})	789	68.1	850	1.8
AME intake	614	54.0	664	2.5
TME intake	674	54.4	722	6.1
A metabolizability	0.78	0.005	0.78	0.003
T metabolizability	0.86	0.012	0.85	0.007
AME (kJ g^{-1})	13.2	0.09	13.3	0.05
TME (kJ g^{-1})	14.6	0.19	14.4	0.12

SEM, Standard error of mean.

Prediction of ME from Chemical Composition

Water-insoluble cell wall material

Carré (1990, 1991; Carré and Brillouet, 1989; Carré *et al.*, 1984) argued that terms such as 'fibre', 'crude fibre' and various subdivisions (acid detergent fibre, neutral detergent fibre) were not sufficiently precise. He proposed that a more useful description was 'plant cell wall', which describes the botanical origin of the material. Soluble non-starch polysaccharides were extracted in aqueous solution and starch and protein were extracted enzymically. The water-insoluble cell wall material (WICW) was then isolated by filtration. N-corrected AME (AME_N) was calculated by the following equation, where GE is gross energy.

$$AME_N = 0.629\ GE^{1.1} - a\ (\%\ WICW) - b\ (\%\ protein)$$

The coefficient for protein varies among different protein sources, but a mean value can be calculated for closely related ingredients.

EC Equation

There have been many equations designed to predict ME content from a (necessarily) limited chemical description of the diet. Several were listed and reviewed by Fisher (1989) and Carré (1990). The following equation (originating from the work of Härtel *et al.*, 1979) is used for regulatory purposes within the European Community. The final form of the equation was based on data sets from five laboratories. All the data came from adult birds, mainly cockerels. This means that differences due to species and age are ignored. A full critique of this equation was published by Fisher and McNab (1989). Although there are clearly reservations on biological grounds, the equation (EEC, 1986) does provide an agreed standard of comparison:

$$AME_N\ (MJ\ kg^{-1}) = 0.3431\ (\%fat) + 0.1551\ (\%CP) +$$

$$0.1301\ (\%total\ sugar\ [expressed\ as\ sucrose]) + 0.1669\ (\%starch)$$

Both the Carré and EC equations gave good agreement with measured values when tested by Bourdillon *et al.* (1990b). The EC equation agreed to within < 0.2%, while predictions from the cell wall equation were low by about 1.6%. Bourdillon *et al.* (1990b) observed that the most likely problem with the use of prediction equations is not their inherent accuracy but rather the accuracy of the chemical analyses on which they depend (Fisher, 1982, 1983). However, variation in the metabolizability of raw materials is due not only to the major chemical components but also to anti-nutritive factors such as non-starch polysaccharides (NSP), enzyme activity, tannins, alkyl resorcinols, protease inhibitors, α-amylase inhibitors, phytohaemagglutinins, alkaloids, saponins and lathyrogens (Hughes and Choct, 1999).

Near-infrared (NIR) Spectroscopy

NIR spectroscopy is increasingly being used as a rapid technique for the analysis and evaluation of raw materials and compound foods (Givens *et al.*, 1997). Examples of its potential for use with poultry diets have been given by Valdes and Leeson (1992a,b, 1994) and Leeson and Valdes (1996). Until now, the technique has been used mainly for the estimation of major chemical components, such as fat, protein and water content. However, as research continues, it is likely that computational and analytical methods will reach the point where minor nutrients and more esoteric chemical properties can also be estimated (Table 12.5).

Heat Production and Net Energy

Measurement of energy utilization

A previous book in this series (Morris and Freeman, 1974) included a number of papers related to the measurement of energy metabolism (Balnave, 1974; Farrell, 1974; van Kampen, 1974). Since that time, there have been major developments in avian calorimetry, mostly related to the use of computerized automation and recording (Lundy *et al.*, 1978; MacLeod *et al.*, 1985; Geraert, 1987; Buyse *et al.*, 1998). Stable isotope techniques have also come into use for measuring the metabolic rate of free-living birds (Ward and MacLeod, 1991).

NE Measurement and Prediction

Net energy is that part of the gross energy of a feedstuff which is used by the animal for maintenance and production (Fig. 12.1; Table 12.1). In other words, it is equal to ME intake minus the heat increment of feeding. Heat increment is the total increase in heat production which occurs when a substance is ingested; it therefore represents the total energy-metabolism response to the stimulus provided by the substance. It is a summation of the

Table 12.5. Near-infrared reflectance (NIR) spectroscopy; some positive and negative points. The negative features are rapidly disappearing with continuing development.

FOR	AGAINST
Rapid	Laborious calibration procedures
Non-destructive physical method	Complexity in data treatment
Minimal sample preparation	Lack of sensitivity for minor constituents
No reagents	Expensive instrumentation
Simple to use, when calibrated	Non-portability of calibrations between instruments

energy costs of ingestion, absorption and metabolism, along with any more direct effects on the bird's metabolic rate. The heat increment, and hence the net energy, of a feedstuff varies with its chemical composition; in general terms, the net energy values of fat and protein per unit of ME are respectively 20% higher and 20% lower than that of carbohydrate. The net efficiency of utilization of ME (or net availability of ME) can be defined, for calculation purposes, as the increase in energy retention which occurs per unit increase in the quantity of food energy ingested; it can, therefore, be calculated as the slope of the relationship between retention and intake over the appropriate range of intakes (possibly just two intakes within the range of interest). This slope (net efficiency) is conventionally given the symbol *k* (Table 12.1).

$$k = dR_E/dI_{ME}$$

k is often given a subscript to indicate the fate of the retained energy: k_m, maintenance; k_o, egg production; k_f, fat deposition; k_p, protein deposition. Because of the differing energetic efficiencies of the chemical transformations involved in these processes, *k* for a given feedstuff can vary, depending on intake and on the physiological state of the animal. For instance, because birds control their body temperature, there are strong interactions between dietary energy and the thermal environment. The interaction is clearest in the case of energy requirement for maintenance and, therefore, gross energetic efficiency. However, there are reasons to think that ambient temperature would also affect partial (net) efficiency of energy utilization. One hypothesis related to this is that the heat increment of feeding would substitute for (facultative) thermoregulatory heat production, leading to higher partial efficiency of energy retention as ambient temperature decreases, until a temperature is reached where the entire heat increment of feeding substitutes for thermoregulatory heat production, giving a measured partial efficiency of 1.00 (100%). This type of pattern has been shown in pigs, but has not been demonstrated so clearly in poultry. When fasted or underfed at any temperature, fowl and turkeys allow their metabolic rate to decrease, and make greater use of heat-conservation mechanisms, even letting their body temperature decrease rather than increasing thermoregulatory heat production (MacLeod *et al.*, 1993). The effect of this is that the heat increment of feeding, and hence net energy, may change little over a wide range of temperatures (MacLeod, 1990, 1992). (Conversely, at high ambient temperatures, poultry seem to be slow in reducing thermoregulatory heat production to allow the heat increment of feeding to be expressed, so that a reduction in food intake is the only rapid mechanism for reduction of metabolic rate (Francis *et al.*, 1990; MacLeod and Hocking, 1993; MacLeod *et al.*, 1993)).

The relative sensitivity of net energy to the state of the animal may be seen as an advantage or a disadvantage, depending on the viewpoint taken. It is obviously a more complete indicator of response than ME but is therefore more prone to variability, for real biological reasons.

MODELLING THE UTILIZATION OF DIETARY ENERGY BY POULTRY[1]

The perception that it is relatively inexpensive to do experiments with poultry has had both positive and negative effects on the development of a modelling approach. The positive effects are that there is a large body of data to underpin modelling and also that validation should be less costly than with other species. The negative effect is that there may be a temptation to do a new experiment for every new set of circumstances. Digestibility and metabolizability measurements should probably be exempted from this charge, since bio-assay is sufficiently rapid and precise to remain the method of choice over predictive methods (Sibbald, 1976; Farrell, 1978; McNab and Blair, 1988; Bourdillon *et al.*, 1990a,b). However, adherence to an empirical approach in poultry nutrition has meant that relatively few scientists have made an active contribution to the development of predictive models. This section of the chapter briefly describes some empirical and mechanistic models, as well as models which have some characteristics of both (see France and Thornley, 1984, for definitions).

Advances in energy evaluation for poultry are likely to come from taking into account the differing biochemical efficiencies with which the chemical constituents of the diet are utilized (Millward *et al.*, 1976). This is most likely to be achieved by a modelling-based approach, of which there have already been attempts at varying levels of mechanism and aggregation (Nehring and Haenlein, 1973; de Groote, 1974a; Emmans, 1994; MacLeod, 1994). Although demonstrably capable of distinguishing between the energetic effects of feeding different classes of chemical substrate (Tasaki and Kushima, 1979), or between grossly different compound feeds (MacLeod, 1990, 1992), calorimetric experiments need copious replication to detect the effects of more subtle changes in diet composition. These relatively small effects, which may still be of biological and commercial significance, are more likely to be detected by a valid predictive model than by any but an extremely large experiment.

Empirical Predictive Modelling of Net Energy

Net energy (NE), as defined above, is that part of the dietary energy which is available to the animal for maintenance and production (i.e. NE = ME – heat increment, where heat increment is the increase in heat production which occurs when food is ingested). A 'productive energy' system (in most respects analogous to NE) was used commercially from about 1946 to about 1960, following the work of Fraps and Carlyle (1939), and Fraps (1946). This system was based on 'comparative slaughter' measurements of energy retention and estimates of maintenance energy requirement calculated from body weight.

[1]Much of the material in the remaining part of this chapter has been published previously in a different form (MacLeod, 2000).

Productive energy was discarded in favour of ME because of its lack of precision (ranges of up to ± 20% for a single feedstuff). Much of this variability may have resulted from the measurement technique, in which inter-individual variation was combined with errors inherent in the comparative slaughter method and in the method of calculating maintenance requirement from bird weight.

The Rostock Net Energy (NEF) model

Work at Rostock (Nehring, 1967; Schiemann, 1967; Schiemann *et al.*, 1971; Nehring and Haenlein, 1973) demonstrated that excessive variability is not an inevitable characteristic of net energy systems. Extensive measurements with several species showed that net energy (for fat deposition) can be predicted from digestible fat, protein and carbohydrate contents of feedstuffs with a coefficient of variation of about ±5%. The Rostock NEF system (net energy for fat deposition) was based on a large number of calorimetric trials on the main agricultural species. The multiple-regression models derived from these trials predict NEF from measurements of digestible crude protein (P), digestible crude fat (F) and digestible crude fibre + digestible N-free extract (largely carbohydrate, C). The model for the domestic fowl (derived from adult cockerels) is:

$$\text{NEF (kJ g}^{-1}) = 10.8\text{P} + 33.4\text{F} + 13.4\text{C} (\pm \text{ cv } 5.2\%) \quad (1)$$

The corresponding equation for ME is:

$$\text{ME (kJ g}^{-1}) = 17.8\text{P} + 39.8\text{F} + 17.7\text{C} \quad (2)$$

The coefficients relating NEF to ME (and therefore equivalent to k_f, net efficiency of energy utilization for fattening) are 0.60, 0.84 and 0.78 for protein, fat and carbohydrate, respectively. The NEF model was based specifically on the utilization of energy for fat deposition, a decision cogently argued by Nehring (1967). The NEF system appeared to satisfy many of the criteria for a practical NE system. Its non-use in most countries may have stemmed from its reliance on digestibility coefficients, which would have been identified as a particular problem in the case of poultry.

A modification of the Rostock NEF Model for poultry

De Groote's (1974a) proposed NE method for poultry by-passed the uncertainty associated with digestibility coefficients by deriving NE values from existing ME data. This was done for each feedstuff by multiplying its ME value by the relative proportions (by weight) of crude protein, crude fat and starch+sugar, each of which was in turn multiplied by an experimentally estimated utilization coefficient. The coefficients were 0.60, 0.90 and 0.75 for protein, fat and carbohydrate, respectively. The calculation was therefore:

$$\text{NE} = \text{ME}(0.60\text{P} + 0.90\text{F} + 0.75\text{C})/(\text{P}+\text{F}+\text{C}) \quad (3)$$

where P, F and C are protein, fat and carbohydrate contents in g kg^{-1}.

In striving for simplicity, de Groote's system neglected differences in the digestibility of the protein, fat and carbohydrate fractions both within and between feedstuffs. Perhaps more importantly, by using proportions of feedstuff by weight, it appeared to neglect the fact that fat has about twice the ME value

of protein and carbohydrate and that the utilization coefficients (0.60, 0.90, 0.75) referred to ME and not to mass. De Groote (1975) rationalized the low ME value assigned to fat by quoting the low digestibility coefficient (about 0.5) cited by the Rostock group. The use of this low value does seem to detract from the de Groote method; it appears also to be inconsistent with the ME values the author quotes for individual animal and plant fats (de Groote, 1974a, Table 12.6). The NE values in the latter publication can be recalculated on the basis of proportions of ME rather than weight by the following equation:

$$NE_1 = ME(0.60\ H\ 17.8P + 0.90\ H\ 39.8F + 0.75\ H\ 17.7C)/(17.8P + 39.8F + 17.7C) \quad (4)$$

where 17.8, 39.8 and 17.7 are ME coefficients from the Rostock model (see equation (2)).

Table 12.6 shows that the original NE values in de Groote (1974a), calculated on a weight basis, have a negative error which increases with fat content. Table 12.7 allows comparison of the relative energy values of ingredients calculated as ME, NE or NE_1. In each case, wheat is given a value of 100. The most important feature of the comparison is the magnitude of the difference between relative replacement values expressed as ME and as NE or NE_1. The disagreement varies according to the concentrations of fat and protein and the ratios of these concentrations to one another. Few independent tests of the de Groote NE model have appeared in the literature. In survey data from chickens 0 to 28 days old, Fisher and Wilson (1974) calculated that, of the 39% variation in growth rate unexplained by variation in dietary ME concentration, 18% could be accounted for by recalculating intake on NE by the de Groote method. In the case of food conversion efficiency, the improvement was even greater; unexplained variation was reduced from 27% to 8%. In the finisher stage, ME and NE scales gave similar correlations with production responses. De Groote's (1974b) own comparison of ME and NE systems claimed improved performance and higher returns over food costs in diets formulated on the basis of NE. As Farrell (1979) pointed out, however, the NE diets almost always contained more fat than the ME-formulated diets; preliminary tests of de Groote's system by Farrell and co-workers gave variable results for the economic advantages of the NE system. It can be argued, of course, that the first level of validation of an NE system should be at the biological level, in terms of predictive power; a diet formulated on the basis of NE need not automatically be less costly. The long-term economic benefits would come from more precise formulation of diets. It is, therefore, surprising that more attention has not been given to the de Groote model. It would at least have obviated *ad hoc* adjustments applied to ME by practical nutritionists.

PREDICTION OF ENERGY VALUE BY METABOLIC SIMULATION

Nehring (1967) alluded to the use of ATP (adenosine triphosphate) synthesis and breakdown as measures of the energy value of the diet or its chemical constituents. This principle was described in greater detail by later authors (Schulz, 1975, 1978; Scheele *et al.*, 1976; Livesey, 1984, 1985; Chudy, 1999). Although

Table 12.6. A comparison of net energy values calculated by the de Groote model on the basis of proportions of ingredient as protein, carbohydrate and fat by weight (NE, Equation 3 in the text) or on the basis of proportional energetic contribution to ME (NE_1, Equation 4). The net efficiency associated with NE_1 is tabulated as k_1. The same raw materials, with the same names, are used as in the original paper (de Groote, 1974a).

Ingredient	ME	NE	NE_1	NE-NE_1 difference (%)	k_1
Wheat	12.9	9.4	9.5	–1.0	0.74
Maize	14.4	10.6	10.8	–1.3	0.75
Rye	12.0	8.7	8.8	–0.8	0.74
Barley	11.4	8.4	8.4	–0.0	0.74
Oats	11.0	8.0	8.2	–2.2	0.75
Sorghum	13.9	10.2	10.3	–1.1	0.74
Wheat shorts	8.5	6.0	6.2	–2.7	0.73
Wheat flour middlings	11.1	7.9	8.1	–2.0	0.73
Wheat bran	5.4	3.9	4.0	–3.1	0.73
Wheat white middlings	12.4	9.0	9.1	–1.0	0.73
Tapioca	12.4	9.3	9.3	–0.3	0.75
Rice germ meal	11.5	8.6	9.0	–4.5	0.78
Dried whey	8.0	5.8	5.8	–0.4	0.73
Molasses	8.2	6.0	6.0	–0.0	0.73
Sugar	15.5	11.5	11.6	–0.8	0.75
Groundnut meal	11.1	7.1	7.1	–0.7	0.64
Sesame meal	8.3	5.4	5.5	–2.0	0.66
Fish meal	13.0	8.4	9.0	–6.5	0.69
Soybean meal (440 g kg^{-1} CP)	9.4	6.0	6.1	–0.8	0.65
Soybean meal (500 g kg^{-1} CP)	10.3	6.5	6.7	–1.7	0.64
Sunflower meal	7.2	4.6	4.6	–1.4	0.65
Herring meal	13.4	8.5	9.0	–5.3	0.67
Blood meal	12.0	7.2	7.3	–0.7	0.61
Meat-and-bone scraps	8.5	5.5	5.9	–6.5	0.69
D,L-methionine	14.7	8.8	8.8	0.0	0.60
L-lysine.HCl	11.0	6.6	6.6	0.0	0.60
Dried skim milk	10.5	7.1	7.2	–0.5	0.68
Brewer's yeast	8.7	5.6	5.7	–1.6	0.65
Lucerne (160 g kg^{-1} CP)	4.8	3.2	3.3	–2.9	0.70
Lucerne (180 g kg^{-1} CP)	5.7	3.8	4.0	–3.7	0.70
Lucerne (200 g kg^{-1} CP)	6.3	4.2	4.4	–4.3	0.70
Maize gluten meal (420 g kg^{-1} CP)	11.5	7.5	7.6	–1.8	0.66
Maize gluten meal (620 g kg^{-1} CP)	14.4	9.3	9.5	–2.6	0.66
Hydrocarbon yeast (BP)	10.6	6.5	6.6	–1.6	0.62
Cottonseed meal	7.6	4.8	4.8	–1.0	0.63
Lard	34.0	30.6	30.6	0.0	0.90
Tallow	31.0	27.9	27.9	0.0	0.90
Soybean oil	37.7	33.9	33.9	0.0	0.90

Table 12.7. Relative substitution values of raw materials on the basis of ME and on the basis of net energy calculated by the 2 NE equations (3 and 4) given in the text. NE is based on proportions of protein, fat and carbohydrate by weight, NE_1 on proportions by ME value. Wheat is given a relative value of 100 units as a standard for comparison. The same raw materials, with the same names, are used as in the original paper (de Groote, 1974a). The practical economic importance of using different models of feeding value may lie in the effects on the substitution values of diet ingredients.

Ingredient	ME	NE	NE_1	NE_1/ME
Wheat	100.0	100.0	100.0	1.00
Maize	111.4	113.0	113.4	1.02
Rye	92.9	93.0	92.8	0.99
Barley	88.6	88.9	88.8	1.00
Oats	85.1	85.3	86.3	1.01
Sorghum	107.5	108.3	108.4	1.01
Wheat shorts	65.6	64.2	65.3	1.00
Wheat flour middlings	85.7	84.1	85.0	0.99
Wheat bran	42.2	41.2	42.0	1.00
Wheat white middlings	96.4	95.6	95.7	0.99
Tapioca	96.4	98.8	98.2	1.02
Rice germ meal	89.0	91.1	94.3	1.06
Dried whey	62.0	61.5	61.2	0.99
Molasses	63.6	63.9	63.3	0.99
Sugar	120.1	122.6	122.2	1.02
Groundnut meal	85.7	75.0	74.8	0.87
Sesame meal	64.6	56.9	57.5	0.89
Fish meal	100.6	89.5	94.4	0.94
Soybean meal (440 g kg^{-1} CP)	72.7	64.0	63.9	0.88
Soybean meal (500 g kg^{-1} CP)	80.2	69.5	70.0	0.87
Sunflower meal	55.5	48.7	48.9	0.88
Herring meal	103.5	90.3	94.2	0.91
Blood meal	92.9	76.8	76.6	0.82
Meat-and-bone scraps	66.1	58.9	62.1	0.94
D,L-methionine	113.6	93.4	92.5	0.81
L-lysine.HCl	85.2	70.1	69.4	0.81
Dried skim milk	81.2	75.7	75.3	0.93
Brewer's yeast	67.2	59.2	59.5	0.89
Lucerne (160 g kg^{-1} CP)	36.9	34.2	34.9	0.95
Lucerne (180 g kg^{-1} CP)	43.8	40.5	41.6	0.95
Lucerne (200 g kg^{-1} CP)	48.7	38.8	46.2	0.95
Maize gluten meal (420 g kg^{-1} CP)	89.3	79.6	80.3	0.90
Maize gluten meal (620 g kg^{-1} CP)	111.4	98.6	100.2	0.90
Hydrocarbon yeast (BP)	82.5	68.7	69.1	0.84
Cottonseed meal	59.1	50.6	50.7	0.86
Lard	263.6	324.7	321.9	1.22
Tallow	240.3	296.3	293.4	1.22
Soybean oil	292.2	360.3	356.8	1.22

much of this work was not targeted at avian species, similarities between taxonomic groups are more important than the differences at this level of biological organization. The model of Livesey (1984) calculates energy yield (as ATP) from carbohydrates, fats and proteins which have been absorbed across the gut wall and are available for cellular catabolism. It treats all substances purely as energy sources and therefore corresponds with an ME form of evaluation. In fact, by assuming oxidation of amino acids it simulates the correction of ME to zero nitrogen retention as discussed earlier in the case of practical ME measurements. ATP yields calculated stoichiometrically for individual amino acids, fatty acids, glycerol and glucose were related to gross energy contents of proteins and fats calculated from bond energies to give values for the gross chemical energy corresponding with a molecule of cytoplasmic ATP. Errors in this relationship are potentially large, depending on the stoichiometry selected for mitochondrial oxidative phosphorylation, on the degree of uncoupling of oxidation and phosphorylation, and on the proportion of amino acids oxidized via gluconeogenesis. Taking account of these sources of error reduces the error range in ATP yield to about $\pm 10\%$. Most of the variation in ATP yield from protein was explicable in terms of real differences in heat of combustion depending on amino acid composition. Relatively little of the variation was due to differences in efficiency of ATP generation (compare Schulz, 1975). The residual uncertainty in food energy equivalents of cytoplasmic ATP could probably be reduced by better information about the energy costs of absorption from the gut and translocation across other membranes. A further paper by Livesey (1985) examined the effects of oxidation-phosphorylation uncoupling on the relationships among the energy equivalents of carbohydrate, fat and protein. For a food evaluation system, in which the prime objective is to obtain reliable relative replacement values for ingredients, these scientifically important finer details may not be of critical importance.

A Simulation Model to Predict Dietary Net Energy Yield for Poultry

Previous models suggested for a net energy system have been highly aggregated in chemical terms (Nehring and Haenlein, 1973; de Groote, 1974a). Such systems may have the advantage of simplicity but, with better description of diet composition, we can use more information to predict response. MacLeod (1994), therefore, set out to develop a mechanistically based model of nutrient metabolism as a basis for a net energy type of food evaluation. The design specifications were that:

1. Variations in efficiency of utilization (heat increment) should be taken into account.
2. The system should, wherever possible, be based on the chemical composition of ingredients.
3. It should allow for different rates and compositions of product synthesis.
4. It should avoid incorporating more assumptions than necessary, but should allow for refinement as more information accumulates.
5. It should provide, in the first instance, a reliable standard for substitution of ingredients for one another, i.e. a standard which is correct in *relative* terms. This relative standard should, however, lay the foundation for *absolute* correctness.

A simplified flow diagram of the model is shown in Fig. 12.4. The simulation is structured as a number of independent program units, which can be refined individually without interacting unpredictably with other parts of the program. The model incorporates several empirical relationships predicting whole-animal responses (e.g. food intake, maintenance requirement). Since this is primarily a food evaluation model, the main reason for predicting intake is to ensure that energy intake is within reasonable limits. All the model would be able to do with a large excess of energy above requirements and above its capacity for fat deposition would be to simulate 'burning it off' as heat (regulatory diet-induced thermogenesis). This would be biologically unrealistic because the domestic fowl does not usually exhibit a capacity for regulatory diet-induced thermogenesis.

Whenever possible, the simulation uses experimentally determined values of the digestibilities of chemical entities within the ingredient (Heartland Lysine, 1990; Longstaff and McNab, 1991; Shafey and McDonald, 1991; Zuprizal *et al.*, 1993).

The stoichiometric foundation of the simulation was derived largely from Schulz (1978). Because of the differences between mammalian (urea-excreting, ureotelic) and avian (uric acid-excreting, uricotelic) amino acid metabolism, however, different stoichiometric coefficients were used for amino acid breakdown. The energy cost of uric acid synthesis is accounted for. Amino acid compositions of proteins in body, feathers and egg were compiled from various sources (Lunven *et al.*, 1973; Hakansson *et al.*, 1978; Blair *et al.*, 1981; Nitsan *et al.*, 1981; Hurwitz *et al.*, 1983).

The original information on the existing ingredients was derived largely from analyses at the Roslin Institute (e.g. Blair *et al.*, 1981; McNab and Scougall, 1982), but new ingredients can be added and existing analyses edited by the user. If an amino acid analysis for a new ingredient is not available, the simulation can make estimates from a measurement of crude protein concentration and a statement of the most closely related feedstuff for which a full analysis exists (e.g. another legume species, another grain species). The model predicts how consumed nutrients are partitioned between different biological processes (e.g. body growth, egg production, body maintenance) and how efficiently the nutrients are used, in energy terms. The model has been designed particularly with food evaluation in view, so the prediction of utilization efficiency is given priority in constructing the model. For instance, hierarchies of biological processes (e.g. maintenance, followed by egg synthesis, followed by body growth) are usually imposed, rather than allowing simultaneous competition for resources on the basis of different affinity constants. The exception is to set up a competitive interaction between protein accretion and oxidation in the metabolic fate of amino acids; the comparative affinities of these two processes are summarized by a 'growth potential' term determined by the age and genotype of the bird being simulated. The hierarchies used in the simulation are hypotheses based on the interpretation of published experiments; it is implicit in their use that, functionally if not intellectually, the animal also has priorities in the use of nutrients. Such priorities have developed through natural (or artificial) genetic selection. The physiological control (endocrine, neural, etc.) of these priorities is sometimes known, but is outwith the scope of this model.

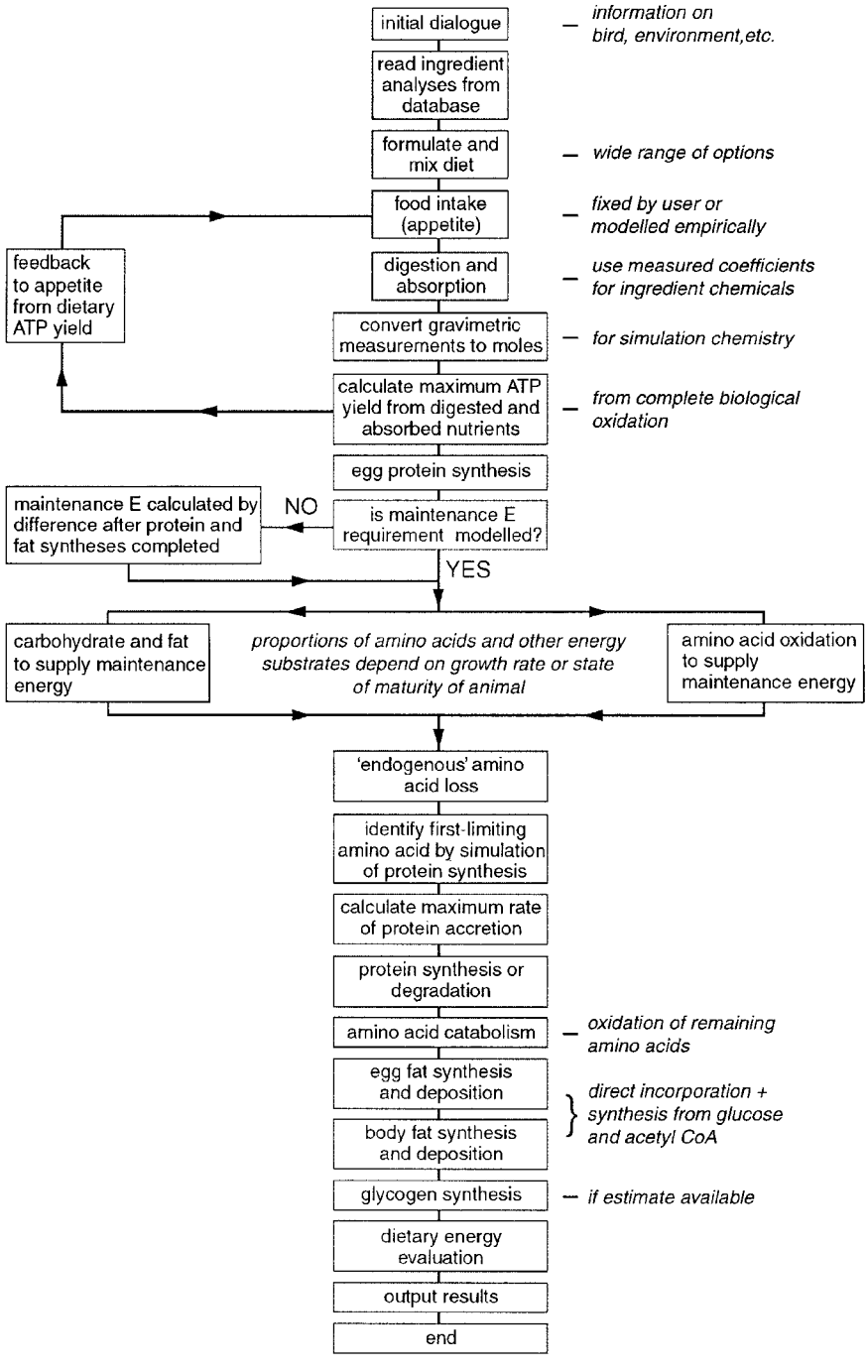

Fig. 12.4. Outline flowchart of the simulation model of MacLeod (1994). The figure shows the key program units and their hierarchical arrangement. Throughout the program, energy utilization and deposition are recorded as the breakdown and synthesis of ATP. This is not explicitly shown in the flowchart, to avoid overloading it with repetitive detail.

One of the effects of building amino acid oxidation into maintenance, even when there is sufficient carbohydrate and fat to satisfy energy requirements, is to simulate a maintenance requirement for protein, removing the need to specify an endogenous loss. It also simulates, or may even explain, a generalized protein requirement over and above that for specific amino acids, which could be ascribed to 'inevitable amino acid catabolism' (Moughan, 1994). The simulation can be used to generate energy values for individual feedstuffs, although it is intended to simulate the use of realistic mixtures. Using single-ingredient values additively may lose some of the advantages of simulation but is still likely to give a better prediction of animal response than ME values used additively. Validation experiments and sensitivity analysis (Table 12.8) of the simulation were briefly described by MacLeod (1994, 1997).

In practice, it may be difficult to demonstrate large differences in net efficiency unless a change occurs in the ratio of fat to protein deposited (Scheele *et al.*, 1985; Huyghebaert *et al.*, 1989). However, this is not an argument against evaluating individual ingredients in terms of their ability to supply net energy; we want a system that gives ingredients their true relative energy value and therefore their true protein:energy ratio.

Table 12.8. Sensitivity of the simulation model of MacLeod (1994) to food intake, maintenance energy requirement and rate of protein accretion. k_g (net efficiency of energy utilization for growth) and NE (net energy per g of food) were predicted while food intake, maintenance energy requirement and rate of protein accretion were varied in turn, with the two other parameters held constant. It is clear that, as in experiment, the net availability of energy is strongly related to the proportion of energy supplied as protein and, particularly, the proportion used for protein synthesis (MacLeod, 1997). The maximum rate of protein accretion permitted by the simulation was similar to the rate recorded in MacLeod (1990) on a similar food intake. The relatively low sensitivity of the simulation model to the selected key parameters, combined with a higher sensitivity to diet composition, suggest that the simulation may be quite robust in its application to feed evaluation.

	Food intake (g)	TME intake (kJ day^{-1})	Maintenance (kJ day^{-1})	Protein retention (g day^{-1})	Proportion dry matter gain	k_g	NE (kJ g^{-1})
Food intake	79	987	525	5.88	0.49	0.732	9.14
varied	92	1149	525	7.39	0.55	0.735	9.18
	105	1311	525	8.74	0.44	0.737	9.20
	118	1474	525	8.74	0.37	0.743	9.28
	131	1636	525	8.74	0.33	0.747	9.33
Maintenance	105	1311	394	8.74	0.38	0.741	9.26
varied	105	1311	460	8.74	0.41	0.739	9.23
	105	1311	525	8.74	0.44	0.737	9.20
	105	1311	591	8.62	0.46	0.734	9.18
	105	1311	656	8.34	0.49	0.733	9.15
Protein retention	105	1311	525	6.68	0.35	0.745	9.31
varied	105	1311	525	7.71	0.39	0.741	9.26
	105	1311	525	8.74	0.44	0.737	9.20
	105	1311	525	8.89	0.44	0.736	9.20
	105	1311	525	8.89	0.44	0.736	9.20

EFFECTIVE ENERGY

Emmans (1984, 1994) has published a well-argued method for estimating the 'Effective Energy' of a diet or ingredient, in which ME is adjusted for the heat increment of feeding by applying linear coefficients to five measurable components of the interaction between the animal and its diet. The coefficients were empirically derived but are broadly interpretable in relation to biological mechanisms. 'Effective energy' is equal to ME less predicted heat increment. The concept, therefore, falls within the category of 'net energy' systems but the term 'effective energy' avoids some of the semantic and logical problems associated with using 'net energy' to describe diets, when it more correctly describes the animal's response. 'Effective energy' can be applied across species, but I will try to draw attention to aspects that are particularly relevant to poultry.

Metabolizable energy (ME) is chosen as the starting point for the effective energy calculation. ME_c (subscript c for conventional) is defined as the gross energy (GE) of the diet less energy losses as faecal energy (FE), urinary energy (UE) and combustible gases (MTHE):

$$ME_c \text{ (kJ day}^{-1}) = GE - (FE + UE + MTHE). \quad (5)$$

The production of combustible gases (largely methane) by poultry is negligible, which allows simplification of the equation to the form used in poultry feed evaluation. Correction of ME to zero nitrogen retention (NR), to give ME_n, is also permissible, corresponding with standard procedure in the case of poultry.

$$\begin{aligned} ME_n(\text{kJ day}^{-1}) &= ME_c - a(6.25\ NR) \\ &= (h_p - a).PR + h_l.LR + H \end{aligned} \quad (6)$$

where PR and LR are the rates of retention of protein and lipid (g day^{-1}), h_p and h_l are the heats of combustion of protein (23.8 kJ g^{-1}) and lipid (39.6 kJ g^{-1}) and H is heat production (kJ day^{-1}). Although poultry excrete uric acid rather than urea, the trans-species nitrogen correction (a) of 5.63 kJ g^{-1} protein retained (35.2 kJ g^{-1} N) agrees closely with the 36.5 kJ g^{-1} N conventionally used for poultry.

The ME obtained from the diet must either be retained in the animal's body or lost as heat. If we know or can predict the performance of the bird in terms of protein and fat retention (carbohydrate deposition being negligible in the long term), prediction of ME requirement needs only a prediction of heat production. Most of Emmans (1994) is concerned with this. Heat production is described as having two components, fasting heat production (FHP) and heat increment of feeding (HIF). FHP is given by:

$$FHP = -(h_p - a).PR - h_l.LR \quad (7)$$

where PR and LR are protein and fat retentions (which are negative in the fasted bird). Maintenance heat production (MH) is calculated with the simplifying assumption that the fasted bird is catabolizing only lipid:

$$MH = FHP - w_u.FUN \quad (8)$$

where w_u (kJ g^{-1} N) is the heat production associated with the synthesis and excretion of urinary N and FUN is urinary nitrogen loss during fasting (g day^{-1}).

Heat increment for maintenance (HIM, kJ day^{-1}), ignoring methane production in the case of poultry, is given by the equation:

$$HIM = w_d.FOM + w_u.UN \quad (9)$$

where w_d (kJ g^{-1}) is heat production associated with the production of faecal organic matter, FOM.

Maintenance ME requirement (MEM, kJ day^{-1}) is given by

$$MEM = MH + HIM \quad (10)$$

Under conditions of positive protein and fat retention (and ignoring methane production),

$$HIF = w_d.FOM + w_u.UN + w_p.PR + w_l.LR \quad (11)$$

(where w_p and w_l are the heat productions associated with protein and lipid deposition, respectively) and ME requirement (kJ day^{-1}) is given by:

$$ME = ER + MH + HIF \quad (12)$$

(where ER is energy retention).

For poultry, the coefficients for the different components of heat increment were estimated from the feeding and comparative slaughter experiments of Hakansson *et al.* (1978).

Effective energy requirement (EERQ, kJ day^{-1}), taking into account the energy contents of protein and lipid, the energy costs of depositing them and the energy cost of nitrogenous excretion was shown to be given by:

$$EERQ = MH + 50PR + 56LR \quad (13)$$

The effective energy of an ingredient can be expressed as:

$$EE = ME_N - w_d.FOM - 0.16\ w_u.DCP + 12.z.DCL \quad (14)$$

where DCL is digestible crude lipid (g g^{-1}) and z is the proportion of retained lipid which comes directly from feed lipid. Although the concept of 'effective energy' is applicable across species and genetic lines, the actual values for raw materials do differ between genotypes and the *de novo* measurement of the effective energy of individual ingredients can be time-consuming (Farrell *et al.*, 1997).

CONCLUSIONS

Poultry industry economics are highly sensitive to relatively small changes in costs and production efficiency. It is, therefore, important to be able to predict as accurately as possible the biological and financial consequences of changes in feed formulation. The intention of this review was to provide a basis for discussion on what type of energy evaluation system can help towards this objective. Are scientific knowledge and techniques at a point where we can now make a soundly based attempt at using a net energy system and is such

a system a worthwhile advance? The cost-effective and biologically supportable formulation of diets depends on the use of a reliable and accurate feed-evaluation system. In its simplest form, such a system should be reducible to two lists for each nutrient or resource, one of requirements and one of bioavailability from a list of ingredients. The adoption of such a simple scheme carries the penalty of ignoring the existence of between-ingredient interactions when mixtures are fed. Hesitancy in the commercial uptake of net energy (NE) models is worth mentioning. Because of the potential for biochemical interactions, the degree of linear additivity of NE values of individual ingredients is often mentioned as a barrier to their use in feed formulation. However, if ME values are linearly additive, while NE values are not, it can only be because ME does not detect the interactions which are seen as complicating a NE system. Emmans (1994) appears to have chosen his words carefully in stating that 'As effective energy values … are additive *to the extent that ME values are additive*, they can be used to formulate diets using linear programming' (my italics). The same might well be said for other NE systems: even if the additivity is not perfect, prediction of animal response might still be more accurate than with a system (such as ME) that takes little account of post-absorptive metabolism. A possible criticism is that NE is a property not of the food but of the bird's response to the food. This is clearly true, but must be true of any meaningful feed evaluation system. The accessibility of computer facilities encourages the suggestion that mathematical simplicity should not take priority over predictive power and biological consistency. A system with a degree of complexity can still be easy to use if it is well-programmed and well-documented. Also, the greater the potential control over diet composition, the more likely it is that complexity in diet description and analysis of bird response will be worthwhile: poultry, and poultry diets, meet this criterion well.

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PART IV
Factors influencing nutritive value

CHAPTER 13
Non-starch polysaccharides: effect on nutritive value

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INTRODUCTION

The 'fibre' component of monogastric feed has received enormous attention in recent years due partly to the finding that soluble non-starch polysaccharides (NSP) have anti-nutritive effects on nutrient digestion and absorption in poultry (Antoniou *et al.*, 1981; Choct and Annison, 1990; Bedford *et al.*, 1991). The negative correlation between NSP of the diet and its nutritive value has been clearly demonstrated in poultry (Choct and Annison, 1990; Annison, 1991), in pigs (King and Taverner, 1975) and in cats and dogs (Earle *et al.*, 1998). Another important factor influencing this increased research output is the use of glycanases in monogastric diets to enhance animal performance. There is an urgent need to develop a clearer understanding of the published data on the effect of NSP on nutritive value and to apply this knowledge in practical feed formulations.

This chapter discusses: (i) the anti-nutritive effects of NSP in chickens and their relationship with structures of the polysaccharides; and (ii) the possibility of using NSP-related characteristics of feed to predict its nutritive value for poultry.

THE QUALITY AND QUANTITY OF NSP IN FEEDS

There is a great deal of confusion in the feed and food industries in terms of the terminology and classification used to describe the 'fibre' in plant ingredients. The terms include fibre, dietary fibre (DF), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), pentosans, hemicellulose, gums and mucilage and so on. These all describe one of two nutrient entities: a fraction of NSP or that fraction plus lignin, a polyphenolic compound. Polysaccharides present in feedstuffs include starch and NSP. The term NSP covers a large variety of polysaccharide molecules. The term CF is still widely used to describe the remnants of plant material after extraction with acid and alkali and it includes variable portions of the insoluble NSP. NDF is composed of the insoluble portion of the NSP plus lignin, whereas ADF refers to a portion of insoluble

 Poultry Feedstuffs: Supply, Composition and Nutritive Value (eds J.M. McNab and K.N. Boorman)

NSP comprised largely, but not exclusively, of cellulose and lignin. Hemicellulose refers to the non-cellulosic component of NSP whereas gum and mucilage represent various types of soluble NSP.

In recent years, the term NSP has been widely adopted, in particular, in the monogastric industry to cover all polysaccharide molecules excluding starch. The classification of NSP is shown in Fig. 13.1.

Different ingredients contain not only different amounts of soluble and insoluble NSP, but also the structure and physiochemical characteristics of the NSP differ widely. This is of paramount importance to the selection of enzymes for improving the nutritive value of the compound feeds. Table 13.1 shows the types and amounts of NSP present in cereal grains and their by-products.

The constituent sugars for the NSP in leguminous ingredients are similar to those found in cereal grains. However, they are not necessarily linked in the same way as in cereals. Most legumes contain pectic polysaccharides as their main NSP, but these polymers also differ widely in terms of their molecular structures. For example, the major NSP in soybean are pectic polysaccharides, more precisely polyrhamnogalacturonans, arabinogalactans and xylogalacturonan with various side chains (Voragen *et al.*, 2001), whereas in canola the occurrence of β-(1–3,6)-linked galactose polymers, which are free of β-(1–4)-linked galactose residues, as part of the pectic polymers has been reported (Siddiqui and Wood, 1972). The amounts of soluble and insoluble NSP including separate cellulose values and monosaccharide composition, and starch in selected Australian legumes are shown in Table 13.2. The amount of NSP also differs widely within a species due to cultivars and growing conditions. Table 13.3 shows some published data on the soluble and insoluble NSP levels for selected European leguminous crops.

What is important from a nutritional point of view is the understanding of the vastly different effects of soluble and insoluble NSP on nutrient digestion and absorption in monogastric animals. The classic system of 'fibre' analysis is not capable of providing vital information on the physicochemical characteristics of NSP and subsequent effects on digestion and absorption.

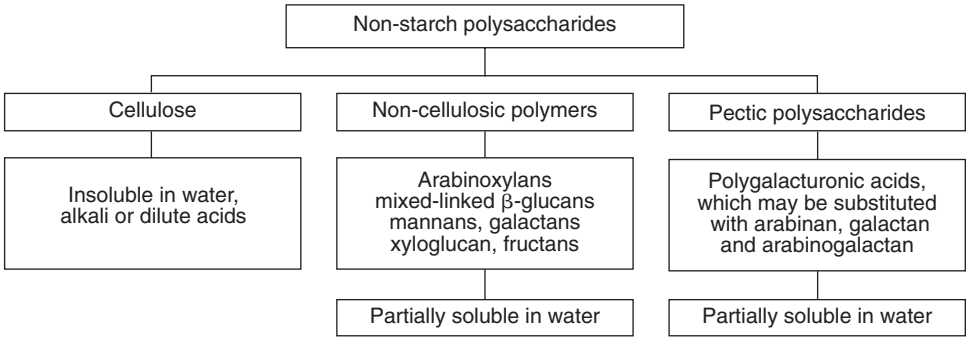

Fig. 13.1. Classification of non-starch polysaccharides.

Table 13.1. The types and levels of NSP present in some cereal grains (% dry matter).

Cereal		Arabinoxylan	β-Glucan	Cellulose	Man	Gal	Uronic acid	Total
Wheat[a]								
	Soluble	1.8	0.4		t	0.2	t	2.4
	Insoluble	6.3	0.4	2.0	t	0.1	0.2	9.0
Barley[a]								
	Soluble	0.8	3.6		t	0.1	t	4.5
	Insoluble	7.1	0.7	3.9	0.2	0.1	0.2	12.2
Rye[a]								
	Soluble	3.4	0.9		0.1	0.1	0.1	4.6
	Insoluble	5.5	1.1	1.5	0.2	0.2	0.1	8.6
Triticale[b]								
	Soluble	1.3	0.2		0.02	0.1	0.1	1.7
	Insoluble	9.5	1.5	2.5	0.6	0.4	0.1	14.6
Sorghum[b]								
	Soluble	0.1	0.1		t	t	t	0.2
	Insoluble	2.0	0.1	2.2	0.1	0.15	t	4.6
Maize[b]								
	Soluble	0.1	t		t	t	t	0.1
	Insoluble	5.1		2.0	0.2	0.6	t	8.0
Rice (pearled)[b]								
	Soluble	t	0.1		t	0.1	0.1	0.3
	Insoluble	0.2		0.3	t	t	t	0.5

[a]Englyst (1989); [b]Choct *et al.*, unpublished data.
t, trace amount.

ANTI-NUTRITIVE ACTIVITIES OF SOLUBLE NSP IN POULTRY DIETS

The adverse effects of soluble NSP on nutrient digestion and absorption in monogastric animals, especially in poultry, are due to their ability to increase the viscosity of the digesta, to modify the physiology of the gastrointestinal tract and to change the ecosystem of the gut. The net effects may include altered intestinal transit time, increased endogenous losses of nutrients, and a change of nutrient digestion and absorption patterns, i.e. enzymatic digestion vs. microbial fermentation.

Viscosity

The viscosity of NSP depends on their solubility, molecular weights, and configurations in solution. Solubility of NSP, in turn, depends on their chemical structures and association with other cell wall components. Viscosity, however, is not specific to the sugar composition or linkage types present in the NSP. Furthermore, the physical effect of viscosity at a given level on nutrient digestion and absorption also appears to be similar regardless of the NSP sources. Generally, high gut viscosity is related to poor bird performance. It is thought that soluble NSP interact with the glycocalyx of the intestinal brush border and thicken the rate-limiting

Table 13.2. The types and levels of carbohydrate polymers and monomers in selected legumes (% dry matter).

Sample	Starch	Total NSP	Cellulose	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose	U.Ac[a]
Soybean[b]											
Soluble		2.7	–	0.1	t	0.5	0.1	0.2	0.6	0.2	1.1
Insoluble		16.5	4.4	0.2	0.3	2.4	1.7	0.7	3.9	0.3	2.5
Total	1.0	19.2	4.4	0.3	0.3	2.9	1.8	0.9	4.5	0.5	3.6
Angustifolius lupin[c]											
Soluble		4.6	–	0.3	t	t	t	t	3.3	0.1	0.8
Insoluble		32.0	1.2	0.5	t	3.9	0.7	0.1	22.9	0.2	2.5
Total	0.4	36.6	1.2	0.8	t	4.9	0.7	0.1	26.2	0.3	3.3
Faba beans[c]											
Soluble		0.62	–	0	0	0.16	0.07	0.16	0.15	0.12	ND
Insoluble		12.36	ND	0	0	2.65	1.75	0	0.74	8.68	ND
Total	37.4	12.98	–	0	0	2.81	1.82	0.16	0.89	8.80	–
Chickpeas[c]											
Soluble		0.88	–	0.09	0	0.21	0.15	0.15	0.27	0.13	ND
Insoluble		14.59	ND	0.07	0	3.81	1.32	0.44	0.70	9.99	ND
Total	32.2	15.47	–	0.16	0	4.02	1.47	0.59	0.97	10.12	–
Field peas[c]											
Soluble		0.51	–	0.03	0	0.16	0.04	0.12	0.12	0.10	ND
Insoluble		15.85	ND	0	0	5.69	2.05	0	0.76	9.28	ND
Total	40.0	16.36	–	0.03	0	5.85	2.09	0.12	0.88	9.38	–
Mungbean[c]											
Soluble		0.70	–	0.01	0	0.14	0.06	0.09	0.18	0.17	ND
Insoluble		8.17	ND	0	0	2.00	1.91	0.06	0.41	3.78	ND
Total	45.8	8.87	–	0.01	0	2.14	1.96	0.15	0.59	3.95	–

[a]Uronic acids; [b]From Irish and Balnave (1993); [c]Choct *et al.*, unpublished data. ND = not determined.
t, trace amount.

Table 13.3. Soluble, insoluble and total NSP contents of some European grain legumes (% dry matter).

Legume	Soluble NSP	Insoluble NSP	Total NSP	Reference
Chick peas	3.3	7.4	10.7	Englyst and Cummings, 1988
Field peas	2.5	32.2	34.7	Graham and Åman, 1987
Navy bean	5.7	1.7	17.4	Chang *et al.*, 1989
Pinto bean	6.3	13.1	19.4	Chang *et al.*, 1989
Rapeseed	11.3	34.8	46.1	Carré, 1990b

unstirred water layer of the mucosa, which reduces the efficiency of nutrient absorption through the intestinal wall (Johnson and Gee 1981). The enzymes cleave the large molecules of NSP into smaller polymers, thereby reducing digesta viscosity and increasing the nutritive value of the feed (Bedford *et al.*, 1991; Choct and Annison, 1992a). In the work of Bedford *et al.* (1991), the relationship between gut viscosity, molecular weight of NSP and performance of birds fed rye was clearly demonstrated. Choct and Annison (1992b) depolymerized an isolate of wheat arabinoxylan (mol.wt 758,000 Da) using a xylanase *in vitro* so that its viscosity was reduced fourfold. When these depolymerized NSP were included in broiler diets, they did not exhibit strong anti-nutritive effects on nutrient digestion compared with intact NSP included at the same level (Table 13.4). However, there are occasions where enzymes elevate digesta viscosity in the ileum as well as AME of the diet (Choct, 1998). In a recent work, we (Waters *et al.*, unpublished data) examined three different glycanases on their effect on the AME of a normal wheat and a low-ME wheat in 3–4-week-old broilers. As shown in Table 13.5, all three enzymes increased AME values of both wheats, especially that of the low-ME wheat, although their effect on digesta viscosity was vastly different, with Enzyme B increasing digesta viscosity in the duodenum, jejunum and ileum. Further studies are certainly required in this area of work.

