Protein nutrition, requirements and dietary supply.

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Energy-Protein Interrelationships

Although these details are focused on Protein Sources for the Animal Feed Industry the utilisation of dietary proteins must be put in the context of the available energy supply. Energy is the main driving force of metabolism. If energy is limiting dietary protein will be used inefficiently as another source of energy instead of being converted into body protein.
Figure 1a. Relationship between increasing protein intake of constant amino acid composition and protein deposition in the

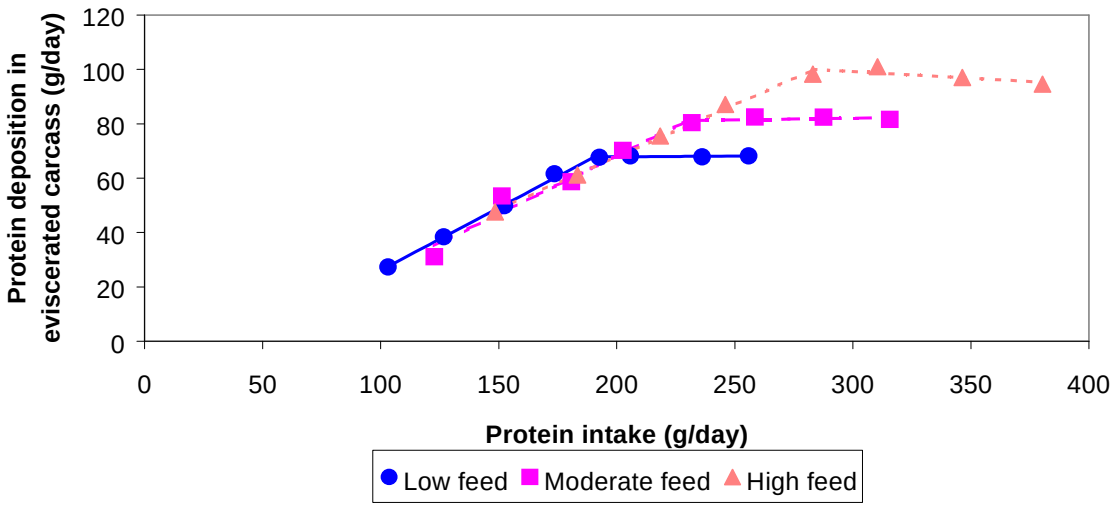

Fig 1.a.

carcass of pigs between 20 and 45 kg live weight. The same feeds were fed at three fixed levels of feeding, low, moderate and high. Data from Campbell et al 1985.

Figure 1a shows the response of growing pigs given diets in which the amount of protein, with a constant amino acid profile, was varied while maintaining a constant energy supply by replacing starch with protein. In addition, the diets were given at three levels of feeding which increased both the protein and energy supply in a fixed ratio. Increasing protein from low and limiting levels at constant energy increased protein deposition in the carcase until energy limits the response. Giving more feed increased the energy supply and allowed the response to dietary protein to continue until the new energy level again becomes limiting. This will repeat until the genetic potential of the animal or some other factor limits further protein accretion.

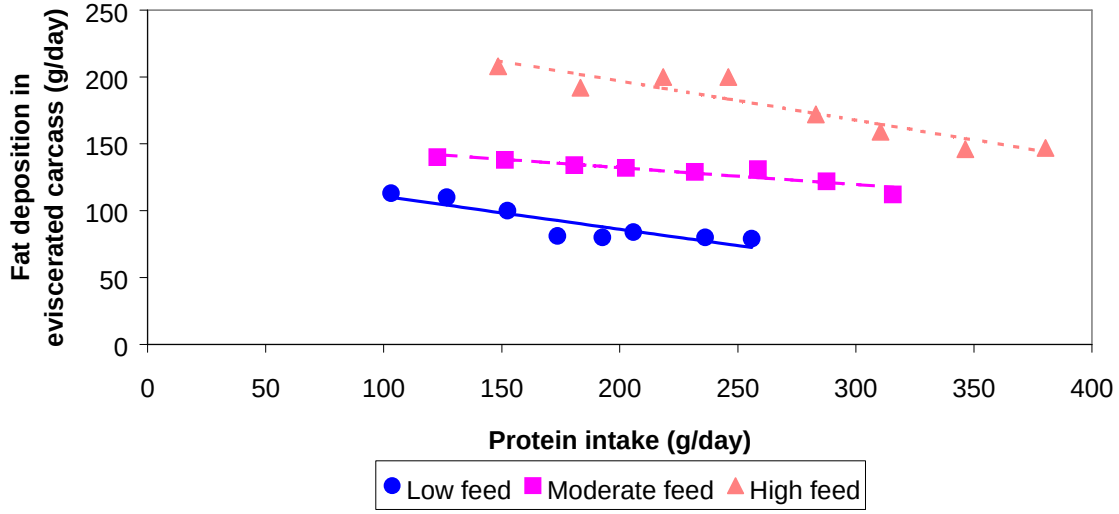

Fig 1.b.

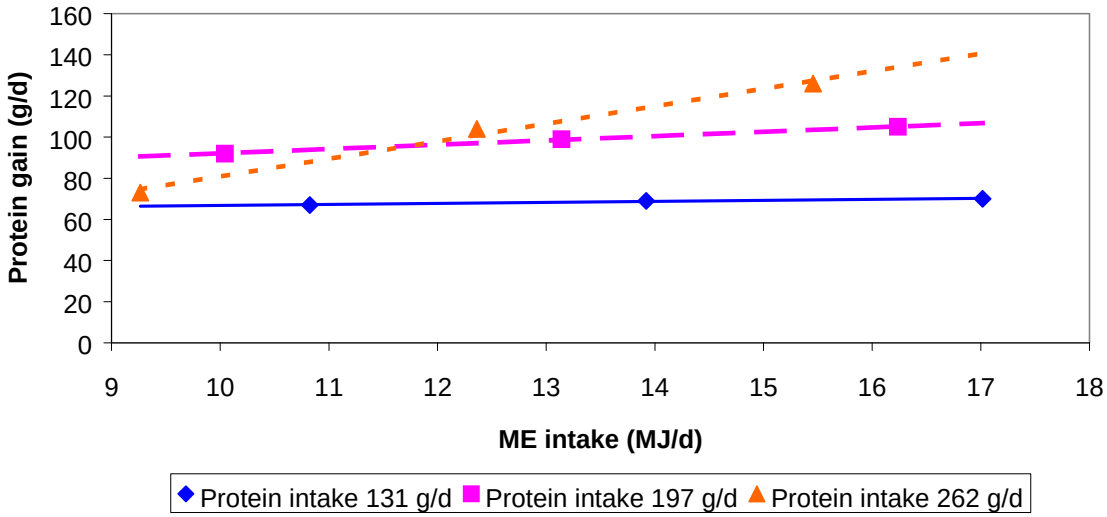

Fig 2.a.

Figure 1b. Relationship between increasing protein intake of constant amino acid composition and fat deposition in the carcass of pigs between 20 and 45 kg live weight. The same feeds were fed at three fixed levels of feeding, low, moderate and high. Data from Campbell et al 1985.
Increasing the protein level also reduced the fat deposition in the carcass, indicating less net energy even though metabolisable energy (ME) was maintained constant (Figure 1b).

In Figure 2a, pig diets supplied a constant amount of protein with an increased ME intake by feeding extra starch.

Figure 2a. Effect of increasing energy by adding starch to a constant protein at three levels of protein intake on protein retention of male pigs over 6 weeks from 12 kg live weight. Data from Kyriazakis & Emmans (1992).

This was repeated with three levels of protein, low, medium and high. At the low and medium levels of protein, providing extra energy had very little effect in increasing N retention. Protein supply was the limiting factor and an increase in protein supply increased protein deposition. At the high level of protein additional energy gave a marked increase in protein deposition.