Table 13.4. Effect of adding soluble NSP, depolymerized NSP or pentoses (15 g arabinose plus 15 g xylose kg^{-1}) to broiler diets on weight gain (WG), FCR, feed intake (FI), digestibility of starch and nitrogen in the ileum, and apparent metabolizable energy (AME) (n=8).

Diet	WG (g $week^{-1}$)	FCR (g g^{-1})	FI (g $week^{-1}$)	Starch (%)	N (%)	AME (MJ kg^{-1} DM)
Control	430[a]	1.589[a]	681[a]	98[a]	69[ac]	16.13[a]
3% Intact NSP	325[b]	1.960[b]	622[a]	92[b]	59[b]	14.53[b]
3% Pentoses	394[ac]	1.695[ac]	658[a]	98[a]	72[a]	16.23[a]
3% DepolNSP[1]	404[ac]	1.649[a]	661[a]	94[ab]	69[ac]	15.74[a]
Pooled SE	24	0.087	26	2	3	0.28
F-value	2.95	3.70	0.61	3.85	5.14	9.78
Significance	0.05	0.01	ns	0.05	0.01	0.001

[1]Depolymerized soluble NSP from wheat.
[abc]Means within a column followed by the same letters do not differ at $P<0.05$.
Choct and Annison (1992b).

Table 13.5. Duodenal, jejunal and ileal viscosity of broiler chickens fed diets containing normal wheat and low-ME wheat with or without enzymes.

Wheat	Enzyme	Duodenum cP	Jejunum cP	Ileum cP	AME of wheat MJ kg^{-1} DM
Normal-ME wheat	Control	3.29 ± 0.46 [c]	8.00 ± 1.24 [c]	23.3 ± 4.0 [bc]	13.65 ± 0.50 [ab]
Normal-ME wheat	Enzyme A	2.16 ± 0.31 [d]	4.06 ± 0.65 [d]	6.8 ± 1.1 [c]	14.50 ± 0.41 [a]
Normal-ME wheat	Enzyme B	3.59 ± 0.46 [bc]	12.25 ± 2.38 [b]	39.7 ± 6.7 [b]	13.94 ± 0.91 [ab]
Normal-ME wheat	Enzyme C	2.32 ± 0.61 [d]	3.33 ± 0.80 [d]	7.7 ± 4.2 [c]	14.15 ± 0.71 [ab]
Low-ME wheat	Control	3.99 ± 0.77 [b]	9.44 ± 0.81 [c]	28.3 ± 5.8 [b]	12.66 ± 0.59 [c]
Low-ME wheat	Enzyme A	2.41 ± 0.36 [d]	5.15 ± 1.75 [d]	8.3 ± 2.7 [c]	13.62 ± 0.92 [ab]
Low-ME wheat	Enzyme B	4.65 ± 0.61 [a]	18.35 ± 4.53 [a]	84.1 ± 42.6 [a]	13.28 ± 0.44 [bc]
Low-ME wheat	Enzyme C	2.23 ± 0.58 [d]	3.43 ± 0.61 [d]	7.2 ± 2.0 [c]	13.93 ± 0.83 [ab]
Source of variance		Probability of greater *F* value in analysis of variance[1]			
Wheat		***	***	**	***
Enzyme		***	***	***	***
Wheat*Enzyme		NS	***	***	NS

[a,b,c,]Values with unlike superscripts differ significantly ($P < 0.05$).
[1]** $P < 0.01$, *** $P < 0.001$.

NSP and Gut Microflora

The small intestine of the chicken is usually colonized by facultative anaerobes whereas the caeca are dominated by strict anaerobes (Salanitro *et al.*, 1978). Choct *et al.* (1996) recently demonstrated that diets rich in soluble NSP markedly elevated fermentation in the small intestine of broilers and that subsequent depolymerization of the soluble NSP *in vivo* using glycanases largely overcame this problem. It is not known whether a sudden change of gut ecology is detrimental to the efficiency of nutrient utilization. In a recent study, we (Choct and Tukei, unpublished data) found that addition of 3% soluble NSP in a control diet resulted in a significant loss of energy as heat and as volatile fatty acid (VFAs) in the excreta. When the NSP were depolymerized using a xylanase, the AME and net energy (NE) were increased by 29.1% and 37.3%, and heat production and energy loss as VFAs were decreased by 11% and 61%, respectively. It is interesting to note that the increases in AME and NE were not proportional, indicating that NSP not only interfere with digestive processes, but also have strong negative effects on availability of energy and nutrients to the animal. This is perhaps not surprising considering that: (i) feeding of barley, a grain rich in soluble NSP, to chickens can lead to increased incidence of necrotic enteritis and elevated numbers of *Clostridium perfringens* in the ileum (Hofshagen and Kaldhusdal, 1992; Kaldhusdal and Hofshagen, 1992); and (ii) anaerobes can deconjugate bile salts which are essential for the digestion of fat (Krogdahl, 1986).

It is understood that utilization of nutrients through microbial conversion of digestible carbohydrates, such as starch, to volatile fatty acids is less efficient than direct absorption of glucose released from enzymatic digestion (Müller *et al.*, 1989; Carré *et al.*, 1995). Even in ruminant animals, use of starch as glucose is 42% more

efficient than as VFAs (Feedlot Manual, 1997). Another factor that may contribute to the negative effect of a high microbial load in the small intestine is increased turnover of intestinal cells. According to LeBond and Walker (1956), a 100 g rat gaining 5 g day^{-1} synthesizes 1 g mucosal cells daily, which represents a 20% additional tissue synthesis without showing up as weight gain. Extrapolating this to a 2 kg bird gaining 60 g daily, the bird would synthesize 12 g of mucosal tissue to maintain the integrity of its small intestine. Increased microbial load can exacerbate this loss (Abrams *et al.*, 1963; Lesher *et al.*, 1964), since some microbial fermentation products, e.g. putrescine, have been shown to significantly enhance small intestinal and colonic mucosal growth rates (Seidel *et al.*, 1985; Osborne and Seidel, 1989). Furthermore, antibiotics can significantly improve performance of birds fed high-NSP diets (MacAuliffe and McGinnis, 1971; Misir and Marquardt, 1978a,b). Whatever the reason, excess fermentation in the small intestine of the monogastric animals may be detrimental to nutrient digestion and absorption, and enzyme supplementation is able to alleviate this problem. Perhaps, reducing the viscosity of the digesta in the small intestine hastens digesta passage and nutrient digestion rate (through removal of the diffusional constraint of viscous gums), thereby giving less substrate and time for the fermentative organisms to proliferate. This may in turn restore the normal and efficient digestion (enzymatically) of starch and protein in the small intestine. Interactions between nutrition and animal welfare will be an important consideration for the future livestock industry (McNab, 1998) as fewer, if any, antibiotics will be allowed to be used as feed additives. Therefore improvement of gastrointestinal health without the use of antibiotics will be of immense interest to producers and consumers alike.

NSP and Endogenous Losses

The soluble NSP can not only act as a physical barrier to nutrient digestion and absorption by increasing gut viscosity, but also change gut functions by modifying endogenous secretion of water, proteins, electrolytes and lipids (Johnson and Gee, 1981; Angkanaporn *et al.*, 1994). In the case of protein losses, the study of Angkanaporn *et al.* (1994) clearly demonstrated that endogenous losses of amino acids in chickens were significant even with relatively low levels (1.5%) of soluble NSP in the diet. At higher inclusions (3%), the true amino acid digestibility was affected. Furthermore, viscous NSP can enhance bile acid secretion and subsequently result in significant loss of these acids in the faeces (Ide *et al.*, 1989; Ikegami *et al.*, 1990). In addition to the modification of the gut physiology, certain NSP can also bind bile salts, lipids and cholesterol (Vahouny *et al.*, 1980, 1981). The net effect may be an altered lipid metabolism in the intestine, resulting in increased hepatic synthesis of bile acids from cholesterol to re-establish the composite pool of these metabolites in the enterohepatic circulation. The continued 'drain' of bile acids and lipids by sequestration, and increased elimination as faecal acidic and neutral sterols, may ultimately influence the absorption of lipids and cholesterol in the intestine. These effects could lead to major changes in the digestive and absorptive dynamics of the gut, with consequent poor overall efficiency in nutrient assimilation by the animal.

Although the anti-nutritive effect of soluble NSP has been discussed under three separate headings, it should be borne in mind that these mechanisms are all interrelated. They are all dependent on the polymeric nature of the NSP because once the polymers are cleaved into smaller fragments, their anti-nutritive activity is largely eliminated.

THE ROLE OF INSOLUBLE NSP IN POULTRY DIETS

Excreta Consistency

The insoluble NSP make up the bulk of the total fibre in diets, but they have little or no effect on nutrient utilization in monogastric animals (Carré, 1990a). Begin (1961) showed no detrimental effect, other than simple nutrient dilution, when up to 21% of cellulose was added to poultry diets. The insoluble NSP, however, are not inert and their roles in monogastric nutrition should not be underestimated. One of the most important attributes of insoluble NSP is their ability to absorb large amounts of water and maintain normal motility of the gut (Stephen and Cummings, 1979). This is essential for the consistency of the excreta in monogastric animals.

Small Intestinal Transit Rate

Elevated levels of insoluble fibre in the diet shorten the residence time of digesta (Kirwan *et al.*, 1974) and some argue that this may lead to a lower nutrient digestibility. The rationale is that the longer the feed is exposed to the digestive processes in the gut, the more complete its digestion. This, however, may not be true under all circumstances. As discussed earlier, soluble NSP can increase gut viscosity and slow digesta transit time in chickens, which allows the proliferation of fermentative organisms in the small intestine in a detrimental manner (Choct *et al.*, 1996). Depolymerization of the soluble NSP with enzymes can greatly increase the digesta passage time (Choct *et al.*, unpublished data). It is believed that when gut viscosity is decreased and nutrient digestion and absorption are enhanced, the indigestible feed materials pass through the gut quickly and insufficient time is available for anaerobic microflora to establish in the upper part of the gut. In Australia, some wheats have very low apparent metabolizable energy values when fed to broiler chickens (Mollah *et al.*, 1983). The poor ME values are due to increased levels of soluble NSP in the wheats (Annison, 1991; Choct *et al.*, 1995). Rogel *et al.* (1987) demonstrated that adding coarsely ground oat hulls (more than 90% NSP, of which 99% is insoluble) to low-ME wheat diets largely ameliorated poor nutritive quality of the wheats. The effect of the oat hulls on digesta transit time was demonstrated with addition of 10% oat hulls increasing the rate of digesta passage significantly (Rogel, 1985). Fine-grinding of the oat hulls, however, rendered them ineffective. In studies with humans, a coarse wheat bran preparation significantly shortened the digesta transit time and showed beneficial effects on colonic function, whereas a fine bran preparation was completely without effect

(Kirwan *et al.*, 1974). These authors demonstrated that the beneficial effect of bran was dependent on the water-holding capacity, which is a function of particle size. Thus, milling the coarse bran to a particle size of 1 mm almost halved the water-holding capacity from 6.15 g to 3.54 g of water per g of bran. It is possible that the effectiveness of the coarse fibre was due to its ability to hold large amounts of water, thereby preventing increased solublization of NSP. The net effect was an increased rate of digesta passage, giving little time for fermentative organisms to establish in the gut, especially in the small intestine. This highlights the possibility that perhaps the relative proportions of soluble and insoluble fractions influence the anti-nutritive effect of the soluble NSP on bird performance.

Cannibalism in Laying Hens

Cannibalism is considered as a major problem in layers kept in open-sided, conventional sheds, in barns or free range systems, in particular, when the birds are not beak-trimmed. In a recent study, Hartini *et al.* (2001) reported that diets high in insoluble NSP were very effective in preventing the onset of cannibalism in laying hens. It was hypothesized that the bulking nature of fibre and its subsequent effect on digesta transit rate may be responsible for this.

PREDICTION OF THE NUTRITIVE VALUE OF GRAINS FROM THEIR NSP CHARACTERISTICS

NSP Contents

The NSP content is a major determinant for the nutritive value of grains for monogastric animals. Carré (1990a) advocated the use of prediction equations based on water-insoluble cell wall content, i.e. insoluble NSP, of raw ingredients. He noted that consistent equations existed for adult birds but prediction of energy of ingredients for use in broiler diets continued to be problematic. To date it is difficult to use NSP values as a predictor of the nutritive value of poultry feed. There are two major reasons for this: firstly, the NSP assay is tedious and complicated, and, hence, is not ideal for use in quality-control laboratories; secondly, the relationship between soluble NSP content and nutritive value obtained on one ingredient may not be applied to another ingredient. Thus it is difficult to use a universal equation for compound feed. This is because the effect of the NSP depends on their molecular size and structure, which cannot be determined using quantitative measurements such as fibre analysis.

Extract Viscosity

A ground sample of ingredients or compound feed can be extracted in water or a buffer for extract viscosity measurement. The viscosity of the extract comes predominantly from soluble NSP in the feed. Since soluble NSP content

of a diet is related to its nutritive value, it is possible that extract viscosity can be used to predict nutritive value. Indeed, a number of studies have demonstrated a strong correlation between soluble NSP and extract viscosity (Izydorczyk *et al.*, 1991; Saulnier *et al.*, 1995). Furthermore, the growth responses of chickens fed barley diets were accurately predicted by an extract viscosity method (Rotter *et al.*, 1989). Choct *et al.* (1993) developed an extract viscosity assay to distinguish wheats with very low AME from normal samples. More systematic studies with promising outcomes have since been reported on the use of extract viscosity as a predictor of nutritive value for poultry (Carré and Melcion, 1995; Wiseman and McNab, 1995; Choct and Hughes, 1997; Dusel *et al.*, 1997, 1998).

The extract viscosity method is simple and rapid. More importantly, it gives qualitative information on the characteristics of the NSP. However, the reliability of the assay may be influenced by the extraction method and the endogenous enzyme activities in the diet or the individual ingredient.

Gut Viscosity

The relationship between gut viscosity and the nutritive value of barley in poultry was first described by G.S. Burnett in 1966. It is only during the past 10 years that the importance of his work has been recognized, and a wealth of information on viscosity of the gut contents and its effect on nutrient digestion and absorption has emerged (Bedford *et al.*, 1991; Bedford and Classen, 1992; Choct and Annison, 1992a; Steenfeldt *et al.*, 1998). Whether gut viscosity can be used to predict nutrient digestibility depends on a number of factors. Firstly, the section of the intestine where the digesta sample is taken will influence the viscosity value, because nutrients are digested and absorbed as digesta moves down the gut, thus leaving the indigestible portion, mainly NSP, to accumulate. On a relative basis, the soluble NSP content per ml of digesta supernatant therefore goes up; secondly, digesta viscosity decreases as the bird gets older (Dusel *et al.*, 1998; Steenfeldt *et al.*, 1998). This also relates to the finding that older birds cope better with low-ME wheats (Rogel *et al.*, 1987) or with barley (Salih *et al.*, 1991). In addition, the relationship between gut viscosity and bird performance is not always apparent (Wiseman and McNab, 1995). For example, Hughes and Zviedrans (1999) reported that two groups of birds fed wheat had ileal viscosity values (mPa s) of 12.5 and 49, but the AME contents (MJ kg^{-1} DM) for the wheat were 10.6 and 12.0. This, however, does not necessarily mean that the viscosity has no effect on nutrient digestion and absorption. Rather, it highlights the complex nature of interaction between digesta viscosity and gut microflora. It is hypothesized that as the bird ages, its gut microflora becomes more established and it can adapt to the environment, e.g. it can cope with the viscous digesta better by producing small amounts of NSP-degrading enzymes. In the case of short-term studies, the sudden introduction of diets high in soluble NSP causes drastic changes to the gut microflora, leading to proliferation of fermentative organisms in the small intestine, especially in the

ileum. The consequences of such a change have been discussed earlier in this paper, but it is worth reiterating that some of these organisms may also produce enzymes capable of degrading some NSP, thus resulting in reduced viscosity as noted in the study of Hughes and Zviedrans (1999). All in all, it is difficult to predict nutritive value of a wide range of ingredients used in poultry diets from gut viscosity.

CONCLUSION

The type and amount of NSP differ widely depending on ingredients, hence their nutritive/anti-nutritive activities in chickens. Generally, only soluble NSP elicit anti-nutritive activities in poultry diets, which are closely related to their polymeric nature and ability to increase digesta viscosity. Extract viscosity has great potential to be used as a simple and rapid method to predict the nutritive value of poultry diets. Further studies on NSP need to address their effect on nutrient utilization in terms of relative efficiency of enzymatic digestion vs. fermentation, and of muscle growth vs. gut cell turnover.

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CHAPTER 14
Secondary plant metabolites in poultry nutrition

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SECONDARY PLANT METABOLITES

In addition to the macro- and micronutrients contained in plant products used in human and animal foods, there are many substances that, while not serving as nutrients themselves, do, or may, have either a beneficial or adverse effect on the animal or human (Table 14.1). The roles of the macro- and micronutrients in poultry nutrition have been exhaustively studied over many decades, and it is quite right that these should have been and still are the main focus of avian nutritional research, since they account for most of the cost of poultry production. However, as knowledge of nutrition has progressed and become disseminated throughout the highly competitive industry, smaller and smaller increments of performance have assumed more importance. It may well be that the effects of some of the secondary plant metabolites will provide a key to small, but significant, advantages that will prove worthwhile to the poultry industry. Many of the secondary plant metabolites that have gained the interest of nutritionists are those that have become known as anti-nutritional substances or anti-nutritional factors; materials that should be avoided or eliminated, or whose effects must be ameliorated. However, even among the substances that are relegated to this category, it is increasingly found that, in appropriate circumstances,

Table 14.1. Common secondary plant metabolites.

With potentially adverse effects	With potentially beneficial effects
Protease inhibitors	Allicin and thiosulphinites
Lectins	Phyto-oestrogens
Tannins	Saponins
Phytate	Glucosinolates
Glucosinolates	Lectins
Saponins	
Alkaloids	
Cyanogenic glycosides	
Phyto-oestrogens	

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they may also have beneficial effects, for example the potential anticarcinogenic effects of glucosinolates (Nugon-Baudon and Rabot, 1994) and phyto-oestrogens (Rickard and Thompson, 1997), the antioxidative and antiatherogenic effects of allyl thiosulphinate and disulphide (Eilat *et al.*, 1995) and the potential coccidiostatic action of artemisin (Allen *et al.*, 1997). Whilst these potentially beneficial effects have been studied mainly with human health in mind, it is quite possible that some of these substances may be of value in poultry nutrition as well.

The most common anti-nutritional secondary plant metabolites are well known, and are either avoided or rendered harmless by processing. Nevertheless, it is prudent to maintain an awareness of these substances and to continue to build on our knowledge of them. Whilst they may be well known in their familiar milieu, it has been known for anti-nutritional substances to arise quite naturally, yet unexpectedly. For example, a letter to *Food Chemistry and Toxicology* (Kirschman and Suber, 1989) describes cases of food poisoning of humans that occurred following consumption of traditionally bred squash or courgettes. The poisoning is thought to have been caused by abnormally high concentrations of cucurbitacin, as a result of either a mutation or of chance pollination from wild bitter species of cucurbits.

Those properties of secondary plant metabolites that confer adverse effects, when consumed by animals, in many instances also provide the plant with resistance to predatory insects and animals and plant pathogens (Fig. 14.1). For that reason these substances are obvious substances to consider when seeking 'natural' means of protecting plants, thereby reducing agrochemical use by building insecticide resistance into crop plants. It is very unlikely that a toxin or anti-nutrient would arise inadvertently during genetic manipulation of plants; overtly toxic substances are hardly likely to be used in transgenic crops. Nevertheless it is important to bear in mind that even apparently innocuous substances such as

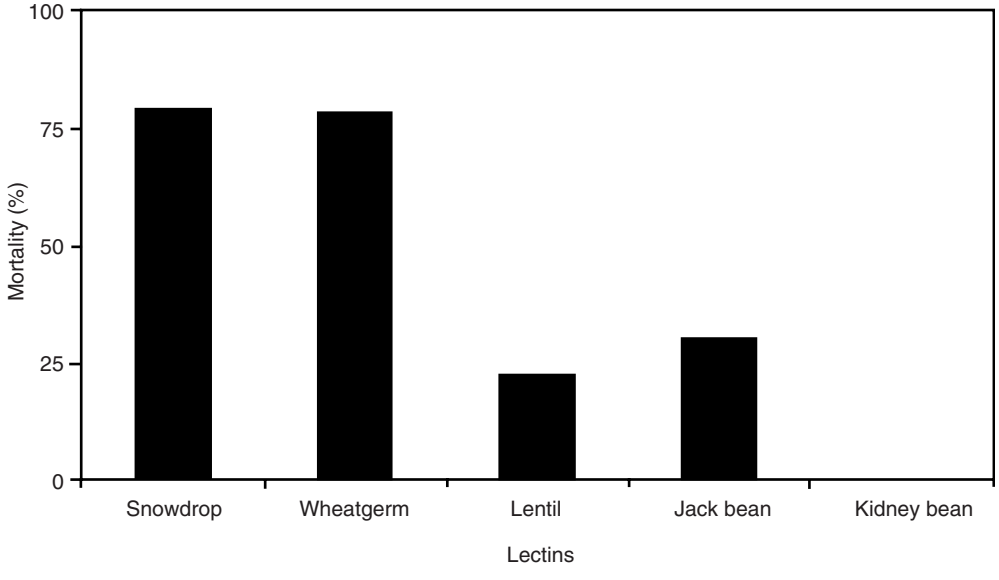

Fig. 14.1. Effect of lectins on brown plant hopper mortality (adapted from Gatehouse *et al.*, 1995).

wheat germ agglutinin, which is normally present at about 300 mg kg^{-1}, if incorporated at insecticidal levels (7000 mg kg^{-1}) will produce pancreatic enlargement, inefficient utilization of dietary protein, thymus atrophy and depressed growth in experimental animals (Pusztai *et al.*, 1995).

Much of what is known of the digestion, absorption and metabolism of the macro- and micronutrients has been gained from studies in non-avian species, but often that knowledge can be applied with relatively little modification to the nutrition of birds. In the case of the non-nutrient secondary plant metabolites, not only has there been less experimental study of their effects in birds, but it is quite clear that in some instances the responses of birds may be quite different from that experienced by other animals. A probable instance of a species difference in susceptibility to the secondary plant metabolite, coniine, is given in the descriptions of the Exodus in the 13th century BC. In the biblical Book of Numbers (Chapter 11, verses 31–33) we read the dire consequence for the Hebrews: '*And there went forth a wind from the Lord, and brought quails from the sea, and let them fall by the camp … And the people … gathered the quails. … And while the flesh was yet between their teeth, ere it was chewed, the wrath of the Lord was kindled against the people, and the Lord smote the people with a very great plague.*' Sergent (1941) offers historical and experimental evidence to suggest that the 'very great plague' occurred after the Hebrews ate quails which, during their northern migration, had been feeding on hemlock seeds. Coniine (Fig. 14.2), the toxic alkaloid in hemlock, is acutely poisonous to humans but quails are able not only to adapt to and accommodate coniine in whole seed, but are also able to accumulate the toxin in body tissue at levels that are toxic to mammals. Thus it is clear that any extrapolation to avian species of knowledge of the effects of secondary plant metabolites gained from mammals must be undertaken with caution.

The tropical legume *Leucaena leucocephala*, is known as ipil ipil in the Philippines where ipil ipil meal is included in poultry rations with no obvious ill effects. Yet the non-nutrient amino acid, mimosine (Fig. 14.3) that is present in *Leucaena* is held responsible for poor growth of cattle consuming high levels of *Leucaena* herbage and whose rumen microflora are not adapted to mimosine detoxification. However, when the animals were inoculated with rumen microflora from Hawaiian cattle the adverse effects were eliminated. Because of its effect on hair follicles, mimosine has even been used in 'chemical shearing' experiments.

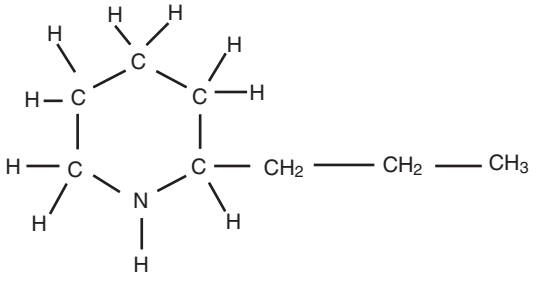

Fig. 14.2. Coniine.

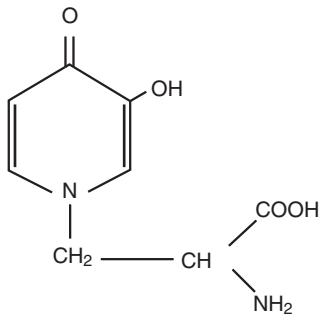

Fig. 14.3. Mimosine.

Before proceeding further, there is one more cautionary note that needs to be sounded. Invariably, more than one secondary plant metabolite with nutritional or other consequences for nutrition and production occur together in the one plant. For example, *Vicia faba* may contain tannins, lectins, phytates, vicine, convicine and heat-labile protease inhibitors, and each of these may affect digestion, absorption or metabolism. Thus the effects of one plant metabolite may obscure the effects of others.

The nutritional effects of secondary plant metabolites are achieved by a wide variety of mechanisms, some bind specifically to and inhibit digestive enzymes, some bind to proteins generally, some chelate trace metal nutrients, others disrupt membrane structures, whilst the phyto-oestrogens achieve their effects by mimicking the structure of a natural hormone.

PHYTO-OESTROGENS

The phyto-oestrogens are compounds of plant origin that have sufficient structural similarity to oestradiol (Fig. 14.4) to allow them partially to imitate the natural oestrogenic hormone. The phyto-oestrogens include the isoflavones, such as genistein and daidzein (Fig. 14.5), and the coumestan, coumestrol, that are present in soybeans and other legumes. The lignans, another group of phyto-oestrogens, characterized by a dibenzyl butane skeleton, occur widely in plants but have come to be of particular interest because of their presence in linseed (Fig. 14.6). They are normally present in plant tissue as glycosides that are hydrolysed and in some instances further metabolized in the gut. The natural oestrogen, as well as phyto-oestrogens and the synthetic oestrogens, act by virtue of their molecular shape, and the hydroxyl groups at either end of the molecules function by binding to specific oestrogen receptors. However, because of differences in molecular shape and different polarities of the hydroxyl groups, the phyto-oestrogens only imperfectly mimic the natural oestrogen, eliciting a weak oestrogenic effect (Table 14.2). However, in addition to partial mimicking of oestrogen, the phyto-oestrogen is also able to compete with the natural hormone, resulting in antioestrogenic consequences.

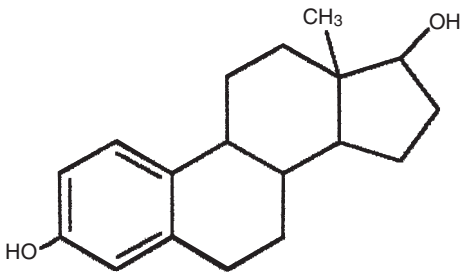

Fig. 14.4. 17 β-oestradiol.

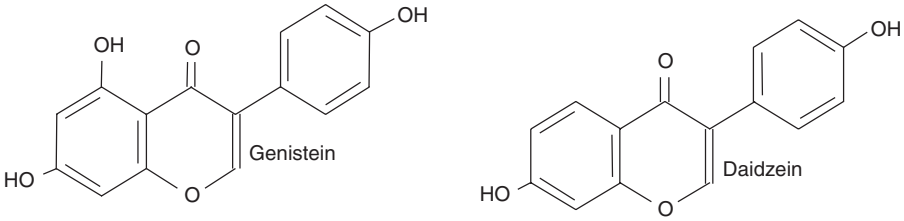

Fig. 14.5. Isoflavones on soybeans.

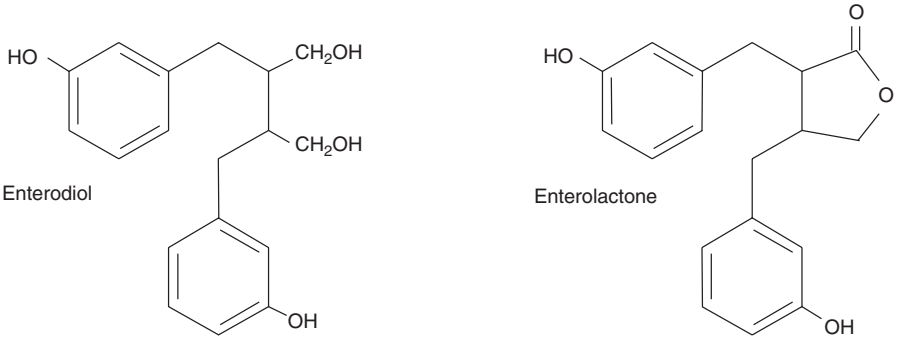

Fig. 14.6. Lignans.

Table 14.2. Oestrogenic potencies relative to oestradiol (adapted from Knight and Eden, 1996, and Franke *et al.,* 1994).

Oestrogen	Relative potency	Concentration in soybeans (mg kg^{-1} DM)
Oestradiol	100	
Coumestrol	0.202	
Genistein	0.084	950–1100
Equol	0.061	
Daidzein	0.013	670–1000
Biochanin A	<0.006	
Formononetin	<0.0006	

Most of the recent interest in these compounds has focused on possible anti-cancer and antiatherogenic effects in humans (Mazur *et al.*, 1998) and this stimulus to phyto-oestrogen research has led to their surprising discovery in such foods as wine and beer (Gehm *et al.*, 1997; Lapcik *et al.*, 1998). However, these substances first came to prominence because of their adverse effect on fertility of ewes (Bennetts *et al.*, 1946). Among the many manifestations of 'clover disease', low conception rates, uterine prolapse, and pseudo-lactation in rams and wethers all point to disruption of normal reproductive processes. The reduced fertility led to lambing performances as low as 46 lambs per 100 ewes (Davenport, 1967) and estimates of ovulation rates 25% lower than normal among ewes grazing on oestrogenic pastures (Lightfoot and Wroth, 1974). In rats, prenatal exposure to genistein affects sexual differentiation (Levy *et al.*, 1995) and in mice coumestrol reduces ovulation rates (Fredericks *et al.*, 1981). In humans, the intake of weakly oestrogenic isoflavones is high in countries that have a low incidence of oestrogen-dependent cancers, such as breast cancer and prostate cancer (Mazur *et al.*, 1998). Also, the longer menstrual cycle in Japanese women (32 days), compared with that of Westerners (28–29 days), has been associated with phyto-oestrogens, and numerous experimental investigations have produced evidence to support hypotheses that link phyto-oestrogens to hormonal effects in post-menopausal women (Setchell and Cassidy, 1999). Despite the clear evidence of oestrogenic effects in mammals and postulated anti-oestrogenic effects in humans, there appears to have been little interest in their potential effects in an industry that exists to produce eggs. The natural oestrogen in laying hens may be regulated, with consequent effects on weight and composition, by altering the fat in their diets (Whitehead *et al.*, 1993); so it is not inconceivable that phyto-oestrogens also might influence egg formation.

Linseed (also called flaxseed) has always been a valuable crop to supply the surface coatings industry, but it has assumed greater nutritional importance because of the potential health benefits of omega-3 polyunsaturated fatty acids that it contains. In poultry nutrition, linseed has been used to produce so-called healthy eggs, but little account has been taken of the potentially oestrogenic effects of the lignans. Studies of effects of potentially oestrogenic linseed on egg production by hens has produced conflicting results. RothMaier *et al.* (1998) found that inclusion of 200 g linseed kg^{-1} diet, either intact or ground, depressed laying performance by about 10% and daily production of egg mass by about 11%. Inclusion of linseed at up to 100 g kg^{-1} diet had no adverse effect on laying performance, but at 150 g kg^{-1} the feed consumption per kg egg mass increased (Eder *et al.*, 1998). On the other hand, Scheideler and Froning (1996) found that up to 150 g kg^{-1} linseed in the diet decreased feed consumption, weight gain, and egg weight compared with control diets. However, linseed significantly improved egg production (88.9%) when compared with a control diet based on maize and soybean (83.1%); it should be noted that soybean also contains isoflavones. Oruseibo (1995) compared the nutritive value of various protein sources for laying hens and concluded that a mixture of sunflower meal plus meat meal was superior to an equivalent soybean/meat meal diet in terms of several egg production variables. This was attributed to the quality of the protein, but the question of what role, if any, the soy phyto-oestrogen may have

had was not raised. Even if it were possible to assert unequivocally that linseed does affect egg production, the role of phyto-oestrogen still would not be resolved, since linseed contains several other nutritionally active substances. Even when a study was made of the effect of added coumestrol, the most potent of the plant oestrogens, the diet contained soybean meal (Béguin and Kincaid, 1984). In addition to the potential effects of phyto-oestrogens on reproductive processes, it is possible that they may also have effects on growth; for many years the synthetic oestrogen, stilboestrol, was used as a growth promoter in ruminants. There is one brief, relevant report that, when isoflavones extracted from red clover were added to diets of male broilers, they yielded a 5% increase in body weight gain and a 9% increase in feed conversion (Wang and Han, 1998). Although this report stands unsupported for the time-being, it may be a pointer to a possible natural growth promoter that may find greater favour with consumers disenchanted with products of chemical plants. Although the evidence for significant effects of phyto-oestrogens in poultry is very slender, biochemical studies which show that phyto-oestrogens inhibit several steroid-metabolizing enzymes *in vitro* (Aldercreutz, 1998) suggest that it would be negligent to assume that they have no effect on birds.

PROTEINACEOUS ANTI-NUTRITIONAL SUBSTANCES

The existence in foods of heat-labile substances that detract from nutritional value was demonstrated as early as 1917, when Osborne and Mendel (1917) showed that heat treatment improved the nutritional value of soybean protein. The later discovery of two protease inhibitors appeared to offer the explanation for the beneficial effect of heating soybeans (Bowman, 1945; Kunitz, 1945). However, 7 years later, Liener and Pallansch (1952) found in soybeans a haemagglutinating substance which had no antitryptic activity, but which was lethal to rats when given by intraperitoneal injection. This substance, which they named Soyin, was identified as a protein (Liener, 1953). Growth studies with chicks demonstrated a relationship between the haemagglutinating activity of heat-treated soybean meal and the nutritional improvement that was gained from the treatment; this suggested that protease inhibitors might not be the sole heat-labile anti-nutritional substance present (Liener and Hill, 1953). Further support for this idea was provided by growth studies in which isolated soybean haemagglutinin was added to autoclaved soybean meal (free from antitryptic activity) at a level equivalent to its natural level in raw soybean meal; this produced growth inhibition in rats roughly equal to half that caused by raw soybean meal containing both active protease inhibitors and haemagglutinin (Liener, 1953). In experiments with conventional and germ-free chicks, neither navy bean trypsin inhibitor nor its haemagglutinin impaired growth of germ-free chicks, but both did in conventional chicks (Hewitt *et al.*, 1973). Whilst a diet containing raw navy beans was lethal to conventional Japanese quail, the same diet merely impaired growth of germ-free birds (Jayne-Williams and Hewitt, 1972).

In many of the early production studies of the heat labile anti-nutritional substances in soybeans, there was no attempt to isolate the effects of the individual anti-nutrients. Furthermore, many conflicting results were reported. Among the studies where raw and heated soybean products were studied in diets of hens for egg production, and where no advantage was derived from heating (Carver *et al.*, 1946; Fisher *et al.*, 1957; Griminger and Fisher, 1960; Saxena *et al.*, 1963a,b) it is possible that the heat treatment applied to the heated soybean was inadequate to achieve protein denaturation. In contrast, Hill and Renner (1963), and Rogler and Carrick (1963) both demonstrated that heat treatment of soybean products resulted in improved egg production!

Protease Inhibitors

Protease inhibitors are widespread in plants, including cereals, cucurbits and solanums, but have been most studied in the legumes, especially soybeans. Their common characteristic is an ability to bind to and inhibit proteolytic enzymes, particularly the digestive enzymes. However, they differ in the proteases that they inhibit, in the nature of the binding site and in their primary amino acid sequences (Whitaker, 1995). The protease inhibitors act by binding with the enzyme at its active site in the manner of a substrate peptide. However, because the inhibitor binds more firmly than the normal substrate, and possibly because the inhibitor is unable to adopt the appropriate spatial conformation, the peptide bond is not hydrolysed. The protease inhibitors from soybeans differ in their ability to withstand the digestive processes in the small intestine; in the rat only about 5% of ingested Bowman-Birk inhibitor survives passage through the small intestine, but about 75% of the Kunitz inhibitor remains intact (Hajós *et al.*, 1995). It is possible that this ability to survive digestion is one factor that contributes to the adverse effects of raw soybeans. It is probably significant that in experiments in which the more robust Kunitz inhibitor was added in crystalline form to chick diets feed intake was decreased, whilst the less refractory Bowman-Birk inhibitor had no significant effect on feed intake (Garlich and Nesheim, 1966; Gertler *et al.*, 1967). The Bowman-Birk type inhibitors are characterized by independent trypsin- and chymotrypsin-inhibiting sites, whereas the Kunitz-type inhibitor has a single trypsin-inhibitor site, and is only weakly chymotrypsin-reactive at two other reactive sites (Guegen *et al.*, 1993). When added to chick diets based on heated soybean, neither of the two types of inhibitor resulted in growth depression, but they did induce enlargement of the pancreas, whereas a comparable diet based on raw soybean depressed growth (Gertler and Nitsan, 1970). The inhibitors also increased the activities of amylase, trypsin, chymotrypsin and pancreatopeptidase E in the pancreas, but depressed the activities of the first two of these peptidases in the gut lumen. Protease inhibitors stimulate enlargement of the pancreas by lowering trypsin activity in the gut, with consequent cholecystokinin release (Green and Lyman, 1972).

For many years, nutritional scientists interested in the anti-nutritional effects of protease inhibitors had to content themselves with the knowledge that these substances increase faecal excretion of nitrogen, but could only speculate on whether the additional excreted nitrogen originated in undigested dietary protein or came from increased secretion of endogenous nitrogen as, for example, saliva, gastric and pancreatic secretions, bile, intestinal secretions, mucosa and sloughed mucosal cells (Souffrant, 1991). However, the introduction of the ^{15}N- and ^{15}N-leucine isotope dilution techniques has made it possible to measure the contributions of dietary and endogenous protein to nitrogen in ileal digesta of pigs (de Lange *et al.*, 1990, 1992), but so far no one has published any comparable studies in poultry. In studies comparing the ileal digestibilities of soybean products, both dietary and endogenous protein contributed to the higher excretion of nitrogen from pigs fed on toasted soybean meal or a mixture of toasted and untoasted soybean meal, compared with a soybean concentrate (Table 14.3; Grala *et al.*, 1998).

A surprising effect, not evident in similar studies, was observed by Santidrian *et al.* (1981); with chick diets containing raw soybean meal (500 g kg^{-1}) or kidney bean meal (300 g kg^{-1}) they found increases of 84% and 63% in food intake by chicks compared with a diet based on heated soybean meal!

Lectins

The lectins (L. *legere*: to choose), which are multivalent saccharide-specific binding proteins (Table 14.4), have been reviewed recently by Liener (1997).

Because of their capacity for binding to glycoproteins, many are able to bind strongly to blood group-specific proteins at more than one site and thereby bring about agglutination of erythrocytes. For that reason they are often known as haemagglutinins. The haemagglutinating capacity of lectins provides one simple means of assaying their activity in foods (Tan *et al.*, 1983). Lectins are widely distributed in nature, with sources ranging from common gorse (*Ulex europaeus*) and banana (*Musa paradisiac*) to the land snail (*Octala lactea*) and to bacteria (e.g. *Pseudomonas aeruginosa*). The lectins located on bacterial pili or fimbriae aid in the attachment of bacteria to epithelia, thereby contributing to

Table 14.3. Distribution of ileal nitrogen between endogenous N and dietary N in pigs fed on diets based on soybean products with different trypsin inhibitor activities (DMI, dry matter intake).

	Soya concentrate	Toasted soybean meal	Mixed soybean meal
Trypsin inhibitor activity	0.45	1.62	3.90
Nitrogen at the ileum (as g N kg^{-1} DMI)			
Total N excreted	2.81	4.27	9.19
Endogenous N	2.15	2.53	3.75
Dietary N	0.66	1.74	5.44

Table 14.4. Saccharide specificity of some lectins.

Species	Common name	Predominant specificity
Arachis hypogeae	Groundnut	Gal-β1,3-GalNAc
Dolichos biflorus	Horse gram	GalNAc-α1,3-GalNAc
Glycine max	Soybean	α- and β-GalNAc
Phaseolus vulgaris	Kidney bean	Gal-β1,3-GalNAc-β1,2-Man
Psophocarpus tetragonolobus	Winged bean	α-GalNAc
Canavalia ensiformis	Jack bean	α-mannose
Galanthus nivalis	Snowdrop	α-mannose
Lens esculenta	Lentil	α-mannose
Pisum sativum	Pea	α-mannose
Vicia faba	Fava bean	α-mannose
Ricinus communis (toxin)	Castor bean	α- and β-Galactose

the virulence of pathogens (Gilboa-Garber, 1994). Although they occur in so many diverse sources, the lectins that are generally of most interest to nutritionists are those that are present in the legumes. The toxicity that some lectins may possess became well known when ricin, a lectin from the seed of castor bean (*Ricinus communis*), one of the most toxic substances known, was used in a notorious assassination in London of an Eastern European diplomat. Many lectins, such as that present in the garden pea (*Pisum sativum*), are quite innocuous when eaten. On the other hand, the lectin of kidney beans (*Phaseolus vulgaris*) has been responsible for a number of accidental food poisonings of humans (Anonymous, 1976). In common with many other anti-nutritional plant metabolites, lectins serve to protect the seed from predation (Peumans and Van Damme, 1995), but other functions related to the symbiosis of legumes and their symbiotic bacteria have also been postulated. In fields other than nutrition, lectins are viewed more positively, particularly as possible bioadhesive adjuvants for precise drug delivery (Lehr, 1996). Even in the field of animal nutrition, positive applications of lectins as growth promoters have been proposed (Pusztai *et al.*, 1997). These proposed strategies derive from the beneficial effects of short-term exposure of the gut to the agglutinin, which results in faster renewal of the gut surface! Thomke and Elwinger (1998) suggested that advantage may be taken of the binding specificities of lectins to prevent adhesion and proliferation of pathogenic species of bacteria to the gut wall.

Two of the main attributes of the different lectins that determine how much they affect the gut are the ability to survive intestinal proteolysis, and the avidity and selectivity with which they bind to the brush border glycosyl units. Some lectins are very resistant to proteolysis; 1 hour after intragastric administration of lectins to rats, almost all (>90%) of the lectins from kidney beans (*Phaseolus vulgaris*) and snowdrops (*Galanthus nivalis*) could be found bound to the small intestinal mucosa, but they still remained immunoreactive. Other lectins are less resistant; less than 60% of the lectins from soybeans (*Glycine max*) and elder bark (*Sambucus nigra*) resisted degradation, but even fewer (25%) from broad bean (*Vicia faba*) survived. Neither broad bean lectin nor snowdrop lectin (both of the mannose-specific group of lectins) bound to the

mucosa (Pusztai *et al.*, 1990). On chronic exposure of rats to the lectins (7 g lectin kg^{-1} diet) in the diet, broad bean lectin did not impair body weight gain in the animals nor did it stimulate growth of the small intestine. On the other hand, impairment of body weight gain by the other lectins closely paralleled their effect on the hyperplastic growth of the small intestine. The lectins from kidney beans and soybeans (both of *N*-acetylgalactosamine specificity) bind strongly to the brush border membranes of the intestinal epithelium; this stimulates hyperplastic growth, increasing crypt cell proliferation, and the lectins can also disrupt the morphology of the brush border (Pusztai *et al.*, 1990; Bardocz *et al.*, 1990, 1995). When kidney bean lectin was administered to rats, it almost doubled the weight of the small intestine and increased the content of protein, RNA and polyamines in the mucosa (Table 14.5; Pusztai *et al.*, 1993). In the intestinal mucosa, the full complexity of the saccharides of the mucosal glycoproteins develops as the cells mature. When cell turnover is high, there is a higher proportion of juvenile cells in the villus membrane and simple mannosyl and polymannosyl residues are the predominant glycosyl units, whereas in the mature cells there is a higher proportion of *N*-acetylgalactosamine units. By increasing cell turnover in the small-intestinal epithelium, kidney bean lectin alters the surface glycans in favour of mannose units. When kidney bean lectin was given to rats, there was a dose-dependent increase in growth and attachment of *Escherichia coli* to the small intestine (Table 14.5; Pusztai *et al.*, 1993); *E. coli* binds to the intestinal epithelium by means of a mannose-specific bacterial lectin (Sharon, 1987). However, when snowdrop lectin, which also is mannose-specific, was intubated alone in the same manner it had no significant effect on the gut itself, but when administered together with kidney bean lectin it did have the potentially beneficial effect of reducing *E. coli* proliferation, presumably by competing with *E. coli* for the mannose-specific binding sites on the epithelium. Hajós *et al.* (1995) speculated that when cells are extruded from the intestinal villi in animals that receive lectins in their diet, the villi may be degraded in the gut, releasing the lectin to bind again to the mucosa further along the intestine, thereby extending the effects that the lectins wreak in the gut. In addition to the effects on the intestine itself, the adsorption of lectin onto the mucosa stimulates pancreatic enlargement, but not via the negative feedback mechanism characteristic of trypsin inhibitors (Pusztai *et al.*, 1995).

Table 14.5. Effect of lectins from kidney beans and snowdrops on the small intestine of the rat (adapted from Pusztai *et al.*, 1993).

	Control	Kidney bean lectin	Snowdrop lectin	Kidney bean and snowdrop lectins
Lactose fermenting coliforms[a]	3.3	8.7	3.1	6.5
Weight (g dry wt. g^{-1} dry body wt.)	0.57	1.11	0.56	1.02
Protein (mg 20 cm^{-1})	84.2	106.5	68.4	111.3
RNA (mg 20 cm^{-1})	3.7	11.8	2.6	9.9
Putrescine (nmol 20 cm^{-1})	34.6	317.8	71	301.2

[a]$\log_{10}$ bacterial counts g^{-1} wet tissue.

Treatments

Because lectins and protease inhibitors are proteins, treatments of foods to ameliorate or eliminate their effects are often effective for both types of anti-nutritional substance. The long-established means (Osborne and Mendel, 1917) of improving the feed value of foods containing proteinaceous anti-nutritional substances is heat treatment (and there have been many variants on combined heat and mechanical treatment). For example, Coates *et al.* (1970) found that heat treatment of soybean meal improved the growth of chicks, but the presence of a conventional gut microflora exacerbated the adverse effect of raw soybean meal on both chick growth and pancreas weight (Fig. 14.7). Studies of the effects of feeding ground raw and heated soybeans to goslings and chicks revealed some subtle species differences; both species suffered depressed weight gains (71% for goslings, 58% for chicks) when the raw food was given, but food intake was much more severely depressed in the goslings (51% vs. 7%; Nitsan and Nir, 1977). Also, although the raw soy caused a doubling of relative pancreas weight (g kg^{-1} body weight) in both species, it had different effects on enzyme concentrations in the pancreas; amylase was much more severely depressed in the goslings (91% vs. 20%) and chymotrypsin was lower (90%) in the gosling, but unaffected in the chick. In neither was the trypsin concentration of the pancreas affected.

In extraction of oil from oilseeds, preliminary roasting of the seed is commonly used to improve the yield of oil, and this heating may serve also to denature proteinaceous anti-nutritional substances. In addition, further heating of the extracted meal has sometimes been used to drive off volatile anti-nutrients, such as glucosinolate degradation products from rapeseed meal.

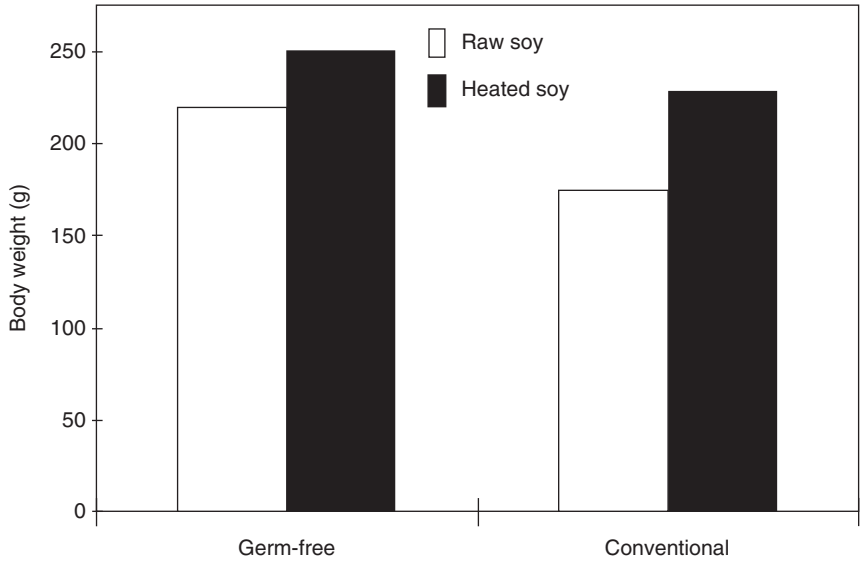

Fig. 14.7. Body weight at 3 weeks of germ-free and conventional chicks fed on diets containing (500 g kg^{-1}) raw or heated soybean meal (from Coates *et al.*, 1970).

One novel attempt to reduce protein anti-nutrients in soy albumin employed pepsin hydrolysis at pH 2.2, followed by enzymic transpeptidation with methionine ethyl ester (Hajós *et al.*, 1996). Even on a laboratory scale, the method met with only very limited success, achieving a modest reduction in the activities of Kunitz inhibitor and lectin, whilst the Bowman-Birk inhibitor was barely affected (Fig. 14.8). In terms of nutritional value, the treatment succeeded in preventing weight loss in animals fed on untreated soybean albumin, but did not achieve the positive growth that the control protein (lactalbumin) did. There are several aspects of this approach that would need to be addressed before it could be developed into an industrial process. Although proteolysis and transpeptidation are low energy operations, they were conducted in dilute solution and subsequent removal of the large proportion of water in the reaction mixture to produce a solid food would have a high energy cost. Furthermore, the soy albumin on which the experiment was conducted is not a normal poultry food. What is needed is a process that could successfully act on meal rather than a protein sol; this would require penetration into the solid feed material and would probably require an appreciably longer incubation time. This might expose the meal to fungal colonization, and would still require the material to be dried afterwards. Nevertheless, it is possible that this experiment might stimulate the search for alternatives to mere heat treatment.

A still more unusual approach to the lectin in soybean is to use the lectin to enhance the efficiency of nutrient absorption. This approach attempts to

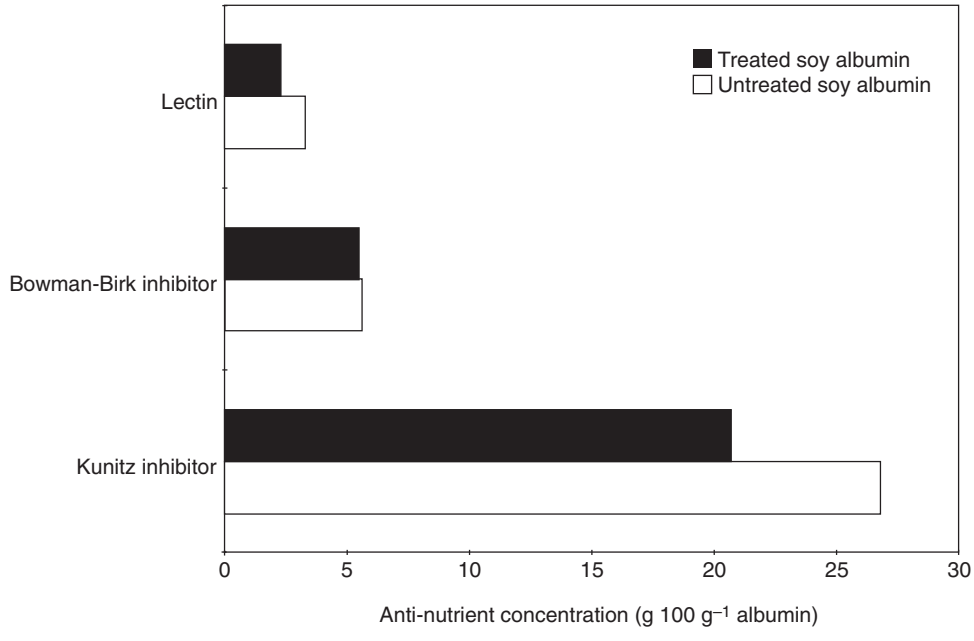

Fig. 14.8. Effect of peptic proteolysis and transpeptidation on anti-nutrient concentrations in soy albumin (adapted from Hajós *et al.*, 1996).

take advantage of the stimulus to cell growth and turnover induced by the binding of lectins to receptors on the intestinal epithelium (Pusztai *et al.*, 1997). Whilst this growth process has a nutritional cost, the hypothesis on which the strategy is based is that the renewed epithelial surface absorbs nutrients more efficiently. However, to avoid the serious intestinal damage and malabsorption of nutrients occasioned by continual exposure to lectin, the dietary strategy comprises alternating phases of lectin-free and lectin-containing feeds. To date, this hypothesis has been tested only in rats. Groups of rats were fed either alternating soybean albumin and lactalbumin (test and control), or they were continually pair-fed the lactalbumin-based diet (control only). During each of the periods of test feeding, the soy-fed group of rats lost weight, while the lactalbumin group made small gains. However, during periods when both groups were being fed on lactalbumin, the test rats gained weight more rapidly and converted the feed more efficiently than the control group (Fig. 14.9). The fact that the final body weights of the alternate-fed rats were significantly lower than those of the group continuously fed the lactalbumin diet may appear to detract from the hypothesis, but that would be to ignore the differences in, for example, protein quality of the two diets. A shorter-term adjunct to the experiment offers support for the effectiveness of the strategy: a third treatment, equivalent to the alternate feeding scheme but with lectin-depleted soy albumin was included. The weight gains of the rats fed the high-lectin diet were significantly higher than those of the rats fed the low-lectin diet (129.9 g vs. 125.5 g). Perhaps fine tuning of such a strategy might lead to genuine benefits.

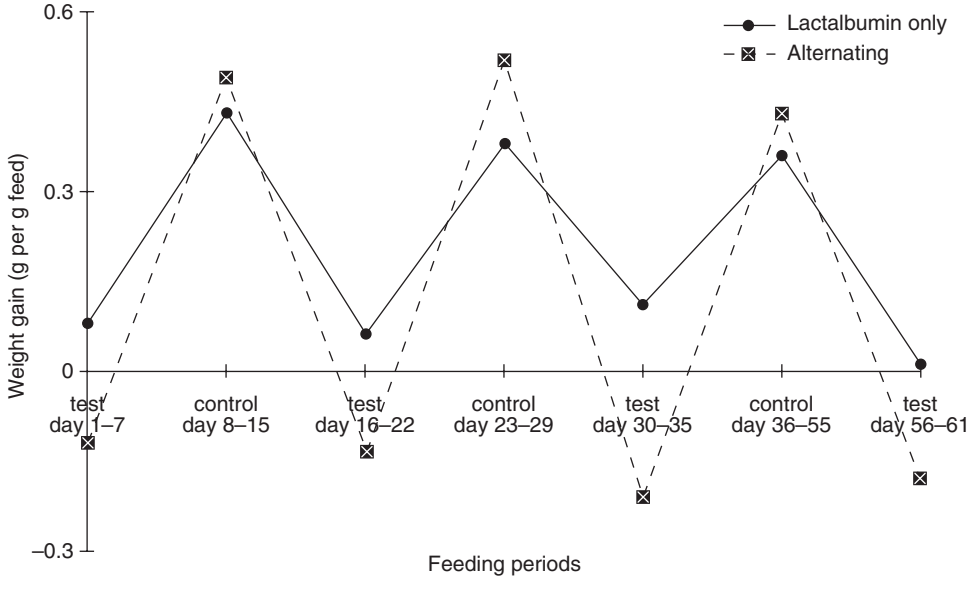

Fig. 14.9. Effect of alternating lactalbumin and soy feeding on gain/intake of rats (adapted from Pusztai *et al.*, 1997).

TANNINS

Tannins are high molecular mass (500–3000) polyphenolic substances that were valued originally for their ability to preserve animal hides as leather. The preservation, achieved largely through the cross-linking reaction of the tannin with the protein of the skin, points to their most readily identifiable nutritional effect, namely that of combining with soluble proteins. On the palate of the wine drinker this manifests itself in the astringency of a well-oaked red wine. Among ruminant nutritionists, the focus of studies on tannins has been related to a quest for the means of protecting protein from degradation in the rumen (Aerts *et al.*, 1999) so that high-quality protein can pass to the small intestine. In poultry, dietary tannins have been blamed for low digestibilities of protein and consequent poor growth performance of birds. Tannins are usually classified into two categories, condensed and hydrolysable tannins. Condensed tannins (procyanidins; Fig. 14.10) are the more chemically refractory of the two classes, being composed of polymers of flavanol units linked by direct carbon–carbon covalent bonds that are resistant to hydrolysis. As a consequence of the high degree of hydroxylation, procyanidins with molecular masses up to about 3000 are soluble.

Hydrolysable tannins (Fig. 14.11) consist of polyphenolic acids esterified to a central monosaccharide unit. Despite the susceptibility of the ester linkages of hydrolysable tannins to hydrolysis by enzymes in the digestive tract, *in vivo* they produce effects similar to those of the hydrolysis-resistant condensed tannins.

Tannins are able to bind with proteins, particularly proline-rich proteins present in the saliva of some animals (Mehansho *et al.*, 1985), and to inhibit enzymes (Milic *et al.*, 1972; Daiber, 1975; Griffiths and Jones, 1977; Horigome *et al.*, 1988). In the rat, but apparently not in the hamster or the vole (Dietz *et al.*, 1994), dietary tannins induce the synthesis of proline-rich salivary proteins, thereby affording the rat an adaptive mechanism that reduces the anti-nutritional

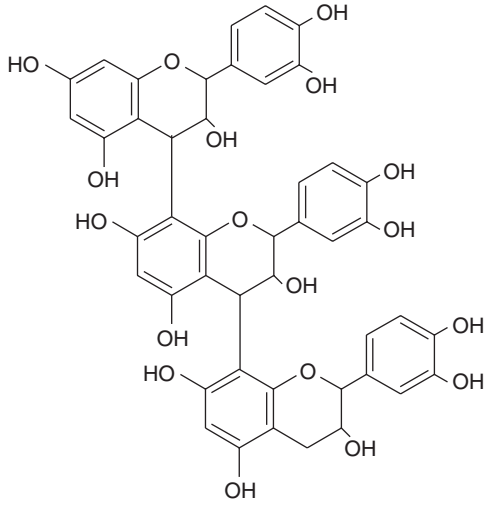

Fig. 14.10. Characteristic of a condensed tannin.

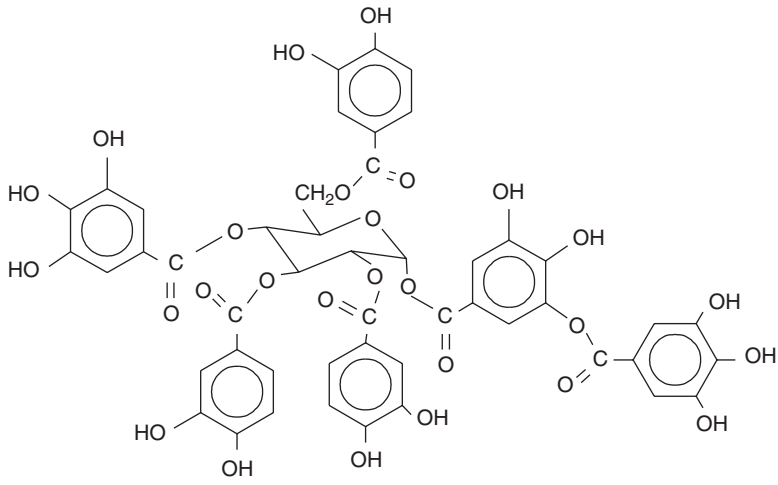

Fig. 14.11. Characteristic of a hydrolysable tannin.

impact of tannins in the digestive tract (Mehansho *et al.*, 1987). In the human, the salivary proline-rich proteins (PRPs) account for about 70% of the total protein in saliva; they can be classified as acid, glycosylated and basic PRPs. Of these, only the basic PRPs show substantial tannin-binding; these bind tannins even more avidly than gelatin, which is generally regarded as one of the most effective tannin-binding proteins. The condensed tannin–PRP complexes are robust and remain insoluble even in conditions similar to those of the stomach and small intestine, indicating that, apart from the biosynthetic cost of PRP synthesis, PRPs offer an effective defence against tannins (Lu and Bennick, 1998). The survival of these stable tannin–PRP complexes as they pass through the gut may account for much of the elevated faecal nitrogen excretion in animals capable of PRP synthesis when fed on high-tannin diets. Mole *et al.* (1990) illustrated the effects of tannins and PRPs in rats by studying the accretion of body nitrogen in relation to the feed nitrogen apparently digested by rats fed on low-tannin sorghum and high-tannin sorghum, with or without the β-antagonist, propranolol, which blocks PRP synthesis (Fig. 14.12). The apparent digestibility of nitrogen in the high-tannin sorghum was far lower than that in the low tannin sorghum, but when the protective effect of the proline-rich salivary protein was eliminated by propranolol, the digestibility was reduced even more. Assuming that the only effect of the propranolol is to inhibit PRP synthesis, this might suggest that the highly efficient binding of tannin by the PRP wastes less protein than when other dietary and endogenous proteins react with tannin. The effect of tannin on the efficiency with which the digested nitrogen was accreted as body nitrogen was even greater than the effect on digestibility; nitrogen accretion became highly negative, in other words, the animals lost body protein when high tannin was combined with absence of proline-rich protein. These results clearly demonstrate how effectively the proline-rich proteins protect from tannins animals which synthesize PRPs. The interactions of tannins with PRPs provide a useful insight into the effects of

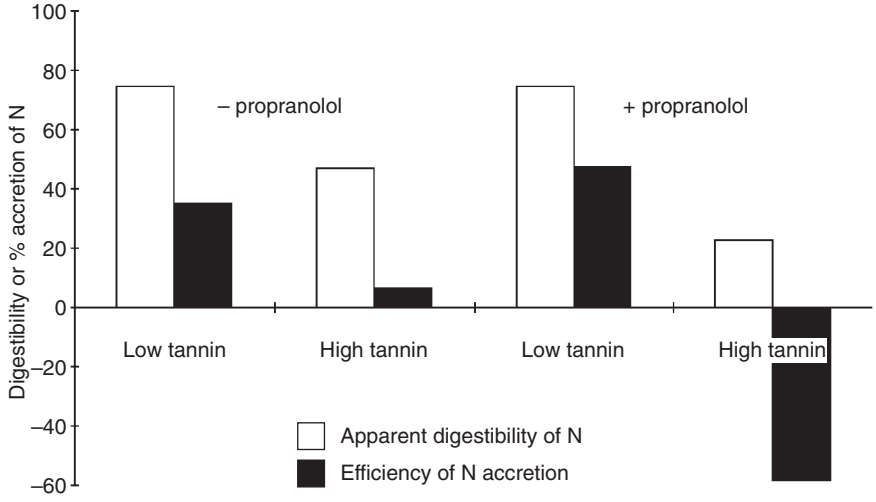

Fig. 14.12. Effect of low- and high-tannin sorghum and propranolol of apparent N digestibility and efficiency of N accretion (adapted from Mole *et al.*, 1990).

tannins in the diets of many mammals, but whether such interactions are of significance in birds remains uncertain, for although the literature contains many references to PRPs in the saliva of mammals, there appears to be no comparable account relating to birds.

Administration of ^{14}C-labelled tannins or polyphenols and sugars to chickens resulted in the detection of label from the low molecular-mass compounds in the liver, femur and plasma, but showed no evidence of absorption of high molecular-mass vanillin-positive substances (Jiminez-Ramsey *et al.*, 1994). This suggests that the effects of high-tannin feeds are elicited either by the action of tannin in the gut, or by other substances that are present together with the tannin, acting either in the gut or following absorption.

Among the angiosperm genera, 54% contain condensed tannins and 13% contain hydrolysable tannins (Swain, 1979), but in poultry diets tannins are most likely to be encountered in field beans (*Vicia faba*), sorghum (*Sorghum bicolor*) and Brassica oilseeds (mainly *Brassica napus*). Whilst there are reasonably consistent reports of diminished growth when chickens are fed on diets containing high-tannin rather than low-tannin sorghum (Chang and Fuller, 1964; Rostagno *et al.*, 1973a,b), the evidence for adverse effects of faba bean tannins is less consistent. Because tannins are not homogeneous compounds, the first difficulty encountered in their study and in the evaluation of the published research is that of quantitation. Some of the classical tannin assays are dogged by a lack of specificity; this and the number of different assays that have been used from time to time makes it difficult to make comparisons between experimental findings.

Sorghum is the world's fifth most abundant cereal (Doggett, 1988) and in Asia it is the third most common cereal, after rice and maize (Ravindran and Blair, 1991). In some countries where wild bird predation of crops is common, it is usual to grow so-called bird-proof cultivars of sorghum; these are

characterized by high contents of tannin. In studies of the role of tannins in the nutritional value of sorghums, one approach that has been used to determine effects of tannins on the growth performance of chickens is the formulation of diets with graded tannin contents by manipulation of the proportions of high- and low-tannin sorghum and maize in the diets. However, some results obtained have been rather equivocal (Pour-Reza and Edriss, 1997). Although these authors present results to show that, in terms of weight gain, there appears to be little difference in the performance value of sorghum and maize, as long as the tannin content, measured according to the Folin-Denis method (AOAC, 1990), is no more than 2.6 g tannin kg^{-1} diet, their results also seem to suggest that the quality of the bird may be compromised by increased deposition of abdominal fat, especially when the fat content of the diet is increased.

Another approach has been to formulate diets, each containing single cultivars of sorghum with different tannin contents. Early studies comparing the nutritional value of high- and low-tannin sorghums clearly associated poorer growth with the high-tannin varieties (Chang and Fuller, 1964; Rostagno *et al.*, 1973a), but in later work high- and low-tannin sorghum supplemented with soybean meal and safflower meal supported growth equally well (Elkin *et al.*, 1978a). However, these authors did find that when only soybean meal was used to supplement the diets, the high-tannin sorghum diet resulted in poorer performance. On the basis of results obtained by supplementing these diets with amino acids, the authors ascribed the contrasting results to the role of methionine as the first limiting amino acid in the diets that contained no safflower meal. In the same set of experiments, nitrogen digestibility, nitrogen retention and dry matter utilization were all lower in a diet containing high-tannin sorghum than in one containing low-tannin sorghum. Other work demonstrated a higher incidence of leg abnormalities among birds given a diet containing high-tannin sorghum when a methionine supplement was included to counter the adverse effect of tannin on growth (Armstrong *et al.*, 1973). However, the abnormalities were not evident among birds fed a low-tannin sorghum diet, when it was supplemented with either tannic acid or methionine, although tannic acid supplementation did induce depression in growth rate. Previously leg abnormalities had been reported in birds fed tannin-containing diets with sub-optimal protein content (Rostagno *et al.*, 1973a). When these authors analysed bone from chicks with normal and bowed legs, they concluded that the sorghum had not impaired bone mineralization. Rather, they suggested that it was the protein matrix that had been affected. In the absence of any knowledge of the ability, or otherwise, of birds to synthesize PRPs, but given the known avidity with which proline-rich proteins bind to tannins and the vital role that proline plays as precursor for the hydroxylated residues responsible for the cross-linking in collagen, it is tempting to speculate that the tannin might impair collagen formation by competing for proline that might be needed for bone. It is conceivable that such interference could take place at the absorption stage. In an experiment to ascertain the effect of dietary tannin on digestibilities of amino acids in chick diets containing sorghum, addition of tannic acid to a protein-free diet resulted in a fourfold increase in endogenous amino acid excretion (Rostagno *et al.*, 1973b). Whilst loss of proline by this route might be consistent with the putative mechanism, excretion of proline was no more severely affected than that of

other amino acids. Furthermore, Elkin *et al.* (1978a) found that more collagen was solubilized from femurs of chicks fed low-tannin sorghum than from those fed the high-tannin cultivar!

On the basis of a number of studies, methionine has been ascribed a special palliative role in diets containing high-tannin sorghum (Armstrong *et al.*, 1973; Elkin *et al.*, 1978b), but differences in dietary protein content, and different supplemental amino acid sources make this difficult to substantiate. Rostagno *et al.* (1973b) found that the apparent digestibility of methionine was no worse affected by high-tannin sorghums than that of other amino acids, but the apparent digestibility of cystine was especially low; perhaps the latter may account for effects attributed to methionine supplementation.

In a tube-feeding experiment (Elkin *et al.*, 1996), 20 cultivars of sorghum, ranging in tannin content (according to the vanillin assay; Price *et al.*, 1978) from 0 to 38.8 g catechin equivalents kg^{-1} grain dry matter, were used to determine the effect of tannin content on digestibilities of amino acids and energy. Although results were scattered, the regression of digestibility on tannin content suggested a depression of 0.84 percentage points in lysine digestibility for each 1 g catechin equivalent kg^{-1}. However, the correlation coefficient ($r = 0.46$) indicates that the tannin content only partly explains the observed variations in amino acid digestibilities. Similar effects were observed for digestibilities of other amino acids and true metabolizable energy (TME_N); again no substantial difference was found between methionine and the other amino acids. The authors, who also observed an inverse relationship between essential amino acid digestibilities and α-kafirin concentration, suggested that variation in the concentration of this poorly digestible storage protein of sorghum is a further contributor to the differences in digestibility. α-Kafirin is a comparatively proline-rich protein and the question arises whether some of the various soaking treatments applied to improve the nutritional value of sorghum might permit the binding of tannin to the kafirin, thereby diminishing tannin effects in the gut; an alternative is that soaking might permit some degree of preliminary hydrolysis of the α-kafirin. Other potential factors contributing to the adverse biological effects of high-tannin sorghum include low molecular-mass polyphenols that occur as intermediates in the biosynthesis of tannins (Jung and Fahey, 1983) and which might be absorbed and impair major metabolic pathways (Butler and Rogler, 1992).

High-tannin sorghums have the advantage of resistance to bird damage. In areas where predation by birds is not a serious problem, the superior market value of the low-tannin varieties makes them the obvious choice for the grower. However, in areas susceptible to bird damage, plant breeding has been taking a different direction. Since bird damage to sorghum crops is at its worst during the earlier stages of grain growth, plant breeders have sought cultivars that contain tannin during the early stages of growth to provide repellence, but which undergo biochemical processes during ripening to produce grain that is palatable and of high nutritional value (Bullard and York, 1996). One unexpected outcome of this quest has been the discovery of a variety which is tannin-free, yet is avoided by birds; the authors suggest that non-tannin polyphenols in this cultivar affect feeding behaviour and digestion in birds, since this variety also showed poor feed efficiency.

Evidence for adverse effects of tannins in *Vicia faba* are somewhat equivocal. Pairs of near-isogenic lines of field beans (*Vicia faba*) with condensed tannin concentrations in the range 4.9–7.4 g condensed tannin kg^{-1} seed (assayed by vanillin-sulphuric acid reaction; Kuhla and Ebmeier, 1981) were used to study the effect of tannins on the nutritional value of field bean-based diets, measured in terms of growth performance. Although a small improvement (about 2.5%) in the feed intake/live weight gain ratio was detected in chicks given the tannin-free beans, compared with those fed on tannin-containing beans, there were no significant effects on feed intake or live weight gain (Helsper *et al.*, 1996). Since half of each diet comprised faba beans, the tannin content of the tannin-containing diets would have ranged from 2.5 to 3.7 g kg^{-1}. Broiler chicken diets formulated to contain different amounts of tannins (0.4–16.58 g condensed tannin kg^{-1} DM, measured by Folin-Ciocalteu reagent; Wareham *et al.*, 1991), by varying the proportions of hulls from high- and low-tannin cultivars, elicited significant decreases in AME_N and AMN as tannin content increased, but no such relationship was evident when tannin content was lower (0.44–7.85 g condensed tannin kg^{-1} DM; Wareham *et al.*, 1993). These authors did not find any consistent effect of dietary tannin content on carcass nitrogen retention when they used near-isogenic lines of faba beans included at rates from 200 to 600 g kg^{-1}.

Because of its high content of tannins, salseed meal is not normally used in poultry diets, but it has been used to formulate diets for the purpose of studying the effects of tannin-containing diets in birds. The gross effect of substituting salseed meal for barley (508 g kg^{-1} diet) on feed intake and growth are shown in Fig. 14.13. Even when the effect of lower voluntary feed intake was eliminated by restricting all intake to that of the salseed group, there remained a severe depression in growth (Mahmood, 1993). In birds fed salseed meal, apparent nitrogen digestibility was lower, the activity of trypsin in the small intestine was 62% lower and the activity of α-amylase was 86% lower. In addition to the effects on the enzyme activities in the lumen of the small intestine, the activities of

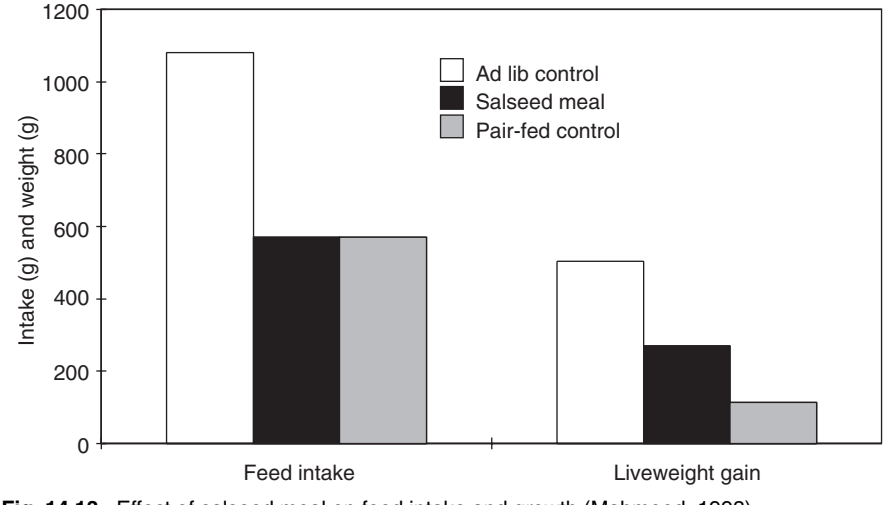

Fig. 14.13. Effect of salseed meal on feed intake and growth (Mahmood, 1993).

the mucosal enzymes, dipeptidase, sucrase and maltase were also lower in the birds fed on salseed meal. Soaking of salseed meal, in water, or acidic (0.67 M acetic acid) or alkaline (0.67 M sodium hydrogen carbonate) solution, followed by air drying (37°C), yielded products with apparently lower tannin content and lower anti-trypsin activity. However, when assayed, in colostomized hens fed diets containing 500 g salseed meal kg^{-1} diet the differences in digestibilities of starch and lipid were negligible, and only the alkali treatment made an appreciable effect on nitrogen digestibility (untreated 0.418 vs. alkali 0.551). Nevertheless, when the treated meals were included in chicken diets (300 g kg^{-1} diet) the water and alkali treatments substantially improved growth and gain/feed (Fig. 14.14).

Bearing in mind the lower activities of the starch digesting enzymes in the small intestine reported above, and the reported effects *in vitro* on carbohydrase activities and on glucose uptake (Carmona *et al.*, 1996), it is surprising that the diet containing untreated salseed meal (500 g kg^{-1} diet) lowered the digestibility by only 6% compared with a diet based on wheat (Mahmood, 1993). It would appear that, in the bird at least, carbohydrate digestion is quite robust in the face of dietary tannins whereas protein digestion is quite susceptible.

SAPONINS AND GLYCOALKALOIDS

Saponins in poultry diets often pass unremarked. Yet saponins are present in one of the most common of feed ingredients, namely soybeans. Also, lucerne (alfalfa) cultivars with various contents of saponins have been evaluated in chicks (Pedersen *et al.*, 1972), saponin extract from *Yucca schidigera* has been proposed as a potentially useful feed additive (Rowland *et al.*, 1976; Anthony *et al.*, 1994) and meal from *Vitellaria paradoxa*, which is high in saponin, has been studied for its potential as a poultry feed component (Annongu *et al.*, 1996a).

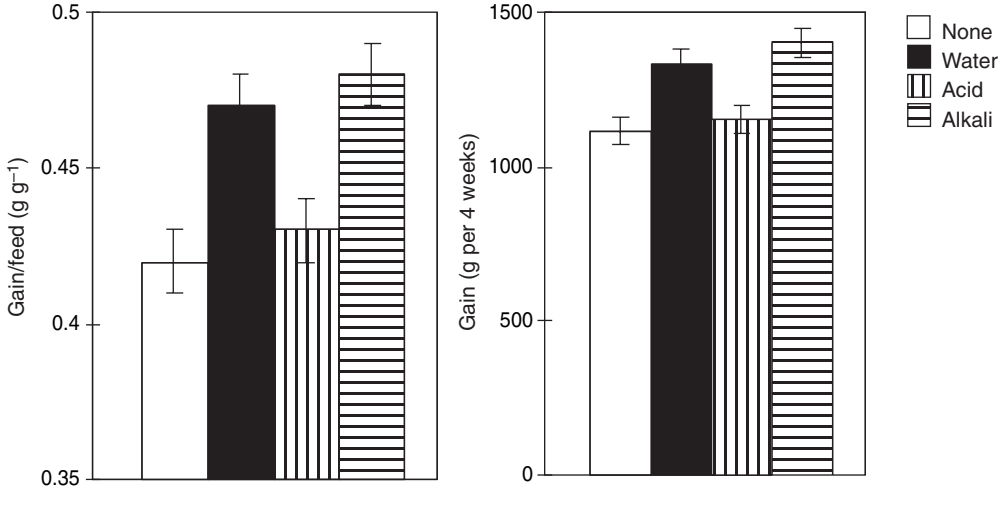

Fig. 14.14. Effect of soaking treatments on gain/feed and growth (Mahmood, 1993).

The saponins derive their name from their soap-like ability to produce foams; their amphiphilic behaviour stems from the combination of the hydrophilic saccharide units and the hydrophobic steroid or triterpene unit (Fig. 14.15). Commercial advantage is taken of the surface active properties of the *Quillaja* saponins as a foaming agent in the food industry, and in more-primitive circumstances the ability of saponins to interact with membrane lipids has allowed the use of saponin-containing plant extracts to poison fish. The glycoalkaloids of the *Solanaceae* are structurally similar to saponins inasmuch as they too are glycosides of steroid-like alkaloids (Fig. 14.16). The chemistry and biochemistry of these substances have been comprehensively reviewed by Price *et al.* (1987). The saponins occur in many plants and the fungicidal (Crombie and Crombie, 1986), molluscicidal (Hostettman *et al.*, 1982) activities as well as their bitter taste suggest that they play a role in protecting plants from predation and infection. Although the structures of the many different saponins share the essential features of this group of compounds, the differences in the aglycone and saccharide moieties make it difficult to generalize about their biological effects. At times the nutritional significance of the saponins has been dismissed as merely that of having a bitter taste. However, the surface activity of some saponins and the glycoalkaloids confers on them the ability to interact with the lipid bilayer of membranes; this makes the membranes permeable to molecules that would otherwise be excluded (Johnson *et al.*, 1986). For example, serum β-lactoglobulin in rats given this protein in their diets increased from a baseline value of 21 ng ml^{-1} to 530 ng ml^{-1} when it was given simultaneously with saponin from *Gypsophila* sp. (Gee *et al.*, 1997). Furthermore, *in vitro* studies on the uptake of glycinin, the main storage protein of

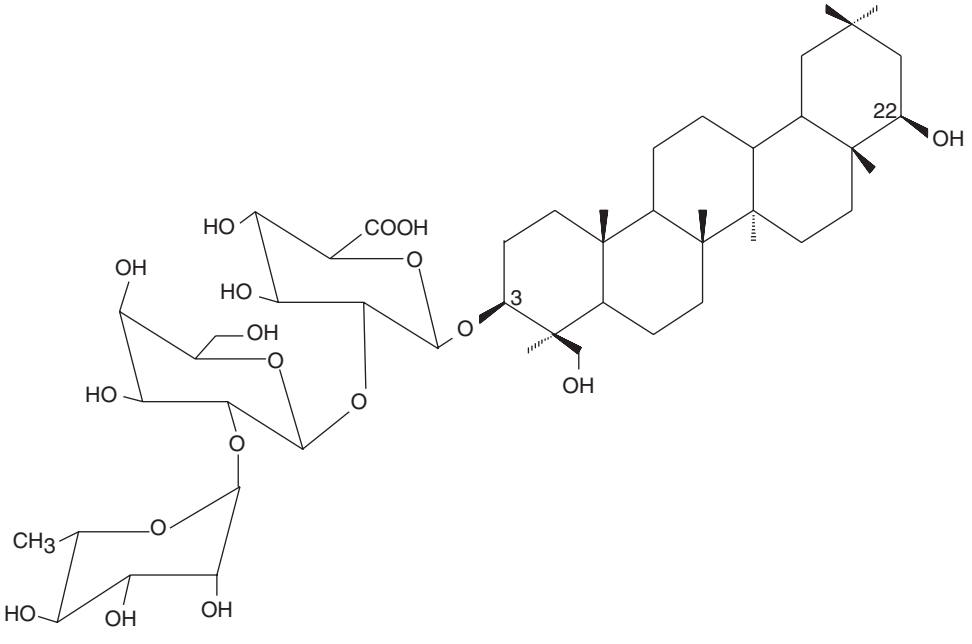

Fig. 14.15. Soysaponin I.

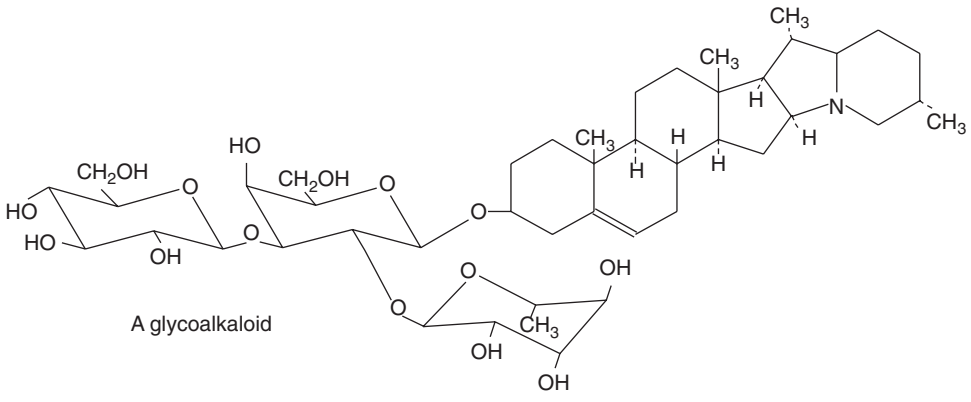

Fig. 14.16. α-Solanine.

soybeans (a sizeable protein molecule, 320,000 Da, 110×110×75 Å; Badley *et al.*, 1975) have shown a strong synergism between soysaponin and soybean lectin, whereby a tiny concentration of lectin (50 μg ml^{-1}) more than quadruples the effect of saponin alone (1 mg ml^{-1}) on membrane permeability (Alvarez and Torres-Pinedo, 1982; Fig. 14.17). One of the most important functions of the intestinal mucosa is that of providing a barrier between the body and the outside world, while still providing passage for nutrients. One of the consequences of increased intestinal permeability will therefore be to compromise this function, possibly allowing entry of toxins, microbes or allergens into the circulation. However, whilst increased intestinal permeability may be a response to dietary saponins shared by different species, *in vitro* evidence (Gee *et al.*, 1996) suggest that there are quite pronounced species differences, and extrapolation to poultry should be undertaken with due caution. Although soysaponins of the common leguminous

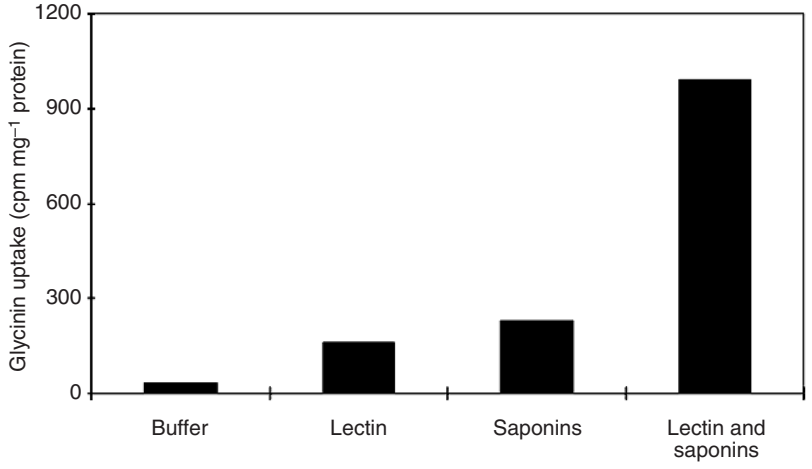

Fig. 14.17. Synergistic effect of soybean saponin and lectin on membrane permeability (adapted from Alvarez and Torres-Pinedo, 1982).

foods share, in common with other saponins, an amphiphilic structure, they appear to have little membranolytic activity on the gut (Gee *et al.*, 1989). Comparison of the cytotoxicity of *Gypsophila* saponins and glycoalkaloids, in terms of lactate dehydrogenase leakage, indicates that the glycoalkaloids have about three times the potency of the saponins (Gee *et al.*, 1996).

Whilst glycoalkaloids are unlikely to be encountered in the familiar poultry feeds, the temptation for feed compounders to use by-products from the food industry, and the possibility of glycoalkaloids being used by plant breeders to enhance pathogen resistance, make encounters more of a possibility than would once have been thought. Glycoalkaloids are well known to be toxic, but food poisoning from potatoes is, thankfully, rare. Glycoalkaloids are potent membranolytic agents and *in vitro* studies suggest that they aggregate with cholesterol in cell membranes to distort, and eventually disrupt the membrane as shown by Keukens *et al.* (1995). In the elegant hypothesis of these authors, apolar attractions between the steroidal moieties and cholesterol in the membranes result in assimilation of the glycoalkaloid into the bilayer structure, and subsequent polar attractions and hydrogen bonding between the saccharide moieties draw the glycoalkaloid molecules closer together, thereby distorting the lipid bilayer (Fig. 14.18). Complementary bonding between different saccharide moieties of the glycoalkaloids plays an important role in the synergism between different glycoalkaloids. Although α-solanine has very little tendency

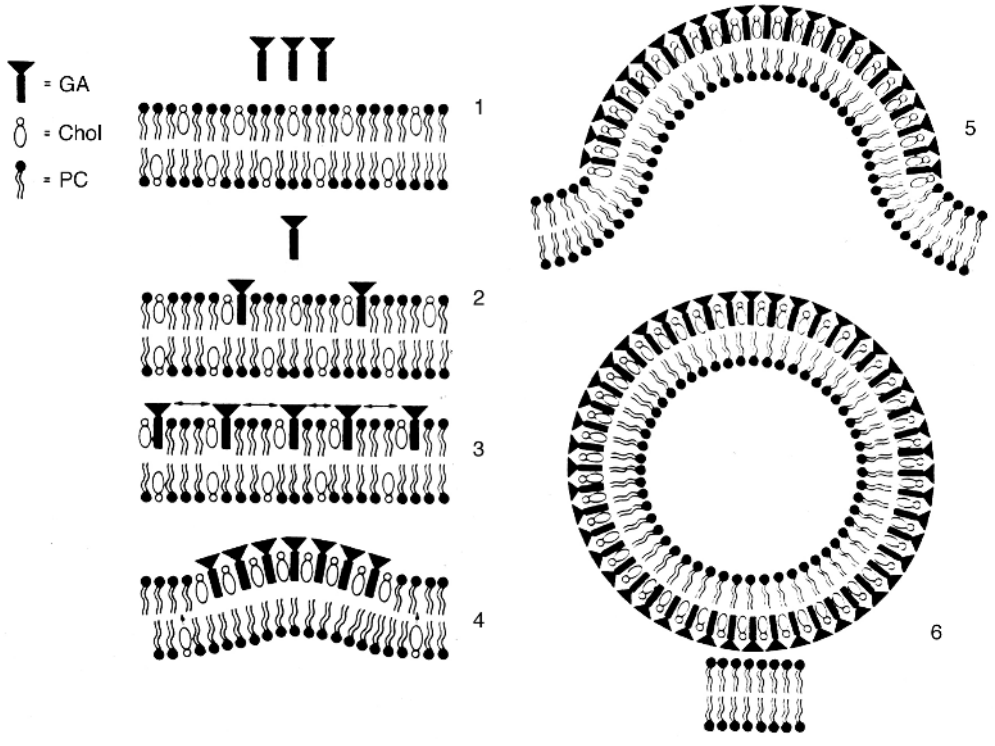

Fig. 14.18. The proposed model for glycoalkaloid-induced membrane disruption.

to form complexes with cholesterol, it very effectively does so when α-chaconine is present; 120 μM α-solanine alone causes almost no damage *in vitro* to membrane vesicles, but in the presence of 30 μM α-chaconine the permeability of membrane vesicles is ten times higher than with 30 μM α-chaconine alone.

Saponins are widely distributed in the plant kingdom and the saponin contents of several legumes used in human and animal foods are shown in Table 14.6. Although there appears to be well-founded evidence that saponins enhance the permeability of the intestinal mucosa to large protein molecules, there appears to be equally good evidence that a variety of dietary saponins exert a hypocholesterolaemic effect (Malinow *et al.*, 1977; Oakenfull *et al.*, 1979). Sidhu and Oakenfull (1986) proposed that the formation of bile salt-saponin micelles of around 10^6 Da renders the bile acids unavailable for the resorption phase of the enterohepatic circulation, thereby diverting cholesterol into compensatory bile acid synthesis.

Although saponins are sometimes said to affect palatability, because of their bitterness, in studies with geese, turkeys, quail and chickens fed on high- and low-saponin lucerne meal (1–20% of diet), it was only at the highest inclusion rate (20%) that any discrimination was made between the different cultivars of lucerne, and then only by geese (Cheeke *et al.*, 1981). In a related study, all four species of poultry discriminated against lucerne-containing diets when maize-based diets and maize-based diets that contained very small additions of quinine sulphate were offered as alternatives. Bearing in mind a suggested potential for meat improvement by lowering the birds' plasma

Table 14.6. Saponin content of seeds and sprouts of some legumes (from Fenwick and Oakenfull, 1983).

Feed material	Saponin content
Seeds	
Soy (*Glycine max*)	43
Mung (*Phaseolus aureus*)	5.7
Green pea (*Pisum sativum*)	11
Lentil (*Lens culinaris*)	3.7–4.6
Chick pea (*Cicer arietinum*)	56
Green bean (*Phaseolus vulgaris*)	13
Haricot bean (*Phaseolus vulgaris*)	19
Kidney bean (*Phaseolus vulgaris*)	16
Field bean (*Vicia faba*)	4.3
Lupin (*Lupinus albus*)[a]	0
Lupin (*Lupinus angustifolius*)[a]	0.3–0.5
Oats (*Avena sativa*)	1
Groundnut (*Arachis hyogaea*)	6.3
Sprouts	
Lucerne sprouts	87
Mung bean shoots	27

[a]Values from Muzquiz *et al.* (1993).

cholesterol, it may be of interest to note that when saponins were fed to chicks and adult roosters there was a reduction in plasma cholesterol (Griminger and Fisher, 1958). However, although addition of 6 g saponin kg^{-1} to the diet of 1-year-old roosters reduced plasma total cholesterol by 25%, the nature of the saponin was not clearly delineated. When *Gypsophila* and *Quillaja* triterpenoid saponins were included (9 g saponin kg^{-1} diet) in chick diets, feed intake, lipid digestibility and weight gain were depressed (Fig. 14.19). Furthermore, despite dramatic increases in cholesterol excretion, there were no changes in total blood cholesterol or high density lipoprotein cholesterol, but liver and blood levels of vitamins A and E were reduced (Jenkins and Atwal, 1994). The steroidal saponin from *Smilax ornata* (sarsaponin) had none of these effects. There have been claims that saponins may have a beneficial effect in poultry production, but these appear to have been based on rather slender evidence. Supplementation of broiler diets with extracts from *Yucca schidigera* (equivalent to 900 μg active saponin kg^{-1} diet) produced a small (3%) but significant improvement in body weight gain over 51 days (Johnston *et al.*, 1981) but no improvement in feed efficiency; when the extract was administered together with monensin there was no comparable improvement in growth, but there was an improvement in feed efficiency (Johnston *et al.*, 1982). When *Yucca* was included (up to 250 mg saponin kg^{-1} diet), under the enigmatic designation of 'urease inhibitor', in chicken diets, it failed to elicit any effect on feed intake, weight gain or feed efficiency. However, from 3 weeks application onwards, there was a startling reduction in mortality due to ascites (Anthony *et al.*, 1994). Although these experiments provide very slender evidence for beneficial effects of *Yucca* saponins in poultry, benefits to other species have been attributed to the antimicrobial activity of saponins. Athough extracts from *Yucca schidigera* may affect microflora *in vitro*, it appears unlikely that all of its *in vivo* effects can be attributed solely to the saponin component (Killeen *et al.*, 1998).

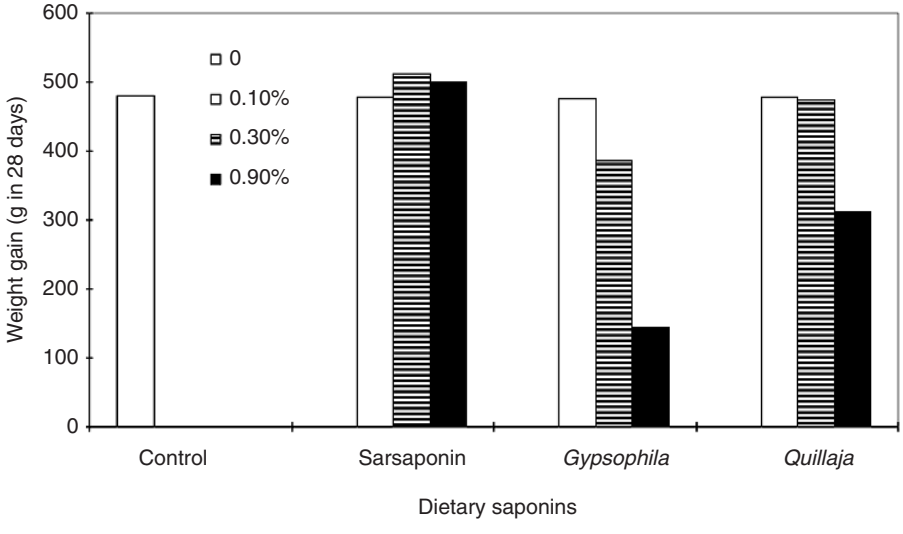

Fig. 14.19. Effect of saponins on chick growth (adapted from Jenkins and Atwal, 1994).

In Nigeria, efforts are being made to utilize indigenous plant products as components of poultry diets. Among these is the residue from the fruit of *Vitellaria paradoxa* after extraction of the edible fat, sheabutter. However, the presence in sheabutter cake of anti-nutritional substances, particularly saponins, discourages its use. Annongu *et al.* (1996a) have investigated the anaerobic incubation of wet sheabutter cake as a means of improving its nutritional value. Compared with untreated sheabutter cake, the fermented material used at up to 200 g cake kg^{-1} diet produced no apparent adverse effects, whereas the untreated cake affected plumage and resulted in 'emaciation with match-stick legs'. Although the authors reported that a diet containing untreated sheabutter cake induced areas of congestion and necrosis in the liver, no comparable observations have been made about the fermented cake (Annongu *et al.*, 1996b). Although no rationale was given, the positive results of supplementing the diet with polyethylene glycol or iron II sulphate in other groups in the same study suggest that the authors may have considered tannins to be the causative agent.

GLUCOSINOLATES

Glucosinolates occur in the Brassicas and in the past have been of most significance in rapeseed, largely *B. napus* and *B. campestris*. However, the value to industry of the erucic acid-containing lipids from *Crambe abyssinica* has led to interest in the by-product meal which contains glucosinolates. Mortality of laying hens, associated with liver damage, and fishy taint of eggs precluded the use of high-glucosinolate varieties of rapeseed in poultry diets (Butler *et al.*, 1982), but development of low-glucosinolate cultivars has made this grain a more attractive option. The name by which the low-glucosinolate varieties are sometimes known, Canola, acknowledges the work of Canadian plant breeders in producing many of these varieties. Useful reviews of many aspects of glucosinolate effects are available (Nugon-Baudon and Rabot, 1994; Shahidi *et al.*, 1997). Interest in these compounds in human nutrition has recently focused on their potential as anticarcinogenic agents (Shapiro *et al.*, 1998). There appears to be general agreement that the glucosinolates themselves exert little serious nutritional effect, but the degradation products generated when glucosinolates undergo hydrolysis and rearrangement catalysed by thioglucoside glucohydrolase (myrosinase) or by enzymes from bacteria in the bird gut (Marangos and Hill, 1974; Miguchi *et al.*, 1974) have well-documented physiological effects. Myrosinase occurs in idioblasts in the plant tissue and acts on glucosinolates when plant cell structure is disrupted. Progoitrin (2-hydroxybut-3-enyl glucosinolate), the principal glucosinolate of rapeseed, and its isomer *epi*-progoitrin, the principal glucosinolate of *Crambe abyssinica*, are degraded to 5-vinyloxazolidine-2-thione (Fig. 14.20), more commonly known as goitrin. These and other glucosinolates also produce a variety of other compounds including isothiocyanates and nitriles as well as the thiocyanate ion. The thiocyanate ion impedes the uptake of iodide by the thyroid gland and goitrin hinders the biosynthesis of thyroxine (Elfving, 1980). Slominski *et al.* (1988) showed that in poultry the caecum was the major site of glucosinolate degradation, suggesting an important role of gut microorganisms in the disappear-

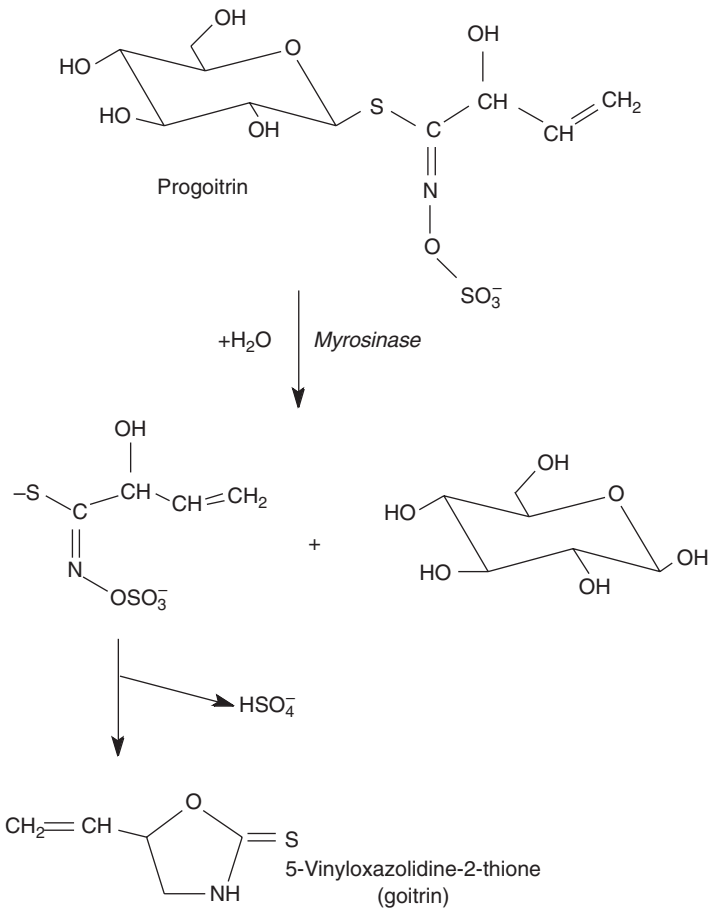

Fig. 14.20. Degradation of progoitrin.

ance of intact glucosinolates during their passage through the gut. When diets containing glucosinolates were fed to germ-free and conventionally reared chickens, adverse effects were observed only in the conventionally reared birds (Nugon-Baudon *et al.*, 1988). This illustrates a further role of gut bacteria in the induction of the physiological effects associated with ingestion of glucosinolates, but it also emphasizes that the intact glucosinolates are merely precursors of the physiologically active substances. Nevertheless, incubation either with homogenates of stomach or intestinal wall or with contents from the stomach or small intestine of rats provided no evidence of hydrolysis of the principal glucosinolates of rapeseed (Michaelsen *et al.*, 1994). However, loss of intact glucosinolate occurred on incubation with colonic and rectal contents, but surprisingly only when the rapeseed meal had not been heat-treated, suggesting that even in the large intestine hydrolysis was dependent on the plant myrosinase. *In vitro* investigation of transport by everted intestinal sacs from rats showed that glucosinolates are not actively transported, but can be passively absorbed (Michaelsen *et al.*, 1994).