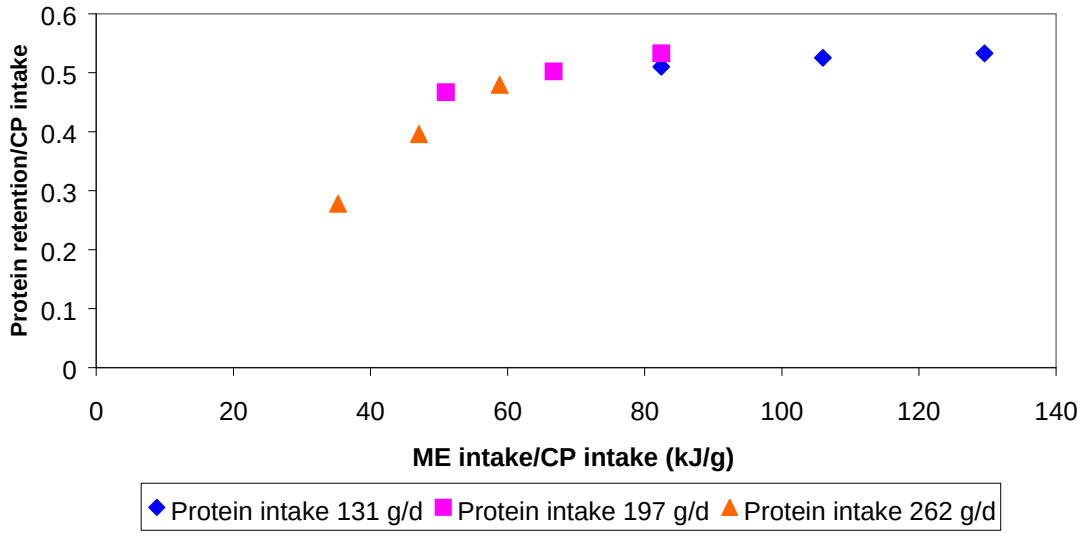

Fig 2.b.

Figure 2b. Effect of increasing energy by adding starch to a constant protein at three levels of protein intake on protein retention per unit protein intake of male pigs over 6 weeks from 12 kg live weight. Data from Kyriazakis & Emmans (1992).

The low protein deposition at high protein but low energy indicates energy was the limiting factor and that the excess protein was used with less efficiency and provided less net energy. When the data is scaled by the protein intake, the efficiency of use of the diet N for N retention in seen to increase with the energy to protein ratio in the diet (Figure 2b). A high energy:protein ratio is needed to make the most efficient use of the dietary protein.

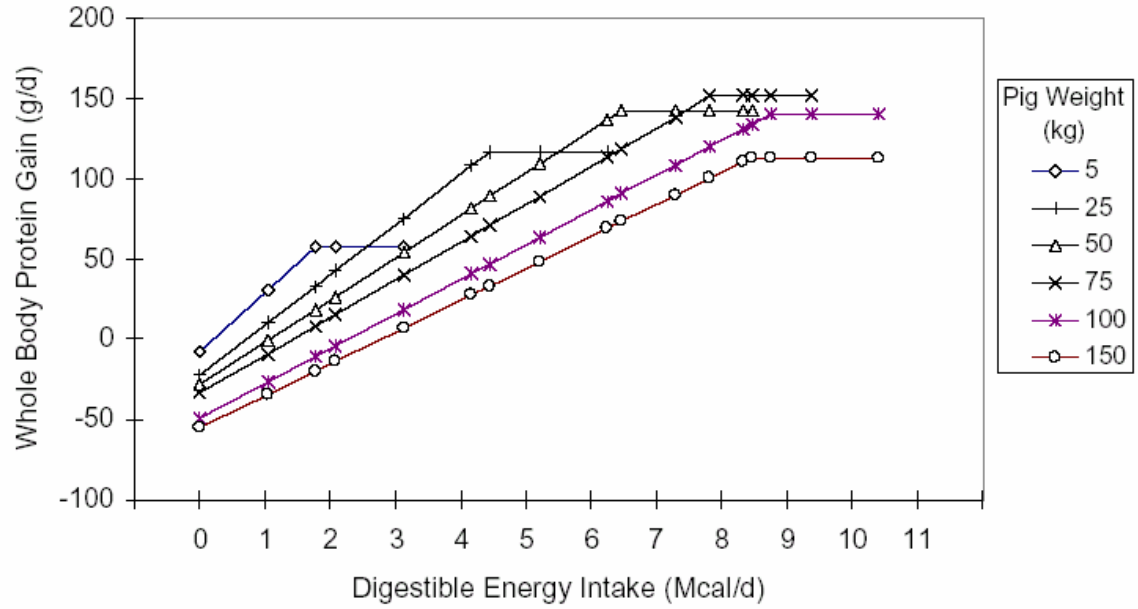

Fig 3

Figure 3. Theoretical relationship of whole body protein gain and digestible energy intake in pigs from 5 to 150 kg body weight when fed diets with adequate protein. (NRC 1998).