The adverse effects of rapeseed in poultry diets include reduced feed intake, depressed growth rate and enlargement of thyroid and liver (Butler *et al.*, 1982). Goitrin may also aggravate another condition that arises in certain susceptible hens, namely that of fishy taint in eggs. The fishy taint arises from accumulation of trimethylamine (TMA) from another compound present in rapeseed, sinapine. Normally TMA is oxidized to trimethylamine oxide, but goitrin inhibits microsomal oxidation of trimethylamine allowing its incorporation in the ova (Butler *et al.*, 1982).

For rapeseed, breeding to reduce the glucosinolate concentrations has been the most successful means of avoiding the adverse effects. However, extraction has been used successfully to reduce both the glucosinolates and degradation products in crambe meal (Kloss *et al.*, 1994). When the extracted meals (100 g meal kg^{-1} diet) were used in a short-term feeding experiment, there were significant correlations between weight gain and glucosinolate intake; for example, over the first week the weight gain was depressed by about 4.3 g for each mg glucosinolate intake per g body weight. Although the extraction processes, particularly water-washing, were very effective at removing glucosinolates, none of the diets containing crambe meal gave weight gains as high as that for the control diet; the depressed gain could be attributed almost entirely to reduced feed intake.

THIOSULPHINITES

As well as plant metabolites whose presence in poultry diets is incidental, there may in future be some that are added deliberately to feed either for the benefit of the bird, or to add value (real or imaginary) to the animal product (egg or meat), or for other reasons that affect consumer perceptions. Already in agriculture there has been a considerable decline in the use of agrochemicals in response to pressure from activists of various persuasions; it is not improbable that similar pressures will come to bear on the poultry industry as well. Antibiotic use is already under challenge and, misguided or not, we may well find that herbal products are more acceptable to many laymen than the products of the synthetic chemist. Manipulation of animal product quality by dietary control is far from new. In the late 1960s, attempts to produce polyunsaturated dairy and beef products centred around the use of oils protected from rumen biohydrogenation. In poultry, egg lipids have been manipulated to enhance levels of *n*-3 polyunsaturated fatty acids (*n*-3 PUFA) and the effects of the 'healthy eggs' in the human diet have been evaluated (Farrell, 1998).

Garlic has long been a subject of folk remedy, but only comparatively recently have its claims been granted scientific respectability. Reduction in serum cholesterol has been reported as a response to garlic preparations in the diets of rats (Bordia *et al.*, 1975), and allicin (Fig. 14.21), the flavour principle of garlic, caused a beneficial alteration to the lipid profile of hyperlipidaemic rabbits (Eilat *et al.*, 1995). However, in clinical trials with human subjects the outcomes have been less convincing (Neil *et al.*, 1996; McCrindle *et al.*, 1998). Nevertheless, garlic products have been fed to chicks, resulting in lower hepatic cholesterol

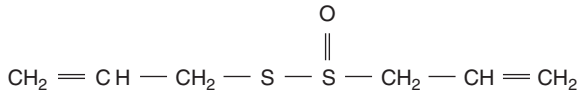

Fig. 14.21. Allicin.

(Sklan *et al.*, 1992) and adding garlic powder to a broiler diet decreased the cholesterol content by 30% in thigh muscle and by 40% in breast muscle (Konjufca *et al.*, 1997; Table 14.7). No matter how statistically significant such reductions may be when expressed in this fashion, when considered in terms of the contribution that they would make to a mixed human diet, it must be conceded that they are probably more of cosmetic than of nutritional significance. Nevertheless, reduced cholesterol chicken meat may find a niche market for a producer seeking to exploit added value as an alternative to quality.

CYANOGENIC GLYCOSIDES

Although the cyanogenic glycosides (Fig. 14.22) are frequently discussed as constituents of bitter varieties of *Prunus amygdalis* (bitter almond), it is not in that form that they are likely to be of most interest in poultry nutrition. Instead, much of the nutritional interest in cyanogenic glycosides has centred around their presence in the tropical root vegetable, *Manihot esculenta* Crantz, or cassava. In addition to their potential for direct toxicity through the liberation of hydrogen cyanide, they have also been implicated in tropical calcific pancreatitis (Tandon, 1998) and as a possible contributing factor to the malnutritional syndrome of kwashiorkor (Kamalu, 1993). Traditional processing, for example by grating, fermenting and heating, removes much of the cyanide, but when cassava is a staple food, chronic consumption of even the residual amounts may be sufficient to induce ill-effects. Cyanogenic glycosides are present also in some pulses, particularly Lima beans (*Phaseolus lunatus*), and in some grasses such as sorghum and Sudan grass. However, it has been the increased interest in linseed largely because of the presence of omega-3 fatty acids that has renewed enquiry into the effects and detoxification of these substances. Levels of cyanogens in linseed are quite high (365–550 mg 100 g^{-1}

Table 14.7. Effect of dietary garlic on muscle cholesterol and hepatic enzyme activities[a].

Added garlic	Muscle cholesterol (mg g^{-1} wet tissue)				Hepatic enzyme activities			
	Thigh muscle		Breast muscle		HMG CoA reductase		Cholesterol 7-α-hydroxylase	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	149	7	55	4	458	198	1.08	0.19
3 g $100g^{-1}$	105	9	32	4	272	81	0.65	0.07

[a]Konjufca *et al.* (1997).

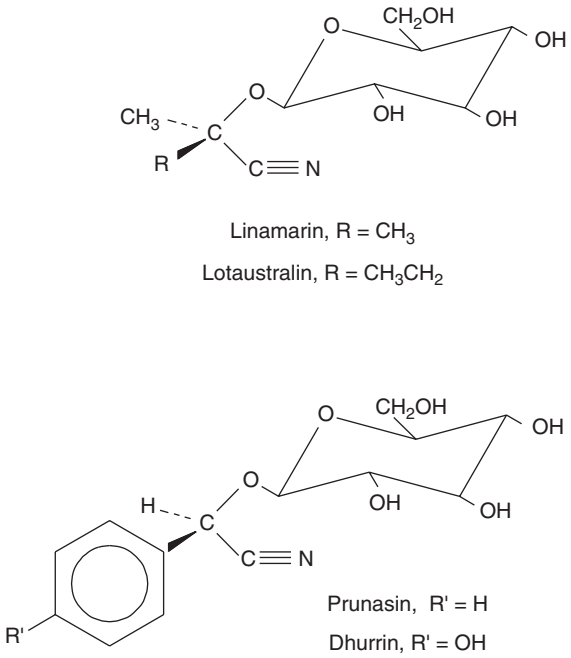

Fig. 14.22. Cyanogenic glycosides.

seed), even when compared with the traditional sources (Shahidi and Wanasundara, 1997). When plant tissue containing cyanogenic glycosides is crushed, the glycosides undergo hydrolysis catalysed by the β-glucosidases that are normally present but separated from the glycoside. The saccharide is first removed to leave the cyanohydrin and this subsequently hydrolyses further to the corresponding aldehyde or ketone and hydrogen cyanide. It is the latter which is the active toxin. The cyanide ion is a powerful complexing agent for the transition metals, and it readily complexes the iron in cytochrome oxidase, thereby uncoupling cellular respiration. In the liver, the toxicity of the cyanide ion is reduced by conversion to the thiocyanate ion (Fig. 14.23). However, it should be borne in mind that thiocyanate competes with iodide for uptake by the thyroid gland and under conditions of marginal iodine intake this could precipitate thyroid insufficiency.

When linseed cake was included in diets of chicks from hatching through to their 18th week, lower feed intakes and live weights were recorded for birds fed diets supplemented with the linseed cake. Elevated plasma thiocyanate and higher thyroid weight indicated that cyanide from the cyanogen was absorbed and metabolized (Richter *et al.*, 1998a). In laying hens too, serum thiocyanate was higher in birds fed diets containing 100 g linseed cake kg^{-1} diet. There was a tendency towards reduced laying, but the diet did raise the linolenic acid content of the eggs (Richter *et al.*, 1998b). On the other hand, Eder *et al.* (1998) reported no adverse effects when they used 100 g linseed (ground or whole) kg^{-1} diet to increase linolenic acid in yolk lipids.

$$CN^- + S_2O_3^{2-} \longrightarrow SCN^- + SO_3^{2-}$$

Fig. 14.23. Hepatic detoxification of cyanide.

PHYTATE

The ready availability of phytase with which to supplement livestock diets in the last few years has stimulated research interest in phytate and may in part have given rise to three recent excellent reviews on the subject (Kornegay, 2000; Maenz, 2000; Selle *et al.*, 2000). In the past, most interest in phytate has centred around the low bioavailability of the phosphorus that is built into its structure. If nutritional value of foods containing phytate is estimated purely by chemical analysis, this can lead to underestimation of requirement for supplemental phosphorus. Recently, concern has been expressed at the environmental burden of phosphorus that may result from the passage of undigested phytate though livestock, finding its way into waterways. The ability of phytate to form coordination complexes with cations (Fig. 14.24), particularly with divalent ones, such as Zn, Fe, Mn, Ca, but also with K ions (Biehl *et al.*, 1995) is also well known. The comparative ease with which phytate may be reduced or eliminated from feeds due to the relatively recent availability of phytate hydrolysing enzymes has made it easier to demonstrate the effect of phytate on other nutrients in foods. For example, Biehl *et al.* (1995) supplemented chick diets with phytase to demonstrate that phytate may detract from the mineral micronutrient nutrition of animals. They showed that there was an appreciable improvement in the utilization of zinc and manganese when microbial phytase is incorporated into the diet (Table 14.8).

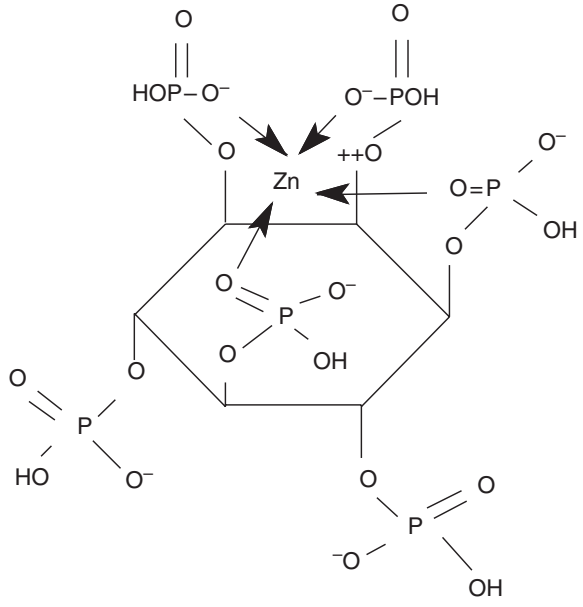

Fig. 14.24. Phytate–Zn complex.

Table 14.8. Growth performance and incorporation of zinc and manganese in tibia by chicks fed on a phosphorus-deficient diet supplemented with microbial phytase (adapted from Biehl *et al.*, 1995).

			Tibia results						
					Zn			Mn	
Dietary addition	Weight gain g in 12 days	Food intake g in 12 days	Weight (mg)	(μg)	(ng g^{-1} tibia)	(ng g^{-1} food)	(μg)	(ng g^{-1} tibia)	(ng g^{-1} food)
None	193	300	667	95	142	317	1.55	2.32	5.16
1200 U phytase	231	340	848	147	173	432	2.53	2.98	7.44

Phytase addition to broiler chicken diets has also revealed that phytate may enhance the digestibilities of some amino acids in female broilers (Sebastian *et al.*, 1997). Several other publications indicate positive effects of phytase on amino acid or protein digestion (van der Klis and Versteegh, 1991; Yi *et al.*, 1996). Whilst Sebastian *et al.* (1997) speculated that either low solubility of protein– or amino acid–Ca-phytate complexes as reported by Saio *et al.* (1967) or inhibition of proteolytic enzyme activities as reported by Camus and La Porte (1976) and by Caldwell (1992), are responsible for the lower amino acid digestibility in diets not supplemented with phytase, they could not explain the absence of a comparable effect in male broilers. The publications do not indicate whether the phytase supplement also exhibits proteolytic activity.

EPILOGUE

Although the common anti-nutritional substances are well known to the poultry industry and have been generally accommodated or their effects ameliorated, it is clear that a number of aspects still need to be resolved. Much of the nutritional behaviour of secondary plant metabolites remains to be understood at a fundamental level, and their effects remain to be explored, and possibly exploited.

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CHAPTER 15
Visual and tactile cues perceived by chickens

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INTRODUCTION

Feed particles are what a bird actually sees and touches in its diet. Broilers and layers eat using their own sensory perception of the food, ignoring in the short term all the chemical analyses done by nutritionists. It is the aim of this chapter to elaborate on the sensory perception of chickens with special attention given to the feed.

Pecking is a precisely controlled process, with time lapses between two actions that allow the chicken to observe and choose the next particle that will be seized or simply touched or moved by the beak. The strength of the peck and opening of the beak are adjusted to the target (see Yo *et al.*, 1997a, for review). Birds are very fast and these decisions are integrated in less than half a second. Only precise observation, using for example slow-motion video, allows the measurement of the intimate and apparently accurate relationship existing between a bird and its food. Humans are relatively slower than birds, having no beak, seeing with human eyes and touching with their fingers. However, humans prepare the chicken's food and measure the growth, breast yield, egg output and food conversion which are the final results of numerous decisions taken by chickens during their short life.

Diet formulation is mainly based on chemical analyses of raw materials and on estimates of chicken requirements measured in terms of production yields. More-detailed attention is given now to food technology, which acts on the physical structure of food particles. Measurement of the overall effect of food structure on production may be biased in experimental conditions because environmental constraints (space, troughs, rhythms of feed distribution, social effects, etc.) interact with the short-term decisions taken by the birds. Food intake behaviour can be observed both at experimental and at production levels and provides short-term information about the adaptation of the chicken to its environment complementary with the usual production measurements (Picard *et al.*, 1999).

The sensory perception of chickens is the crucial link between food technology and behavioural responses, a direct connection between the feed mill and the farm. The feed cues perceived by chickens must be described,

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classified and ranked according to their prevalence in terms of adaptation of the chickens to the farming conditions and in terms of productivity. New directions of research on food management could emerge from a better knowledge of the behavioural adaptation of chickens to the food characteristics.

SENSORY PERCEPTION

Food identification is one of the first essential challenges for day-old chicks. Pecking particles is at first independent from nutrition and rapidly learned by association between the sensory characteristics of the particles and their positively-perceived effects (Hogan-Warburg and Hogan, 1981; Hogan, 1984; Rogers, 1995). Sensory identification of food uses most sensory channels (Gentle, 1985), especially specific visual and tactile cues (Fig. 15.1).

Vision

Two large eyes, positioned on the side of the skull, convey a broad picture of the environment. Within this panoramic view, a narrow central area (approximately 25°) can be used for binocular vision. The monocular lateral fields seem specialized for the detection of distant moving items, whereas the binocular area would be more used for close-up observation of static objects (Hodos, 1993). It is still unclear whether or not the two types of visual cues have the same significance for birds. Within the monocular fields, angular zones are preferred for detailed observations. These are within the angular distances from the beak of 34–39° and 61–66°, corresponding probably to more-efficiently used areas of the retina (Fig. 15.2).

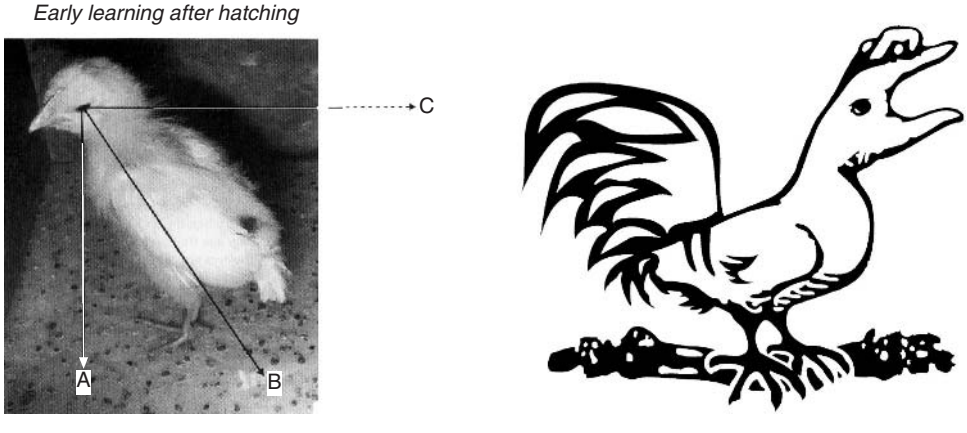

Fig. 15.1. Vision of details and tactile sensitivity of the beak are the two major tools used by chickens to detect and categorize food. The beak is the hand of birds. Just before a peck, several successive fixed positions of the head give access to a precise vision of details of a particle (A), while checking another particle on the floor (B) and any predator approach in an overview of the surroundings (C). Adapted from Rogers (1995).

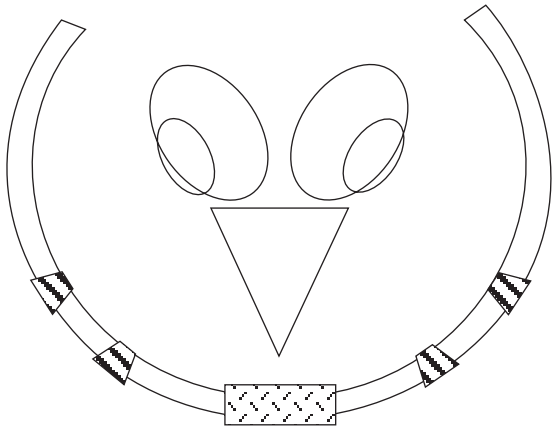

Fig. 15.2. Schematic representation of the visual fields of chickens. = Binocular vision zone. = Preferred angles (34–39° and 61–66°) used for detailed lateral observation. Adapted from Meyer (1987), Andrew and Dharmaretnam (1993).

The two hemispheres of the brain have distinct functions which might be more-efficiently stimulated by the corresponding eye (opposite side). This problem of lateralization can be illustrated by the role of the left hemisphere (right eye) for categorization of food versus non-food items and by the 'detailed analysis of the properties of one stimulus' which would be a specific activity of the right hemisphere (left eye). A number of studies demonstrate side preferences in several tasks varying with sex and age (Andrew, 1991; Dharmaretnam and Andrew, 1994; see Rogers, 1995, for review). However, Guiton (1972) demonstrated that monocular occluded chicks peck at food particles efficiently and that, after a period of occlusion, the deprived eye needs several days before being efficiently used again.

For brain lateralization, as for the use of frontal or lateral fields, chickens may have visual preferences but also large capabilities for adaptation. The distance at which a stimulus is presented seems to be a critical factor for identification. Adult hens discriminate more efficiently images when presented at 5–25 cm compared with 120 cm (Dawkins and Woodington, 1997). In the same experiment, the colour of the stimulus (red or blue) was more discriminant than its shape (saw or hammer). The peak sensitivity of the chicken's eye to colour is between 500 and 700 nm. Recent results suggest that vision in the blue and UVA range (380–500 nm) is greater than in human eyes (Prayitno and Phillips, 1997; Prescott and Wathes, 1998, 1999b). Chicks have tetrachromatic colour vision (Osorio *et al.*, 1999). UVA light might be important for social identification and reproduction (Prescott and Wathes, 1999a; Jones *et al.*, 2001). Chickens prefer to eat at high light intensity and, more generally, they require various types of lights for different activities. Taking into account the specificity of vision in poultry, novel lighting systems in poultry farms could be developed (Prescott, 1999).

Vision is primarily concerned with the detection of food in the environment and then with the observation of food particles at a close range between two consecutive pecks (Hutchinson and Taylor, 1962a,b; Yo *et al.*, 1997a). In pigeons, the grasping movement in itself is controlled by distinct neural pathways (Jäger *et al.*, 1992; Bermejo *et al.*, 1994). Visual cues are used to identify the food particles and to condition the peck localization and the opening of the beak, but the belief that the eyelid is closed during the peck limited the implication of visual control. In fact, in pigeons, the eyelid is not completely closed but leaves a 1.5 mm slit which would, conversely, improve the focal depth of retinal images during the peck (Ostheim, 1997).

Visual perception of food particles by chickens involves complex and precise tasks performed quickly. Spectral sensitivity of colours is slightly different to that in the human. Between two pecks, close observation of the details of the feed particles conditions and prepares the grasping actions.

Touch

Birds have a complex jaw apparatus compared to mammals, with more bones and joints (Bühler, 1981). The tip organ of the lower beak of a chicken includes 15–20 specialized dermal papillae containing highly specialized mechanoreceptors (Merkel and Herbst corpuscles) used for fine tactile palpation (Gentle and Breward, 1986). The beak is a multi-purpose tool used by the chicken for essential tasks, such as eating, drinking, touching the environment, preening, fencing against predators and conspecifics, mating in males and nesting in females (see Megret *et al.*, 1996, for review).

Tactile perception is used for learning about food at the beginning of life (Reymond and Rogers, 1981). The early pecking preferences are influenced by reward gained from tactile cues rather than from nutritional feedback. When nutrition is established, tactile perception contributes to the identification of the food. Beak-trimming induces post-ingestional perception to substitute for the decreased touch (Workman and Rogers, 1990). Beak-trimming decreases the risk of feather pecking, cannibalism and of feed wastage under some circumstances but raises ethical problems (see Cunningham, 1992, for review). When limited beak-trimming is performed at an early age in chicks (1–10 days), no behavioural or histological evidence of pain is measured (Gentle *et al.*, 1997). However, the loss of sensoriality is obvious. Beak-trimming is often performed at an age older than 10 days to be effective in the prevention of feather pecking.

The need for a certain amount of beak-related activities has been suggested in turkeys (Hughes and Grigor, 1996) and laying hens (Bubier, 1997). Pecking activities can be redirected after a change in the environment such as the floor type (Blokhuis, 1989). Feeding is just one activity of the beak. As such, the food is part of a larger system of adaptation of the bird to its environment, the beak being one of the major media used by chicken to relate to this environment.

More attention should be paid to the functions of the beak. There is no real mastication in birds, the tongue is rigid and the tactile cues are mainly per-

ceived when the particles are grasped and/or touched by the extremity of the beak. Fast growth in broiler chicks makes the length and the width of the beak double within 2 weeks. There is limited precise information on the food particle requirements of beak-trimmed birds, for example, food intake is reduced more by hard pellets than by mash when roasters are beak-trimmed at 50 days (Deaton *et al.*, 1988).

As the major exploratory tool for chickens, the beak is used for several activities. During the grasping of a particle, the beak perceives precise sensory information. However, the exact nature of this information is not known. The physical characteristics of feed particles which are optimal may change when chickens are beak trimmed.

Other

Olfaction is used in learning about food (Turro *et al.*, 1994). A number of molecules are detected by the olfactory system in chickens (see Jones and Roper, 1997, for review). The observed responses to odours depend on the dose. Increasing intensities of an odour are successively ignored, increasingly perceived as a positive and then as a negative factor (Burne and Rogers, 1996). The observed dose–effect response may be responsible for inconsistent results with odours, due to difficulty in monitoring precisely the dose of an odour or, alternatively, a failure to understand how food odours are used by chickens (Picard and Porter, 1998).

Young chickens perceive tastes (Ganchrow *et al.*, 1990). The lack of mastication and the mode of deglutition limits the practical importance of taste in feeding birds. Taste cues are combined with odours to form flavour, which is part of the post-ingestive information used by chickens to identify a food. In some instances, flavour may become a dominant signal, more important in avoiding an aversive item than visual cues such as colour (Roper and Marples, 1997). Sensory cues such as taste and colour may be combined or associated separately, depending on the context of learning (Franchina, 1997).

A number of signals are also perceived by the digestive tract after deglutition. For example, crop repletion (Savory, 1979, 1985, 1999) and duodenum content (Jackson and Duke, 1995) have direct regulatory effects on food intake; gut signals such as cholecystokinin are involved in the memorization process of a new food colour in Japanese quails (Berthelot *et al.*, 1996). Food identity depends on a combination of sensory, digestive and metabolic information, of which visual and tactile cues are the first perceived. Their dominant influence is on the recognition process which leads to ingestion, and they participate in the determination of the amount of food eaten on a short-term basis.

Food odours are perceived by chickens but their exact influence on ingestion is not clear. Food flavour (taste plus odour), digestive tract and metabolic signals are combined with visual and tactile cues to build progressively the identity of the food.

FOOD CUES

Chickens eat a mixture of particles issued from various technological treatments such as grinding, cooking or drying, solvent extraction, pelleting, adding liquid enzymes or coating with fat, crumbling, cooling (and many more if trace elements are included in this description). Furthermore, foods are processed through various systems of storage and distribution before entering feeding troughs. Normal foods are indeed a relatively heterogeneous mixture of particles when they reach the bird's accurate eye and beak. A real challenge to the food industry is to select the characteristics that are predominantly important in seeking to improve the performance of chickens and their adaptation to the environment. Some of the major cues are discussed below, considering successively the predominantly visual (movement, size, shape, colour), and tactile cues (hardness, roughness, elasticity). In addition, two general factors which affect perception of the cues, homogeneity of the feeding experiences of the bird and context of the feed, are briefly presented.

Movement

Movement is part of the feeding behaviour of chickens. Pecking can be stimulated by a moving arrow as it can be by other birds eating close by. Live insects are actively hunted by wild birds but also by domestic chickens. A moving target is a strong stimulus to eat. A bunch of white string was demonstrated to be attractive for pecking in chicks (Jones *et al.*, 2000). However, static devices were found more attractive than those incorporating occasional movements (Jones, 2001). Movement, like most sensorial cues, can have opposite effects depending on its 'dose' and the substrate to which it is applied. Even if 'jumping pellets' are still to be invented, movement is not absent from systems of feeding laying (moving hoppers in front of the cages) or growing fowl (sequential feed delivery in troughs which repeat noisy and moving stimuli several times daily). The situation may be quite different from farm to farm and especially when, under experimental conditions, troughs are not touched for days. Feed management will be considered at the end of this chapter.

Size, Shape, Colour

Of these three major visual cues, colour is probably the only one not concerned with the beak or post-ingestive mechanisms. Young chicks are attracted by brightly coloured particles appropriately sized to the shape of their beaks. There is a large variability in the published evidence about colour preferences (e.g. Rogers, 1995; Weeks *et al.*, 1997). A key question is the relative intensity of colour actually perceived, taking the spectrum of the chicken's eye into account (Prayitno and Phillips, 1997). Recently, results obtained with broilers suggest that although the birds can easily adapt to a wide range of coloured foods, they prefer a familiar colour which has been coupled with nutritional experience

rather than an unknown one (Picard *et al.*, 2000). Quail chicks exhibited an accurate visual memory of colours expressed by pecking at coloured beads with habituation and dishabituation capabilities (Aoki *et al.*, 2000). The shape of the beads (ball or triangle) was also memorized, but asymmetrically: chicks distinguished the triangle as a novel object after habituation to the ball, but did not respond to the ball after being adapted to a triangle (Sakai *et al.*, 2000).

A body of published evidence confirms that chickens select relatively large particles to eat first (i.e. 1.18 to 2.36 mm at 8 and 16 days of age and >2.36 mm in older broilers, Portella *et al.*, 1988). The preference for a given size seems to be relatively independent of food composition (Wauters *et al.*, 1997). Particle size can influence the nutritional choice of broilers offered a choice between maize (whole, cracked or mash) and a protein concentrate (pellets or mash). From 2 to 6 weeks of age the ratio varied from 27% of mash concentrate offered with mash maize to 37% of pellets offered with whole grain (Yo *et al.*, 1997b). When a diet is diluted with fibre-rich raw materials, the effects of combining the particles into pellets are emphasized in growing and laying fowl (Savory, 1980; Newcombe and Summers, 1985; Vilariño *et al.*, 1996). The predominant effect of the size of particles on performance was demonstrated in broilers and turkeys by Plavnik *et al.* (1997). When steam-pelleted diets, supplemented with various concentrations of carbohydrates or fat, were reground, the improvement in growth and feed efficiency compared with the corresponding mash diets was completely abolished.

Size of ground particles can be expressed as median diameter or geometric mean diameter, which express, respectively, the central tendency of the particle size distribution or the geometric standard deviation, giving a dispersion tendency of the distribution. Both factors act on broiler performance (Nir *et al.*, 1994a). The type of mill used (i.e. hammer mill or roller mill) and its parameters, and also the moisture content of the materials, change the precise characteristics of particles of the same size (Nir *et al.*, 1990, 1994b). Another expression of food particle characteristics is the specific surface, which represents the external surface area including open pores and cracks. The specific surface of particles of the same size may differ according to the technological history of the material (Melcion and de Monredon, 1987). Both grinding and pelleting methods act on food intake (Nir *et al.*, 1995). This can be due to a variation in the particle size actually available to the chickens. However, pellet processing includes effects other than size, such as heating, solubilization and crystallization of feedstuff components (Thomas and van der Poel, 1996), which may act on food utilization in addition to food intake stimulation due to size (Hamilton and Proudfoot, 1995).

Size of the particles eaten is almost immediately evaluated by birds (Nir *et al.*, 1990). After probing, they can adjust progressively their behaviour to the offered particles. A balanced diet was prepared using screened (0.5–3 mm) particles of maize (46%) and peas (30%) as parts of a grain mixture, or crumbs made from the same batch of screened parts. The two resulting foods had exactly the same composition and particle size. To the human eye, the two feeds could not be visually distinguished without amplifier. Under a binocular microscope, the crumbs had a slightly more faded colour than the other mixture

and their shape was ovoid with less sharp angles. Thirteen-day-old broilers immediately identified them (Picard *et al.*, 1997a). Their reactions epitomize three major features of food intake behaviour in chickens (Fig. 15.3):

- Instantaneous reaction to a variation in physical characteristics different from that previously consumed (Nir *et al.*, 1990);
- Minimal consumption of a 'new' food at first, which is maintained even if the food is not preferred subsequently, to provide information about edible material in the environment (Hogan-Warburg and Hogan, 1981);
- Progressive switch of preferences according to sensory and nutritional testing of the food. For example, pieces of grains are preferred to crumbs at first reaction because pieces are more similar to the previous control diet than crumbs. After 2 h of consumption there is no clear choice, and after 24 h a clear preference for the crumbs is evident.

Particle size is a major factor in food perception under visual control linked to the efficiency of pecking and probably also of deglutition. However, factors other than morphology modulate identification and prehension of the food particles.

Hardness, Roughness, Elasticity

Wheat kernels or pellets of the same average size differ in colour and other visual details, such as brightness, shape (ovoid or cylindrical), angularity (kernels are smooth, extremities of pellets are rough), texture, which can be expressed by hardness (seeds are much harder than pellets) and probably by 'elasticity'. However, this last parameter requires standardized methods of evaluation. When chickens peck at grains or pellets, the characteristics of their

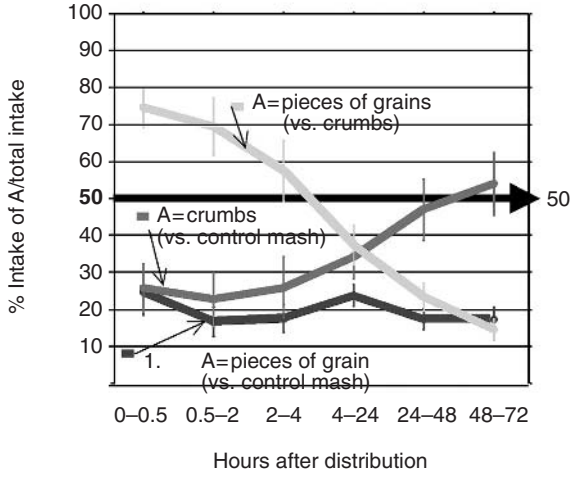

Fig. 15.3. Choice between food particles of the same size and composition offered as pieces of grains (maize and peas) or crumbs of the same ingredients or control mash diet received by 13-day-old chicks prior to the test (Picard *et al.*, 1997a).

pecking rhythm and pecking strength differ significantly (Yo *et al.*, 1997a). However, the precise reason for the differences remains unclear. Any or a combination of the variables listed above and/or the post-ingestive information can induce distinct behaviour.

The raw material composition of the food and the process used for preparing pellets both influence the hardness (or resistance to crushing strength) and the durability (or resistance to abrasion) of the particles obtained (Thomas and van der Poel, 1996; Thomas *et al.*, 1997, 1998). The methods of evaluation of 'pellet quality' used under practical conditions actually evaluate a combination of both characteristics. For chickens, durability is probably related to the 'size' information of the food particles in the trough. Hardness is a completely different type of information given to the beak, and it may be related also to the ease of digestion in the upper part of the digestive tract. When the particles offered are of the same size, hard pellets are eaten faster than soft pellets by broilers, irrespective of the size of mash particles before pelleting (Picard *et al.*, 1997b). It seems important for improvement in technology to distinguish between the effects on feed intake behaviour of the hardness of a particle and the presence of a proportion of small particles caused by abrasion even if, at feeding trough level, these factors are usually present together.

When various proportions of mash, soft pellets and hard pellets of the same food are given to broilers, feed efficiency is stimulated by the size of the particles, although hardness may reduce intake when the feed is pelleted twice (Fig. 15.4). The same experiment showed that development of various sections of the gut and of enzyme activity are distinctly affected by size (mash or pellets) and hardness of the particles (Nir *et al.*, 1994c). Thus effects on digestive tract may interact quickly with feed intake behaviour in addition to cues perceived by the beak. More severe treatment conditions are required to produce harder pellets. A higher hardness possibly means subsequent higher modifications of the seed components which induce changes in the digestive tract. Furthermore,

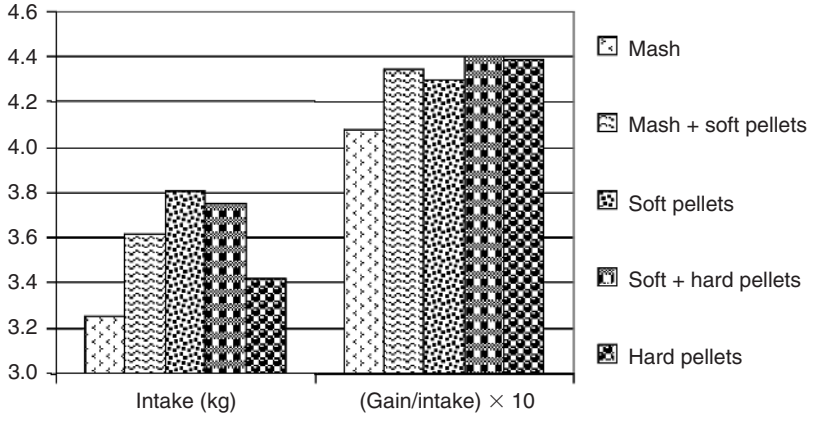

Fig. 15.4. Effects of the physical structure of feed particles on food intake and food conversion in 7- to 42-day-old broilers. 'Soft' pellets were pelleted once, 'hard' pellets were pelleted twice successively. Mixes were in equal proportions (50/50, w/w). (Taken from Nir *et al.*, 1994c.)

the results obtained in individual cages where the feed is found with few social interactions and activities may differ from that underlying the results obtained in field conditions. From several evaluations in practical poultry sheds it seems that hardness of the feed particles is a major factor modulating the speed of eating. This factor may become more critical during the finishing period in broilers and turkeys when bird density is high.

Recent unpublished results show that when the edges of a cylindrical pellet are slightly smoothed, broilers are able to detect the variation and prefer to eat the less rough particles. Testing preferences is useful in providing information on detection. However, it does not mean that an improvement in production may be expected when the preferred item is offered alone. Methods, which might be used to characterize a food particle further, must relate the detailed bird reactions to physical measurements performed on food particles. New choice procedures and methods to describe detailed patterns of feed prehension are under development. These will be coupled with an artificial vision system, in order to characterize objectively the appearance of the feed. Numeric images of the feed can be produced using a CCD camera connected to a computer. Precise computed data on colour, size, shape and texture of feed particles can be compared with animal choices and patterns of pecking.

Hardness of feed particles modulates the speed of eating and possibly the strength of pecks independently of their size. Other parameters such as roughness and elasticity are potentially important to the beak but they require further research.

Homogeneity, heterogeneity

Novelty of a feed can be both attractive and fear-inducing (Hogan, 1965). Food neophobia (fear of novelty) seems to be observed more frequently in fast-growing than in slow-growing hybrids (Jones, 1986). Familiarization to visual cues such as red coloured water facilitates the later consumption of a red solution of vinegar (Franchina and Slank, 1989; Franchina, 1991). This suggests that food identification is based on a combination of learned cues or that a familiar cue has a reassuring effect which might reduce fear (Jones, 1996). Familiarization with the colour of the food has been described as 'feed imprinting' by Bessei (1980). Familiarization with one food may reinforce the memory of cues used by chickens for its categorization. More complex experiences during development can reduce the reactions of chicks toward an environmental change (Broom, 1969). Homogenization of the environment of broilers and turkeys under practical conditions may 'over-familiarize' them to a limited number of environmental cues, a situation where neophobia toward slight changes might be favoured (Picard, 1997; Picard *et al.*, 1999). Because eating is the major activity of chickens under usual farming conditions, homogeneity or heterogeneity of the cues perceived from the food itself are potentially important in the monitoring of fear reactivity. Food is probably the best medium to vary something important for the chickens in their environment.

Sensory cues from the food can also be evaluated in term of homogeneity and heterogeneity which might modulate the reactions of chickens toward novelty.

Context

Fundamental research studying visual pattern recognition by pigeons suggests that the 'context' of a task is important. Objects placed close to a target reduce correct identification scores of the target by the bird compared to the target alone (e.g. Donis *et al.*, 1994). The way chickens classify and organize the sensory cues perceived from their environment may be of real practical importance, but it is still more hypothetical than actually understood. For example, chickens approached a video image of feeding chicks sooner than one of a food dish, but adding a soundtrack of feeding chickens to both images did not significantly change the latencies (Clarke and Jones, 2001). Chickens can react to a variation of their environment by changing one or several behaviours in ways that do not always seem logical to humans, e.g. a change of floor type may induce redirected pecking toward feathers in other birds (Blokhuis, 1989). Food is part of the environment of the chickens, but it is unclear if they have a concept of what 'food' represents. As for several other animal species, a cognitive representation of food in the mind of a chicken is still a matter of scientific debate (Haskell *et al.*, 2001). Thus, sensory perception of a food cannot be dissociated from the 'context'. In practical terms, context means trough, shed, litter, light, mates, management of the food distribution, etc.

IMPLICATIONS AND PERSPECTIVES

A brief review of the implications and consequences of sensory food cues is presented considering their three major behavioural effects on speed of eating, food selection or choice and food identification. Specific sensory requirements and consequences for food management are discussed. This part aims at giving some insights rather than an exhaustive view of application.

Speed of Eating

When a food is easy to peck, it is eaten faster. Speed of eating can be measured by relating the number of accesses to the trough, pecks at the food, and time spent eating, to the weight of feed consumed (Fig. 15.5). The easiest parameter to measure on the farm is the time spent eating, by means of scan sampling (Picard and Faure, 1997). Several factors independent of the feed itself, such as social context, can be responsible for variation in eating speed (Nielsen, 1999). However, food intake behaviour is not reducible to the measurement of eating speed; food quality, such as hardness or size of the particles, does change the time allocated to eating. Thus, it is true that social factors modulate eating patterns, but several feed characteristics are also involved and the observed characteristics of 'speed of eating' are typically an interaction between the diet and the other environmental factors. This last obvious observation is not a good reason to give up with such measurements. On the contrary, this kind of variable can be useful on the farm to analyse practical situations. The idea of the 'meal' (or 'feeding bouts') is open to criticism in chickens, because the minimal durations

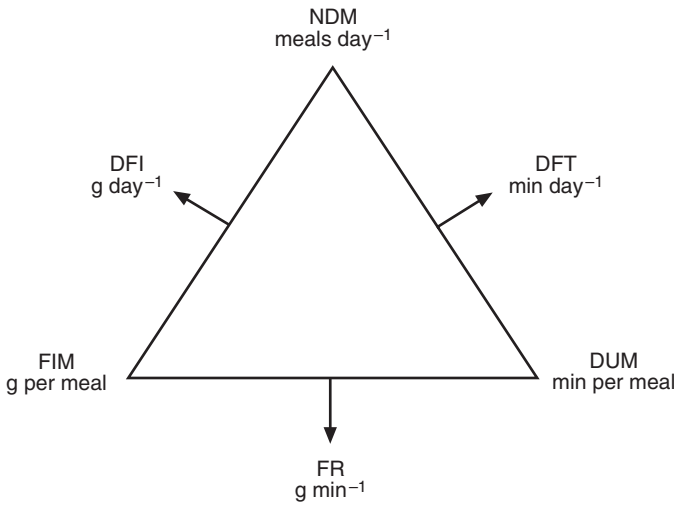

Fig. 15.5. Components of feeding rhythms and their interrelations. NDM = number of daily meals, FIM = average feed intake per meal, DUM = average duration of a meal; DFI, daily food intake; DFT, daily feeding time; FR, feeding rate, averages are daily within animal (from Nielsen, 1999).

of a bout and of an interruption are dependent on the recording equipment and on large individual variability (Picard *et al.*, 1992; Yo *et al.*, 1997a).

Average feeding rate (g min^{-1}) can be further dissected by splitting between successful or consummatory pecks versus unsuccessful or exploratory pecks and duration of inter-peck activities (observation, head movement, mandibulation). Digitalized video recording is now routinely used in the laboratory to analyse detailed time patterns of food pecking. A part of pecking gesture can be described as stereotyped movement (Van den Heuvel and Berkhoudt, 1998). A change of food texture induces disorganization of the time patterns of food pecking by increasing the duration of the observation periods and the proportion of exploratory pecks. More specifically, the change of diet changes the proportion of time spent in consistently organized patterns and the time spent in less structured exploration of the food (Martaresche *et al.*, 2000). This can be used to measure reactions of chickens to feed cues coupled with detailed analyses of particle selection.

Speed of eating can be measured on the farm by scan sampling. It is the result of interactions between the food texture and environmental factors (i.e. bird density). Effects of feed cues on detailed time patterns of pecking require further research.

Selection of Food Particles

Food particle selection is the favourite sport (and main occupation) of chickens on the farm. Layers eat particles in decreasing size order after the passage of the hopper in front of their cages (Rousselle and Rudeaux, 1994). Composition

of the food in terms of raw materials may change the nutrient content of particles of different size and change protein intake in broiler chicks (Wauters *et al.*, 1997). Nutritional consequences of food selection vary from bird to bird, and may therefore increase variability and consequently 'safety margins' used in feed formulation. Feed management practices aim at reducing bird selection by obliging them to finish their allocation. These practices result in achieving a flock average intake but, conversely, they may increase bird competition and the efficiency of some individuals at particle selection when food is delivered. Social stimulation at the trough stimulates more the number of pecks given to the feed than the actual amount of food eaten (Keeling and Hurnik, 1996).

Selection of food particles is the elementary expression of choice. The mechanisms of diet selection lead, in the medium term, to an approximate nutritional balance (Emmans, 1991; Emmans and Kyriazakis, 2001). On a short-term basis, fluctuations can be large and choices reflect sensory preferences and punctual stimulation (Rogers and Blundell, 1991; Kyriazakis, 1997). The time-window and mechanisms used by chickens to harmonize their short-term pecking choices with medium-term nutritional wisdom are still largely ignored (Collier and Johnson, 1990). Hedonic sensory attraction stimulated by social competition is not the only drive to food particle selection. Specific appetites for nutrients such as calcium follow the requirement of a laying hen during the last hours of the day and make the birds specifically select calcium carbonate particles at that time (Mongin and Sauveur, 1979). Selection of a specific nutrient is learned by association with sensory cues from the sources (Hughes, 1979).

Chickens first eat the large particles because of sensory preferences reinforced by social competition (or stimulation). When a nutrient is strongly lacking at the metabolic level, sensory hedonism is tuned to fit nutritional wisdom.

Identification of the Feed

Sometimes a minor change of diet can induce periods of non-eating (of several hours). Are these transitory reactions due to the fear induced by the change, neophobia (see above)? Alternatively, do the chickens not eat for a time because they do not recognize the food as being an edible material, a reaction that could be distinct from fear? Inhibitions, escapes and fights, which might be associated with fear reactions, are not frequently observed immediately after the change. Birds seem to ignore the new batch of food in the trough and become more active only when hunger increases their motivation to eat. Visual, and maybe tactile, characteristics of the food particles seem to be mainly responsible for this lack of identification. However, their occurrence is not easily predictable from the food cues as they typically result from an interaction between the past experiences of the chicken and the novel food cue(s).

Young broiler chickens are adaptable to a large range of constraints and feed cues (Turro-Vincent, 1994). With increasing age, broiler chickens reduce their exploration of the environment and their ability to detect hidden food (Vilariño *et al.*, 1998). Whether this reduced ability is due to a reduction of

locomotion linked to fast growth, or to an insufficiently stimulating environment that favours routine behavioural patterns, is open to debate. Broadening the sensorial experience of a chicken by prior exposure to a variety of food colours, for example (Jones, 1986), can reduce the latency to peck at a new diet. This enrichment of the environment or of the sensorial experiences of a chicken is known to have fear-reducing capacities (Jones, 1996). Feral chickens have to probe a variety of food items in a changing environment from the time of hatch. Sensory stimulation is appreciably lower under commercial conditions. In terms of sensory cues, practical requirements might be considered as a time-dependent process to facilitate adaptation.

Transitory lack of identification of a new batch of food by birds might be reduced by enrichment of the environment including experiencing of feed cues before the new delivery.

What Requirements?

The goal to produce meat and eggs efficiently for human consumption is central to most determinations of 'requirement' for farm animals. Sensory satisfaction of chickens may be seen as a secondary preoccupation. However, adaptation to farming conditions is a common currency between the bird and the farmer, and sensory detection is a major clue to understanding the reaction of birds. This chapter has already described several situations where the consequences of a food cue depend on other environmental factors. A second difficulty in defining a sensory requirement is the rapid adjustment of the chickens, who learn the cue and include it in their knowledge of the environment. When a diet is deficient in methionine, the metabolic paths to correct that methionine deficiency are limited and the effect of the diet on growth is relatively consistent over time. A requirement can be measured. When a diet is made of soft pellets coloured purple, the bird will first be surprised and then adjust its behaviour to the new diet if this does not induce a malaise after consumption. However, the colour of the pellet may induce transitory inhibition of eating, or the soft pellet may reduce pecking efficiency. Both reactions can depress performance under some farming conditions but not all. A 'requirement', for such variables appears to be flock dependent.

Poultry diets are usually prepared to stimulate intake and production. When given free choice, chickens select in general an almost balanced diet, but one that is slightly less growth-stimulating (e.g. Munt *et al.*, 1995; Picard *et al.*, 1997c). A food considered as good quality because the pellets are eaten easily and fast and because the growth performance is high may also increase the mortality rate of broiler males from sudden death syndrome (Proudfoot and Hulan, 1989) or ascites (Nir *et al.*, 1995). There is a need in broiler and turkey production to monitor growth and food efficiency at different stages of development. Sensory cues might very well be used to reduce or stimulate food intake or activity of the chickens according to daily records of a flock in the same way as the lighting programme is manipulated.

Finally, although our scientific work is based on relatively crude comparisons (i.e. meal versus pellets), reports of refined measurement of hardness or even precise determination of size of the particles eaten are scarce. Accurate information about food characteristics is insufficient to allow definition of requirements. Manipulation of the sensory cues of a diet is a tool to adjust the short-term adaptation of chickens to farming conditions and to monitor food intake and activity of the birds. It does not match universal norms. More precise data on food characteristics are needed.

Feed Management

Feeding chickens is not only about formulating a balanced diet with carefully controlled ingredients in a clean modern plant. Feed distribution on the farm becomes an increasingly critical and technical step in the process. In Europe, the use of whole grain wheat and maize is growing (see Forbes and Covasa, 1995; Noirot *et al.*, 1998, for reviews). In practice, whole grain can be included in the pellets, mixed on the farm with a complementary diet, or given alternately with a complementary diet. The three techniques are all effective. Including whole grain in pellets is visually perceived by the chickens and the incorporated grains facilitate fragmentation of pellets. Much experience of mixing on farm exists in northern Europe. Chickens receiving a mixture of pellets and grains usually select the cereal grain first. Alternate distribution of wheat and complementary pellets during successive periods within a day is also an efficient method (Rose *et al.*, 1995). This last technique has been successfully tested in French farms. It opens new routes because the use of two different feeds allows more flexibility in the day-to-day adjustment of the diet in order to monitor growth. It also enriches the sensory experiences of chickens by reducing the homogeneity of their environment.