Figure 3 is a theoretical model of the response to energy in the presence of adequate protein for pigs of different weights presented by National Research Council (1998). The response is linear up to the maximum potential protein deposition and then reaches a plateau. The maximum potential protein deposition increases to a maximum at about puberty and then decreases as maturity is approached. Figure 4 demonstrates the change in maximum potential protein deposition for different sexes of improved European pig breeds.

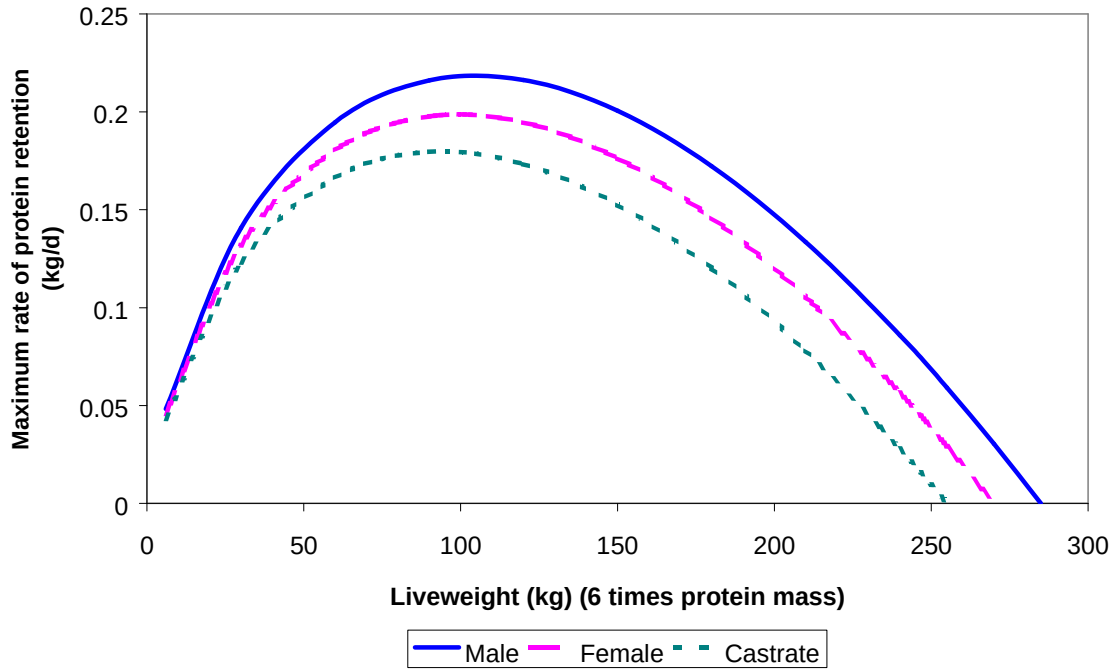

Fig 4

Figure 4. Prediction of the maximum rate of protein retention male, female and castrate pigs of an improved breed type at different stages of growth from $Pr_{max} = B_p.Pt.\ln(A_p/Pt)$ where Bp is the growth coefficient for protein mass, Pt is protein mass at different growth stages, A_p is the mature protein mass. The independent x axis is shown as liveweight, calculated as six times protein mass. Equation from Whittemore et al (2001).

Dietary protein is not used efficiently as a source of energy. Although the gross energy of protein is greater than that of carbohydrate (23.6 kJ/g v 17.4 kJ/g for starch), when protein is used as an energy source the N has to be excreted as ammonia (fish), urea (mammals) or uric acid (birds). The ME value of protein at zero N retention takes into account the loss of energy in the excreta such that the ME of protein and carbohydrate are approximately similar.

The ME value for mammals and birds, however, does not take into account the energy costs of synthesising urea or uric acid and cost of excretion in the kidney. Net energy (NE) of the diet represents the useful energy used to replace the losses of maintenance and the net deposition of energy as new tissue in growth or milk secretion during lactation after subtracting the heat losses of metabolism. For the pig Noblet and Henry (1991) reported NE/ME ratios of 0.50, 1.0, 0.61 and 0.76 when ME was derived from protein, fat, fibre and carbohydrate, respectively; but such efficiency values are only indicative values for some given combination of energy use for maintenance, protein deposition and lipid deposition. Nevertheless, the net energy value for protein is less than that of carbohydrate and fat and when dietary protein is exchanged for carbohydrate at equal ME the net energy decreases, protein deposition may be increased but fat deposition is decreased.

The protein requirements of animals are in terms of an amount of protein and their constituent amino acids per unit of time - usually the amount to be fed each day. However, this value continually changes as the animal grows, so is not convenient to use (see Figure 4). Instead we express protein requirements in terms of protein concentration of the diet, usually as g/kg diet as fed. Since most animals eat to meet their energy requirements an alternative method of expressing protein needs is in relation to the energy concentration as g/MJ of ME or, using 17 kJ ME/g protein, as protein energy % (1.7 x g CP/MJ ME). Typical protein contents of diets, expressed in these three ways, for various livestock classes are shown in Table 1. In these terms young growing animals have greater requirements for protein than older animals. As the animal grows more energy is needed for maintenance of the bigger body and to support an increasing proportion of fat deposition in the body. Thus the protein % of the diet and protein:energy ratio declines. In addition, voluntary intake increases so the increased amount of protein required to meet the increased daily protein need can be accommodated within a lower protein concentration in the diet.

Table 1. Typical dietary crude protein and metabolisable energy concentrations (/kg air dry feed) and dietary protein expressed relative to energy (g/MJ ME and as protein energy %)

	CP g/kg	ME MJ/kg	CP g/MJ	Protein energy %
PIG				
Starter 3 week weaning 5-10kg 240	14.1	17.1	29	
5 week weaning 10-20kg210	13.7	15.3	26	
Grower 20-60kg	165	12.6	13.1	22
Finisher 60-90kg	140	12.5	11.2	19
Sow lactating	176	12.5	14.1	24
pregnant	130	12.0	10.8	18
BIRDS				
Broiler starter 0-2 weeks	230	12.8	18.0	31
Broiler grower 2-4 weeks	210	13.0	16.2	27
Broiler finisher 4-7 weeks	190	13.2	14.4	24
Rearing pullets 0-6 weeks	210	11.5	18.3	31
6-12 weeks	145	11.5	12.6	21
12-18 weeks	120	11.5	10.4	18
Laying hens	160	11.5	13.9	24
Turkey starter 0-6 weeks	300	12.6	23.8	40
Turkey grower 6-12 weeks	260	12.6	20.6	35
Turkey finisher 12 + weeks	180	13.0	13.8	24
Breeding turkeys	160	11.3	14.2	24
DOGS Growth / Lactation	250	>14	17.8	30
Maintenance	130-220	>13	10.0-16.9	17-29
CATS Growth / Lactation	>310	>16	19.4	33
Maintenance	>220	>14	15.7	27
FISH[1]				
Salmonids Fry, fingerlings	550	17	32.4	70
Smolt	400-460	15-17	26.6	58
Catfish and Cyprinids	320-360	12-13.5	26.6	58

1. Energy values in digestible energy (DE MJ/kg), CP/MJ DE and Protein energy % as 100 x digestible energy from protein/DE.

Differences between species in their digestive system also affect the required concentration of protein. Carnivores have no ability to digest fibrous feed and even a limited ability to digest starchy carbohydrates. Consequently, the diet has to contain more of both protein and fat, but the protein:energy ratio is not greatly increased compared with pigs and poultry. Fish appear to have much higher protein needs than mammals and aquaculture diets (a very important area in developing countries) are high in protein. To a large extent this is not due to a greater need for protein but a smaller need for energy. Poikilothermic animals (fish, reptiles) do not need energy to maintain their body temperature, whereas homiothermic animals (birds and mammals) expend a considerable amount of energy (partly reflected as basal metabolic rate and maintenance energy and partly as shivering or panting) to maintain a constant body temperature different to the environmental temperature.