Sensory cues need to be evaluated in an integrative approach studying for example, the lighting programme and the texture of food particles (Hamilton and Kennie, 1997). The mode of distribution of the food according to its sensory cues is part of the decisions which require more dialogue between the nutritionist, the food plant manager and the farmer. Food intake behaviour can be quantified on the farm just as major chemical and physical characteristics of raw materials and food are controlled at the food mill today.

CONCLUSIONS

'Chickens *peck* at *particles* of their *environment*'. The three keywords of this obvious sentence recall that:

- *Pecking* is a precise hi-tech activity with accurate sensory perception of the food using vision of details and sensitive touch of the particles.

- *Particles* are the elementary feedstuff for the chicken and their size and hardness are the only measured variables to date, although a more precise definition might emerge from a reinforced dialogue between nutritionists and technologists.
- *Environment* means other chickens, light, trough, feed, human, etc., which have been homogenized to standardize and optimize growth, but, by reducing the sensory experiences of the chicken, homogenization might have induced over-reaction (or lack of identification) when minor changes occur.

New farming conditions raise new problems of adaptation. Because feeding is a dominant occupation for chickens, food intake behaviour is an excellent tool to measure and solve these problems. Implication of sensory cues of the food and food technology depends on the phase of food intake behaviour involved (Fig. 15.6).

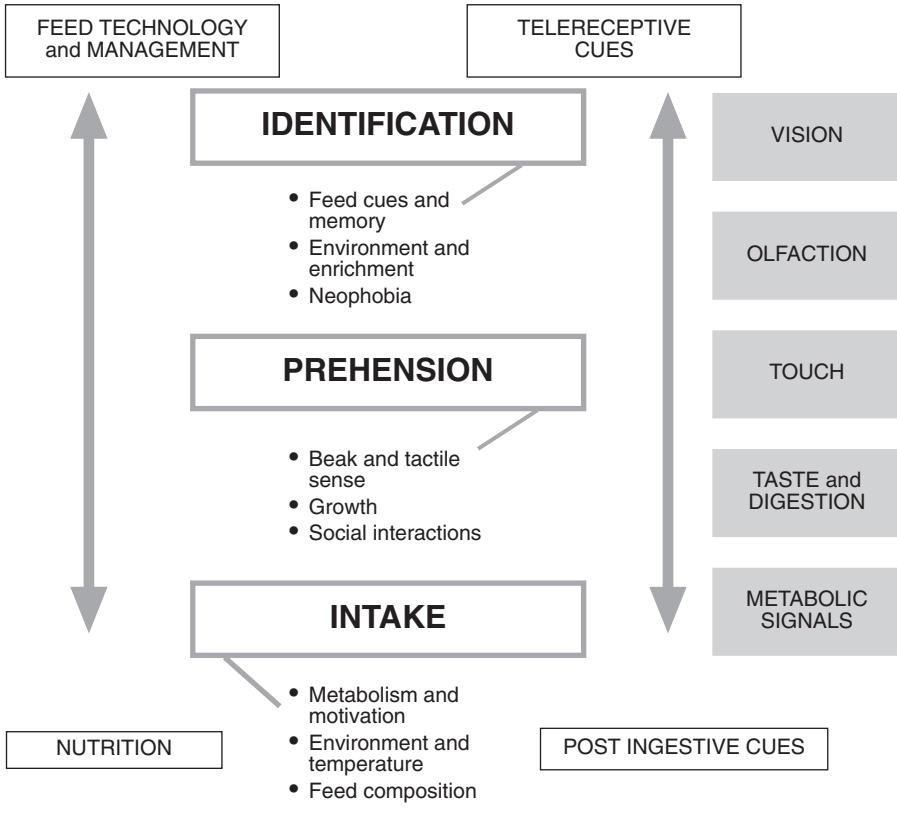

Fig. 15.6. The three phases of feed intake behaviour. The three phases are regulated by different factors illustrated on the right of the figure. Genotype, age and environment of the chickens act on all three phases but by distinct paths. In practice, feed identification and prehension can be manipulated by feed technology and management, although medium-term feed intake remains mainly regulated by nutritional factors. Adapted from Picard *et al.* (1999).

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CHAPTER 16
Effects of physical processing on the nutritive value of poultry diets

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INTRODUCTION

Commercial production of poultry diets normally involves the admixture of a number of different feed ingredients. Some of these, particularly the protein supplements, will have undergone some degree of physical processing prior to receipt at the feed mill. For example, oil extraction, either by hydraulic press or solvent extraction, is applied to most oilseeds. In addition to removal of most of the oil, anti-nutritive factors such as protease inhibitors are inactivated to varying degrees during such processes. However, for the purposes of this paper, the term 'physical processing' is interpreted as relating to the treatment of materials during, or immediately following, admixture with the purpose of providing a balanced diet suitable for consumption by poultry. This process normally involves some degree of grinding of the material which improves uniformity of admixture, provides particles of a size perceived to be suited to the target group and may make nutrients more available for digestion in the bird. Subsequently, the feed may be subjected to heat conditioning and/or pelleting by one of several processes. Heat conditioning has been adopted widely as a means of reducing spoilage and pathogenic microorganisms (Cox *et al.*, 1986) and pelleting has become widespread, particularly for broilers, as a means of improving growth and feed efficiency when compared with mash diets.

The term 'nutritive value' is not easy to define. In effect it describes the interaction between the diet and the target species for the promotion of a particular physiological function, such as growth or egg production, under particular environmental conditions. Clearly two major components of any diet are energy and protein (amino acids) and the nutritive value of diets may be broadly defined in terms of these. However, for poultry, energy may be described in terms of true metabolizable energy (TME), apparent metabolizable energy (AME) or productive energy (PE). Equally, protein may be described in terms of crude protein (N × 6.25), the amino acid profile, ileal digestible amino acids, etc. Ultimately, a wide range of minerals and vitamins also contributes to nutritive value and should perhaps be considered within the framework of this title. However, for the purposes of this chapter the author will focus on aspects

 Poultry Feedstuffs: Supply, Composition and Nutritive Value (eds J.M. McNab and K.N. Boorman)

relating primarily to energy and protein. Furthermore, since, in the commercial sense, nutritive value embodies both the diet and animal interactions which affect feed (energy) intake and nutrient partition, consideration will also be given to parameters such as feed intake, body-weight gain and feed conversion efficiency whilst recognizing the difficulties inherent in interpretation of these 'chicken and egg' variables.

PHYSICAL PROCESSING

Grinding of ingredients is routinely a part of feed manufacture. This can either be before or, more frequently, after mixing, normally using a hammer mill or, less frequently, a roller mill. In a hammer mill, the screen size can be varied (normally in the range 3–10 mm) depending on the coarseness of the grind required. In a roller mill, the material is passed between two rollers, the gap between being adjusted to modify the fineness of the grind. Traditionally, roller milling has been applied mainly to produce coarser feeds, but fine grinds of similar uniformity to those produced by hammer mills can be produced in roller mills (Appel and Behnke, 1988). In either case, a range of particle sizes is produced depending on a number of factors including the type of grain and the speed of grinding.

Pelleting of mash feeds increases the bulk density, and hence reduces handling and transport costs, but is expensive in terms of the equipment required and the energy costs of the process. Hussar and Robblee (1962) reported a 24% increase in density on pelleting and most of this effect was lost when pellets were reground. Pellets are produced by forcing the meal through circular orifices in a metal die. 'Dry' or 'cold' pelleting refers to the manufacture of pellets without steam conditioning of the meal. Although the meal enters the die at ambient temperatures, the frictional resistance of the meal to compression causes the die and the meal to be heated. Depending on the nature of the material, the size of the die etc., temperature increases of as much as 40°C can occur, giving rise to pelleting temperatures of 50–65°C (Skoch *et al.*, 1983a,b; Wood, 1987).

Whilst cold pelleting is frequently used in laboratory-scale operations, steam pelleting is almost universally used in commercial practice. There are several reasons for this. First, steam-conditioning improves pellet quality and throughput. Of even greater importance in recent years is the benefit conferred in terms of reduced microbiological contamination (Cox *et al.*, 1986). It is often stated that pellet durability is dependent upon a degree of starch gelatinization during steam conditioning. However, Wood (1987) has pointed out that high moisture contents (30–50%) are required for efficient gelatinization. Skoch *et al.* (1983b) showed that a greater degree of starch damage occurred during cold pelleting than with steam pelleting, presumably due to the higher shear forces during passage through the die.

In commercial mills, the extent and nature of the steam conditioning process varies widely (Garland, personal communication) in terms of temperature and moisture content of the steam and the contact time. There are many types of conditioner, e.g. Kettle, Ripener, conventional turbulator, inclined turbulator, high volume turbulator, SIRT, Extrutech, Expander, Boa Compactor.

All have different characteristics and the duration and degree of heating can be varied. Conventional turbulators are typically short duration while the SIRT system, for example, can provide residence times of up to 10 minutes. The degree of heating can be anywhere in the range 50–85°C in a turbulator, or up to 120°C for very short periods in expanders or Friction Compactors. A typical example of conditioning of a broiler/breeder diet in the UK is 80–85°C (15–20 s) with frictional heat increase through the die of up to 10°C. However, much lower temperatures may be used in combination with longer contact time. Compounders routinely aim for Holman pellet durability of 92 and this is affected by diet composition, heat conditioning and throughput. Although pellet durability is important, the results of Nir *et al.* (1994a,b) indicated that extremely hard pellets could negatively affect feed intake and weight gain with no benefits in terms of gain : feed ratio. The effect of pellet quality on performance was reviewed by Moran (1989).

One major problem in seeking to assess the current literature on the effects of pelleting on the nutritive value of poultry feeds is the lack of information provided about the conditions employed. In some cases it is difficult to decide whether 'cold' or 'steam' pelleting has been used, while in most other cases authors restrict themselves to a vague reference to the fact that the material was steam-pelleted with no information about the conditions used, the temperatures achieved or the durability of the pellets produced.

In recent years, a number of alternative ways of heat-processing have been introduced into animal feed production. These include extrusion and expansion, in which the feed is heated to relatively high temperatures for a short time while being forced by a revolving screw through a heated die (Marsman *et al.*, 1997) and the rapid pressure decrease on exit from the die ruptures cell structure.

The main difference between extrusion and expansion processes is that, with expansion, the feed is pre-conditioned to temperatures of 80–90°C before entrance to the screw, whereas with extrusion there is no pre-heating but higher pressure is exerted by the screw (Plavnik and Sklan, 1995). Other developments include the Anaerobic Pasteurising Conditioning system which involves the ignition of gaseous fuel in the presence of water and uses the resulting hot gases to heat the meal to about 80°C, with less increase in moisture content than occurs in normal steam-conditioning (Kratzer *et al.*, 1994; Bielby, 1995), and Friction Compacting (Leeson *et al.*, 1998).

A further important aspect of the commercial pelleting process, which has apparently received little attention in the scientific literature, is the use of post-pelleting spray applications of fat to obtain good pellet quality with high inclusion rates of fat.

WHOLE VERSUS GROUND CEREALS

Cereals comprise the major component of most poultry feeds, but need to be admixed with protein supplements, minerals and vitamins to provide a balanced diet. Whole grains have been fed to poultry probably throughout the history of poultry production and there is continued interest in their use for sound

economic reasons. They may be offered in one of several ways, e.g. choice feeding where grains and high-protein balancers are offered *ad libitum* in separate feeders, sequential feeding (cereal and balancer offered in the same trough at different times) or loose mixtures (cereal grain plus pelleted balancer). Choice feeding has been the subject of much research and has been reviewed by Rose and Kyriazakis (1991) and more recently by Forbes and Covasa (1995). The loose mixture option has been used with high proportions of whole wheat inclusion for broilers (Rose *et al.*, 1995; Salah Uddin *et al.*, 1996) and layers (Blair *et al.*, 1973) with no effect on productive performance, suggesting that the nutritive value of the whole grain is as high as that of the ground grain. This is supported by the summarized results for AME content of whole grains or their ground equivalents (Table 16.1). Whilst there is some variability in the results, particularly those of McIntosh *et al.* (1962), it is clear that, at least for wheat and barley, poultry can obtain as much AME from whole grain as from mash.

However, in respect of comparisons between whole and ground grains, a caveat needs to be applied for very young chicks. First, the beak dimensions mitigate against prehension of large particles and, second, the gizzard is less well developed than in older birds. The subject of grinding and the implications of particle size for feed consumption and utilization have been actively researched, particularly during the past 10 years. The focus has been mainly on feed intake, body-weight gain, feed conversion efficiency and, in some cases, organ growth. Furthermore, the picture is very confusing, but inferences can be drawn from some of the gain : feed ratio data about the impact of particle size on nutritive value. Probably the most definitive study was that of Nir *et al.* (1995), who ground maize in a hammer mill with an 8 mm screen and then sieved the material into three fractions (coarse, medium, fine), the geometric mean diameters (GMD) being 2.0, 0.90 and 0.53 mm, respectively. During the first week after hatching, feed intake and gain : feed ratios for the diet based on the coarse fraction were respectively 0.95 and 0.90 of the value for the

Table 16.1. Comparison of AME and TME values for whole grains, mash or pellets (values relative to whole grain = 100).

	Authors	Main cereal	Mash	Pellets
AME	McIntosh *et al.* (1962)	Wheat	106	106
	McIntosh *et al.* (1962)	Wheat	91	92
	McIntosh *et al.* (1962)	Wheat/maize	99	103
	McIntosh *et al.* (1962)	Barley	100	101
	Salah Uddin *et al.* (1996)	Wheat	–	100
	Preston (1997)	Wheat	97	97
TME	Sibbald (1976)	Wheat	–	103*
		Barley	–	101*
		Oats	–	97*
	Sibbald (1977)	Various	–	100

*Pellets reground before feeding.

medium-fraction diet. From 1 to 21 days intake was higher (1.02) for the coarse fraction but gain : feed ratio was lower (0.91) and similar values were recorded for the original ground material which contained approximately 50% of the coarse particles. It would seem therefore that the coarse particles had been poorly digested by these young birds.

A similar trend was observed by Hamilton and Proudfoot (1995a), who produced three diets (fine, coarse, very coarse mash). Over the first 21 days feed intake was greater for coarser diets (1.05 and 1.08 for the coarse and very coarse diets relative to fine, respectively) and gain : feed ratio was poorer (0.97 and 0.93, respectively). Notably, up to 42 days of age, body weight increased from fine to very coarse (1.03) and gain : feed ratio was unaffected, suggesting that the older birds were able to deal efficiently with the larger particles. Douglas *et al.* (1990) used diets based on maize or sorghum as the cereal, ground either in a hammer mill or a roller mill to produce fine and coarse particles, respectively. With maize the GMD were respectively 0.95 and 1.47 mm and with sorghum the values were 0.84 and 1.80 mm. Intake and gain : feed ratio with the diets of larger particle size were both reduced irrespective of cereal (0.97 and 0.96 relative to fine diets, respectively).

However, the above results contrast with those of Reece *et al.* (1985) who used a roller mill or hammer mill with maize, producing GMD of 1.34 and 0.81 mm, respectively. In this case the coarser diet improved feed intake (1.07 relative to the finer diet), gain : feed ratio (1.02) and body-weight gain (1.10).

MASH VERSUS PELLETS

Over 60 years ago, Patten *et al.* (1937) reported that birds given pelleted diets, as opposed to mash, showed improved feed conversion efficiency and weight gain. Since that time many studies have been conducted and the vast majority have shown improvements in feed intake, feed conversion efficiency and weight gain. Calet (1965) reviewed the literature and concluded that there were no measurable differences in ME content or N retention with pelleted and mash feeds when account was taken of differences in feed intake. In the final paragraph he stated:

> it is regrettable that our information on the subject shall have to be so restricted, despite the number of publications which have been devoted to it. On the scientific level the reasons for the efficiency of pellets are far from being fully understood, and the reasons for the differences between authors have not been elucidated.

Many more studies have been reported since 1965. Some of the main reports over the past 12 years are summarized in Table 16.2. They clearly indicate the same pattern as earlier studies, i.e. increased feed intake, weight gain and gain : feed ratio. However, the differences in the extent of the effects are remarkable – 1 to 26% for feed intake, 3 to 39% for gain and 0 to 12% for gain : feed ratio. In view of the earlier discussion on the effects of particle size it would seem that part of the differences is due to differences in the particle size of the mash diet and possibly the type of cereal. However, only in three of the papers listed

Table 16.2. Increases (%) in feed intake, body-weight gain and gain : feed ratio with pelleted feed relative to mash.

Authors	Period (days)	Feed intake	Weight gain	Gain : feed ratio
Choi *et al.* (1986)	1–28	5	5	0
Zatari and Sell (1990)	1–49	9	13	4
Douglas *et al.* (1990)	1–21	6	16	10
		5	13	8
		12	19	6
		2	8	6
Pettersson *et al.* (1991)	1–20	20	27	6
		25	29	3
Deaton (1992)	21–42	3	4	2
Kratzer *et al.* (1994)	1–24	12	18	6
	1–22	20	26	5
Hamilton and Proudfoot (1995a)	1–42	3	8	5
McCracken *et al.* (1994a)	7–28	22	27	4
Munt *et al.* (1995)	21–42	3	7	4
Nir *et al.* (1995)	1–49	1	3	2
Plavnik *et al.* (1997)	28–49	15	20	4
		9	13	4
Preston (1997)	7–28	26	39	12

(Douglas *et al.*, 1990; Hamilton and Proudfoot, 1995a; Nir *et al.*, 1995) is there any information on particle size. The interpretation of gain : feed ratio as a measure of nutritive value becomes difficult when differences in feed intake occur, since gain : feed ratio tends to improve with young poultry up to maximum feed intake. On the other hand, gain : feed ratio declines with increasing weight. Since all of the studies reported are based on fixed time periods, higher feed intakes are associated with higher final weights. For these reasons, a few of the studies in Table 16.2 are of particular interest, namely those of Deaton (1992), Nir *et al.* (1995), Munt *et al.* (1995) and Hamilton and Proudfoot (1995a) where feed intakes were only increased 1–3% with pellets but significant improvements in gain : feed ratio, of 2–5% were recorded. These results indicate an intrinsic improvement in the nutritive value of the pelleted feeds.

However, the data shown in Table 16.1 do not indicate any improvement in AME or TME content when cold pellets are compared with mash. Similar results were obtained in two other studies on AME where the main cereal was respectively wheat or maize (Blakely *et al.*, 1963; Reddy *et al.*, 1963). On the other hand, a number of studies comparing steam-conditioned pellets with mash tended to show an improvement in AME content (Table 16.3) though not all the effects were statistically significant. The reduced value reported by Francesch *et al.* (1994) for wheat was statistically significant but is particularly unusual in that a similar value was obtained with feed enzyme addition, contrary to the almost universal improvement in AME content with feed enzyme addition (Bedford and Morgan, 1996).

Table 16.3. Relative values for AME content of steam-pelleted diets relative to mash (100).

Authors	Main cereal	Relative value
Hussar and Robblee (1962)	Wheat	103
Farrell *et al.* (1983)	Maize	104
	Barley	102
	Wheat	105
Zatari and Sell (1990)	Maize (starter)	106
	Maize (finisher)	103
Francesch *et al.* (1994)	Wheat	96
	Barley	98
Preston (1997)	Wheat	104

A recent study by BOCM PAULS Ltd (Garland, personal communication) examined the effects of steam conditioning without the complication of pelleting. Birds fed the heat-treated mash increased intake, gain and gain : feed ratio by 7%, 14% and 8%, respectively. There was no effect of heat treatment on AME measured in adult cockerels, but digestibilities of most amino acids, particularly lysine (7%) and threonine (6%), were increased. Unfortunately there is a lack of data on amino acid digestibility in most of the scientific studies reviewed.

Data on aspects of nutritive value other than AME are scarce. A rare exception is that of Pettersson *et al.* (1991), who fed a diet based on barley (40%), wheat (25%) and rye (7%) as a mash or dry- or steam-pelleted. Pelleting resulted in small increases in water-soluble starch and crude protein but had no effect on any of the measured fibre components. Whole tract digestibilities of organic matter, crude protein and crude fat were not improved by pelleting but ileal digestibility of organic matter, measured at day 21, was significantly increased (7% and 5% for cold and steam, respectively). Such a difference, if real, could have implications for the efficiency of use of absorbed energy. This is an aspect which needs further investigation although it is recognized that the problem of accuracy of measurement of ileal digestibility is a major limitation to interpretation.

Graham *et al.* (1989) reported that steam-pelleting (93°C) of a barley-based diet for pigs increased the solubility of mixed-linked β-glucan from 45% to 62% but had no other measurable effects on diet composition. They observed a significant increase in ileal starch digestibility and a numerical increase for energy digestibility, and concluded that pelleting had disrupted the endosperm cell walls. Such a conclusion is supported by the earlier studies of Saunders *et al.* (1968, 1969) on the effect of steam-pelleting on wheatbran, where the number of empty aleurone cells, in both the lower small intestine contents and excreta of chickens, was increased.

One further aspect of the effects of pellets versus meal relates to the energy expended in physical activity. Jensen *et al.* (1962) reported that birds offered pellets visited the feeders as frequently as those offered mash but spent much less time in consuming similar amounts of feed. They suggested that part of the

improved efficiency of use of pelleted diets resulted from less energy expenditure in prehension of feed. Savory (1975) observed that hybrid and Brown Leghorn chicks spent approximately twice as much time feeding when offered reground pellets instead of pelleted feed.

Taking all of these factors together, there would appear to be good reason why pelleting should improve the productive energy of a feed and particularly why steam-pelleting should do so. There is, however, a notable lack of data and the one report (Reddy *et al.*, 1963) indicates a much larger improvement (30%) than would seem to be justified on the basis of all the other accumulated evidence.

In contrast to the situation with growing poultry, there is considerable evidence that laying birds perform equally well whether their food is supplied as mash or pellets (Morgan and Heywang, 1941; Temperton and Dudley, 1948; Blount, 1949; Black *et al.*, 1958), but in most studies there was a tendency for birds fed *ad libitum* to eat rather more food and to gain more weight when offered pellets. Contrary results were obtained by Deaton *et al.* (1987) and by Hamilton and Proudfoot (1995b) who observed lower intakes of pellets compared with mash using hens that had been beak trimmed. When feed intake was controlled, however, birds offered pellets showed significantly higher egg production and weight gain (Black *et al.*, 1958). This result is all the more remarkable since, for the most part, the mash was created by grinding the pellets to minimize problems of differences in chemical composition. As discussed above, the effect might be attributed to differences in energy expenditure associated with eating or, as suggested by Black *et al.* (1958), due to differences in the length of time feed was retained in the crop with potential effects on nutrient digestion. However, the study of McCracken *et al.* (1993) showed no differences in AME content of a diet offered as mash or pelleted after steam-conditioning at 75°C for 1 min, so it would seem that the latter suggestion (Black *et al.*, 1958) is extremely unlikely.

DEGREE OF HEAT TREATMENT

As discussed earlier, there is a wide range of temperature/time combinations used in commercial feed formulation and a variety of methods of applying heat. Whilst it is generally considered that steam heat treatment improves the nutritive value, and the studies discussed above would tend to support this view, there is considerable evidence that excessive combinations of temperature/time and shear pressure can cause damage, particularly to protein, and adversely affect nutrient absorption (Björck and Asp, 1983; Wiseman *et al.*, 1991). Relatively few studies are available for poultry but there is some evidence that heat-conditioning, within the commercial range, can lead to small reductions in AME concentration and in gain : feed ratio. McCracken *et al.* (1993) observed a significant (3%) reduction in AME of barley and wheat-based broiler diets conditioned to 85°C for 15 min prior to pelleting and McCracken *et al.* (1994b) observed a similar effect with layers. A recent study by BOCM PAULS Ltd (Garland, personal communication) compared heat con-

ditioning at 85–88°C for 2–3 min with standard processing (85°C for 20 s). The weight gains of both groups were similar but gain : feed ratios were respectively 0.62 and 0.66 ($P<0.001$). These results may tend to explain those of Pepper *et al.* (1968), who observed better performance of layers given mash, as opposed to pelleted or crumbled diets, in contrast to most other reports. They used a high pelleting temperature (90°C, time not specified) and concluded that some degree of protein damage may have occurred. In view of the trend towards use of higher temperatures these results suggest the need for caution and more research using carefully defined experimental conditions.

Extrusion is widely used with oil seeds and legumes to minimize the effects of anti-nutritive factors (Björck and Asp, 1983; Hendriks *et al.*, 1994; Marsman *et al.*, 1997), but recently extrusion and expansion have been introduced into the processing of diets for pigs and poultry (van der Poel, 1997a). These processes lead to increased gelatinization of starch and result in improved physical pellet quality and decreased energy costs during subsequent pelleting (Vande Ginste and De Schrijver, 1998). Relatively few published comparisons on poultry are available. Plavnik and Sklan (1995) compared unheated maize/soy, maize/wheat/soy and maize/barley/soy diets for broilers with the same formulations extruded (125°C) or expanded (90°C). All diets were milled (1.5 mm) before feeding. With the maize/soy diet, N and starch digestibility were unaffected by processing but fat digestibility was increased (7% and 5% for extrusion and expansion, respectively) and AME concentration tended to be higher (1.5%). With the other two diets, there were no significant effects on digestibility of N, fats or starch but AME concentration was higher with both heat treatments. However, the effects were no greater than those shown in Table 16.3. McCracken *et al.* (1997) compared an expanded diet for broilers with a pelleted diet heat-conditioned at 80°C for 10 min. The expanded diet was exposed to 80°C for 15 s followed by 100°C for 3 s. No differences in performance or AME concentration were observed, but *in vivo* viscosity tended to be higher with the expanded diet and gizzard weight (g kg^{-1}) was increased. In a recent study (Garland, personal communication), expanded feed gave an inferior (not significant) gain : feed ratio (0.6) compared with standard processing (0.64). Studies with pigs (Laurinen *et al.*, 1998) tend to confirm the observation on broilers. Average expander temperatures were 115°C and the diets were based on barley and wheat bran/middlings. Expansion increased fat digestibility (10%) but tended to reduce dry matter and energy digestibility (1.2%).

Vande Ginste and De Schrijver (1998) compared mash, pelleted, expanded and expanded/pelleted barley/wheat/soy diets. Expanded feed gave similar results to mash but tended to be inferior to pelleting for ileal and total tract N and DM digestibility. Significantly, ileal and total tract digestibility of Ca and P were reduced with the expanded feed relative to both mash and pellets, presumably due to destruction of endogenous phytase activity in the diets. All three heating processes tended to improve available lysine indicating that there was, at least, no negative effect on protein quality. Furthermore, the recoveries of free amino acids were in good agreement with the concentrations of free amino acids included in the diets.

A number of trace ingredients, such as some vitamins, antibiotics and feed enzymes, are susceptible to heat. For example, vitamins A, D_3 and E were reduced to approximately 80% recovery after expansion and 2 months' storage, and vitamins C and K showed recoveries of 25% and 20%, respectively (Albers, 1996). Van der Poel (1997b) warned that expander processing can seriously denature enzymes in the feed and that phytase is particularly sensitive.

Taking all of these results together, it would seem that appropriate use of extrusion or expansion will not result in any negative effects on nutritive value of diets, but that these processes do not confer any improvement in nutritional value over that obtained with conventional methods of heat conditioning. However, the effects of any heat treatment on certain trace ingredients must always be borne in mind.

WET FEEDING

Although feeding of wet mashes was common practice 50 years ago (Robinson, 1948), it has not generally been considered to be suited to large-scale intensive production, although Thorne *et al.* (1989) developed an automated wet-feeding system for laying hens to permit the use of high-moisture by-products. Soaking of materials containing anti-nutritive compounds is a cost-effective way of eliminating the detrimental effects (D'Mello *et al.*, 1985; Melcion and van der Poel, 1993), and it has been suggested that a high moisture diet might be useful for layers during heat stress (Tanor *et al.*, 1984; Tadtiyanant *et al.*, 1991).

The situation in relation to the use of wet-feeding to improve nutritive value is somewhat confused. Fry *et al.* (1957, 1958) showed that soaking diets based on barley or pearled barley increased feed intake, weight gain and gain : feed ratio of chicks and turkey poults. No effects were seen with maize and only minor effects on feed intake with wheat-based diets. Misir and Marquardt (1978) showed that water extraction of rye reduced beak impaction and improved excreta condition but had little effect on feed intake or gain : feed ratio. However, inclusion of the water extract of rye in wheat-based diets depressed performance indicating that 'rye contains a water-soluble factor which reduces nutrient utilization'. In these studies the feed was redried prior to feeding so the effects were not attributable to the use of wet feed *per se* but to changes that occurred during soaking. In the light of current knowledge of the effects of feed enzymes in improving the nutritive value of barley and rye-based diets for poultry, it seems clear that the effects reported were associated with changes in the non-starch polysaccharide (NSP) fractions of the cereals used.

More recently, Yalda and Forbes (1995, 1996) and Yasar and Forbes (1999) have studied the effects of feeding the complete diet as a wet mash on performance of growing broilers. Yalda and Forbes (1995) observed large increases in food intake, weight gain and gain : feed ratio when a pelleted grower diet was mixed with water (1 : 2, feed : water) prior to feeding over the period 28–49 days of age. In a further study from 14–35 days, increases in feed intake and weight gain occurred but there was no change in gain : feed ratio

despite a reported 12% improvement in 'digestibility' (feed minus excreta). Yalda and Forbes (1996), using a similar diet, reported more-modest increases in feed intake and weight gain, again with no improvement in gain : feed ratio but a 14% improvement in 'digestibility'. Unfortunately no description of the diet composition is available, but the low ME content and high declared crude fibre content of the grower diet, coupled with the poor performance of the birds on the air-dry diet, would suggest that the feed may have contained a high level of barley. Thus the results would appear to agree with those of Fry *et al.* (1958).

Later studies (Yalda and Forbes, 1996) used a different grower diet of lower declared crude fibre content, and feed was soaked for 0, 12 or 24 h. In all three cases feed intake, gain and gain : feed ratio were increased but 'digestibility' was only improved (5%) when feed was offered immediately (0 h) after soaking. Yasar and Forbes (1999) used diets of known composition containing high proportions of wheat, barley or oats. In this case the dry diets were fed as mash. Marked increases in feed intake and gain occurred with wet feeding for all three cereals. In one experiment gain : feed ratio was improved only with the barley-based diet and was reduced for both wheat and oats, and in the other experiment there were no significant effects of wet-feeding on gain : feed ratio. In both experiments 'digestibility' was unaffected by feeding method.

Preston (1997) offered a wheat-based diet as dry mash, pellets or wet-fed to broilers from 14 to 48 days. Contrary to the results of Yalda and Forbes (1995), intake of wet feed was substantially lower for the first week than for birds fed pellets, and overall intake and gain were only 0.91 and 0.95 respectively of those given pellets, though gain : feed ratio tended to be improved (4%). There was no effect of feed form on AME concentration and it was concluded that the improvement in gain : feed ratio may have been related to the effects of voluntary restriction of intake (Nir *et al.*, 1986) which occurred between 14 and 21 days. Taking all of these reports into consideration, it would appear that wet-feeding may give some improvement in nutritive value with barley-based diets but probably not with wheat or maize-based diets. Differences in methods of soaking the feed and in other aspects of diet composition may have contributed to some of the apparent inconsistencies in the studies discussed above.

SUMMARY AND CONCLUSIONS

One major observation arising from the above is the multifactorial nature of the whole area of physical processing, e.g. diet constituents, type and extent of processing, target species. In particular, there is major diversity in the type of equipment used for heat conditioning and the range of time, temperature and moisture combinations employed. There is need for much more stringent reporting and evaluation of the conditions used in scientific studies. Furthermore, there is a dearth of good information on the effects of processing on protein quality and amino acid availability. It would seem that, from as early as 2–3 weeks of age, poultry can effectively digest and metabolize whole grains at relatively high inclusion rates, though studies up to 21 days indicate that

large particles, let alone whole grains, are less efficiently digested. Numerous studies have demonstrated improvements in feed intake, gain and gain : feed ratio when pellets, as opposed to ground mash, are offered to broilers or turkey poults, and with steam-pelleting there is some evidence of improvements in nutritive value for broilers, as represented by AME concentration, though no benefits are apparent for layers. There is some evidence of detrimental effects of heat-conditioning within the range of treatments currently used commercially, and a need for careful evaluation of new procedures such as expansion, APC and Friction Compacting, coupled with definitive information on the conditions used. The effects of wet-feeding remain somewhat controversial, though it would appear that the nutritive value of barley-based diets may be improved and that wet-feeding, in some circumstances, leads to higher feed intakes and weight gain than feeding as pellets or dry mash.

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PART V
Dietary enzymes

CHAPTER 17
The role of carbohydrases in feedstuff digestion

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ABSTRACT

Carbohydrases, specifically xylanases and β-glucanases, are used routinely in wheat- and barley-based diets, respectively, throughout Europe, Canada, Australia and many parts of the Middle East. Whilst their use is well accepted, there is still considerable debate regarding their mode of action. Use of such enzymes results in increments in performance that far exceed the nutritive value of the sugars they release from cell walls. Evidently, the substrates they degrade are either anti-nutrients or reduce the efficiency of the digestive process. As a consequence, both the viscosity and the cell wall encapsulation hypothesis are discussed as likely candidates for the mode of action of carbohydrases. The bulk of the evidence supports the former, although the cell wall mechanism cannot be ruled out entirely. Whilst enzyme use in wheat and barley diets is well established and accepted, it is only recently that maize- and sorghum-based diets have been shown to respond to the relevant enzymes. Maize starch, in particular, is incompletely digested by the time it exits the terminal ileum. In addition to the cell wall degrading enzymes, amylases and proteases have been found to enhance the rate of starch digestion in the ileum of the broiler and hence improve overall performance. Carbohydrase enzymes effectively increase the rate of diet digestibility whether the target grain is barley, wheat or maize. The net result is more nutrients for the bird and fewer for the resident intestinal flora. As a result, the use of carbohydrases has been shown to reduce the populations of ileal flora. This response in itself may well be responsible for the bulk of the response to feed enzymes. Carbohydrases also produce many oligomeric and smaller products, which act as substrates for many bacteria in the caecum. This results in an increase in total fermentation and an alteration in volatile fatty acid profiles towards propionate, which has implications both for energy conservation and pathogen control. The response to addition of a carbohydrase is dependent upon many different factors that are discussed, and as more is understood of their mode of action, it is likely that new enzymes will be designed rather than empirically derived.

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 Poultry Feedstuffs: Supply, Composition and Nutritive Value
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INTRODUCTION

Carbohydrases are a class of enzymes that are responsible for the hydrolysis of carbohydrates. Carbohydrates, in turn, are a very broad group of compounds built up from one or many of a multitude of different sugars, either present as single entities (monosaccharides) or linked in an almost infinitely variable manner (in terms of sequence, chain length and side chain complexity). They are present in virtually all ingredients used in animal feed manufacture, and in order for the animal to succeed in its bid to extract energy from such feedstuffs, it must first break down all complex carbohydrates into their simple sugars for absorption. To this end, the digestive system of the bird produces an array of enzymes which cooperate to reduce the complex structures of starch, the most abundant of cereal storage polysaccharides, to the most useful form – glucose – in as little time as possible. Whilst starch is by no means the only carbohydrate present in cereal grains (which in turn provide the bulk of a poultry diet), it is the most abundant and, fortunately, the most easily degraded of the carbohydrates present in the grain. As a result, the strategy of the bird has been to focus the attentions of its digestive system on starch and ignore the remaining carbohydrates, since the effort involved in deriving energy from the latter is disproportionately high compared with that involved for starch. A summary of the sources of plant carbohydrates along with their suitability as energy sources is presented in Table 17.1.

Some forms of starch, however, are not susceptible to degradation by the carbohydrases (amylases and further starch-reducing enzymes) produced by the bird and as a result are termed resistant starches. Consequently, a considerable amount of ingested carbohydrate enters the digestive tract but escapes degradation by enzymes derived from the bird. A simplistic view would thus be that supplementa-

Table 17.1. Plant carbohydrates – their suitability as sources of energy for monogastrics.

Polymer	Sugar components	Abundance	Issues influencing choice for energy source	Primary enzymes required
Cellulose	Glucose	High	Insoluble, resistant to digestion	Cellulase, cellobiohydrolase
β-glucans	Glucose	Moderate/low	Relatively resistant to digestion	Cellulase β 1–3,1–4 glucanase
Arabinoxylans	Arabinose, Xylose	Moderate/low	Relatively resistant to digestion	Xylanase Arabinofuranosidase
Pectins	Uronic acids, Rhamnose, Galactose, etc.	Moderate/low	Some resistance Some sugars of poor nutritive value	Many in the pectinase family
Oligosaccharides	Glucose, Fructose, Galactose	Moderate/low	Some sugars of poorer nutritive value	α-Galactosidase
Starch	Glucose	Very high	Minor resistant fraction	Amylase and debranching activities

tion of a poultry diet with enzymes capable of degrading those carbohydrates which the bird ordinarily cannot, should increase the nutritive value of the diet. The reality is a little more complex than this, however, since some plant carbohydrates are clearly anti-nutritive and thus their enzymatic degradation yields far more benefit than can be accounted for by the value of their constituent sugars. This chapter will review the literature relating to benefits derived from addition of enzymes which target carbohydrates that the bird cannot process.

CELL WALL CARBOHYDRATES

A great deal of research has focused on the anti-nutritive properties of the cell wall carbohydrates present in rye, wheat and triticale (principally the arabinoxylans) and barley and oats (principally the β-glucans), and on the means to limit the detrimental effects of such compounds (Antoniou *et al*., 1980; Antoniou and Marquardt, 1981; White *et al*., 1981; White *et al*., 1983; Broz and Frigg, 1986; Edney *et al*., 1989; Choct *et al*., 1999; Bedford, 2000). The use of the appropriate enzymes was shown in these studies to significantly improve the performance of birds fed diets based on such grains. The responses observed could not be explained simply by release of the constituent sugars from the targeted polysaccharide, rather they must have been derived from the release of a constraint on digestion, which was due to their presence. As a result, the benefits from enzyme use were seen to be disproportionate to their activity if their target substrate were viewed in isolation. In order to explain such a response, two separate, and not necessarily exclusive, hypotheses on mode of action were put forward, namely the cell wall encapsulation and the viscosity theories. Each is discussed in brief detail.

VISCOSITY THEORY

In many cereal endosperm cell walls there exists a fraction of the structural carbohydrate component (mostly arabinoxylan in wheat, triticale and rye and β-glucan in barley and oats) which is both soluble in the small intestines of the bird and of high molecular weight (White *et al*., 1981, 1983; Choct *et al*., 1992; Bedford and Classen, 1992a). The combination of these two properties results in a solute that interferes with the free movement of other solutes. The viscosity of the intestinal contents increases in a geometric manner with increasing concentration of viscous cell wall fractions. Since digestion is a dynamic process which is reliant upon diffusion of enzymes, substrates and products, any interference with free molecular movement will undoubtedly reduce the efficiency of the entire process. *In vitro* studies have clearly shown that viscous solutions reduce the diffusional rates of sugars and salt (Fengler and Marquardt, 1988), the effect being greater with increasing solute molecular weight. Figure 17.1 shows the results of a study in which bradykinin (mol. wt 1063), a protein of nine amino acids, was placed in a dialysis bag containing solutions varying in viscosity, and then the bag was placed in a beaker of distilled water. The amount of bradykinin diffusing into the surrounding water was determined after 1 hour.

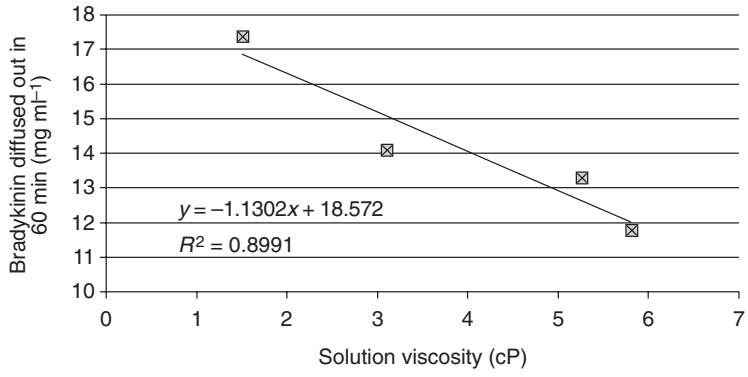

Fig. 17.1. Influence of solution viscosity on the rate of diffusion of bradykinin.

It is clear that even very small increments in solution viscosity have significant effects on the mobility of a reasonably small molecule.

It is important to recognize that a relatively insignificant increase in viscosity from 2–6 centipoise (the difference between an average and good quality wheat when determined at the jejunal level) can result in a 25% reduction in the rate of diffusion of bradykinin. Whilst this will certainly reduce the rate of digestion of such a substrate, the effect is even more dramatic for larger substrates. Fat digestibility is influenced by more than the diffusional constraints of increased viscosity. For rapid digestion, it is essential that fats are properly emulsified into micelles in the small intestine. This requires the involvement of emulsifiers and vigorous mixing of the fat and aqueous intestinal phase for production of a stable micelle structure. It is clear that as aqueous phase viscosity increases, the success of micelle formation is dramatically decreased (Fig. 17.2; Pasquier *et al.*, 1996).

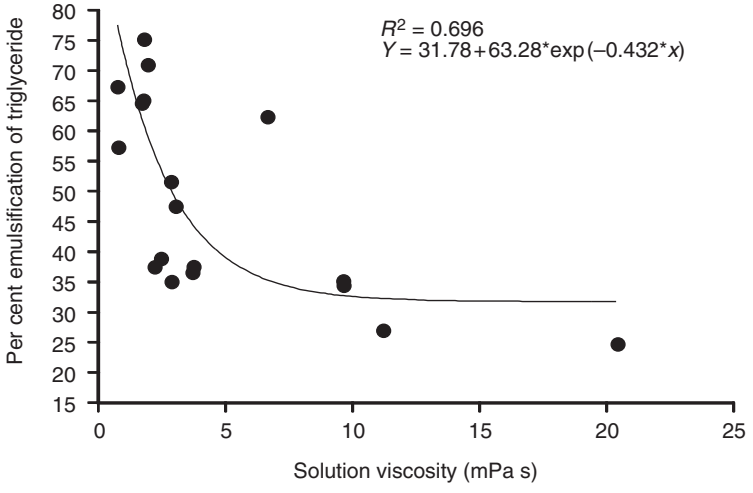

Fig. 17.2. Influence of aqueous phase viscosity on per cent emulsification of triglycerides. (Pasquier *et al.*, 1996.)

Thus, the effects of increased viscosity on the rate of fat digestion and absorption are much more evident compared with other nutrients. Numerous studies have demonstrated that reduction of viscosity through the use of the relevant enzyme increases the digestibility of fat more so than any other nutrient (Choct and Annison, 1992; McCracken *et al*., 1996; Allen *et al.*, 1997; Silva and Smithard, 1997; Steenfeldt *et al*., 1998; Danicke *et al*., 1999, 2000) which lends credence to the *in vitro* data presented in Figs 17.1 and 17.2. As a result, the carbohydrases of interest in reducing viscosity are the xylanases and β-glucanases that target wheat and barley cell viscous cell wall components, respectively.

Viscosity is geometrically related to the concentration of high molecular weight, soluble cell wall carbohydrates (White *et al.*, 1983; Bedford and Classen, 1992b) which infers that very large reductions in viscosity will be achieved with very few, well targeted hydrolytic events. Provided the enzyme attacks the polymer randomly, and so, on average, towards the centre of the polymer, each hydrolytic event will substantially reduce the molecular weight of the soluble polymer. Such enzymes are endo-hydrolases rather than exo-hydrolases. The soluble polymers found in the intestines of the bird are not simple structures by any means (Bedford and Classen, 1992b). As a result, the enzymes used must either be impervious to obstacles on the backbone structure which they target, or else accessory enzymes that can remove such obstacles must be present in sufficient quantities. An example is the effect of arabinose side chains linked onto the xylan backbone on the activity of various xylanases. Some endo-xylanases are more dependent on such accessory enzyme activity than others for efficient depolymerization of the native structure (Poutanen *et al*., 1991; Sunna and Antranikian, 1997). A xylanase that is independent of such side activities is evidently a better choice for usage in animal feed. Such parameters, in addition to many others, are under investigation so that better products can be designed for the animal feed industry in the near future.

CELL WALL THEORY

Simply stated, the cell wall theory relies on the fact that the feed manufacturing process of grinding and pelleting does not break open all cell walls of the endosperm. With ground and pelleted feed, the gizzard fails to develop fully, and as a result many complete, intact particles of feed enter the small intestine (Svihus *et al.*, 1997). As a result, the contents of such cells escape digestion by virtue of the fact that they are physically separated from the digestive system. Unlike viscous carbohydrates, which are dealt with effectively by relatively few, well-placed hydrolytic events, cell walls require a concerted and prolonged attack by many different enzymes to effect total dissolution. In practice, however, small puncture holes, large enough for digestive enzymes to enter the cell and degrade the substrates such that they can escape through the same hole, would be sufficient for this process to improve diet digestibility. Even such a puncture-hole process would require large amounts of enzymes and, moreover, many different enzymes to complete the process since the cell wall of cereals is a complex structure (Forrest and Wainwright, 1977; Ward and Moo-Young, 1989). The fact that pure

xylanases alone have been shown to be almost equal or better than a complex mixture of cell wall degrading enzymes (Grootwassink *et al*., 1989; Cowan *et al*., 1993) in promoting performance of enough broilers suggests that the cell wall mechanism seems unlikely, even if complete dissolution is unnecessary.

Furthermore, xylanases give much greater responses in expanded diets, compared with pelleted or mash diets (Nissinen, 1994). Expansion of a feed results in almost total cell wall destruction, which would suggest that the role of the enzyme is limited in such circumstances. Expansion, on the other hand, leads to greatly increased intestinal viscosity (Nissinen, 1994). These observations clearly favour the viscosity theory over that of the cell wall. Finally, virtually all feed enzyme-related work, in which fat digestibility has been determined, has suggested that improvements in fat digestibility are responsible for much of the improved performance seen on use of an exogenous enzyme (Campbell *et al.*, 1983; Classen *et al.*, 1985; Choct *et al*., 1992; Viveros *et al*., 1994; McCracken *et al*., 1996; Danicke *et al*., 1997, 1999).

Nevertheless, the micrograph presented in Fig. 17.3 demonstrates that there is a considerable amount of such 'encapsulated' material in the small intestine of wheat-fed chickens (Bedford and Autio, 1996) which is largely removed on addition of a xylanase. Such images are also found for barley-, rye- and even maize-fed birds. As a result, there is an opportunity for cell wall degradation by exogenous enzyme addition and hence improved nutrient utilization by the bird. It is clear from Fig. 17.3 that there is an apparent and substantial disappearance of endosperm, but not aleurone cell wall material, on use of the enzyme that suggests that the enzyme is directly degrading some cell wall structures.

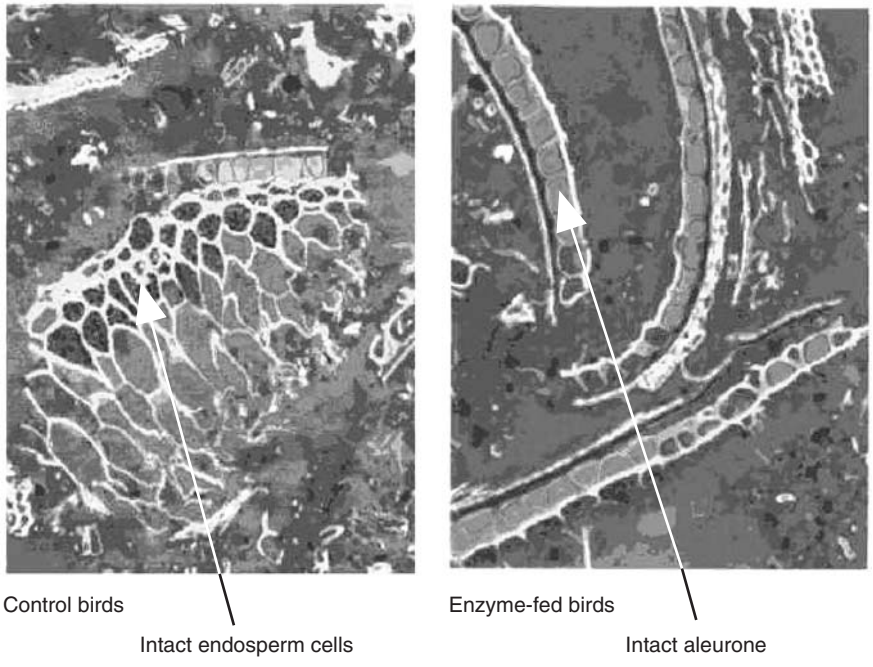

Fig. 17.3. Micrograph of material at the terminal ileum of wheat-fed birds.

Such observations run counter to *in vitro* work, which suggests that the amount of enzyme required to achieve such a degree of cell wall disintegration is far in excess of the amounts used commercially (Parkkonen *et al*., 1997). Further doubt is cast on the ability of feed enzymes to degrade endosperm cell walls to such an extent when the time constraints of the bird's digestive tract are taken into account and compared with the incubation times used in the *in vitro* studies (Parkkonen *et al*., 1997). Furthermore, the *in vivo* studies to date (Bedford and Autio, 1996) indicate that a considerable amount of cell wall degradation has already taken place by the time the feed has reached the gizzard. This presents an enormous challenge to the exogenous enzyme if such an effect is to be achieved at such an early stage in the digestive process, particularly since the pH of the proventriculus and gizzard is much lower than the optimum of most enzymes used commercially today.

A potential explanation to the above, lies in the fact that the activity of enzymes results in the production of sugars from cell wall carbohydrates that are fermented in the caeca, a topic that will be dealt with later. Research with rats has shown that enhanced fermentation in the colon elevated enteroglucagon concentrations, which in turn depressed gastrin concentrations markedly, thereby slowing gastric emptying but increasing motility (Gee *et al*., 1996). If a similar mechanism exists in chickens then it would explain why enzyme addition appears to have degraded cell walls considerably at the point of the gizzard. Presumably the enteroglucagon signal encourages increased gizzard activity per unit mass of feed, with the result that the physical grinding effect of the gizzard, rather than the hydrolytic action of the enzyme action *per se*, is responsible for cell wall degradation. Regardless of mechanism, the end result is that the contents of more cells are open to attack by digestive enzymes. The cell wall theory, therefore, whilst unlikely to be of great significance from the viewpoint of direct enzyme activity, may play a very large role via an indirect mechanism.