Alteration in energy needs from those considered during normal maintenance and production also alters the protein requirement expressed as a proportion of the diet. Reduced energy expenditure during heat stress results in reduced voluntary feed intake and to maintain the same protein intake to support growth the dietary concentration needs to be increased. Increased energy expenditure as in additional exercise (e.g. cattle walking increased distances to find water or adequate grazing) can mean a lower proportion of protein is needed in the feed so long as energy need can be satisfied by increased voluntary feed intake.

Protein synthesis in the body involves a considerable expenditure of energy to create the activated amino acids to be linked together. In addition, protein tissues are constantly being turned over. For every one unit of net accretion of protein about 5 units of protein are synthesised. Some tissues are turning over faster than others. Indeed some of the fastest turning over

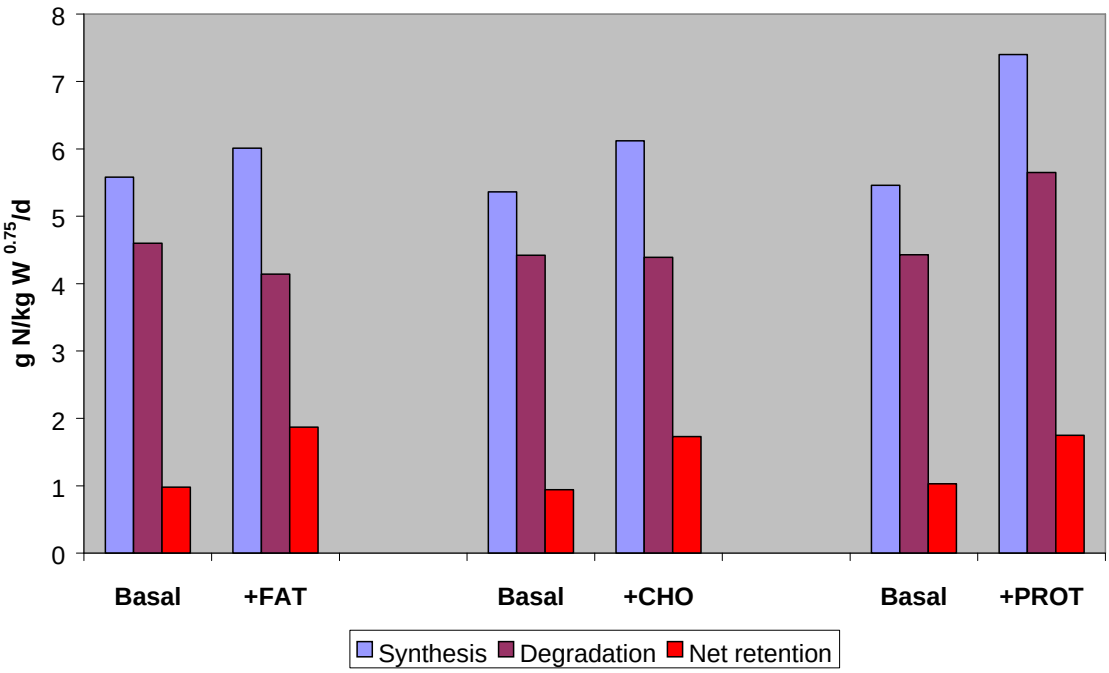

Fig 5

tissues such as the intestinal epithelium and liver lead to little or no net accretion.

Figure 5. Effect of supplementing a basal diet with either fat or carbohydrate at constant protein or with protein at constant energy on protein synthesis, protein degradation and net protein retention in pigs. Data from Reeds et al (1981).