RESISTANT STARCHES

The targets of most carbohydrases used commercially to date are the cell wall carbohydrates from the 'viscous' grains. It is accepted that carbohydrases used in diets containing such grains tend to improve the nutritive value of diets containing poorer (or more viscous) quality grains more so than high quality grains. As a result, enzyme use has effectively reduced the variation between the best and worst quality viscous grains (Classen *et al*., 1995; Bedford *et al*., 1998; Choct *et al.*, 1999; Scott *et al.*, 1999). Until recently, it has been accepted that the feeding value of non-viscous grains such as maize or sorghum does not vary as greatly as wheat or barley. As a result, many ignore these grains as targets for exogenous enzymes. Recent data suggest that the variability of maize (Leeson *et al*., 1993; Collins *et al*., 1998) can be as great as that determined in wheat (Classen *et al*., 1995) and barley (Scott and Boldaji, 1997). Maize starch digestibility at the terminal ileum has been reported to be as low as 85% (Noy and Sklan, 1994) and our work has shown it to drop below 80% with some

samples of maize (Finnfeeds 1998, unpublished). Since intestinal viscosity of birds fed maize or sorghum is very low compared with wheat or barley, it is unlikely that viscosity plays a major role in describing the variation between samples reported (Leeson *et al*., 1993; Collins *et al*., 1998). The direct cell wall mechanism is again unlikely to play a role for the reasons described above, but this does not preclude the indirect mechanism. In fact, it is ironic that so much debate has centred around the mechanism of action of enzymes on 'viscous' grains, since if the cell wall mechanism were shown to be correct for viscous grains then it should hold true for non-viscous grains. There is ample evidence that maize and sorghum (and indeed wheat and barley) are not completely digested by the terminal ileum, and as a result a considerable amount of starch escapes utilization by the bird. Such starch is termed resistant starch and presents an opportunity for use of feed enzymes. Whilst the section below focuses on maize, the issues raised are just as valid for wheat and barley. Viscosity, as a criterion of quality of these grains is so over-riding, however, that it must be dealt with first before considering resistant starch as a characteristic of quality.

There are three classes of resistant starch, RS1, RS2 and RS3 (Brown, 1996):

1. RS1 is that portion of the maize starch which is not digested for reasons of accessibility. After grinding and pelleting, a large number of cereal endosperm cells remain intact. Many of these cells pass through the digestive tract without being exposed to the contents of the digestive tract. As a result, the starch within is shielded from digestion by the cell walls of the endosperm (see Fig. 17.3). This fraction of dietary starch is referred to as resistant starch 1. Microscopic analysis of the ileal digesta suggests an appreciable amount of starch escapes digestion by this route, but the relative lack of such particles in the faeces suggests much of this resistant starch may be fermented in the large intestine or caeca (Bedford, 1996a). This classification of resistant starch is the same as that which has been discussed under the cell wall hypothesis heading.

2. RS2 is that portion of the maize starch that is not digested due to the physical and chemical structure of the native granule. The first consideration is the crystalline pattern of the α-1–4 glucose linear polymers, which make up the starch itself. There are two major patterns in cereal starches, the A and B patterns, which are illustrated in Fig. 17.4. The view of the structure is from the end of the each of the seven (A) or six (B) linear polymers. The A pattern is actually more rapidly digested than the B, which contains much more water.

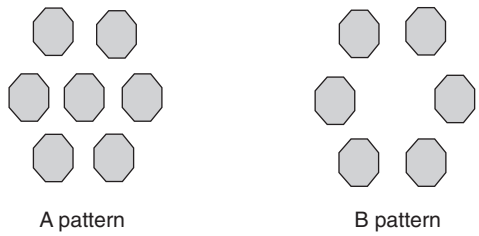

Fig. 17.4. Starch granule types.

While most cereals possess the A pattern, potatoes, bananas and high-amylose maize possess the B pattern. As a result, high amylose maize will be more slowly digested than its normal counterpart. The second consideration with respect to starch granule structure is the relative content of the linear polymer, amylose, compared with the highly branched polymer, amylopectin. Amylopectin is more readily digested due to its amorphous nature, which allows more rapid entry of moisture and thus enzymatic degradation. As a result, the higher the amylose content, in general, the slower the rate of digestion and hence the greater the content of RS2 (Brown, 1996).

3. RS3 is produced as a result of high-temperature processing of starch followed by subsequent storage at lower temperatures for hours or even days. After processing, a proportion of the starch is gelatinized, i.e. the crystalline structure is destroyed and an amorphous, hydrated gel is created. If this is subsequently stored at a low temperature, over a period of time a proportion of the gelatinized material can re-associate into crystalline complexes with proteins and cell wall structures and form an indigestible complex known as retrograde starch. Starches rich in amylose, while they are more resistant to gelatinization than amylopectin-rich starches, once gelatinized are much more likely to retrograde. Retrograde or RS3 is impervious to pancreatic amylases, but is susceptible to fermentation in the large intestine and caeca. Gelatinization occurs most often when water is in excess. Annealed or heat-processed starch are forms that are generated in moderate or limited water content, respectively. Such forms of starch are equally prone to modifications on cooling which significantly alters their rates of digestion by pancreatic amylases (Annison and Topping, 1994; Jacobs *et al.*, 1998; Jacobs and Delcour, 1998). The classical measurement of RS3 is through use of an *in vitro* method, a method that may not identify the same resistant fraction as that found in the terminal ileum (Annison and Topping, 1994). Although there is a terminology and several methods for the determination of RS3, the relevance of the values generated in these assays is therefore questioned (Annison and Topping, 1994).

It is important to note that whilst retrograde starch (RS3) is classically considered to be formed as a result of high-temperature processing, it is also likely that similar processes occur during wet harvest years when the grain is dried vigorously in forced-air driers. In addition, storage of the recently harvested grain in vertical silos with metal sides can result in the silo temperature exceeding 50°C (FFI internal data), which over a period of time may lead to significant annealing of starch.

Protein encrustation of starch granules has also been suggested as a possible factor in limiting exposure to amylases (McAllister *et al*., 1993; Oria *et al.*, 1995; Elkin *et al.*, 1996). Removal of this protein mask increases the rate of *in vitro* starch digestion (McAllister *et al.*, 1993).

RS1, as discussed above, may well be subject to exposure to attack as a result of the use of cell wall-degrading carbohydrases feeding back on gizzard activity through production of sugars in the caecum. RS2 and RS3 escape digestion by the bird but are to a large extent exposed to attack by the caecal microflora. Feeding high levels of such starches significantly alters

the populations in the caeca (Gibson *et al*., 1996; Silvi *et al*., 1999). Amylases from organisms such as *Aspergillus fumigatus* have been shown to digest much of the chemically defined resistant starches (Planchot *et al.*, 1995), which suggests they have different mode of action to pancreatic amylases. If they are shown to work synergistically with pancreatic amylases, they may well be of benefit. Recent evidence suggests that enzymes containing activities which target the cell walls (RS1, through the indirect mechanism), the starch itself (RS2 and RS3) and the protein matrix which surrounds the starch granules have been successful in improving the digestibility of maize- and sorghum-based diets (Wyatt *et al.*, 1997a,b, 1999; Pack *et al*., 1998).

Maize starch appears to be more resistant to rapid digestion than wheat or barley (McAllister *et al*., 1993) in its native form, which may explain why maize responds so well to enzymes in the absence of any viscosity considerations. Evidently, variations in starch digestibility exist in wheat (Rogel *et al.*, 1987), much of which is attributable to variations in viscosity, but some of that is likely to be addressed by consideration of resistant starch. With viscosity largely controlled through the use of the relevant enzymes, it is likely that further improvements in performance of wheat- and barley-fed animals could be achieved by targeting resistant starch. Animal performance could therefore benefit if carbohydrases (and indeed proteases) are used to increase the exposure of starch to pancreatic amylases in the intestinal lumen. Such benefits have been shown in grains that previously were thought to be unlikely candidates for improvement (maize and sorghum).

OLIGOSACCHARIDES

The oligosaccharides, raffinose, verbascose and stacchyose, are present in many feed ingredients but particularly so in oilseed meals and pulses. These carbohydrates are not digested by the bird but are readily fermented by intestinal flora. Extraction of these compounds with ethanol from soybean meal has been shown to improve digestibility (Coon *et al*., 1990; Leske *et al*., 1993), which suggested that these sugars are not only a diluent but an active anti-nutrient. Addition of α-galactosidases has also been shown in some work to increase the TME of soybean meal (Knap *et al.*, 1997). However, the removal of oligosaccharides with use of ethanol also results in the loss of 40% of the mass of the meal, the majority of which is not oligosaccharides. Ethanol is also a denaturant that will undoubtedly alter the structure of the residue, and as a result ethanol-extracted material cannot be used as proof of the anti-nutritive value of oligosaccharides. There is also uncertainty regarding the benefits of α-galactosidases. Almost complete removal of the oligosaccharides from soybean meal by preincubation with α-galactosidases had no beneficial effect on bird growth rate or feed conversion efficiency (Irish *et al.*, 1995). As a result, there is no consensus as to whether the oligosaccharides present in oilseed meals are actually detrimental to performance, particularly when fed at typical commercial levels.

COMMON MECHANISM?

A common effect with use of all carbohydrases in poultry diets is that they remove some of the constraints on digestion and as a result reduce the amount of material remaining in the small intestine. Such remaining material is the 'food' for the resident bacteria. The poorer the digestibility of the feed, the greater the substrate available, the greater the numbers of total bacteria (Wagner and Thomas, 1987; Apajalahti *et al*., 1995; Choct *et al.*, 1996; Bedford and Apajalahti, 2000). Removal of substrate by addition of enzymes is known to reduce bacterial numbers in the ileum. Such an effect is beneficial for the following reasons:

1. Bacteria compete for nutrients in the lumen with the bird. The greater the number of bacteria, the greater the loss of nutrients.
2. Some bacteria secrete enzymes which inactivate bile salts, lecithin and pancreatic enzymes and damage the small intestinal surface (Feighner and Dashkevicz, 1988; Thomke and Elwinger, 1998; Corzo and Gilliland, 1999a,b). As a result, the efficiency of digestion is clearly reduced in the presence of a gut microflora.
3. If numbers of any species approach a significant level, the bird may mount an immune response which in itself is energetically costly.

If diet digestibility is compromised, the bird responds by producing more pancreatic secretions and increasing the absorptive surface area by increasing intestinal mass. In the absence of a gut microflora (i.e. germ-free) such responses can largely, if not completely, compensate for a reduction in digestive efficiency. Impeding digestion of maize-based diets through addition of a viscous pectin or cellulose derivative has been shown to reduce the growth rate and ileal fat digestibility in conventional but not germ-free chicks (Smits and Annison, 1996; Schutte and Langhout, 1999). As a result, it can be stated with reasonable confidence that the response to enzymes, or indeed any additive which improves nutrient digestibility, may well depend upon the microbial loading (i.e. challenge) in the small intestine.

SUGAR PROVISION EFFECTS

Exogenous carbohydrases depolymerize plant carbohydrates to produce smaller polymers, oligomers, mono-, di- and tri-saccharides. Pancreatic or brush-border enzymes do not target most of these products, and as a result they escape digestion and absorption. Many are substrates for bacterial fermentation in the ileum and particularly the caecum. Several papers have shown that use of enzymes significantly alters the population profiles of bacteria (Apajalahti *et al*., 1995; Choct *et al*., 1996; Hock *et al*., 1997; Vahjen *et al*., 1998; Apajalahti and Bedford, 1999; Bedford and Apajalahti, 2000) in both ileum and caecum. Whilst ileal populations tend to be deprived of nutrients on addition of enzymes, the reverse seems to be the case for caecal populations. Cell wall degrading enzyme addition has consistently been shown to increase

the total microbial population in the caeca, alter the relative population distribution and increase the proportion of propionate as a fraction of total volatile fatty acids (Choct *et al*., 1996; Hock *et al*., 1997; Apajalahti and Bedford, 1999). It is interesting that, with the common use of growth promoters, much of this caecal benefit is not realized, since the sugars produced by enzymatic action are presented into an environment where the bacterial populations are held in check and cannot take advantage of these substrates. As a result, the caeca are largely vestigial in comparison with birds unexposed to growth promoters. With the removal of growth promoters, there will be an increase in caecal fermentation which may provide an additional source of energy to the bird (as much as 5–8% of total requirements (Annison *et al*., 1968)) and also lead to some degree of pathogen control through increased propionate concentrations. Enlarged caeca are an obvious result of such a change. Unfortunately, the lack of control of bacterial numbers in the caeca in the absence of growth promoters is also likely to result in many more explosive growths of undesirable bacteria, since there is little to prevent them taking advantage of influxes of nutrients from poorly digested ingredients. Far greater vigilance of raw material quality and correct enzyme dosage are thus essential if the problems predicted with growth promoter removal are to be mitigated to any degree.

CONCLUSIONS – INTERACTIVE FACTORS MAKE INTERPRETATION OF THE LITERATURE DIFFICULT

It is clear from the above that the use of carbohydrases in poultry diets does not just target one problem and produce a simple effect. There are many factors that interact to determine if there will be a response in the first place, and, if so, the scale of the response observed. As a result, the literature is certainly not clear on what would appear to be simple issues such as mechanism of action. Some factors that can interact with carbohydrase response are listed below:

1. Cereal quality. Higher quality samples need less 'help' from enzymes, and as a result do not respond as dramatically as those which contain a large amount of the enzyme substrate (e.g. viscous arabinoxylans in wheat). Most trials are conducted without prior knowledge of the quality of the cereal used. The literature is therefore highly variable. Factors which have been shown to influence cereal quality are variety (Classen *et al*., 1995; Barrier-Guillot *et al*., 1997), environment (Campbell *et al*., 1991; Barrier-Guillot *et al*., 1997; Bedford *et al*., 1998) and processing/drying conditions (Teitge *et al*., 1991; Nissinen, 1994).

2. Fat. Quantity and degree of saturation has conclusively been shown to significantly influence the response to xylanase addition to wheat- and rye-based diets. The greater the quantity and degree of saturation of fat, the greater the response to the enzyme.

3. Microbial status. Comparison of germ-free with conventional chicks suggests that the greater the microfloral challenge, the greater the likelihood of response to enzyme usage. Responses are thus expected to be larger under commercial circumstances, for example, compared with academia.

4. Age. Conventional wisdom has it that the response to enzymes decreases with age (Steenfeldt *et al*., 1998) due to the fact that viscosity tends to fall with age (Petersen *et al*., 1993). As the bird ages, however, the microfloral populations increase, which means that the consequences of higher viscosity are potentially greater. As a result, some reports suggest that the response can increase with age (Bedford, 1996b).
5. Antimicrobial agents: It is clear from the discussion herein that there is a relationship between the microfloral status of the bird and its response to feed enzymes. As a result, any agent which manipulates the microflora, either directly (e.g. growth promoters, coccidiostats, aresenicals, copper, zinc) or indirectly (e.g. live bacterial cultures, fermentable sugars) will influence the result of an enzyme additive. Such interactions can be negative, neutral or positive as viewed simply from the interactions between enzymes and antibiotics (Moran *et al*., 1969; Lund, 1987; Broz *et al*., 1994; Allen *et al*., 1996; Hock *et al*., 1997).

It is essential, therefore, that the above factors are reported or considered for correct interpretation of the literature. It is likely that many reports have been erroneously interpreted due to unconsidered flaws in trial design.

FUTURE

Enzymes are widely used today, primarily on the basis of empirical research which has resulted in the design of 'best-fit' products. The true, native substrate of such products is still poorly understood, and the effects of the products of fibre digestion on microfloral status are only just being understood. With increasing knowledge of the substrate and the products required for optimal microbial fermentation, it is likely that more dramatic advances in enzyme efficacy will be made in the near future. It is hoped that with a better understanding of the substrates, there may be an opportunity to develop rapid screening methods to identify which cereal samples require enzymes and which do not. Whilst this is already a reality for wheat and barley, there are currently no quality parameters that are relevant for describing the nutritive value of maize and sorghum.

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CHAPTER 18
The Influence of lipase, α-galactosidase or multi-component pectinase enzymes on energy and amino acid availability in feedstuffs

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ABSTRACT

Performance and balance trials were conducted to examine the effect of lipase, α-galactosidase and multi-component pectinase on broiler performance. It was found that lipase and α-galactosidase had little effect on broiler performance in fat- or soy-rich diets respectively. Improvements in energy and amino acid availability could be obtained on vegetable proteins using the multi-component pectinase. Feeding studies using diets with altered amino acid levels confirmed the hypothesis that the mode of action of the enzyme was more to improve the availability of amino acids than energy.

INTRODUCTION

The use of xylanase enzymes for increased performance of broilers fed wheat-based diets is well established. The mode of action has been extensively studied, and the beneficial effects of NSP-hydrolysing enzymes on performance parameters are generally explained by softening of a so-called cage effect on the one hand and by decreasing digesta viscosity on the other. The cage effect results in a form of encapsulation of nutrients that prevents access by endogenous digestive enzymes. Viscosity seems to mediate not only an impaired nutrient digestibility, but also modifications in morphology and histology of the intestine, protein and energy metabolism and microbial population in the gastrointestinal tract (Cowan, 1989, 1993, 1994; Bedford and Morgan, 1996; Dänicke *et al.*, 1998). A general review of the use of enzymes in vegetable protein meals (as distinct from cereals) was published by Thorpe and Beal (2001).

To further increase performance, two areas of investigation are possible. The first of these is to consider the supplementation of endogenous enzyme activities. This functions on the assumption that at some stage of the broiler's growth, enzyme secretion is insufficient to match the demands placed upon it

 Poultry Feedstuffs: Supply, Composition and Nutritive Value (eds J.M. McNab and K.N. Boorman)

by modern animal husbandry. Jin *et al.* (1998) reviewed the literature on the development of digestive enzymes and the digestive process in poultry and concluded that lipase and amylase supply was sufficient in young chickens, but that protein digestion was not optimum. This observation was considered to be linked more to levels of intestinal tissue peptidase activity than to an insufficiency of trypsin or chymotrypsin. However, as diet content can influence enzyme secretion, it is clear that more work needs to be done using more standardized diet compositions. A study on the influence of lipase on broiler performance was conducted to try and gain more insight in this area.

A second avenue of investigation is to consider other components of the diet not influenced by xylanases, such as the α-galactosides of soy and the overall digestibility of proteins from vegetable sources. Of the gross energy contained in soy, only 52% is utilized by poultry (Potter and Potchanakorn, 1985). Soybean meal contains substantial amounts of raffinose and stachyose, but also of complex polysaccharides such as xylo-glucan, galactomannan and pectic substances, and this latter group may be as much as 15.5% of the overall dry weight (Eldridge, 1979).

Both groups of saccharides have the potential to contribute to the reduced nutrient availability of soy and should be investigated using enzymes specific for the substrates involved.

MATERIALS AND METHODS

Experiment 1: Influence of Lipase and α-Galactosidase on Broiler Performance

Day-old broiler chicks were allocated to pens with 15 males and 15 females per pen. The floor area of the pens was 1.6 m^2, giving a density of 18.75 chicks per m^2. Two diet formulations were used, one rich in soybean meal (diet A) and one with elevated levels of animal fat (diet B). For each diet, eight pens of broilers were used, giving 32 pens in all. Table 18.1 lists the compositions of the two diets.

Diet A was used for the examination of the effect of α-galactosidase. The enzyme was sprayed on to the pelleted diets so as to provide 700 GALU kg^{-1} diet. Diet B was used for the lipase experiment and the enzyme was also sprayed onto the pelleted diet to provide 45 KLU kg^{-1} feed.

At days 14, 28 and 42, bird weight and feed consumption were determined. Livability was determined on a daily basis and feed consumption corrected for mortality. Statistical analysis of the data was performed using the procedures of the Statgraphics (1992).

Experiment 2: Influence of Multi-component Pectinase on Nutrient Availability in Vegetable Proteins

The availability of amino acids and energy in several vegetable protein sources was determined using a modification of the method of Bourdillon *et*

Table 18.1. Composition of diets used for poultry experiments.

Ingredients (g kg^{-1})	Diet A	Diet B
Wheat	614.0	605.0
Soybean meal	320.0	230.0
Fish meal	–	40.0
Meat and bone meal	–	40.0
Animal fat	30.0	60.0
Methionine (40%)	4.0	5.0
Limestone	10.5	7.0
Dicalcium phosphate	15.5	7.0
Salt	3.0	3.0
Vitamin premix	2.6	2.6
Choline chloride (50%)	0.4	0.4
ME	11.80	12.94
Protein %	22.60	23.38

al. (1990). In this method, the digestibility of nutrients is derived from the differences in these parameters obtained with a basal diet and with a diet in which one of the test proteins has replaced a portion of the basal diet. In the present study, the test materials were included in the diet at a level of 20% (soybean meal) or 25% (sunflower or rapeseed). Diet composition is shown in Table 18.2. Each treatment group (ten in total) consisted of four cages of eight female broiler chicks.

At 14 days of age, birds were assigned to each of 40 cages and the appropriate test diet introduced. After a 10-day acclimatization period, excreta were collected over 96 h, quantitatively per cage. Birds were fed *ad libitum* except for a 6-h fasting period before the start and after the end of the collection period. Excreta samples were pooled, stored at –20°C and analysis performed on the freeze-dried pooled excreta. Enzyme addition was made to one of each of the pairs of diets.

Table 18.2. Composition of basal diet for balance trials.

Component	Content (g kg^{-1})	Component	Content (g kg^{-1})
Maize	500.00	L-lysine HCl	1.10
Soya flour (50% CP)	190.00	D,L-methionine	2.50
Full fat soya	100.00	Limestone	10.20
Fish meal	30.00	Mono-calcium P	9.00
Tapioca	77.50	Salt	3.00
Animal fat	20.00	Vit/mineral mix	10.00
Soya oil	30.00	Diamol*	16.70

*Diamol is a proprietary insoluble inorganic marker used in balance studies.

Experiment 3: Influence of Multi-component Pectinase on Performance in Diets with Differing Lysine and Sulphur Amino Acid Levels

The ability of the enzyme to improve amino acid digestibility was also studied in a broiler performance study. A total of 2070 day-old broilers were sexed and divided into pens, each containing 32 males and 37 females. Broiler diets were formulated to be isocaloric (13 MJ kg^{-1}) but to contain a low (0.98%), medium (1.16%) or high (1.43%) level of lysine. Methionine content was modified in a similar manner. For each diet, one group of five pens received the standard diet, a second group of five pens the same diet plus 500 mg kg^{-1} of the multi-component pectinase. To limit potential inaccuracies in diet composition resulting from too many diet variations, a single diet was used throughout the feeding period for each group. Diet compositions are shown in Table 18.3.

All parameters were subjected to a three-factorial analysis of variance using Statgraphics 6.0.

RESULTS

Experiment 1: Influence of Lipase and α-Galactosidase on Broiler Performance

Addition of α-galactosidase to a wheat-based broiler diet resulted in a numerical improvement in FCR (feed conversion ratio) but no overall improvement in

Table 18.3. Diet compositions for feeding trial (g kg^{-1} diet).

Nutrient	Low lysine	Normal lysine	High lysine
Sorghum	415.0	415.0	415.0
Wheat	100.0	100.0	100.0
Maize	100.0	100.0	100.0
Soybean meal	221.6	220.4	204.0
Rapeseed 00	50.0	50.0	50.0
Fish meal	32.0	37.0	50.0
Animal fat	40.0	30.0	30.0
Soy oil	6.0	10.0	10.0
Di-calcium phosphate	16.6	16.0	14.8
Limestone	4.5	4.6	4.4
Salt	2.9	2.9	2.9
D,L-Methionine	1.4	2.5	3.9
L-Lysine Hl	–	1.5	4.0
Vitamins, premix, growth promoter	10.24	10.24	10.24
Energy (MJ kg^{-1})	12.98	12.93	13.04
Crude protein	190.2	193.2	195.0
Lysine	9.9	12.0	14.0
Methionine	4.5	5.7	7.2
Calcium	7.9	7.9	7.9
av. Phosphorus	3.5	3.5	3.5

weight gain (Table 18.4). Addition of lipase to a fat rich diet produced a small numerical improvement in FCR and weight gain in the initial 14 days of the experiment, but the differences were not significant.

Experiment 2: Influence of Multi-component Pectinase on Nutrient Availability in Vegetable Proteins

The results of dietary amino acid digestibility for all amino acids and for energy level following enzyme supplementation are shown in Table 18.5. Enzyme supplementation increased dietary amino acid and energy availability in all raw materials tested. When the effect on the individual amino acids was examined, it could be seen that the effect on digestibility was a general one and no specific amino acid was increased above the others.

Table 18.4. Performance results with α-galactosidase or lipase addition.

Diet	A	A + α-galactosidase	B	B + lipase
0–14 days				
Weight (g)	471	475	475	482
FCR	1.18^{a}	1.17^{ab}	1.13^{bc}	1.11^{c}
0–42 days				
Weight (g)	2259^{b}	2247^{b}	2316^{ab}	2325^{a}
FCR	1.89^{a}	1.86^{a}	1.73^{c}	1.74^{c}

Means without a common superscript are significantly different at $P < 0.05$, LSD test.

Table 18.5. Effect of enzyme supplementation on diet amino acid digestibility for diets containing different protein sources and derived raw material AMEn.

	Basal – enz	Basal + enz	Soy – enz	Soy + enz
Amino acid digestibility	83.7% ± 0.6	84.1% ± 0.5	80.4% ± 0.5	83.4% ± 1.8
AMEn (MJ kg^{-1})	13.38 ± 0.15	13.39 ± 0.16	8.1 ± 0.4	8.8 ± 0.6
	Sunflower – enz	Sunflower + enz	Rape seed - enz	Rape seed + enz
Amino acid digestibility	82.7% ± 1.0	84.5% ± 0.9	81.2% ± 0.8	83.1% ± 0.6
AMEn (MJ kg^{-1})	5.7 ± 0.7	6.3 ± 0.8	12.3 ± 0.7	13.4 ± 0.7

AMEn, apparent metabolizable energy corrected for N-equilibrium.

Experiment 3: Influence of Multi-component Pectinase on Performance in Diets with Differing Lysine and Sulphur Amino Acid Levels

The results of the growth experiment are shown in Table 18.6. Reduction in lysine levels reduced growth and resulted in a poorer FCR, with the highest efficiency seen at the high lysine addition level. Supplementation with pectinase improved performance for the low and normal lysine level diets, but had no effect on diets containing the highest lysine level. Mortality tended to be higher in the groups receiving the highest lysine levels, but this was not affected by pectinase addition.

DISCUSSION

Supplementation of wheat-based broiler diets with either α-galactosidase or lipase resulted in little change in overall performance. For the α-galactosidase, the relatively low level of substrate present may well have resulted in benefit not being measurable through performance trials. When the influence of α-galactosidase on soy AMEn was determined through a balance trial, an improvement of 7.6% was achieved (Huyghebaert and De Groote, 1995). This level of improvement might not be sufficient to result in a change in performance under the conditions of the trial procedure.

When lipase was added, a similar, small but nonsignificant improvement in performance was observed. This was more pronounced in the first 14-day period, but again the magnitude of the improvement was small. These results are in accordance with those summarized by Jin *et al.* (1998). They also tend to confirm the hypothesis that fat digestibility may be more influenced by the level of emulsification coming from bile salts and feed components than by an insufficiency of endogenous lipase.

In these two experiments, mono-component enzymes were used. This is an important point in that it ensures that all results may be ascribed to the presence of specific enzyme activities and are not due to the influence of unknown or undeclared side or other activities within the enzyme product, such as xylanase or β-glucanase.

Using a multi-component enzyme with pectinase as a main enzyme component, but with no lipase or α-galactosidase activity, made it possible to examine the influence of the non-α-galactoside polysaccharides found in soy and

Table 18.6. Effect of lysine and pectinase on broiler growth in iso-caloric diets containing different levels of amino acids.

	Low lysine	Low lysine + pectinase	Normal lysine	Normal lysine + pectinase	High lysine	High lysine + pectinase
FCR	1.956[a]	1.900[a]	1.855[b]	1.833[b]	1.810[c]	1.819[b]
Final wt (kg)	1.883	1.897	2.051	2.083	2.088	2.081
Mortality	7.4%	9.9%	11.9%	10.5%	17.2%	14.5%

Means without a common superscript are significantly different at $P < 0.05$, LSD test.

other vegetable proteins. Annison (1995) discussed the importance of galactans in lupins, and this polysaccharide may also be found in soy and other vegetable proteins.

Addition of the multi-component pectinase increased AMEn and also amino digestibility in the vegetable protein sources studied. When diets were formulated to take account of these apparent increases, performance was only maintained in the presence of the enzyme complex. Addition of enzyme to diets containing sufficient levels of amino acids to meet the nutritional need, did not result in any further increases in performance.

Thus, performance improvements can be obtained by targeting other feed components in the diet other than xylans or β-glucans and it would appear that supplementing diets with enzyme activities already produced by the animal is of lesser benefit. Although both energy and amino acid availability can be increased in soy and other vegetable proteins, the relative contribution of these raw materials to energy and protein supply is not equal. In general terms, cereals are responsible for >60% of the energy supply of the diet, whereas vegetable proteins contribute approximately the same level of protein and amino acids. Therefore, the influence of endogenous enzymes on protein digestibility in vegetable proteins will exert an overall greater effect than their effect on energy availability in these feedstuffs.

In practical diet formulations, improved nutrient availability of vegetable proteins is more easily observed by concentration on amino acid availability rather than energy. This effect may become more pronounced if levels of readily digestible proteins, e.g. meat and bone meal, are reduced. This is particularly relevant in the current circumstances where animal by-products have been banned in feeds for monogastric animals. The above studies also indicate that the availability of amino acids in vegetable proteins has been overrated. For successful feed formulation in the new circumstances, these factors need also to be taken into account. The main challenge in this area is to increase the efficiency of the current enzyme preparations by the development of specific mono-component enzymes targeted at these raw materials (Ohmann and Pettersson, 2000).

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CHAPTER 19
Recent trends and future developments in the use of feed enzymes in poultry nutrition

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INTRODUCTION

Supplemental enzymes found only limited practical use in animal nutrition before the early 1990s. The main reason was an unfavourable relationship between enzyme costs and their real benefits. However, due to the advance in biotechnology leading to lower production costs, economical use of feed enzymes is now possible.

The purpose of this chapter is to review some recent trends and discuss possible future developments in the use of feed enzymes in poultry nutrition.

NSP-HYDROLYSING ENZYME PRODUCTS

It is now well known that the nutritive value of cereals, such as barley, oats, triticale, rye and wheat, is adversely affected by the presence of certain non-starch polysaccharides (NSP) in their endosperm cell walls. An important feature of the NSP, e.g. mixed linked β-D-glucans and arabinoxylans, is their partial solubility in water, resulting in the formation of viscous gel solutions. These cause a dramatic increase in gut viscosity, which may impair the action of digestive enzymes, decreases digesta flow and absorption of nutrients and, finally, causes sticky droppings. As a consequence, digestibility of nutrients and utilization of dietary energy may be markedly impaired. These anti-nutritive effects have been extensively studied (Choct and Annison, 1990, 1992; Annison and Choct, 1991; Choct *et al.*, 1992) and are reviewed by Choct (Chapter 13, this volume).

RECENT DEVELOPMENTS

In order to enhance the nutritive quality of various low-energy cereals and other responding dietary ingredients, specific enzyme products capable of

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hydrolysing the relevant NSP have been established as a new class of feed additives, in particular for poultry. It has also been confirmed that endo-1,3:1,4-β-glucanase and/or endo-1,4-β-xylanase are the main enzyme activities responsible for beneficial effects. Recent progress in this area provides an excellent example of the way in which some applied research findings may be successfuly implemented in practice. In the meantime, a broad range of NSP-hydrolysing enzyme products has been developed, which can be divided into four groups as follows:

1. Enzyme complexes derived from single strains (e.g. *Trichoderma longibrachiatum*, *T. viride*, *Aspergillus niger*, *Humicola insolens,* etc.).
2. Enzyme mixtures based on two or more fermentation products.
3. Mono-component enzymes derived from genetically modified strains.
4. Combinations of an enzyme complex and mono-component enzyme (GMO-derived).

Good progress has been also achieved in analytical determination and chemical characterization of the relevant NSP, which has allowed the development of more-specific products and a better understanding of their mode of action. It is now obvious that a marked improvement in the apparent metabolizable energy of cereal-based diets by supplemental enzymes is mainly related to an increase in nutrient digestibility (fat, starch) and not due to the NSP itself becoming available to the birds as a substantial energy source (Broz, 1993b; Annison, 1995). In the case of wheat, in which soluble arabinoxylans are present at levels below 20 g kg^{-1}, complete utilization of this fraction would yield energy in the region of 17 kJ g^{-1}, which would eventually increase the AME (apparent metabolizable energy) value of wheat by 0.34 MJ kg^{-1} (Annison, 1995). However, this is much less than the improvement in AME recorded by Choct *et al*. (1995) and van der Klis *et al*. (1995b).

When added to relevant poultry diets, NSP-hydrolysing enzymes usually result in various benefits, as follows:

- improved metabolizable energy
- increased utilization of nutrients (e.g. fat, protein)
- improved feed/gain ratio (by 2–5%)
- increased growth rate (by 2–3%)
- decreased viscosity of intestinal digesta
- modification of intestinal microflora
- reduced incidence of sticky excreta
- improved litter conditions.

Their effects on important performance parameters are fully evident and have been discussed extensively in many earlier reviews (e.g. Campbell and Classen, 1989; Hesselman, 1989; Jeroch, 1991; Broz, 1993a; Jeroch *et al.*, 1995a).

In recent years, however, substantial progress has been also achieved in the understanding of various metabolic and physiological effects of supplemental enzymes (see reviews by Broz, 1993b; Chesson, 1993; Annison, 1995; Bedford, 1995, 1996; Bedford and Morgan, 1996).

Utilization of dietary energy

As a consequence of the mode of action of the NSP-hydrolysing enzymes, increased utilization of dietary energy may well be expected. In fact, increases in metabolizable energy values of either whole diets or the given cereal due to enzyme supplementation have been repeatedly described in the literature. Significant improvements in the AME of broiler diets based on either rye or oats were reported by Broz (1987) and Broz and Frigg (1990). Pettersson and Aman (1989) demonstrated that graded levels of a pentosanase preparation significantly increased the AMEn (AME corrected for N-equilibrium) values of a rye–wheat diet from 10.7 to 11.3, 11.2, 11.2 and 11.6 MJ kg^{-1} dry matter, respectively. Rotter *et al*. (1990) observed that enzyme supplementation had a differential effect on the AMEn values of four different barley cultivars when determined in young broiler chickens (see Table 19.1). The greatest response was noted for Scout barley (+ 25.3%) but no response was found with Bedford barley.

Annison (1992) reported that various commercial enzyme preparations significantly raised the AME of Australian wheat by 7.2–10.2%. There was no significant difference in the efficacy of the enzymes as measured by the AME assay. Friesen *et al*. (1992) also observed that enzyme supplementation increased the AMEn of several test cereals. The corresponding increases in the AMEn of the enzyme-supplemented diets containing 70% Bedford barley, Scout barley, Terra oats or Gazelle rye were 7, 42, 33 and 14%, respectively.

However, for the least-cost formulation of practical type broiler diets, specific information concerning the effects of NSP-hydrolysing enzymes on the AME value of individual cereals is very valuable. For that reason, most relevant experimental data showing the effects of enzyme supplementation on AME values of barley and wheat, as reported in the literature, are summarized in Tables 19.1 and 19.2. All these results were obtained in bioassays conducted with young broiler chickens. It is obvious that the relative improvement in AME values for barley depends on the nutritive value of the cultivar, its content of water-soluble β-D-glucan and the resulting viscosity. The results shown in Table

Table 19.1. Effects of enzyme supplementation on AME values of barley (bioassays conducted with young broiler chickens).

	AME value (MJ kg^{-1} DM)			
Cereal characteristics	− enzyme	+ enzyme	Improvement (%)	Source
Barley (cultivar Bedford)	12.24	12.19	−0.4	Rotter *et al.* (1990)
Barley (cultivar Bonanza)	12.54	13.05	+4.1	
Barley (cultivar Scout)	11.12	13.93	+25.3	
Barley (cultivar Harrington)	12.96	13.27	+2.4	
Winter 2-row barley	12.78	13.14*	+2.8	Fuente *et al.* (1995)
8 Spanish barley cultivars (4 spring and 4 winter)	11.68	12.61**	+8.0 (+4.0–15.7)	Villamide *et al.* (1997)

Significance: * $P < 0.05$, ** $P < 0.01$.

Table 19.2. Effects of enzyme supplementation on AME values of wheat (bioassays conducted with young broiler chickens).

	AME value (MJ kg^{-1} DM)			
Cereal characteristics	– enzyme	+ enzyme	Improvement (%)	Source
Low AME wheat	12.02	14.94*	+24.3	Choct *et al.*
Normal AME wheat	14.52	14.83	+2.1	(1995)
13 wheat cultivars	13.23	14.36**	+8.6	van der Klis *et al.*
			(+4.5–12.4)	(1995)
Wheat (cultivar Ibis)	14.76	14.94	+1.2	Dusel *et al.*
Wheat (cultivar Alidos)	13.98	14.65*	+4.8	(1998)
Wheat	12.35	13.91	+12.6	Hew *et al.*
		14.66*	+18.7	(1998)
Wheat (cultivar Alba)	13.78	14.37*	+4.3	Seskeviciene *et al.*
Wheat (cultivar Sirvinta)	13.29	14.24*	+7.1	(1999)

Significance: * $P < 0.05$, ** $P < 0.01$.

19.2 indicated much stronger AME improvements obtained with Australian low-energy wheats (Choct *et al.*, 1995; Hew *et al.*, 1998), but also suggested that, under European conditions, improvements in AME values in the range of 4–8% might well be expected.

Utilization of nutrients

Marked improvement in the metabolizability of dietary energy by supplemental enzymes appears to have various causes. As mentioned above, part of the additionally metabolized energy may originate directly from sugars released after partial or complete hydrolysis of NSP (e.g. D-glucose, D-xylose, L-arabinose). More important in this respect, however, seems to be the ability of supplemental enzymes to minimize or even eliminate the anti-nutritive activities of cereal NSP and thus exert beneficial effects on the digestibility of dietary nutrients, such as fat, protein and starch.

Significant positive effects of supplemental enzymes on dietary fat retention in rye-based diets were reported by Fengler *et al*. (1988), Broz and Canterranne (1990) and Pawlik *et al*. (1990). Edney *et al*. (1989) observed an improved apparent fat absorption in enzyme-supplemented diets containing either barley or oat groats. Similarly, Salih *et al*. (1991) reported improved fat digestibility in broilers fed hull-less barley. Friesen *et al*. (1992) confirmed the beneficial effects of additional enzymes on apparent fat digestibility in broilers fed diets containing either barley, rye or oats (see Table 19.3). More recently, Viveros *et al*. (1994) found significant improvement of apparent fat digestibilty due to enzyme supplementation in a barley-based diet. Almirall *et al*. (1995) observed that ileal fat digestibility was significantly improved by β-glucanase in young broiler chickens fed a barley-based diet, but the improvement was not statistically significant in adult cocks.

Van der Klis *et al*. (1995b) evaluated the effects of an endo-xylanase on apparent fat digestibility in broilers fed diets containing 13 different wheat

Table 19.3. Apparent lipid digestibility (%) in young broiler chicks when fed enzyme supplemented (+) or unsupplemented (−) diets containing different concentrations of Scout barley, Bedford barley, Gazelle rye and Terra oats.

	Barley				Rye		Oats	
Dietary cereal	Scout		Bedford		Gazelle		Terra	
Inclusion rate (%)	−	+	−	+	−	+	−	+
0*	76.1	79.3	76.1	79.3	76.1	79.3	76.1	79.3
35	63.6	81.1	73.4	82.7	32.0	68.2	52.0	75.1
70	43.2	79.9	75.2	85.0	24.9	48.3	17.4	51.0

*Replacement for HY320 wheat (0 = 70% wheat). Adapted from Friesen *et al.* (1992).

cultivars. On average, digestibility improved from 75.8% to 82.8%, with relative improvements ranging between 4.5% and 14.7%. These results were confirmed recently by Steenfeldt *et al*. (1998b), who also reported a significant improvement in apparent ileal and excreta fat digestibility in broilers fed enzyme-supplemented wheat-based diets.

In addition, supplemental enzymes may also affect dietary protein utilization. In some instances, significant or numerical increases of nitrogen retention were noted in rye-based diets (Broz, 1987; Broz and Canterranne, 1990). The apparent protein digestibility was improved by enzymes in broiler diets based on either barley, rye, oats or wheat (Rotter *et al.*, 1990; Friesen *et al.*, 1992; Almirall *et al.*, 1995). In contrast, only a moderate effect was observed in barley-based diets by Jeroch *et al*. (1990). Bedford (1995) reported that the apparent ileal digestibility of amino acids was significantly improved by supplemental β-glucanase in broilers fed a barley-based diet. This observation was confirmed recently by Dänicke *et al.* (1997b) when using rye-based broiler diets supplemented with a xylanase preparation. However, beneficial effects on the ileal digestibility of amino acids were dependent on fat quality, being much more pronounced in a diet containing tallow. Only marginal positive effects on the apparent digestibility of amino acids measured at the excreta level were noted in broilers fed a diet based on wheat and rye (Langhout *et al.*, 1997). As reported by Hew *et al*. (1998), enzyme supplementation resulted in significant improvements in ileal and excreta amino acid digestibility in Australian wheat.

Edney *et al*. (1989) noted certain improvements in apparent starch absorption due to enzyme addition to broiler diets based on either hull-less barley or oat groats. In barley-based diets, significant improvements in starch digestibility were confirmed in young broilers at both ileal and excreta levels (Viveros *et al.*, 1994; Almirall *et al.*, 1995). Annison (1992) found a significant increase in the ileal digestibility coefficients of starch from 0.88 in control birds to 0.96–0.98 in those fed enzyme-supplemented wheat diets. However, other authors observed only marginal effects of NSP-hydrolysing enzymes on apparent digestibility of starch in wheat-based diets, which is usually very high (e.g. Steenfeldt *et al.*, 1998b).

Viscosity of intestinal digesta

In agreement with the anticipated mode of action, partial or complete hydrolysis of NSP in the gut is directly reflected by changes in the viscosity of intestinal digesta. As reported by Bedford *et al*. (1991), the viscosity of fore- and hindgut contents in broilers fed rye was significantly reduced by a pentosanase preparation derived from *T. longibrachiatum*. Furthermore, an increase in digesta dry matter content was noted. Salih *et al*. (1991) demonstrated that β-glucanase supplementation significantly lowered the viscosity of hull-less barley diets to that of the wheat diets. Reduction in gut viscosity, particularly in jejunum, by supplemental enzymes was confirmed in broilers fed diets based on barley (Almirall *et al.*, 1995; Fuente *et al.*, 1995; Esteve-Garcia *et al.*, 1997), rye (Dänicke *et al.*, 1997a), or wheat (Veldman and Vahl, 1994; Choct *et al.*, 1995; van der Klis *et al.*, 1995a,b; Esteve-Garcia *et al.*, 1997; Steenfeldt *et al.*, 1998a).

As a consequence of the reduced viscosity of intestinal digesta, changes in its passage may be expected. Using a dietary marker, Jeroch *et al*. (1990) and Salih *et al.* (1991) noted an increase in passage rate resulting from enzyme supplementation of barley-based diets in broiler chickens. Subsequently, the birds may increase feed intake, as observed in many performance trials and particularly with mash diets.

Intestinal microflora

When investigating further the mechanism of the anti-nutritive activities of NSP in broiler diets, Choct *et al*. (1996) observed that increased fermentation occurs in the small intestine of birds receiving a large amount of viscous NSP in the diet, which was eliminated by enzyme supplementation. It has been speculated that increased amounts of soluble NSP in the diet lead to the development of an undesirable gut microflora. Vahjen *et al*. (1998) studied the influence of xylanase supplementation on the development of selected bacterial groups in the intestinal tract of broilers. Enzyme addition resulted in significantly lower colony-forming units per gram of wet weight for total presumptive enterobacteria and total gram-positive cocci in luminal and tissue samples in the first 3 weeks. They concluded that the less viscous intestinal environment caused by the xylanase addition slowed proliferation of gram-positive cocci and presumptive enterobacteria in enzyme-supplemented birds during this period.

Water intake, dry matter content of excreta

Due to the modification of gel-forming NSP in the gut, water intake by birds receiving enzyme supplemented barley-based diets may be markedly decreased, as demonstrated under practical conditions by Elwinger and Teglöf (1991). As an indirect measure of lower water consumption, higher dry matter content in excreta of enzyme-supplemented broilers was reported in several papers (e.g. Broz and Frigg, 1986; Pettersson *et al.*, 1991; Francesch *et al.*, 1994; Fuente *et al.*, 1995; Vukic Vranjes and Wenk, 1995).

Viscosity of excreta and incidence of sticky droppings

As mentioned earlier, dietary inclusion of cereals such as barley, oats, and particularly rye, results usually in an increased viscosity of excreta and high occurrence of

sticky droppings, which adversely affect production parameters. Several authors have reported that dietary enzyme supplementation reduces or almost eliminates this problem. Fengler *et al*. (1988) and Pawlik *et al*. (1990) showed that enzyme supplementation (*T. viride* cellulase) dramatically reduced excreta viscosity in broilers fed a rye-based diet. Pettersson and Aman (1989) observed that increasing levels of a pentosanase preparation added to a broiler diet containing rye and wheat decreased the occurrence of sticky droppings from 31% to 22%, 17%, 13% and 11%, respectively. Similar beneficial effects of enzyme addition were demonstrated when using a diet containing barley, wheat and rye (Pettersson *et al.*, 1991). In practical broiler experiments, Elwinger and Teglöf (1991) and Brufau *et al.* (1991) found a significant reduction of sticky droppings by the addition of a *Trichoderma viride* enzyme complex to barley-based diets (see Table 19.4).

Litter conditions

Due to improved nutrient utilization and lower incidence of sticky droppings, enzyme supplementation generally improves litter quality. However, such beneficial effects can be obviously demonstrated only in trials conducted under practical conditions. For example, Elwinger and Teglöf (1991) observed improved litter status using a scoring system and a significant increase of litter dry matter content (see Table 19.4). Improved litter conditions may further reduce the incidence of breast blisters and thus contribute to better carcass grading.

Experimental studies clearly suggested that the response to NSP-hydrolysing enzymes in broiler chickens may interact with several dietary factors. The most important are:

- feed formulation
- type and amount of cereal in the diet
- level of relevant non-starch polysaccharides in the cereal, in particular water-soluble part
- viscosity of intestinal digesta
- type of fat added to the diet
- feed processing conditions.

Table 19.4. Effect of *Trichoderma viride* enzyme complex on the occurrence of sticky droppings, litter conditions and water intake in broilers fed a barley-based diet until market weight.

		Enzyme addition (ppm)		
Parameter	Age (days)	0	100	200
Sticky droppings (%)	7	34.6	4.3	1.0
Litter condition (scores)	20	2.8	1.3	2.0
	34	9.0	6.8	6.5
Litter dry matter (%)	34	52.2	56.6	59.4
Water intake (ml per bird)	1–7	252	238	229
	7–14	452	445	442
	14–21	1012	926	917
	21–25	790	703	681

Adapted from Elwinger and Teglöf (1991).

New Opportunities

In recent years, some beneficial effects of NSP-hydrolysing enzymes have been noted on the nutritive value of vegetable protein sources such as peas, lupins and rapeseed (canola) meal.

Peas

Peas have a relatively high content of non-starch polysaccharides (18% total NSP, 5.2% soluble NSP; Bach Knudsen, 1997), substantial starch content (47–51%) and low but variable levels of galacto-oligosaccharides (raffinose, stachyose, verbascose). Anti-nutritive factors such as tannins, protease inhibitors, lectins and saponins are present in modern cultivars only in small amounts.

Brenes *et al.* (1993a) evaluated the nutritional value of raw, autoclaved and dehulled peas in chicken diets and the effects of added crude enzyme preparations from different sources. Their results indicated that enzyme supplementation does not improve the performance of White Leghorn and broiler chickens fed diets containing 75% or 70% peas. In balance experiments conducted by Van Cauwenberghe *et al.* (1995), the addition of a pectinase preparation to pea-based diets showed no significant effects on AMEn values and N-digestibility in young male turkeys and male broilers. Jeroch *et al.* (1995b) conducted a balance trial with broiler chickens to study the influence of dehulling and enzyme supplementation on digestibility and AME content of peas. Enzyme preparations derived from *Penicillium janthinellum* significantly improved the AMEn value. This observation was confirmed by Broz *et al.* (1996), who reported that supplementation with a *T. viride* enzyme complex resulted in an increase in the AME value for peas from 10.39 to 11.08 MJ kg^{-1} dry matter (+ 6.6%). In contrast, Liebert *et al.* (1996) observed no substantial effects of enzyme supplementation when combined with hydrothermic treatments of peas. Supplementation of unpelleted broiler diets containing high levels of yellow, green, and brown peas with pectinase significantly improved weights and feed consumption but feed conversion was not affected (Igbasan and Guenter, 1996). Jeroch and Keller (1997) reported that a combination of α-galactosidase with a multi-enzyme preparation improved the AMEn values of two varieties of peas. However, the addition of either enzyme alone resulted in very limited effects. Igbasan *et al.* (1997) observed that growth rate, feed intake and feed conversion of broiler chickens fed a pea-based diet supplemented with graded levels of pectinase were not significantly affected. However, with a combination of pectinase and α-galactosidase, both growth rate and the AMEn value tended to be improved. The effects of supplementation with either pectinase or α-galactosidase were also evaluated by Daveby *et al.* (1998) in broiler chickens fed experimental diets based on 70% of dehulled peas. Neither enzyme significantly affected chick performance or apparent ileal digestibility of nutrients.