The energy cost of protein synthesis in protein turnover just to maintain the existing protein has been estimated to account for 15 to 33% of energy needed for maintenance. When additional energy is provided there is an increase in protein synthesis and a decrease in protein degradation and these two effects combine to enhance net protein retention. When additional protein is supplied at constant energy there is an increase in both protein synthesis and in protein degradation resulting in a smaller net increment in protein retention. This is illustrated in Figure 5, which gives the determined synthesis and degradation contributions to the net N retention. With increasing protein in the diet there are frequently small improvements in carcase quality, measured as increased protein and decreased fat content. These changes arise from the decreased net energy value of protein compared with carbohydrate and the increased energy required for increased protein turnover driven by higher dietary protein intake resulting in reduced energy available for fat synthesis.

Indispensible amino acid requirements

Mono-gastric animals do not have a requirement for protein as such but for 9 to 10 amino acids, which the body cannot synthesise, together with a source of amino nitrogen that can be used for the synthesis of the remaining amino acids. The amino acids that cannot be synthesised must be provided by the diet. They are termed indispensable or essential amino acids. In addition two amino acids, Cysteine and Tyrosine, can be synthesised in the body but only from indispensable amino acids Methionine and Phenylalanine respectively. Consequently, they are not indispensable but a dietary supply spares the need for the indispensable parent amino acid. These are termed semi-indispensable or semi-essential. Arginine is an indispensable amino acid for birds and fish but in mammals it is synthesised as part of the urea cycle. However, as most of the synthesised Arginine is broken down to release urea the amount available for protein synthesis may be inadequate and a dietary supply may promote growth in young animals. Similarly Glycine and Serine may not be synthesised in sufficient quantities in certain situations such as young animals and rapidly growing chicks and so are termed conditionally indispensable. Initially practical trials were carried out to determine the requirement for each of the indispensable amino acids in turn from response curves to supplementation of deficient diets with the amino acid under consideration. A typical response curve to lysine supplementation of a deficient diet for chicks is shown in Figure 6.

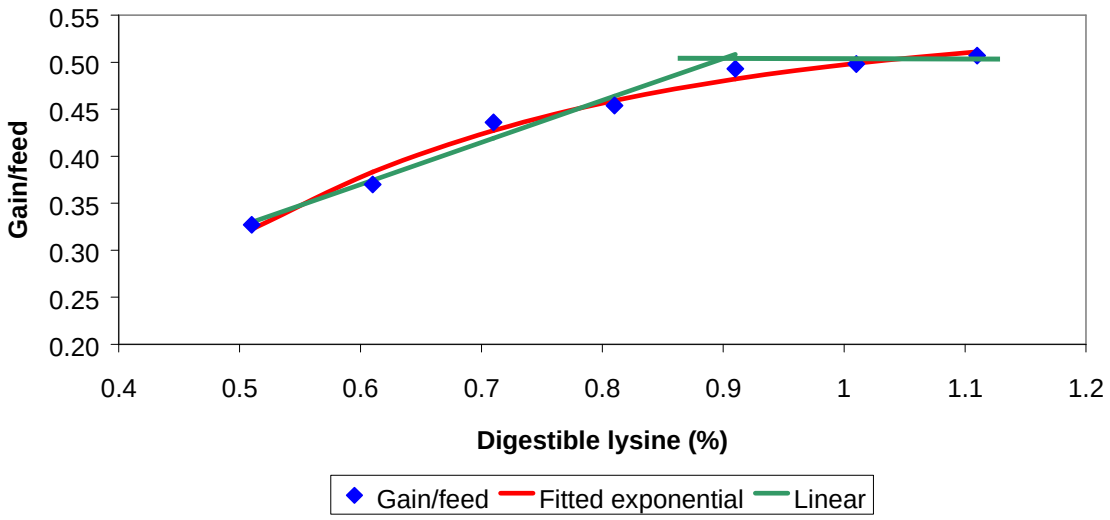

Fig 6

Figure 6. Feed efficiency ratio response to supplementing a lysine deficient diet with increments of lysine in male chicks from 3 to 6 weeks of age. The data are interpreted as either a) as an exponential response over the whole range or b) a linear response to the requirement breakpoint. Data from Han & Baker (1994).

Differences in the size, maintenance requirement and potential growth rate of individual chicks result in the flock response tailing off as the asymptote is reached. Researchers and National Committees have attempted to give a single value as the requirement that feed compounders could use as the target in least cost diet formulation. However, fitting different statistical models to the data can result in quite large differences in apparent requirement with consequences for diet costs. More attention is now being paid to the response curve such that the economically optimum level of each amino acid can be targeted. There is no point in targeting maximum growth rate or production if the last increment is uneconomic.