It may be concluded that multi-enzyme preparations or combinations of enzymes seem to exert certain beneficial effects on the metabolizable energy of peas for broiler chickens. However, when used in practical growth trials, supplemental enzymes were unable to affect significantly growth rate or feed conversion, even in experimental diets containing a high proportion of peas.

Lupins

In general, lupins contain very high levels of non-starch polysaccharides (36–40% total NSP), only traces of starch and about 9% galacto-oligosaccharides such as raffinose and stachyose. However, there are still certain discrepancies in the literature regarding the content of water-soluble NSP, ranging from 4.6% (Choct, 1997) to 13.4% (Bach Knudsen, 1997). Current lupin varieties contain low concentrations of anti-nutritive factors such as alkaloids (Annison *et al.*, 1996).

Brenes *et al.* (1993b) reported that the supplementation of lupin-based diets with crude enzyme preparations and their combinations resulted in substantial improvements in their nutritive value for chickens. Roth-Maier and Kirchgessner (1994, 1995) investigated the replacement of soybean meal by higher proportions of lupins in broiler diets and observed that performance of broilers was markedly improved by supplementation with a *T. viride* enzyme complex. According to Bryden *et al.* (1994), supplementation of a lupin-based diet with a β-galactanase improved its AME by 10.7%, but a second enzyme with α-galactosidase acitivity showed no effect. Brenes *et al.* (1993b) also observed only limited effects when α-galactosidase alone was added to the lupin-based diet. These findings suggest that the NSP fraction rather than the oligosaccharides is responsible for limiting the AME value of lupins in poultry. Recently, Annison *et al.* (1996) conducted two balance feeding experiments to assess the potential of two enzyme preparations to improve the nutritive value of dehulled lupin kernels for broiler chickens. The product containing an enzyme complex derived from *Humicola insolens* significantly increased the AME value of lupins and also markedly reduced ileal viscosity (see Table 19.5).

The results of these studies clearly indicate that NSP-hydrolysing enzyme complexes have a potential to improve the nutritive value of peas and lupins for broilers. However, in order to develop more specific enzymes for such plant protein ingredients, additional information is still needed with regard to the composition of the NSP present.

Table 19.5. Effect of an enzyme complex from *Humicola insolens* on the AMEn values of the basal diet and lupin kernel-containing diets, the calculated AME value of the lupin kernel and ileal viscosity in broiler chickens.

Diet	Enzyme (g kg^{-1} feed)	AMEn (MJ kg^{-1} DM)	AME lupin (MJ kg^{-1} DM)	Ileal viscosity (mPa s)
1. Basal	0.00	14.84^{a}	–	3.3^{a}
2. Basal	1.00	15.03^{a}	–	2.4^{a}
3. Lupin	0.00	12.79^{d}	10.01^{a}	9.5^{c}
4. Lupin	0.25	13.14^{c}	11.05^{b}	6.8^{b}
5. Lupin	0.50	13.39^{b}	11.65^{c}	5.2^{ab}
6. Lupin	0.75	13.36^{bc}	11.65^{c}	4.8^{ab}
7. Lupin	1.00	13.31^{bc}	11.50^{bc}	4.4^{ab}

^{a,b,c,d} Values with unlike superscripts differ significantly ($P < 0.05$).
Adapted from Annison *et al.* (1996).

Soybean meal

Soybean meal is traditionally the major vegetable protein source in poultry diets. Depending on the quality, it usually contains 44% or 48% crude protein. Like other grain legumes, the carbohydrate fraction includes only traces of starch, a relatively high amount of galacto-oligosaccharides (raffinose, stachyose and verbascose), free sucrose and a substantial part of non-starch polysaccharides, which are constituents of cell walls. Due to its specific composition, AMEn values of soybean meal for poultry are relatively low. The total content of NSP in soybean meal can vary between 18% and 22%, of which 2–3% are water-soluble NSP and the remainder is insoluble (Bach Knudsen, 1997; Choct, 1997). Anti-nutritive factors present in raw soybeans (e.g. trypsin inhibitors, lectins, saponins) are to a larger extent destroyed during the processing of soybean meal.

Possible improvement in the feeding value of soybean meal for poultry by supplemental enzymes has already been evaluated by some research groups. In this connection, several concepts involving potentially relevant enzymes have been discussed recently. These approaches include the use of either α-galactosidase, β-galactanase or a pectinase complex, β-mannanase, a combination of xylanase and cellulase, or protease.

An experimental effort based on the dietary addition of α-galactosidase, even in combination with invertase, did not meet expectations (Irish *et al.*, 1994, 1995; De Schrijver, 1996a,b). For example, the experimental results of Irish *et al.* (1995) indicated that the removal of up to 90% of the α-galactosides had no beneficial effect on the nutritional value of soybean meal determined in various assays using either broiler chickens or adult White Leghorn roosters. Similarly, De Schrijver (1996a,b) did not find any significant effects of a crude α-galactosidase preparation derived from *A. niger* on the apparent metabolizable energy, N-retention and fat digestibility determined in adult broiler roosters. However, enzyme supplementation appeared to exert a degradative effect on the oligosaccharides in the small intestine, as indicated by the lower concentrations of sucrose, raffinose and stachyose found in its distal part.

It appears that the whole carbohydrate fraction has to be considered as the target. More recently, Marsman *et al.* (1997) evaluated the effects of thermal processing and enzyme treatments on the nutritive value of soybean meal in broiler chickens. Enzyme treatment (a combination of protease and carbohydrase) significantly improved apparent ileal digestibility of crude protein and NSP, although it did not result in better growth performance of birds.

Based on more precise characterization of the structure of various NSP present in soybeans, more specific enzymes capable of degrading these NSP to their constituent sugars might be developed for practical use.

MICROBIAL PHYTASE – CURRENT RESEARCH EFFORTS

Application of microbial phytase to poultry diets represents another important achievement resulting in improved utilization of phytate phosphorus from plant feed ingredients, and also in markedly reduced environmental pollution. The

effectiveness of supplemental phytase in broiler chickens, laying hens and turkeys is well documented and is reviewed in Chapter 20 by Schöner and Hoppe.

At present, only two phytase preparations are available on the market at costs allowing their practical application. Both preparations are produced by fermentation of genetically modified *Aspergillus* strains. The genetic information originates from *A. ficuum*/*niger*.

Both phytases reveal a low intrinsic resistance to heat inactivation and their pelleting stability is very limited. Current research efforts are therefore focused on the isolation and development of new, heat-stable microbial phytases from other microbial sources. Recently, Pasamontes *et al.* (1997) reported the gene cloning, purification and characterization of a heat-stable phytase from the fungus *Aspergillus fumigatus*. This phytase was able to withstand temperatures up to 100°C for a period of 20 min, with a loss of only 10% of the initial enzymatic activity. The enzyme showed high activity at a pH range of 2.5–7.5 and its substrate specificity and *in vivo* efficacy were comparable to the industrial phytases currently used. Its biological efficacy in piglets and growing pigs has been reported by Simoes-Nunes and Guggenbuhl (1998).

Substrate specificity of new phytases has to be also considered as an important parameter in order to increase their *in vivo* efficacy and achieve an almost complete release of phytase phosphorus.

PRACTICAL USE OF FEED ENZYMES IN POULTRY DIETS

Conventionally, feed enzymes have been added to animal feeds in dry powder/granulate form. However, in recent years, the degree of hydrothermal processing (addition of heat and moisture associated with conditioning and pelleting) applied during feed production in many feed mills has been increased. These more aggressive processing conditions are designed to improve the physical quality of the finished product, to increase the range of raw materials that can be used and to improve the hygiene status (e.g. salmonella kill) of the feed (Pickford, 1992; Beumer and van der Poel, 1997; Thomas and van der Poel, 1997).

Feed enzymes are susceptible to the degree of hydrothermal processing (Gadient *et al.*, 1994; Engelen and van der Poel, 1999). Each enzyme product has a specific 'stability curve' and a critical temperature at which enzyme activity is reduced. The curves will differ depending upon the inherent temperature stability of the enzyme and the formulation of the product, some products being able to withstand higher temperatures than others.

Concern has been expressed over the stability of powder enzymes in some feed manufacturing processes based on low analytical recoveries of the added enzyme activities. As a consequence, interest in the use of liquid feed enzymes applied post-pelleting (bypass the hydrothermal treatment) has increased. Many commercial feed enzyme preparations are marketed in both powder and liquid product forms with comparable efficacy, and application equipment for the accurate application of liquid enzymes is readily available to the feed compounder.

The relationship between pelleting temperature, enzyme recovery and bird performance was studied by Gadient *et al.* (1993). In this trial, as pelleting temperature increased above the critical temperature, powder enzyme recovery decreased with a concomitant decrease in subsequent broiler performance (expressed as feed conversion). Bird performance was returned to the level of the feed pelleted below the critical temperature when the enzyme was applied in liquid format after heat treatment.

However, a low analytical recovery of a powder enzyme in a heat-treated feed does not necessarily reflect a significant denaturation of the added enzyme activity (Engelen and van der Poel, 1999; Steen, 1999). The reasons for this discrepancy are not known – suggestions have included incomplete extraction during analysis, due to the enzyme binding to substrates in the feed matrix, or the possibility of 'interfering factors' in the feed. Analysts in a number of organizations are currently working to improve the analytical techniques for the analysis of powdered enzymes in processed feeds.

In addition, there are a number of possible disadvantages with the use of liquid enzymes which must also be considered (Altemuller and Beardsworth, 1999). Liquid enzymes are less stable during storage than powders; liquids are relatively more difficult to handle and add in the feed mill; special application equipment (with a varying degree of sophistication and cost) is required; care is required to ensure accurate addition and adequate mixing through the feed.

Although there are some practical benefits from using dry products, the aggressive feed processing conditions employed in some feed mills would preclude their use. Trial results and field experience have demonstrated that avoiding the conditioning/pelleting process by the use of liquid enzymes applied post-pelleting is a practical alternative where appropriate.

CONCLUSIONS

The use of NSP-hydrolysing enzymes and phytase in poultry nutrition is now well established. Further information is still required on a wider range of feed ingredients and feed processing conditions. Opportunities exist for some additional enzyme activities to improve the nutritive value of vegetable protein sources. There is also an increasing demand for new products with improved thermal stability, particularly phytase.

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CHAPTER 20
The effects of phytase in poultry nutrition

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INTRODUCTION

Three aspects of the feed enzyme phytase will be considered: (i) its effects on the utilization of dietary phosphorus and calcium and on the utilization of the trace elements manganese and zinc will be considered, which represent the classical purposes for using microbial and plant phytases in poultry diets; (ii) recent work indicates intriguing effects of dietary phytase addition on the utilization of protein, amino acids and apparent metabolizable energy; (iii) future developments concerning phytase, namely as a constituent of genetically modified canola.

EFFECTS OF PHYTASE ON MINERAL UTILIZATION

A look at its structure indicates which nutrients will bind to phytic acid and thus be likely to show improved digestibility on addition of dietary phytase (Fig. 20.1). Binding occurs not only with phosphorus, calcium and trace minerals, but also with proteins and carbohydrates. The effects of phytase on these substances depend on the strength of the association and the accessibility of phytase to the substrate. One example of the effect of microbial phytase on the rate of utilization of phosphorus and calcium in broilers (Table 20.1) was shown by Simons *et al*. (1990). These authors used a maize/soybean meal diet, which is generally accepted as being low in native phytase activity but containing ample phytate phosphorus, making it an ideal model diet on which to study the effects of phytase. In this experiment, graded additions of phytase resulted in marked increases in the utilization of phosphorus and calcium. Notably, the effect on calcium was more pronounced than that on phosphorus. Many further experiments have corroborated these results and there is now unequivocal evidence that phytase is a very efficient means of improving mineral utilization in broilers.

 Poultry Feedstuffs: Supply, Composition and Nutritive Value (eds J.M. McNab and K.N. Boorman)

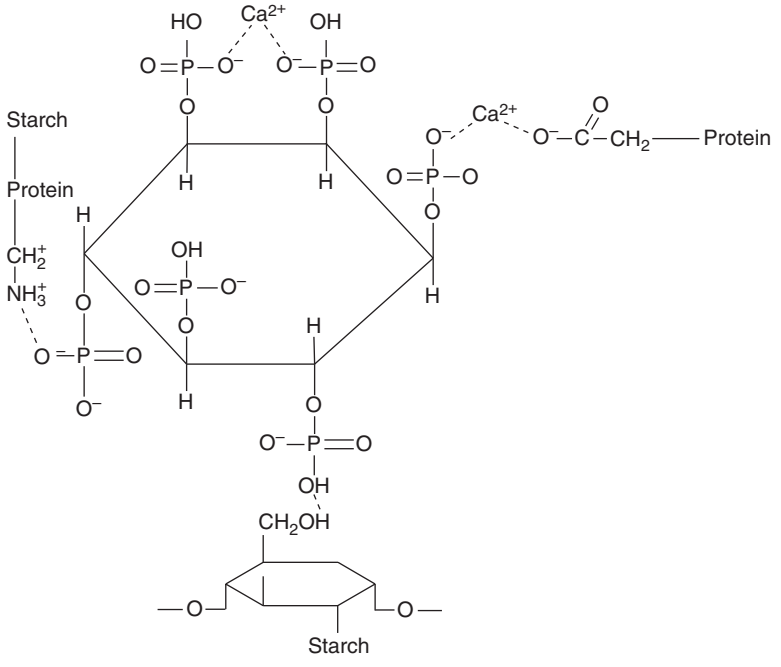

Fig. 20.1. Potential interactions of phytic acid with protein, minerals and starch (Thompson, 1988).

Table 20.1. Effect of added microbial phytase on the utilization of phosphorus and calcium in broilers (Simons *et al.*, 1990).

Phytase added (U kg^{-1})	P (%)	Ca (%)
–	49.8[a]	47.2[a]
250	56.5[c]	57.1[b]
500	59.6[cd]	59.3[bc]
1000	62.5[de]	64.3[cd]
1500	64.5[e]	68.1[d]

Maize/soybean meal diet, containing 6 g of calcium and 4.5 g of phosphorus kg^{-1}.

The question arises as to whether the effect of the phytase on calcium retention is a consequence of the calcium being cleaved from the phytate complex (i.e. a direct effect), or an indirect effect accruing from the enhanced phosphorusutilization. This question was addressed in a broiler trial where a maize/soybean meal diet, adequate in total phosphorus (6.0 g kg^{-1}) but deficient in calcium (4.0 g kg^{-1}) was fed as control (Schöner *et al.*, 1994). To this diet were added calcium and phytase, respectively, at graded concentrations of 0.75, 1.50 and 2.25 g kg^{-1} and 250, 500 and 1000 U kg^{-1}. These diets were fed for 21 days when bird performance and the crude ash (XA) contents in the

phalanges of the birds' middle toes ('toe ash') were monitored. The effects on gain are shown in Fig. 20.2. Addition of calcium resulted in significantly improved weights gained by the birds (Fig. 20.2A). Likewise, additions of phytase caused a significant increase in bodyweight, indicating that phytase caused more calcium to be available (Fig. 20.2B). The effects of calcium and phytase additions on toe ash are shown in Fig. 20.3. Both additives resulted in significant increases in toe ash, corroborating the responses seen for body weight gain.

Based on linear equations relating additions of phytase and calcium to weight gain and toe ash, respectively, the calcium equivalency of phytase was calculated (Table 20.2). Using live weight gain and toe ash, respectively, as criteria, 500 U of phytase were equivalent to 0.35 and 0.45 g of calcium. Thus,

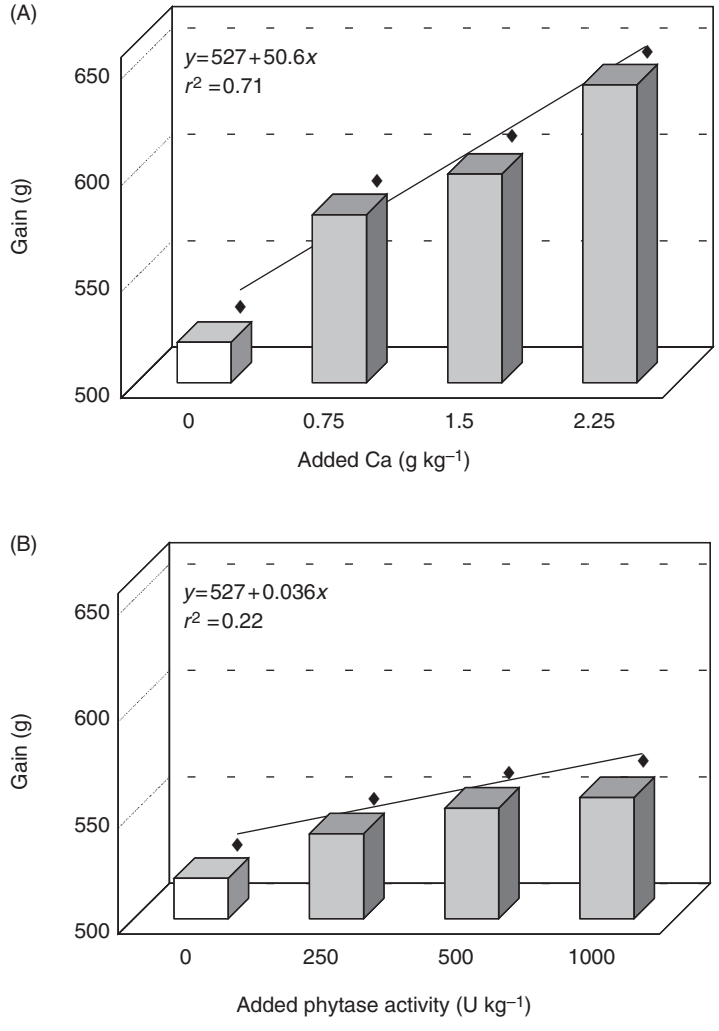

Fig. 20.2. Effects of dietary additions of Ca and phytase on liveweight gain of broilers (21 days) (Schöner *et al.*, 1994).

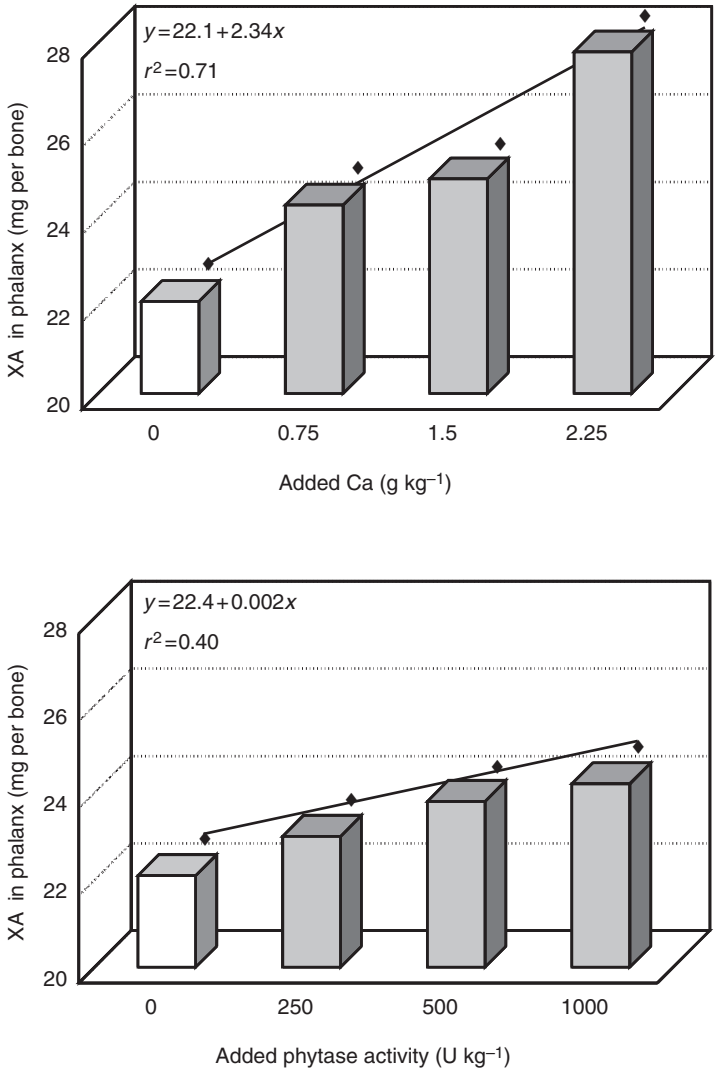

Fig. 20.3. Effects of dietary additions of Ca and phytase on crude ash in toe ash of broilers (Schöner *et al.*, 1994).

the amount of calcium liberated from the phytate complex was rather small. We infer from this result that the marked effects of phytase on the utilization of dietary calcium are probably caused by an indirect effect accruing from the increase in phosphorus utilization.

Improved trace mineral utilization by broilers resulting from the dietary addition of microbial phytase has been reported from many laboratories. For example, an experiment carried out by Thiel and Weigand (1992) is shown in Table 20.3. These authors used two maize/soybean meal diets containing 27 or 45 mg kg^{-1} zinc with or without phytase addition (800 U kg^{-1}). While body weight was not affected sig-

Table 20.2. Regression equations for calculating the calcium equivalency of phytase.

Liveweight gain (g)	$y = 527$	$+ 0.0356\ x_1$	$r^2 = 0.22$
	$y = 527$	$+ 50.6\ x_2$	$r^2 = 0.71$
Toe ash (mg)	$y = 22.4$	$+ 0.00202\ x_1$	$r^2 = 0.40$
	$y = 22.1$	$+ 2.34\ x_2$	$r^2 = 0.71$
Criteria	*Equivalency*		
Liveweight gain	500 U phytase = 0.35 g calcium		
Crude ash of Phalanx I	500 U phytase = 0.45 g calcium		

x_1, phytase activity added (U kg^{-1}); x_2, calcium added (g kg^{-1}).

Table 20.3. Effect of phytase on the utilization of zinc in broilers at 14 days of age.

Feed			Zinc	
Zinc (mg kg^{-1})	Phytase (U kg^{-1})	Liveweight (g)	Retention (mg)	Utilization (%)
27	–	293	3.3	34[b]
45	–	287	4.2	27[c]
27	800	300	3.8	38[a]
45	800	306	4.9	28[c]

(Thiel and Weigand, 1992.)

nificantly, utilization of zinc was improved by phytase at lower dietary zinc concentrations. However, no significance difference was found for zinc retention.

In a further experiment in broilers, Mohanna and Nys (1999) investigated the effect of microbial phytase on the zinc and manganese in the tibia ash of broilers. The diet was adequate in calcium (10.1 g kg^{-1}) and available phosphorus (4.3 g kg^{-1}) and contained 34 mg kg^{-1} zinc and 31 mg kg^{-1} manganese. Addition of phytase resulted in significant increases in the zinc and manganese in the tibia by ≈ 40% and 73%, respectively (Table 20.4).

At the present time the benefit from adding microbial phytase to poultry diets on the utilization of minerals and trace elements is undisputed, because many studies have shown consistent effects with differing diets. Thus, it is widely accepted that microbial phytase is an effective means of improving the economics of broiler production. The use of phytase has become accepted all over the world, especially in countries with intensive animal production on limited acreage, where reduction in phosphorus output from animal manure is of ecological concern. Phytase contributes to alleviating excessive P output.

EFFECTS OF PHYTASE ON DIGESTIBILITY

Recent research has indicated that phytase not only improves mineral and trace element utilization, but may also enhance the protein digestibility and energy utilization. First indications for such effects were found by Schutte and Kies (1995), who fed diets with adequate phosphorus (0.67%) or with reduced phosphorus (0.56%)

Table 20.4. Effects of microbial phytase on zinc and manganese in tibia ash of broilers.

Phytase (U kg^{-1})	Zinc (mg kg^{-1})	Manganese (mg kg^{-1})
0	73[a]	1.66[a]
1200	103[b]	2.88[b]

(Mohanna and Nys, 1999.)

supplemented with phytase (500 U kg^{-1}) to broilers at 6–27 days in three independent trials. The results (Table 20.5) showed improvements in feed conversion rate in the individual trials, but these were not statistically significant. Across all three trials, however, the effect was significant, improving the feed conversion rate by 1.5%.

These results created a great deal of interest in the potential of phytase to improve protein and amino acid digestibility. They also stimulated researchers to investigate in detail the effects of phytase on apparent ileal digestibility in broilers. Ravindran *et al.* (1999) used a complex commercial diet containing several types of grain. The results are shown in Table 20.6. Improvements were

Table 20.5. Effects of dietary phytase on broiler performance (6–27 days of age).

Trial no.	Total P (%)	Phytase (U kg^{-1})	Weight gain (g)	FCR
1	0.67	–	1.135	1.523
	0.56	500	1.120	1.506
2	0.67	–	1.057	1.553
	0.56	500	1.067	1.529
3	0.67	–	0.989	1.589
	0.56	500	1.011	1.559
Mean*	0.67	–	1.060	1.555[a]
Mean*	0.56	500	1.066	1.532[b] (+ 1.5%)

*Across trials.
(Schutte and Kies, 1995.)

Table 20.6. Effects of phytase on apparent ileal digestibility (%) and calculated change in digestible crude protein and amino acids content in the feed.

	Digestibility (%)		
	No phytase	400 U kg^{-1} phytase	Change (g kg^{-1})
Crude protein	81.5	83.9	5.14
Lysine	84.5	86.2	0.2
Methionine	92.7	92.5	−0.01
Threonine	73.4	75.2	0.14
Isoleucine	80.4	81.6	0.11

(Ravindran *et al.*, 1999.)

found for the apparent ileal digestibility of crude protein, lysine, threonine and isoleucine, while there was no effect on methionine. These results were used to calculate the improvement in the ileal digestibility of amino acids and crude protein, respectively (Table 20.6).

The same authors also investigated whether the level of dietary phytate P affects the effects of phytase on ileal digestibility of nitrogen and lysine. They used diets with 2.9 g kg^{-1} (normal), 3.7 and 4.4 g kg^{-1} phytate P, respectively, each supplemented with 0, 400 and 800 U kg^{-1} phytase. As shown in Table 20.7, numerical increases in nitrogen digestibility due to phytase addition were seen at all dietary phytate levels. Likewise, ileal digestibility of lysine was enhanced numerically by phytase addition at all dietary phytate levels. Thus, it appears that the effect of phytase on ileal digestibility of nitrogen and lysine did not depend on the amount of phytate P in the diet.

The same approach was used by Yi *et al.* (1996) in a 20-day study with turkey poults. A corn/soybean meal diet with 22.8% crude protein (= 80% of NRC requirement, 1994), a ratio of calcium : non-phytate phosphorus of 2, and 12 g kg^{-1} calcium was used. As shown in Table 20.8, body weight gain and feed conversion ratio were improved numerically. However, ileal digestibility of nitrogen, methionine, lysine and threonine improved significantly as did N-retention.

A further study was carried out by Kornegay *et al.* (1999), who investigated apparent ileal digestibility of crude protein, lysine, methionine, cysteine and threonine. Compared with the control diet without phytase, 450 U kg^{-1} phytase resulted in increases for crude protein and all investigated amino acids (Table 20.9). The effect on apparent ileal digestibility of crude protein was similar to that found by Ravindran *et al.* (1999), as seen in Table 20.6.

The effects of phytase on apparent ileal digestibility of nitrogen and amino acids cannot fully explain the improvement in feed conversion ratio. Therefore, efforts have also been made to look at the utilization of feed energy. Ledoux (1999) investigated the effects of phytase on nitrogen digestibility and apparent metabolizable energy in turkey poults, using a maize/soybean meal diet sufficient in all nutrients including calcium (1.2%) and total phosphorus (0.92%).

Table 20.7. Effects of phytase on ileal digestibility (%) of nitrogen and lysine in broilers as a function of dietary phytate-P.

		Phytate-P (g kg^{-1})		
Nutrient	Phytase (U kg^{-1})	2.9	3.7	4.4
Nitrogen	0	81.5	81.0	79.3
	400	83.9	82.2	80.9
	800	84.0	82.5	81.0
Lysine	0	84.5	85.0	82.3
	400	86.2	86.0	83.5
	800	85.2	85.9	83.6

(Ravindran *et al.*, 1999.)

Table 20.8. Performance, apparent ileal digestibility of dietary nitrogen and essential amino acids and N-retention in turkey poults.

		Phytase		
Criteria		None	750 U kg^{-1}	
Gain	g	441[a]	471[a]	(+ 6.7%)
Feed intake	g	555[a]	554[a]	
Feed : gain	g g^{-1}	1.256[a]	1.172[a]	(+ 6.7%)
Apparent ileal digestibility				
Nitrogen	%	88.8[a]	91.5[b]	
Methionine		94.1[a]	95.6[b]	
Lysine		92.4[a]	94.4[b]	
Threonine		86.4[a]	89.5[b]	
N-retention	%	58.5[a]	67.8[b]	

(Yi *et al.*, 1996.)

Table 20.9. Effects of phytase on apparent ileal digestibility and calculated change in digestible crude protein and amino acids content in the feed.

	Digestibility (%)		
	No phytase	450 U kg^{-1} phytase	Change (g kg^{-1})
Crude protein	76.4	79.3	4.0
Lysine	80.7	82.8	0.16
Methionine	82.0	83.8	2.01
Cysteine	71.8	76.2	2.01
Threonine	70.1	73.6	3.85

(Kornegay *et al.*, 1999.)

As shown in Table 20.10, nitrogen digestibility was improved by 600 U kg^{-1} phytase, and there was a significant improvement of AME by 2.9%. This indicates that an improved utilization of dietary energy may contribute to the positive effect of phytase on broiler productivity.

A new source of phytase that may become available in future is called PhytaSeed. This is a seed product from canola that carries the identical phytase gene as that in the currently used microorganisms, *Aspergillus niger* (Natuphos). In the canola product, phytase is contained in the plant matrix, whereas it is present in a freely soluble form in the Natuphos formulation. The question arises whether phytase in PhytaSeed has the same efficacy as Natuphos. This was investigated in broilers by Kornegay and Denbow (unpublished). Both sources of phytase were compared at three activity levels (250, 500 and 2500 U kg^{-1}). As shown in Fig. 20.4, there was no difference between the phytase sources in body weight gain. Likewise, a comparison of regression equations for toe ash showed no difference between the sources (data not shown).

Table 20.10. Effects of phytase on nitrogen digestibility and AME in turkey poults.

Phytase (U kg^{-1})	N (%)	AME (MJ kg^{-1})
0	86.9^{b}	12.31^{b}
200	87.3^{ab}	12.30^{b}
400	87.0^{b}	12.48^{ab}
600	88.3^{a}	12.67^{a} (+ 2.9%)

(Ledoux and Firman, 1999.)

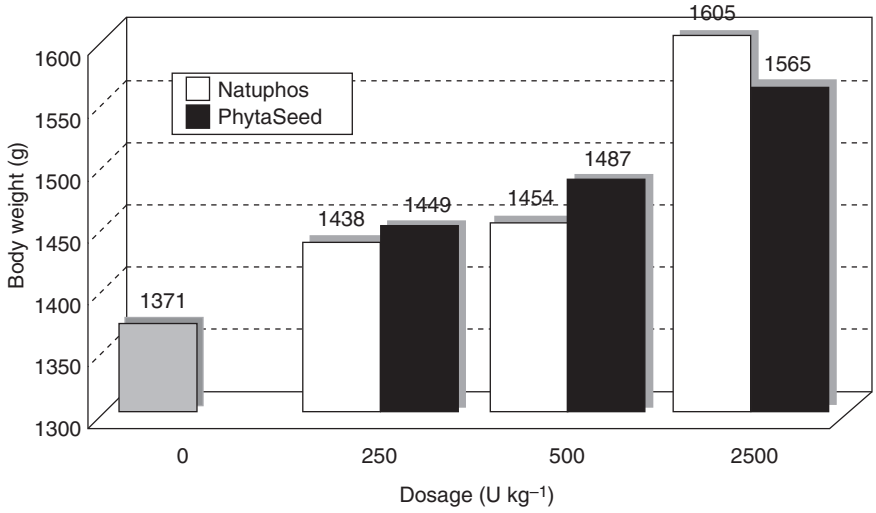

Fig. 20.4. Effect of phytase sources on body weight of broilers (day 35) (Kornegay and Denbow, 1997).

In a similar experiment in turkey poults, Ledoux *et al.* (unpublished) added graded levels of phytase activity (250, 500 or 2500 U kg^{-1}) to the diet and measured tibia ash (Fig. 20.5). At the lowest dose, both phytase sources resulted in a similar increase in tibia ash and a dose–response was found up to 2500 U kg^{-1}. Again there were no significant differences between sources of phytase activity, indicating that the novel source PhytaSeed has the same biological effect as phytase from Natuphos.

CONCLUSIONS

Plant phytate should not only be seen as a potential source of phosphorus, the utilization of which is improved by phytase, but as an anti-nutritional factor that has negative effects on the utilization of nutrients other than minerals and trace minerals, namely, crude protein, amino acids and apparent metabolizable energy.

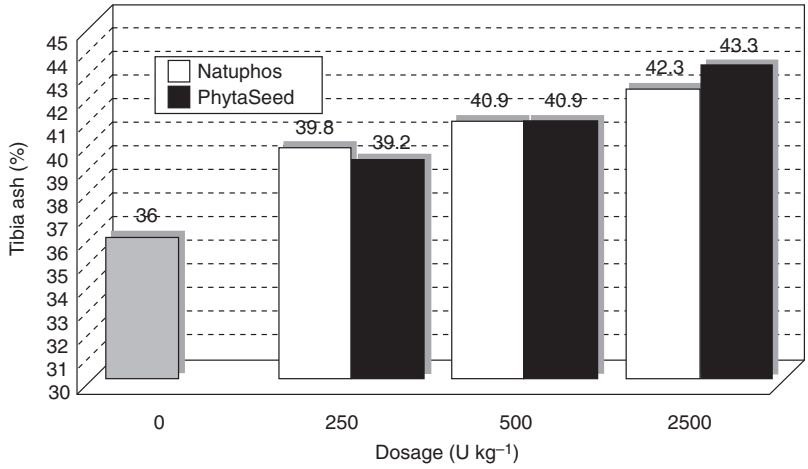

Fig. 20.5. Influence of phytase source on tibia ash in turkey poults (Ledoux *et al.*, 1997).

While phytase effects on the utilization of minerals and trace minerals have been well established, indications for the potential effects on protein and energy utilization have emerged only very recently. In order to evaluate these effects and make use of them in poultry production, further studies are needed.

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CHAPTER 21
The scientific challenges ahead

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INTRODUCTION

All species of commercially produced poultry are now reared far more efficiently than they were even 25 years ago. In Western Europe, as well as in many other places in the world, the gradual introduction of sophisticated production systems, which are capable of strictly controlling the environment and the incidence of infectious diseases, have greatly contributed to this progress. This has meant that poultry now use less energy in movement and temperature regulation and there are far fewer losses from infection and predation. However, the dramatic increase in the volume and efficiency of poultry production has largely been brought about by the application of the sciences of genetics and nutrition. Although the precise proportion of the improvement which can be attributed to each discipline is frequently the subject of heated debate, it is generally acknowledged that the implementation of selective breeding programmes should be credited with the lion's share. Informed opinion appears to be confident that, while the pace of advancement may be slower in the years ahead, genetic changes will continue to enhance both performance and production efficiency. Geneticists are now expected to turn their attention from weight gain to food conversion efficiency and it is believed that ratios of 1.35 g of food g^{-1} of gain are achievable within the next 5 years. Nutrition too has played a key role in the progress of poultry to its present position as one of the most efficient converters of plant materials into high quality animal protein, much sought after by consumers worldwide.

FEEDSTUFFS

In all locations, food represents the major cost of the production of poultry and eggs (somewhere between 65% and 75%) and any improvements in performance that can be related to the diet inevitably have a profound effect on profitability. It is not surprising, therefore, that nutrition in general, and foodstuffs in particular, has played an important part in most national research programmes. This seems likely to continue despite the view expressed in some quarters that the science of nutrition has reached a plateau in terms of effecting economic returns. The principal function of feedstuffs is to provide the poultry farmer with

 Poultry Feedstuffs: Supply, Composition and Nutritive Value (eds J.M. McNab and K.N. Boorman)

cost-effective diets that meet the nutrient requirements of the particular type of stock being fed. To fulfil this demanding role, there will be a continuing need to have knowledge of the dose–response relationships for the major (cost-sensitive) nutrients in relation to different types of output (eggs, meat, progeny) and detailed information on the ability of different raw materials to supply these nutrients. Consequently, compositional analyses and availability assessments are likely to continue to feature strongly as research topics. In the immediate future, it may be unrealistic to aspire to describing raw materials in terms of available nutrients because, with the exception of some vitamins (Whitehead, Chapter 11), no appropriate methods are under development. However, expressing these in terms of digestibility coefficients may be a reasonable compromise; their application to amino acids and some elements is becoming relatively common and encouraging confidence in the formulation of diets containing feedstuffs that are less well digested than maize, wheat, soybean and fish meals. In this context, it is noteworthy that the digestibilities of several important amino acids in a number of by-products are turning out to be higher than had originally been imagined (NRC, 1994), and the consequences of their dietary incorporation may not be as negative as has often been portrayed. This rather sweeping conclusion, surprisingly, does not generally concur with much of the data presented at this meeting (Choct, Chapter 13; Parsons, Chapter 8; Wiseman, Chapter 9), and suggests that care still needs to be exercised when including unconventional ingredients in poultry diets, particularly if their planned concentration is high and the diets are intended for young birds. Future work should focus on establishing reliable data banks of values for as wide a range of raw materials as possible across appropriate ranges of ages and species. Such data will be invaluable in informing not only future plant breeding programmes but the processing conditions required to optimize the nutritional value of by-products. McCracken (Chapter 16) provided many examples where the processing conditions were largely determined by gut-feel rather than as the result of scientific experiment. The current preoccupation with feed biosecurity appears to be driving the temperatures at which diets are conditioned higher and higher, and the times they are held in the conditioner longer and longer, scant regard being paid to the consequences of this action on nutrient stability.

In future studies involving responses to nutrient intakes, it is becoming increasingly more widely accepted that there is a need to look beyond the usual criteria of weight gain and food conversion efficiency to body composition and yield of product (for example, breast meat). A further step would be the consideration of well-being or health. Whether genotype–nutrition interactions merit study is worthy of greater debate. These have certainly not been compelling components of government-funded research programmes during the last two decades. Whether this has happened because the work is seen as not being non-competitive but benefiting particular commercial breeding companies, or because the view that the nutrient requirements of poultry have not been affected by the genotypic changes introduced, has prevailed, is unclear. It would, however, seem prudent to establish the nutrient requirements of new genotypes directly to ensure that the desired performance criteria are being maximized by current dietary specifications. Whether this apparent omission

from recent research programmes is responsible for the increasing and costly incidence of metabolic problems, such as ascites, sudden-death syndrome and tibial dyschondroplasia must probably await their mechanistic elucidation. There is good reason for believing that there is a, as yet unidentified, nutritional factor involved in all these conditions. It seems possible that, particularly as far as the broiler is concerned, the continuing drive for weight gain may have directed finite nutritional resources towards protein and fat accretion at the cost of skeletal growth, cardiovascular development and immune function. In the coming years, research programmes will arguably have to address these issues to protect the welfare of future poultry populations. However, to allow both sides of the argument to be aired, it must be pointed out that there appear to have been few if any changes to the amino acid requirements of broilers between 1977 and 1994, a conclusion which has tended to be supported by results from recent experiments in our laboratory. This would imply that diets with different concentrations of nutrients are not necessarily needed for changed genotypes. Caution is advised, however, as this inference may not apply to all classes of nutrients, and other recent work on breast meat yield suggests that economically valuable returns can be achieved in response to higher inputs of methionine.

Like the UK and EC, most countries seem to avoid setting policies that define priorities for research on feedstuffs. Some general trends can be identified, however. For obvious reasons, there is a trend towards the provision and use of: (i) domestically grown/produced high-protein plant foods (e.g. beans, peas, lupins and rapeseed); (ii) by-products from the human food and beverage industries (e.g. wheat feed, maize germ, distillers' and brewers' wastes) and recycling (particularly from human food preparation). A specific priority could also be to reduce the dependency of poultry on the storage carbohydrates of plants, principally cereal starch, by developing technologies that would allow them to make greater use of structural carbohydrates. Another untapped and plentiful source of energy is chitin, the second (to cellulose) most abundant, naturally occurring organic substance. The application of ascorbic acid to the depolymerization of polysaccharides (Fry, personal communication, 1999) is a potentially exciting, innovative approach to what has proved, at least up to now, an intractable problem, namely the release of the energy locked up in the cell wall complex of plants to monogastric animals. If successful, all these initiatives would have a greater impact in the countries of the Third World where grains, which are used primarily as an energy source for the human population, are almost always in short supply for inclusion in diets for animals.

Although increased imports, given that they are available and affordable, will probably always be the preferred option even in the Third World, it is desirable that databases containing the nutrient compositions of locally available ingredients should be established. Only by taking such a positive approach will new crops, in the form of improved varieties (e.g. sorghum in East Africa, jack beans in Mexico and pond weed in Bangladesh), be developed and exploited, thereby improving the small-scale farmers' production and profitability, encouraging the more widespread availability of poultry products and the expansion of the poultry industry.

ENZYMES

Although they have featured strongly at this Symposium (Bedford, Chapter 17; Broz and Beardsworth, Chapter 19; Cowan *et al.*, Chapter 18) no summarizing crystal ball gazer could omit expressing his views on the future role of dietary enzymes. Without doubt, at least as far as the UK is concerned, they were the nutritional success story of the 1990s. Eight years ago their inclusion in diets was seen as a curiosity, whereas currently almost all broiler diets and many of those for laying hens and turkeys contain enzymes. The addition of β-glucanase has allowed barley to be used again with confidence as a major ingredient in broiler diets and xylanase/pentosanase is now almost a standard component of wheat-based diets in many parts of the world. Although the mechanism of the action of these carbohydrases has still to be unequivocally elucidated, strong empirical evidence has been provided to show that their dietary inclusion results in lower water consumption, less sticky droppings, much improved litter conditions and, consequently, improved bird welfare and carcass quality. Carbohydrases have, however, been relatively unsuccessful in releasing more energy to the bird and this should be a clear objective of future programmes.

The enzyme phytase, which liberates phosphorus from phytic acid, thereby making what was previously unavailable phosphorus to the animal absorbable and metabolizable, has had a striking impact on the formulae of diets for both pigs and poultry in Belgium and the Netherlands. It has been estimated that in the last 10 years the amount of phosphorus excreted by pigs in the Netherlands has been reduced by over 50% as a result of the inclusion of phytase in their diets. It is almost certain that this practice will spread to other nutrients and countries. Additional benefits resulting from the elimination of phytic acid are being realized (release of chelated elements) and these and the future prospects for phytases have already been fully discussed (Coon *et al.*, Chapter 10; Schöner and Hoppe, Chapter 20). At the moment, much industrial thinking is concerned with improving the stability of phytases, which are notoriously heat-labile, whereas that of the academic community is preoccupied with understanding the barriers to the more complete release of phosphorus from phytic acid. Expressing heat-stable phytases in food plants or, arguably better, in the chicken and pig might be ambitious targets of future research programmes.

It seems probable that enzymes capable of degrading other anti-nutritive factors (lignins, tannins, lectins, alkaloids and saponins), one or more of which are invariably present in plant feedstuffs, could also become commercially available, and these will offer an alternative strategy to genetic modification as a means of raising nutritive value. In this context a thioglucoside capable of breaking down glucosinolate in rapeseed has been bred for use in diets for growing pigs. Clearly, the chemical characterization of the anti-nutritive factors in feedstuffs and of their mode of action will be essential to allow the appropriate exogenous enzymes to be identified and developed. At the moment, there seems to be some doubt over the extent of their toxic properties and we have heard of the potential benefits from consuming phyto-oestrogens (Smithard, Chapter 14), a class of compounds the consumption of which I had always associated with the induction of behavioural problems in poultry.

ENVIRONMENTAL FACTORS

Historically, because the supply of many nutrients in the feed has not been particularly cost-sensitive, there has been a tendency to formulate diets with excessive margins of safety, particularly if doubts existed on nutrient availability or if the requirement was uncertain. This rather prodigal policy appears to have stifled research initiatives on some topics (Coon *et al.*, Chapter 10) and is coming under increasing criticism on environmental grounds, because nutrients for which the birds have no need are inevitably excreted and ultimately may become a source of pollution. This is particularly relevant for phosphorus and protein, the permitted manurial contents of which are under legislative control in some countries. Excretion of nutrients in excess of what is required by the birds to ensure optimal performance, or from feeds which have been badly digested, can also cause husbandry and welfare problems. For example, the incidence of carcass defects, such as breast blisters or hock burn, are often attributed to poorly digested fats or to high excretion rates of uric acid, arising from excesses of dietary protein or from dietary proteins with imbalanced (and, hence, poorly available) amino acid profiles.

There is, therefore, a continuing need for research directed at understanding the barriers to complete digestion (particularly of fat and protein) of the kind described by Fry (personal communication, 1999). Chemical structures (e.g. cellulose, retrograde starch), location of nutrients in the feedstuff (e.g. aleurone cell protein behind an indigestible carbohydrate barrier) and interactions with anti-nutritive factors (e.g. tannins, lignins, phytic acid and trypsin inhibitors) are all likely to decrease digestive efficiency and, thereby, increase the excretion of compounds which may end up polluting the environment. In this context, it is probably worth noting that the development of gene transfer by plant molecular biologists (Dean, Chapter 1; Snape, personal communication, 1999) – a practice which is currently the subject of a heated public debate in the UK – could increase the likelihood of previously absent anti-nutritional factors being introduced into potential feedstuffs either adventitiously or deliberately, because of the protective properties these components often confer on plants against possible predators. As was stressed by Snape (personal communication, 1999), collaboration between plant geneticists and animal nutritionists is essential at the later stages of any new plant food's development to ensure its suitability (nutritional quality) and safety to the target species.

GENERAL NUTRITION

Better information on requirements, the gradual but steady increase in the commercial accessibility of synthetic amino acids and more reliable data on amino acid digestibility coefficients are allowing the compounder much better control over protein nutrition. Possible synergy between amino acids of the sort described by MacLeod (Chapter 12) needs further investigation before any advantages can be exploited. More work is probably required to enable the ideal amino acid balance for broilers to be defined in all circumstances; particular emphasis might be placed on clarifying the optimal dietary arginine:lysine ratio, which has been shown to widen as the ambient temperature rises.

There is also much more data on the energy values of raw materials, mainly because of the truly vast amount of work which has resulted from the introduction of rapid methods for the derivation of metabolizable energy, where the debate about the relative merits of the different methods rumbles on. Whether the length of time the birds are exposed to the diet ('conditioning'), as speculated by Duke (Chapter 7), explains differences between apparent and true metabolizable energy values must unfortunately await results from further studies. MacLeod (Chapter 12) drew attention to the fact that there had been an astonishing 301 papers published on the topic of metabolizable energy since 1994. Meanwhile, net energy, a theoretically more sound evaluation system on which to base the energy values of both diets and raw materials, still lurks unfulfilled in the background. Its introduction could result in an improvement in the way fats are described where, despite the assertion of Wiseman (Chapter 9) that fat digestion is adequately understood, I believe there is a clear need by the industry to have a more robust method for assessing their dietary values, especially where younger birds are concerned. At a strategic level, there appears to be a lack of knowledge on the precise role of bile salts in fat digestion and whether the extent of their expression controls the degree to which fats are digested by young birds. Their possible removal from the bird's digestive tract by certain microflora of the hind gut is a topic that has started to receive some research attention (Langhout, 1998) but merits much more. The role of the microflora in digestion is poorly understood yet impinges on a number of issues raised at this Symposium, such as the mechanism of exogenous enzyme action, understanding barriers to digestion and consequently the best way to measure digestibility coefficients.

There is also a need to study the metabolism of relatively unusual sugars, such as xylose, arabinose and galacturonic acid, to allow any nutritional opportunities to be exploited. Although these monosaccharides would appear as a result of cell wall degradation, an important objective proposed earlier here for future dietary enzyme (carbohydrase) addition, there is still considerble uncertainty as to how effectively they are utilized by birds. There is a need to investigate the mechanisms controlling their release, absorption and metabolism, especially any unexpected interactive effects.

More work is required to understand whether the bird has a requirement or preference for particular physical characteristics in its food (Picard *et al.*, Chapter 15), particularly when it is very young. The apparent enigma contained in the results of feeding wet diets was highlighted (McCracken, Chapter 16). Despite the beneficial responses reported, it will prove very difficult to maintain a sufficiently high standard of hygiene to satisfy good husbandry procedures as well as the requirements of regulatory bodies. The apparently popular practice of feeding whole grain (wheat) to broilers alongside their compounded feed, and maintaining performance of the birds but reducing their feed costs, needs to be carefully evaluated. It implies that the composition of the carefully compounded diet is not ideal. The reported outcome, albeit anecdotal, also seems to conflict with the widely held view that controlled heat treatment of feed improves its digestibility (McCracken, Chapter 16). This typifies a common problem in animal nutrition and referred to several times at this

symposium (McCracken, Chapter 16; Parsons, Chapter 8), namely, how to reconcile frequently spectacular improvements in nutritive value of feedstuffs reported from metabolic-type trials with the more modest responses normally found in performance experiments. It may, however, simply mean that we are being careless with our nomenclature as strongly argued by Rosen (personal communication, 1999).

There will also probably be benefits to be gained from continuing research directed at improving the prediction of whole animal responses to nutrients by mathematical modelling. Changes in the environment (e.g. global warming; extensification) can readily be accommodated in these models. New technologies may be required to be developed and collaboration with highly skilled resource centres sought (nuclear magnetic resonance imaging; doubly labelled water techniques).

The compound feed industry continues to seek out methods that can be used to determine nutrient values for the wide range of raw materials at its disposal. These methods must be robust, repeatable and, preferably, rapid. Whether physicochemical techniques, such as infra-red reflectance spectroscopy, will provide this information remains a matter of speculation. However, in order to secure the full confidence of the commercial and research communities, there will have to be a move away from the present empirical to a more logical system based on sound chemical principles. *In vitro* techniques will probably continue to be explored, if only for reasons of speed and ethical constraints on the use of bioassays.

On the more strategic front, the increasing role of nutrients in regulating metabolic control mechanisms is attracting considerable interest and is likely to assume greater importance in future research programmes. Vitamins such as pyridoxine, biotin and vitamin D, respectively, play key roles in triggering steroid hormone production, biotin-binding protein release and bone cell differentiation. Fatty acids have also been shown to affect oestrogen/progesterone responses that impact on egg size. The immune system seems to respond to vitamins A, D and E, to methionine and to mannose. Nutrients may also play a role in the mediation of stress. Vitamin C has always been associated with alleviating the effects of heat stress and recent studies suggest it may play a role in reducing fear and distress. At high environmental temperatures, dietary supplementation with vitamin E has also recently been shown to ameliorate the anticipated drop in egg production, and additional dietary sodium bicarbonate has been reported to result in improved egg quality.

The banning of the inclusion of mammalian by-products from poultry diets in the UK has stimulated debate on the long-term consequences for performance. Changed monovalent cation (Na + K − Cl) balances and increased consumption of phyto-oestrogens (from the higher dietary inclusion rates of soybean meal) are two of the more obvious consequences of this policy that may affect bird performance. However, it was reassuring to learn that dietary phyto-oestrogens are more likely to impart benefits to the stock being fed than induce negative responses (Smithard, Chapter 14). Another ban likely to impact on efficiency is that recently applied to certain growth promoters in

Europe. It has been estimated that, for every million birds produced, the current use of growth promoters saves almost 100 t of feed. At the same time, less manure needs to be disposed of. The consequences of this decision on prices and profitability have still to be fully appreciated but it seems inevitable that it will stimulate research into so-called natural alternatives.

CONCLUSION

Despite these exciting new avenues of research for the next generation of poultry nutritionists, two areas are likely to preoccupy policy makers and scientists in the new millennium: the provision of an adequate supply of foodstuffs to allow the poultry industry to continue to grow and, thereby, supply the anticipated increasing world demand for all type of poultry products. It is an exacting challenge which, I'm sure, will be accepted and, with a little biotechnological help, overcome.

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PART VI
Poster abstracts

POSTER 1

Variation in lysine excretion among birds in the precision-fed rooster bioassay for digestible amino acids

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High variability was noted for the lysine content of endogenous excreta samples in conducting the precision-fed rooster bioassay for digestible amino acids. In unfed birds, the coefficient of variation for lysine excretion was 4–5 times higher than that for most other amino acids. Subsequently, in an attempt to document the extent of variability and delineate the factors causing the variation in lysine excretion, a detailed study of the endogenous amino acid excretion in birds was conducted according to a modification of the protocol of the Sibbald TME precision-fed rooster bioassay. The modifications included the use of a 28-h precollection starvation period and the provision, by gavage, of 25 g glucose to the birds during the precollection period. Type of maintenance diet fed to the birds, precision feeding of a non-nitrogen source of energy (25 g of a 90:10 glucose/canola oil mixture), caecetomy vs. intact status and age of birds were factors that did not markedly influence the degree of variability noted in lysine excretion. In addition, methodology did not appear to be a contributing factor as assessed by interlaboratory determinations of amino acid contents of excreta samples and critical appraisal of sample chromatograms. Lysine excretion appeared to be very consistent, as groups of birds ($n=15$), categorized according to individual lysine excretion and remeasured at several time periods, consistently showed significantly ($P<0.001$) different values for lysine excretion. Furthermore, little or no differences were noted for other amino acids, except for histidine and arginine. Lysine excretion values (mg per 48 h) for birds categorized as normal-lysine or high-lysine groups were 34 and 169, respectively, whereas comparable values for other amino acids (i.e. glutamic acid, leucine) were 89 vs. 91 and 39 vs. 39. Histidine and arginine excretion values for normal-lysine or high-lysine groups were 36 vs. 71 and 34 vs. 48, respectively. The extent to which this variability exists among birds precision-fed various feedstuffs or diets has not been determined at this time. Further study is warranted to determine the implications regarding the precision and accuracy of the amino acid digestibility bioassay. This is of particular concern given that lysine is a very critical amino acid for the determination of amino acid requirements based on the ideal protein concept.

 Poultry Feedstuffs: Supply, Composition and Nutritive Value (eds J.M. McNab and K.N. Boorman)

POSTER 2
The influence of phytase on amino acid digestibility in broiler diets

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The influence of phytase on amino acid availability has previously been studied in a balance trial and was increased through enzyme addition. A feeding trial was set up in order to determine whether diets formulated with reduced amino acid levels but with adequate phosphorus would benefit from phytase addition.

Broiler diets were formulated to be sufficient in phosphorus (0.35% available P) and to contain low, normal or elevated levels of lysine (0.98%, 1.20% and 1.42%, respectively) and sulphur amino acids (methionine + cystine 0.75%, 0.89% and 1.03%, respectively). The three diets were fed with and without a supplementation of phytase at 500 FYT kg^{-1} and performance monitored over 42 days.

The results of the performance trial for the male and female birds are presented in Tables P2.1 and P2.2. Addition of phytase to diets received by the male birds increased growth at all levels of amino acid supplementation and tended to reduce food conversion ratio. For the female birds, the phytase effect was most pronounced in the low amino acid diet, with little effect being shown at the normal and elevated amino acid levels.

It is concluded that when the phytase is added to diets sufficient in phosphorus but deficient in amino acids, it can improve performance by increasing the utilization of dietary protein. There was a difference in response to phytase between male and female birds, which needs to be investigated further.

Table P2.1. Performance of male birds.

Treatment	Growth (g day^{-1})	Food intake (g day^{-1})	FCR 0–42 days
Low AA	42.97	83.5	1.942
Low AA + phytase	44.09	85.81	1.946
Normal AA	50.77	94.05	1.852
Normal AA + phytase	51.23	93.6	1.827
Elevated AA	51.14	91.74	1.794
Elevated AA + phytase	52.69	93.79	1.780

Table P2.2. Performance of female birds.

Treatment	Growth (g day^{-1})	Food intake (g day^{-1})	FCR 0–42 days
Low AA	40.46	80.38	1.987
Low AA + phytase	41.18	82.48	2.003
Normal AA	44.77	84.6	1.889
Normal AA + phytase	44.73	84.78	1.895
Elevated AA	45.85	84.07	1.883
Elevated AA + phytase	46.04	85.41	1.885

POSTER 3
Protein and fat deposition in carcass and organs of young broiler chicks: response to energy and amino acid intake

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Daily intakes of energy and amino acids determine growth and body consumption of broiler chickens. For an accurate estimate of the requirements for energy and amino acids, knowledge is required about the growth response of the birds towards different intakes of both nutrients.

A dose–response trial was used to investigate the distribution of amino acids and energy between carcass and organs in terms of protein and fat deposition. Fifty-four male broiler chicks (10 days old) were assigned to one of 18 treatments: two energy intakes combined with nine amino acid concentrations, expressed as amounts per day. This implies that the animals were feed-restricted. The energy levels were 2.0 and 2.5 times maintenance, expressed as protein-free ME. The amino acid contents were 0.92, 1.04, 1.16, 1.22, 1.28, 1.34, 1.40, 1.52 and 1.64 g digestible lysine per MJ protein-free ME. All essential amino acids were supplied at an overdose of 15% compared with lysine. The birds were individually housed, slaughtered at a body weight of 800 g and divided into carcass and organs. The abdominal fat pad was added to the carcass fraction. Both fractions were analysed for protein and fat content.

Protein deposition increased linearly with an increase in amino acid intake, (Fig. P3.1). Describing this relationship with a linear model, the intercept and slope of this model were similar at the two energy intakes. This means that protein deposition is independent of energy intake. These effects on protein and fat deposition mean that, at this age, amino acid intake stimulates lean growth. The intake of energy and amino acids did not affect the distribution of these nutrients between carcass and organs. This similar response of carcass and organs was confirmed by the fact that, at 800 g body weight, the ratio of organs : carcass was independent of lysine intake.

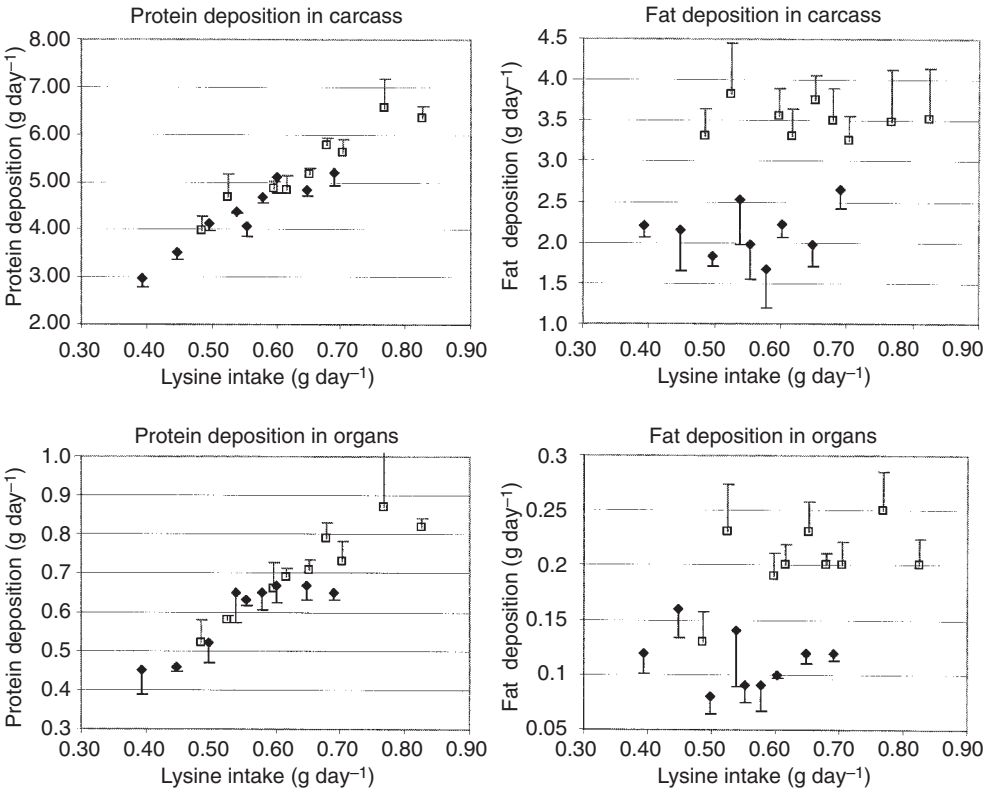

Fig. P3.1. Protein and fat deposition (g day^{-1}) in carcass and organs of broilers; □ = 2.5 × maintenance, ◆ = 2.0 × maintenance.

POSTER 4
Effect of the exclusion of synthetic amino acids and potentially GMO-containing protein sources in vegetable layer diets

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Various restrictions on protein sources are under discussion or are already used in food formulation, mainly in the context of labelling and in ecological programmes. These limitations are often related to the exclusion of animal protein sources, genetically modified components (GMO) and/or synthetic amino acids (SAA). Because of the existing ban of animal proteins from most Swiss layer diets, the aim of this experiment was to determine the effect of an additional exclusion of SAA, possible GMO-protein sources (soybean, maize, gluten) and their combination in vegetable diets for laying hens.

From week 21 to week 64, four vegetable diets were formulated to 11.5 MJ ME and 0.41% methionine per kg feed. In comparison to the control diet (diet A), soybean and maize gluten, as possible GMO-protein sources, were excluded from diet B, and SAA from diet C. Exclusion of both protein sources was combined in diet D. The diets (fed as crumbs) and water were available *ad libitum* during the whole trial. A flock of 1800 white laying hens (H&N) was divided into 10 groups of 180 animals. The hens were kept in a floor system. Diets A and B were fed to three groups while diets C and D were given to two groups each. Egg production was recorded daily and egg weight weekly. Food consumption and mortality were determined every 4 weeks. Faeces were collected in week 46 in order to analyse nitrogen content and to calculate nitrogen excretion by the indicator method (4 M HCl-insoluble ash as indicator).

Formulating diets without possible GMO-components resulted in a small reduction in egg production (−1.2%), whereas food consumption and the food conversion ratio increased by 3.4 and 4.9%, respectively. Due to the exclusion of SAA, crude protein content had to be increased from 18% in diets A and B to 19% in diets C and D (base: 0.41% methionine). Without dietary SAA, an increase in mortality from 2.7% to 4.4% was observed. Egg production improved by 1.9%, whereas egg weight was significantly lowered by 1.5%. Nitrogen content of faeces (+ 13.4% and 11.8%, respectively) as well as daily nitrogen excretion per hen (+ 10.6% and 6.8%, respectively) were higher for diets C and D.

It can be concluded from this trial that further limitation of available protein sources for vegetable layer diets may be followed by considerable effects on productivity as well as on ecology. Therefore, these aspects should also be considered in future discussions about additional restrictions on protein sources.

Diet	A	B	C	D	Significance		
GMO-Protein sources (GMO)	+	–	+	–	GMO	SAA	GMO
Synthetic amino acids (SAA)	+	+	–	–			X SAA
Egg production – % (per hen per day)	86.1	85.3	88.0	86.7	n.s	n.s	n.s
Egg weight (g)	62.8	62.2	61.6	61.6	+	*	+
Food consumption (g day^{-1})	120.3	123.9	120.1	124.6	n.s	n.s	n.s
(g per egg)	139.8	145.5	136.5	143.8	n.s	n.s	n.s
Food conversion rate (kg kg^{-1} egg mass)	2.23	2.34	2.22	2.33	+	n.s	n.s
Mortality total (%)	3.3	2.0	4.2	4.5	n.s	+	n.s
N-content in faeces (g kg^{-1} DM)	52.4	51.1	59.4	58.6	n.s	*	n.s
N-excretion (g day^{-1} + hen)	2.07	2.06	2.29	2.21	n.s	n.s	n.s

* $P \leq 0.05$, + $P \leq 0.10$, n.s., not significant.

POSTER 5
Relative egg yolk pigmenting efficiency of apo-ester versus marigold xanthophylls

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To achieve the consumer's requirement for a golden-yellow yolk colour, it is essential to have a good yellow base as the foundation for the addition of red carotenoids. A good yellow base would be equivalent to 7–8 on the Roche Yolk Colour Fan (RYCF). A poor yellow base can result in 'off' colours that are unacceptable to the consumer. There are two main types of supplementary yellow carotenoids (xanthophylls) commonly used for egg yolk pigmentation: apo-ester and lutein/zeaxanthin. In recent years, Roche has performed several trials with laying hens to confirm the relative efficiency of apo-ester versus lutein/zeaxanthin from marigold products.

Bird (1996) compared apo-ester (in powder form) and marigold xanthophylls (in liquid form) across a wide range of inclusions. The results demonstrated that the deposition rate of apo-ester is approximately three times that of lutein and zeaxanthin in liquid marigold products.

This result was confirmed by Kluenter (1998) in two different experiments using a range of dietary inclusions of apo-ester and marigold xanthophylls. In the first experiment, apo-ester efficiency was compared with that of a powder marigold product (containing 92% lutein and 8% zeaxanthin). In the second experiment, a liquid marigold product (containing 70% lutein and 30% zeaxanthin) was used. The results of both trials indicated that three times the level of apo-ester as lutein/zeaxanthin in the feed was required to achieve similar yolk colours and carotenoid content in the yolk.

The aim of the present trial was to compare apo-ester with two different marigold products (M1 and M2). The dose rates for the M1 and M2 xanthophylls ranged from 3 to 9 ppm, the corresponding levels of apo-ester from 1 to 3 ppm. Yolk colour became more golden-yellow with increasing dosages of the three products. However, at corresponding feed levels of apo-ester and marigold xanthophylls (ratio of 1:3), RYCF values were higher with apo-ester.

To optimize yolk colour, it is essential to have a good yellow base. The use of marigold xanthophylls in layer diets can result in comparable RYCF scores to apo-ester as long as the feed inclusion rate is at least three times

that of the apo-ester. In conclusion, the relative yolk pigmenting efficiency of apo-ester : marigold xanthophylls is at least 3 : 1, regardless of the physical characteristics of the marigold products (liquid or powder) and their lutein/zeaxanthin ratio.

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POSTER 6
Regression analysis to assess the bioefficacy of different methionine sources in broiler chickens

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In several studies on the bioefficacy of liquid D,L-methionine hydroxy analogue (MHA-FA) in broilers, a biological effectiveness of 65% of that of D,L-methionine (DLM) was demonstrated. However, there is an ongoing discussion on this topic as well as on the correct statistical approach to evaluate the bioefficacy of essential nutrients. In the present study, the biological efficacy of liquid MHA-FA was compared with that of DLM in male broilers from 5 to 33 days of age. It was also examined whether regression analysis is the proper tool for determining the biological efficacy of different nutrient sources. Therefore, four graded inclusions of DLM and MHA-FA (0.04%, 0.08%, 0.12%, and 0.16%) were each added to a basal maize–soy diet containing only 0.60% of met-Cys but adequate in all other nutrients and energy. In four additional treatments, DLM, which was diluted in glucose to a methionine content of 65%, was added at the same supplementation levels as pure DLM. The four supplemental concentrations of each of DLM, MHA-FA and diluted DLM were confirmed by analysis. A total of 1170 male Cobb broilers were allotted to the 13 treatments in six replicate groups of 15, i.e. 90 birds per treatment. The birds were housed in battery cages. Pelleted feed and water were supplied for *ad libitum* consumption. Weight gain and feed conversion ratio (FCR) were chosen as performance criteria. The birds responded significantly to the supplements, i.e. bioefficacy was tested in a sensitive range. Regression analysis revealed that the responses in weight gain (Fig. P6.1) and FCR to liquid MHA-FA and diluted DLM (65%) were very similar at corresponding supplement concentrations. Diluted DLM can be regarded as an internal standard to check the validity of regression analysis as well as the bioefficacy of MHA-FA. In order to reach the same performance level, liquid MHA-FA was only 63% (weight gain) and 60% (FCR) as efficacious as D,L-methionine. The bioefficacy of diluted DLM for weight gain and the FCR were 60% and 65%, respectively, relative to DLM. Regression analysis was therefore confirmed to be a statistically valid tool for determining the relative efficacy of nutrient sources. In conclusion, the present study confirmed the outcome of previous experiments showing the bioefficacy of MHA-FA is 65% of that of DLM.

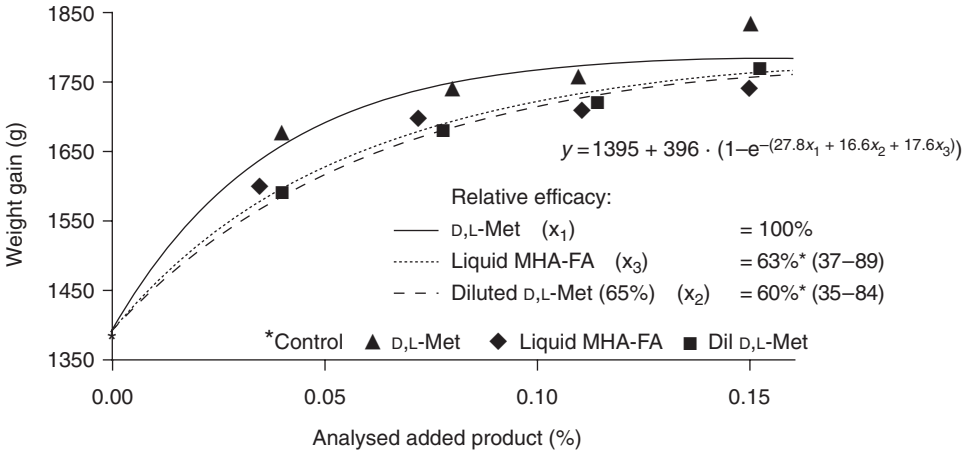

Fig. P6.1. Bioefficacy of diluted D,L-methionine (65%) and liquid MHA-FA as compared to pure D,L-methionine in broiler chicks.* Significantly lower than D,L-Met ($P < 0.05$).

POSTER 7
The ME_N value of wheat cultivars in relation to their chemical and physical parameters

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Wheat is one of the main feed ingredients in many countries around the world. Its nutritional value in terms of ME_N can be highly variable, with reported values ranging from 10.4 to 15.9 MJ kg^{-1}. The reported variation in nutritional value is due to many factors related to the genetics of wheat cultivars and to growing conditions, which affect chemical composition and physical characteristics (e.g. viscosity). The aim of this research was to relate the ME_N value of wheat cultivars to both chemical and physical parameters. The 15 cultivars under study were analysed for their main Weende components (protein, fat, starch, sugar) and both NSP and pentosan (total and soluble, respectively), and for physical characteristics in terms of extract-viscosity (EV) and potential and real applied viscosity (PAV and RAV). The balance was carried out according to the European Reference Method (with broilers of 14–25 days of age; wheat was included at 50%). The ME_N contents were determined for all experimental diets, without correcting for endogenous secretions or metabolic losses. The ME_N value of the wheat cultivars was calculated by difference. Regression analysis was used to relate ME_N to chemical and physical parameters.

There was appreciable variation related to all tested parameters: e.g. the ME_N content of the 15 cultivars varied from 12.6 to 14.1 MJ kg^{-1} DM (a range of 11.1% or 1.5 MJ kg^{-1}). ME_N was negatively correlated with EV and RAV, but positively correlated with starch, fat and sugar (as %).

ME_N (MJ kg^{-1}) = 0.0055* protein + 1.24* fat + 0.148* + 0.297*sugar (R^2 = 0.99; SE = 0.39; P <0.01)
ME_N (MJ kg^{-1}) = 0.107*protein + 0.65*fat + 0.163*starch + 0.18*sugar − 0.45*EV (R^2 = 0.99; SE = 0.25; P<0.01)

ME_N (MJ kg^{-1}) = 0.042*protein + 1.033*fat + 0.154*starch + 0.32*sugar − 0.31*RAV (R^2 = 0.99; SE = 0.36; P<0.01)
ME_N (MJ kg^{-1}) = 7.634 + 0.0886*starch (R^2 = 0.33; SE = 0.37; P<0.01)
ME_N (MJ kg^{-1}) = 14.38 – 0.432*EV (R^2 = 0.38; SE = 0.37; P<0.01)
ME_N (MJ kg^{-1}) = 13.95 – 0.126*PAV (R^2 = 0.07; SE 0.45; P = 0.33)
ME_N (MJ kg^{-1}) = 14.76 – 0.618*RAV (R^2 = 0.57; SE = 0.30; P<0.01)
ME_N (MJ kg^{-1}) = 8.16 + 0.095*starch – 0.463*EV (R^2 = 0.78; SE = 0.21; P<0.01)
ME_N (MJ kg^{-1}) = 11.07+0.0519*starch – 0.494*RAV (R^2 = 0.62; SE = 0.27; P<0.01)

The ME_N predictability from the four main nutrients was quite good. The introduction of the physical parameter slightly improved the accuracy of the regression model. The ME_N predictability from the one parameter was also quite good; in this respect, RAV was a better parameter than both EV and starch. The accuracy of the starch-based regression model was clearly enhanced after introduction of a physical parameter. The accuracy of 'predictability' in terms of SE as a % of mean ME_N varied from 1.6% to 3.3%.

POSTER 8
Effects of method and level of inclusion of feed on performance of broilers given a low-AME wheat diet

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In another study (Preston *et al*., Poster 9, this volume, pp. 400–401) positive effects of feed enzyme addition were demonstrated, although the enzyme activity appeared to have been totally destroyed by the heat-conditioning regime employed. This study was undertaken to examine the effects of different methods and different levels of enzyme addition on the apparent metabolizable energy (AME) content of diets and their effects on broiler performance. The wheat was the same as that used by Preston *et al.* (2000) and the diets contained (g kg^{-1}), wheat 744, casein 142, blended fat 50, minerals etc. 64. Seven diet treatments were used. These were: A, heat-treated (HT), control (no enzyme); B, HT plus enzyme (1 g kg^{-1}); C, HT plus enzyme (2 g kg^{-1}); D, HT plus enzyme (5 g kg^{-1}); E, cold pelleted (1 g kg^{-1}); F, HT, liquid enzyme (1 g kg^{-1}); G, HT, liquid enzyme (0.5 g kg^{-1}). All diets were pelleted (3 mm die). Fifty-six male Ross broiler chicks were randomized with weight blocks to the seven dietary treatments in individual cages and given the diets *ad libitum* from 7 to 28 days. A total collection of excreta was made from days 14 to 21 for determination of the apparent metabolizable energy (AME) contents of the diets. Birds were killed at 28 days and samples of proximal ileal digesta used for the measurement of *in vivo* viscosity. Analysis of the diets for xylanase activity showed expected levels in diets E, F and G. Levels in diets B and C were negligible but diet D (5 g kg^{-1}) showed approximately 10% recovery and, hence, comparable activity to diet G. DM intake was not significantly affected by treatment but tended not to be lower with the cold-pelleted diet (E) which was of poor quality (table).

There were marked diet effects ($P<0.001$) on live weight gain (LWG) and gain:food which were least with diet A and greatest with diet D. ME:GE followed the same pattern ($P<0.001$) as gain:food. Viscosity was greatest with the control diet (A) and diet (B) and fell from 27.2 to 13.5 with increased enzyme

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Table P8.1. Effect of method and level of enzyme inclusion on performance of broilers ($n = 8$).

Diet	A	B	C	D	E	F	G	*P*	SEM
DM intake (g day^{-1})	73.2	70.9	69.3	69.3	63.5	71.1	69.8	NS	2.76
LWG (g day^{-1})	42.4	44.6	50.4	53.2	44.5	52.3	51.9	<0.0001	1.80
Gain : food	0.59	0.63	0.73	0.77	0.70	0.74	0.75	<0.0001	0.017
ME : GE	0.62	0.71	0.76	0.81	0.74	0.77	0.80	<0.0001	0.027
Viscosity (cps)	30.6	27.2	22.0	13.5	12.4	10.3	13.9	<0.0001	3.24

inclusion in the HT diets. Values were similar for diets D, E, F and G, corroborating the measurements of residual enzyme activity. The results with diets B and C corroborate the observations of Preston *et al.* (2000) that enzyme effects occurred during processing. However, the results with the sprayed liquid enzyme were better than for diets B and C and on a par with diet D, indicating that liquid enzyme is a satisfactory means of treating heat-processed diet. The poor results with the cold-pelleted diet are probably attributable to poor pellet quality leading to increased feed wastage rather than to an enzyme effect *per se*.

POSTER 9
Effects of heat processing on feed enzymes and on diet characteristics and subsequent broiler performance

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The experiment was designed to examine the response of a wheat-based diet to suboptimal levels of xylanase inclusion (Avizyme, Finnfeeds International Ltd). The diets, which were based on (g kg^{-1}) wheat 744, casein 142, blended fat 50, minerals 64, were heat-conditioned in a pilot plant mixer using direct injection of steam and pelleted. The levels of enzyme inclusion were 0, 0.05, 0.1, 0.15, 0.2, 0.3, 0.5, and 1.0 g kg^{-1}, the highest inclusion being that recommended by the manufacturer. Subsequent analysis revealed that no measurable enzyme activity was recoverable in the feeds. Sixty-four, male, Ross broiler chicks were randomized, within weight blocks, to the eight dietary treatments and fed *ad libitum* in individual cages from 7 to 28 days. A total collection of excreta was made from 14 to 21 days for determination of the apparent metabolizable energy (AME) contents of the diets. Birds were killed at 28 days and samples of proximal ileal contents used for measurement of *in vivo* viscosity.

Dry matter (DM) intake and liveweight gain (LWG) were not significantly affected by treatment (Table P9.1) but gain:food improved ($P<0.05$) from 0.65 without enzyme to 0.78 with 1 g kg^{-1} addition. Similarly, ME:GE increased ($P<0.05$, linear trend) from 0.69 to 0.78. However, viscosity of the ileal supernatant was high, averaging 45 cps, and unaffected by treatment. Further examination of the diets (total and soluble NSP) and measurements of ileal digestibility using TiO_2 marker have still to be completed.

The results for xylanase activity in the diets and the *in vivo* values demonstrate that the form of heat-conditioning applied had destroyed the enzyme prior to feeding. However, the marked improvements in gain:food and ME:GE, with the 1 g kg^{-1} inclusion of enzyme, suggest that changes in the nutritive value of diet occurred during the heating/pelleting/cooling stage.

Table P9.1. Effect of level of enyme inclusion (g kg^{-1}) on performance of broilers (n = 8).

	0	0.05	0.1	0.15	0.2	0.3	0.5	1.0	*P*	SEM
DM intake (g day^{-1})	79.7	77.2	89.3	78.8	79.2	80.1	78.8	71.5	NS	36.1
LWG (g day^{-1})	51.6	51.5	58.3	54.2	54.5	51.7	55.5	55.5	NS	20.7
Gain : food	0.65	0.67	0.67	0.69	0.69	0.65	0.71	0.78	0.022	0.025
ME : GE	0.69	0.72	0.68	0.74	0.74	0.71	0.73	0.78	0.04(L)	0.028
Viscosity (cps)	47.0	45.3	41.4	41.7	58.4	50.9	40.6	38.9	NS	8.45

POSTER 10
Is development of pecking damage in layer pullets influenced by dietary protein source?

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Modern layer diets rely heavily on plant protein sources, particularly soybean meal. However, anecdotal reports from egg producers suggest that inclusion of animal protein (especially fish meal) can be effective in preventing or halting outbreaks of damaging pecking, a persistent welfare and economic problem. The notion that animal protein is beneficial was accepted by the Farm Animal Welfare Council in its 1997 Report on the Welfare of Laying Hens – 'We recommend further research work to identify and quantify the factors in animal protein responsible for reducing injurious behaviour in laying hens'. Currently, there is no experimental evidence that the presence of fish meal does reduce damaging pecking, and the alternative possibility of detrimental factors in plant protein sources has been ignored. The aim of this experiment, therefore, was to determine if dietary protein source has any effect on development of feather pecking and cannibalism in layer pullets. Day-old ISA pullets were randomly assigned to 'animal' or 'plant' protein treatments, in six penned groups of 12 birds per treatment. Birds were fed starter (0–6 weeks), grower (7–16 weeks) and layer (17–24 weeks) diets based on either fish meal or soybean meal according to treatment. Vitamins, minerals and the amino acid tryptophan (shown previously to influence pecking behaviour) were equalized. Pecking damage was first seen at 6 weeks of age, but no severe pecking occurred until 18 weeks, after which three groups (one animal, two plant) were removed from the experiment because of severe damage. Information from systematic observations of pecking behaviour was divided into weeks 1–4, 5–8, 9–12, 13–16, 17–20 and 21–24. The only significant effect of dietary treatment was in weeks 13–16, when numbers of (non-aggressive) vigorous pecks were higher ($P<0.05$, by *t*-test) in plant protein groups. This increased pecking was not reflected by increased damage scores, which did not differ significantly between treatments in any time period. Also, there was no significant effect of dietary treatment on plasma concentrations of triglyceride and zinc (which increased when ovarian steroid secretion com-

menced at 16 weeks), or on egg production. Although these results do not provide convincing evidence supporting the notion that fish meal in layer diets suppresses pecking damage, there was a trend for higher numbers of vigorous, potentially damaging pecks in plant protein groups throughout the experiment. When applied on a commercial scale, such a trend could account for the perceived worsening of pecking problems in layer flocks associated with increased use of plant-based diets.

POSTER 11
Nutritive values of certain agroforestry products for poultry

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Several agroforestry products have great potential as feedstuffs for livestock and poultry, but are not yet exploited by nutritionists. In order to fulfil the great demand for feedstuffs by the ever-growing feed industry, it is imperative to assess their suitability for poultry and livestock feeds.

The chemical composition and the toxic compounds present in kapok (*Ceiba pentandra*) seeds and oil extracted meal (KS and KSOM), subabul (*Leucaena leucocephala*) seeds and leaves (SS and SL) and tamarind (*Tamarindus indica*) seeds, roasted and decorticated seeds (kernels) as well as the seed testa (TS, TK and ST) were assayed and the values are reported in Tables P11.1 and P11.2. Three biological experiments were conducted to study the nutritive values of differently processed KSOM, SS and TK for commercial broiler chicks from 0 to 6 weeks of age. Based on these experiments, it was concluded that KSOM could be safely used in broiler feeds up to 9%, without any further processing, replacing sunflower meal w/w.

SS could be incorporated in broiler feeds as a protein supplement up to 5%, without any processing. However, after germination, ferrous sulphate treatment or yeast fermentation, SS could be used up to 10%. TK could be used in broiler starter and finisher feeds as an energy and protein source up to 20% without any further processing.

All these agroforestry feedstuffs reduced feed cost considerably without affecting the performance of the broilers.

REFERENCE

Bolton, W. and Blair, R. (1974) *Poultry Nutrition, Bulletin 174*. Her Majesty's Stationery Office, London.

Table P11.1. Proximate composition and A.I.A. (acid insoluble ash) content of kapok, subabul and tamarind seeds and their products.

Ingredient	No. of samples	% Composition ± SE (dry matter basis)						
		Dry matter	Crude protein	Either extract	Crude fibre	Total ash	N – free extract	Acid insol. ash
Kapok seeds – KS	12	90.8 ± 0.55	28.2 ± 1.21	22.6 ± 1.19	25.2 ± 0.82	8.2 ± 0.51	15.8 ± 1.82	4.72 ± 0.78
Kapok seed oil cake – KSOM	12	91.8 ± 0.47	32.4 ± 0.87	9.7 ± 0.82	28.9 ± 0.28	9.4 ± 0.54	19.6 ± 0.82	5.3 ± 0.62
Subabul seeds – SS	12	90.7 ± 0.57	30.1 ± 0.55	4.7 ± 0.59	14.3 ± 0.77	4.9 ± 0.24	46.0 ± 1.38	0.51 ± 0.18
Subabul leaves – SL	12	68.9 ± 1.21	25.3 ± 0.46	2.4 ± 0.36	19.6 ± 1.02	9.1 ± 0.42	43.6 ± 1.13	0.42 ± 0.21
Tamarind seeds – TS	12	90.0 ± 0.92	14.3 ± 0.49	5.6 ± 0.44	20.3 ± 0.76	4.0 ± 0.15	55.8 ± 1.16	0.62 ± 0.31
Tamarind kernels – TK	10	89.6 ± 0.46	18.5 ± 1.15	7.2 ± 0.32	9.1 ± 1.95	2.9 ± 0.29	62.3 ± 2.22	0.48 ± 0.22
Tamarind seed testa – TST	10	89.5 ± 0.25	9.0 ± 0.52	0.0	32.4 ± 0.95	5.2 ± 0.26	53.4 ± 0.58	0.68 ± 0.28

Table P11.2. Some nutrients and toxic principles present in kapok, subabul and tamarind seeds and their products.

Component	Kapok seed	Kapok seed oil cake	Subabul seed	Tamarind seed	Tamarind kernel	Tamarind seed husk
Starch (%)	6.96	8.10	12.06	15.44	43.87	8.80
Sugar (%)	4.50	5.20	3.30	7.24	10.83	0.63
Available carbohydrates (%)	11.06	12.83	15.06	22.03	53.73	9.37
ME* (MJ kg^{-1})	14.5	10.7	9.1	8.2	14.8	3.4
Calcium (%)	0.33	0.38	0.36	0.29	0.33	0.50
Total phosphorus (%)	0.93	1.1	0.41	0.36	0.22	0.35
Cyclopropenoid fatty acids (%)	2.80	1.04	–	–	–	–
Tannins (%)	1.31	1.50	1.55	5.1	2.8	13.3
Mimosine (%)	–	–	1.58	–	–	–
Saponin (%)	–	–	0.47	–	–	–
Trypsin inhibitor activity (units kg^{-1})	7.8	ND	11.6	12.2	ND	ND
Aflatoxin – B1 (ppB)	ND	ND	ND	ND	ND	ND

ND, not determined.
*Calculated according to Bolton and Blair (1974), mean of two samples.

POSTER 12
Amino acid contents of common poultry ingredients of Western India

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The poultry industry is well developed in western India (Gujarat, Maharastra and Rajasthan states). The amino acid profiles of the major feed ingredients are presented here. The ingredients were collected from the field and composite samples were prepared for analysis. The analysis was carried out at Degussa AG, Hanau, Germany. The amino acid profiles are adjusted to 88% dry matter and presented in tabular form.

Maize available in the field has a lower crude protein content than the value usually reported. Maize is a major energy source; however, millets (sorghum and pearl millet) are equally used throughout the year and all are similar in amino acid profile. Rice polish and deoiled rice bran are two by-products of the rice industry and are the cheapest sources of protein and amino acids.

Groundnut industry by-products, namely extraction and expeller cakes, are poor in methionine, containing around 1% of crude protein compared to 2% in other vegetable protein sources, but rich in arginine. Although meat meal (a by-product of the leather industry produced from leather scrap and processed to contain not more than 10% moisture, 6% crude fibre and 2.75% chromium) has very high crude protein content, its digestibility is very low for amino acids compared with fish meal (Shyam Sunder *et al.*, 1998).

REFERENCE

Shyam Sunder, G., Sadagopan, V.R., Rama Rao, S.V. and Reddy, M.R. (1998) Available lysine as a criterion for evaluating the feeding value of commercial meat meals. In: *Abstracts of the 10th European Poultry Conference*, Jerusalem, Israel, Vol. 1, pp. 476–478.

Table P12.1.

		DM (%)	CP (g kg^{-1})	Met (g kg^{-1})	M+C (g kg^{-1})	Lys (g kg^{-1})	Thr (g kg^{-1})	Trp (g kg^{-1})	Arg (g kg^{-1})
Cereals	Maize	88	80.00	1.50	3.20	2.50	2.80	0.60	3.90
	Sorghum/Jowar	88	95.00	1.60	3.30	2.10	3.10	1.10	3.50
	Pearl millet/Bajara	88	76.00	1.70	3.10	2.90	2.90	0.40	3.70
Cereal by-products	Rice/polishings	88	124.00	2.50	5.00	5.60	4.50	1.40	9.30
	Deoiled rice bran	88	154.00	2.90	5.70	6.20	5.50	1.90	11.10
Plant proteins	Groundnut meal, extract	88	380.00	4.00	9.00	13.10	9.90	3.80	40.00
	Groundnut meal, expel	88	313.00	3.10	7.00	10.80	8.20	3.20	33.10
	Sunflower meal	88	272.50	5.80	10.20	9.70	9.70	3.60	21.30
	Rapeseed meal	88	310.00	5.50	13.00	13.60	12.50	4.40	19.80
	Soybean meal	88	463.00	6.00	12.60	27.50	17.50	5.60	33.50
Animal proteins	Fish meal	91	435.00	9.20	12.90	22.30	14.00	3.40	20.80
	Meat meal/leather meal	91	729.00	5.20	6.30	21.80	11.80	0.30	48.90

POSTER 13
Influence of polyenzymes on energy and amino acid utilization from Indian feedstuffs by broilers

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The nutritive value of feedstuffs used for Indian poultry is limited by the high content of non-starch polysaccharides (NSP) and the poor digestibility of crude protein (CP) and amino acids. However, because of population growth and food shortage in general, the nutritionist has to use such ingredients. Their nutritive value may be improved by enzyme addition. A feeding experiment with commercial HubChick broilers was therefore conducted using polyenzymes (xylanase 950 IU, pectinase 2100 IU, cellulase 100 IU, α-galactosidase 80 IU, amylase 1200 IU, protease 12,000 IU and phytase 135 IU g^{-1}) in broiler feed from day 1 to 42.

A total of 1200 broilers was randomly distributed into four treatment combinations with three replications of equal sex ratio. They were kept on deep litter. All the birds were offered a starter diet containing 11.25 MJ kg^{-1} ME and 210 g kg^{-1} CP (days 1–21) and a finisher containing 11.55 MJ kg^{-1} ME and 195 g kg^{-1} CP (days 22–42). Treatment E1 included polyenzymes (1 g kg^{-1} of feed) with E_0 as control. Digestible L-lysine and D,L-methionine amino acids were reduced by 5% in diet 2 (D2) compared with diet 1 (D1). The amino acid content of the diets was adjusted using synthetic amino acids. The ingredients used for D1 were maize, rice polish (rice bran), soybean meal, deoiled rapeseed meal, deoiled sunflower meal, fish meal and meat meal, while D2 contained deoiled groundnut meal in addition to the ingredients listed above.

The effect of the polyenzyme on two separate diets showed (Table P13.1) improvement in economic performance.

A two-way analysis of variance was carried out (Table P13.2) and revealed that inclusion of polyenzymes in treatment E_1 significantly improved food conversion ratio and profit margin, by reducing food intake.

Treatment E_1 reduced food cost per kg body weight gain by about Rs 0.28 (i.e. 4.4% increase in profit) over E_0. A reduction in digestible amino acids in diet D_2 caused poorer performance than D_1. The interactions between enzyme and diet effects were found to be non-significant for all the parameters.

It seems that the polyenzymes used increased the availability of nutrients from ingredients with high concentrations of NSPs such as rice polish, rapeseed meal, sunflower meal and groundnut meal. Detailed assessment of NSP utilization through metabolic trial is in progress.

Table P13.1. Performance of broilers under various treatments at 6 weeks of age.

	Enzyme treatment	BW/body weight (kg)	Food consumption	Food conversion ratio	Rs kg^{-1} BW	
					Feed cost	Profit
Diet 1	E_0	1.468	3.225	2.177	12.454	6.546
	E_1	1.444	3.036	2.078	12.075	6.925
Diet 2	E_0	1.434	3.221	2.227	12.560	6.440
	E_1	1.388	3.036	2.164	12.373	6.627

Table P13.2. Effect of enzyme and diet on performance at 6 weeks of age.

	Treatments	BW (kg)	FC (kg)	FCR	Rs kg^{-1} BW	
					Feed cost	Profit
Enzyme	E_0	1.451	3.223	2.202	12.507	6.493
	E_1	1.416	3.036	2.121	12.224	6.776
	C.D.*	0.029	0.098	0.024	0.139	0.139
Diet	D_1	1.456	3.130	2.128	12.265	6.735
	D_2	1.411	3.129	2.195	12.466	6.534
	C.D.*	0.029	NS	0.024	0.139	0.139

C.D., critical difference, * $P<0.05$.

POSTER 14
Effect of methods of analysis and heat treatment on viscosity of wheat, barley and oats

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Viscosity of grains has been shown to be negatively correlated with nutritive value. However, no standardized method exists for *in vitro* viscosity measurements. Thus 20 varieties each of wheat, oats and barley, grown on experimental plots in 1997, were analysed for fibre content, physical characteristics and viscosity using different methods.

For analysis of water extract viscosity (WEV), an incubation time of 30 minutes at 40°C in distilled water followed by centrifugation (2596 *g*, 10 min) was shown to be appropriate in order to obtain a stable viscosity value. WEV was also measured after heating the samples for 5 min at 100°C in an autoclave. Acid extract viscosity (AEV) was obtained by incubating the sample in a pH 1.5 HCl/KCl solution for 1 h, followed by centrifugation. *In vitro* digestion viscosity (IDV) was measured according to Bedford and Classen (*Poultry Science* 72, 137–143, 1993). IDV was also analysed on ten samples per grain species after heat treatment, and the dietary fibre content was determined on the same samples. To compare viscosity values obtained using the different methods, the following transformation was performed: transformed viscosity = (In(viscosity of sample in solution / viscosity of solution without sample))/(g sample/ml solution).

For wheat, the correlations between WEV and IDV, AEV and IDV and WEV and AEV were 0.68, 0.86 and 0.85, respectively. For barley, the corresponding correlations were 0.95, 0.80 and 0.69. Only the correlation between WEV and IDV (0.69) was significant for oats.

Table P.14.1. Results (mean ± SD).

	Wheat	Oats	Barley
1000 grain weight (g)	40.4 ± 4.89	32.5	41.6 ± 3.54
hl weight (kg)	84.8 ± 1.76	57.4 ± 2.06	72.6 ± 1.94
Insoluble dietary fibre (%)	10.6 ± 0.86	26.2 ± 6.14	15.6 ± 1.22
Soluble dietary fibre (%)	1.6 ± 0.36	2.8 ± 0.53	4.1 ± 0.57
WEV	2.7 ± 0.61	6.9 ± 1.55	8.7 ± 2.74
WEV after heat treatment	3.5 ± 0.85	18.6 ± 7.52	17.7 ± 6.58
IDV	5.6 ± 0.96	28.7 ± 12.05	27.8 ± 6.51
IDV after heat treatment	6.1 ± 0.89	35.9 ± 8.97	28.9 ± 6.69
AEV	4.1 ± 0.85	37.4 ± 6.07	25.5 ± 6.02

POSTER 15
Effects of inorganic phosphates on the quality of eggs and performance in two strains of laying hen

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One hundred and eighty, 36-week-old laying hens of two strains were fed for 14 weeks on one of three diets containing either dicalcium phosphate (17% P, 25% Ca) or two local phosphates, namely: K3 (12.7% P, 36.8% Ca and 139 ppm V) and K5 (14.4% P, 34.3% Ca and 174 ppm V). Half of the laying hens used in this experiment were obtained from a commercial Isabrown strain and half from an experimental cross of Isabrown males with females from a strain selected for low residual food intake. The pullets were grown to 18 weeks of age under similar conditions and transferred to cages in a naturally ventilated laying house. They were fed on a commercial diet until the start of the experiment. The experimental diets were isoenergetic and isonitrogenous and provided 3.5% Ca and 0.42% P. Hen performance, egg weight, shell quality parameters, egg component yield and interior egg quality were measured in 14 day periods.

Significant dietary effects were observed for overall egg production, egg mass and food conversion efficiency. Significant differences between strains were observed in egg production, feed intake, egg weight, egg mass, food conversion efficiency, shell weight, albumen weight and shell quality measurements. Statistical analysis also revealed significant diet × strain interactions for egg production, egg mass and feed efficiency. Hens from the experimental strain fed on the test phosphate sources gained more weight than their counterparts fed on the dicalcium diet. Efficiency of phosphorus utilization measured as g of P intake per g of eggshell mass showed a significant dietary effect. Storage of eggs for 3 weeks at 4°C revealed a significant dietary effect on albumen quality. The overall Haugh Unit changes observed were smaller with the K3-based diet.

The results suggest that local phosphates may replace dicalcium phosphate satisfactorily without effect on food consumption, egg weight, mortality rates or hen body weights. The response of laying hens to diets containing local phosphates may vary between strains.

POSTER 16
Digestible valine requirement of female Nicholas poults during the period 0–4 weeks post-hatching

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Two experiments were conducted, using, respectively, 168 and 192 female Nicholas poults, to determine the digestible valine requirement during the period 8–21 days post-hatching. In a maize–soy mixture, the essential amino acids were fixed at ratios to lysine, with digestible lysine defined at 100%. The diets were isocaloric and were also made isonitrogenous by varying the concentration of L-glutamic acid. Body weight gain, feed conversion and carcass nitrogen responses to increasing dietary valine concentration were measured. The objective of the first experiment was to obtain a growth curve to permit the design of a further experiment if necessary. In experiment 2, the poults receiving 8.2 g kg^{-1} valine were significantly heavier than those given 7.9 g kg^{-1} or lower concentrations. No significant differences for feed conversion (Fig. P16.1) or carcass nitrogen retention were found in response to valine concentration. The mean growth data subjected to broken line analysis (Fig. P16.2) indicated that the digestible valine requirement for maximum body weight gain was 8.2 g kg^{-1}. However, the requirement for optimum food conversion efficiency was 7.4 g kg^{-1}. It was concluded, therefore, that the dietary digestible valine requirement of starting female Nicholas poults is 8.2 g kg^{-1} in a maize–soy diet with an energy concentration of 13.5 MJ kg^{-1}.

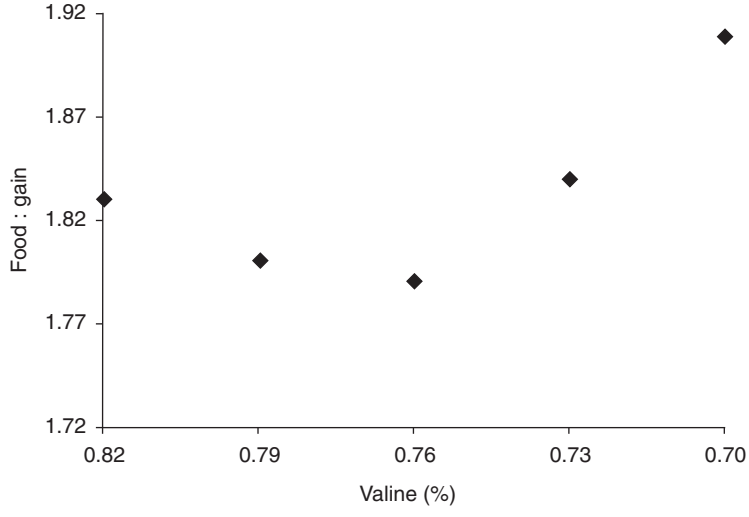

Fig. P16.1. Food : gain ratio of poults fed graded levels of digestible valine.

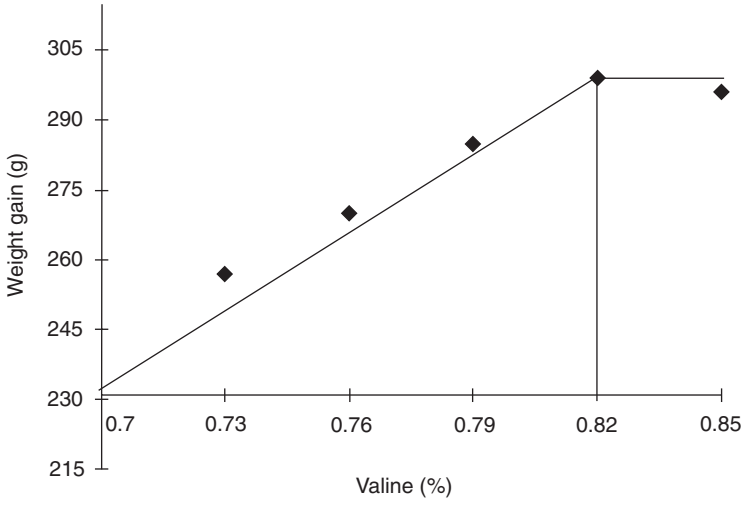

Fig. P16.2. Broken line analysis of weight gain of poults fed graded levels of digestible valine.

INDEX