The practical trials indicated the requirements varied with many different conditions, such as sex, genetic strain, environmental temperature, growth rate and energy supply, in the same way as requirements for crude protein. To shortcut the work needed to study all possible situations with each amino acid the concept of the ideal protein was formulated. Since each protein has a fixed and characteristic sequence of amino acids it follows that the ratio of the amino acids to one another is constant in any one protein. If the proportions of the different proteins in the body during growth also remain reasonably constant then the ratios of the amino acids in the total body proteins will remain constant. Consequently, if the requirement for one amino acid is determined by empirical trial in one situation the requirements for all the other can be estimated by applying the ratio as determined for the ideal protein. Because lysine is normally the first limiting amino acid in most practical diets and therefore the requirements for lysine were the most studied in empirical trials, lysine is used as the reference amino acid and all others are expressed as a ratio to lysine

(Table 2).

A first approximation to the ideal ratio is the amino acid composition of the whole body, or of the tissue protein gained during growth. This makes the assumption that each absorbed indispensable amino acid is used with the same efficiency for protein synthesis. This is not true since some amino acids e.g. Tryptophan and Methionine are used for purposes other than protein synthesis and others such as Cystine and Threonine have large losses in intestinal muco-proteins. Also as different proteins turn over at different rates the ideal pattern changes with change in proportions of the different proteins being synthesised at any one time. For example, as the proportion of protein involved in maintenance of the body compared with accretion of new tissue changes with age so the ideal pattern will change to reflect the different proteins involved. Consequently, the ideal pattern has evolved in recent years as some of these factors have been studied. Given an accurate determination of the lysine requirement in terms of % of diet or g/MJ ME in any given situation the requirement for the remaining indispensable amino acids can be calculated.

Table 2. Amino acid requirement patterns relative to lysine = 100

	Pig [1]	Chick [2]	Rat	Salmonids[3]	Trout[4]	Common carp[4]	Tilapia[4]	Cat
	20-50 kg	0-3 wk						
Lysine	100	100	100	100	100	100	100	100
Met + Cys	51	72	70	50	56	54	63	90
Threonine	64	67	60	42	44	68	73	90
Valine	74	77	85	46	67	63	55	80
Leucine	114	109	110	75	78	57	66	<150
Isoleucine	57	67	70	42	50	44	61	65
Phe + Tyr	114	105	110	110	100	114	108	130
Trypt	17	16	14	13	11	14	20	15
Histidine	36	32	36	33	39	37	34	35
Arginine	(40)*	105	(*)	88	83	75	83	150
Gly or Ser	-	(65)*	-	-				-
Proline	-	(44)*	-	-				-
Taurine	-	-	-	-				6
*Arginine synthesis is not adequate to meet needs, especially in young animals and cats.								
Glycine plus serine synthesis not sufficient for maximum growth rate.								
Proline response obtained with experimental free amino acid diets; practical diets supply sufficient supplemental proline.								
Approximate lysine requirement g/MJ ME								
	0.88	0.91	0.6	1.2[a]	1.2[a]	1.3[a]	1.1[a]	0.4

1. Boisen et al. (2000). 2. Baker & Han, (1994). 3. Bureau & Cho (2000). 4. NRC (1993)
a: g/MJ DE

One problem with this mode of expression is it does not indicate the amount of dispensible amino N needed, i.e. the minimum crude protein content in which the indispensable amino acids must be supplied. If this is known then the ideal pattern can be expressed as a percentage of ideal protein or g/16 gN. In this form the ideal pattern can be used to estimate the biological value of feed proteins through the calculation of a chemical score (CS) based on the proportion of the amino acid in the feed protein compared with that of ideal protein.

$$CS = \frac{\text{Amino acid in test feed (g/16 g N)}}{\text{Amino acid in ideal protein (g/16 g N)}} \times 100$$

The amino acid with the lowest score below 100 is the limiting amino acid. Amino acids present in greater amount relative to the ideal protein than the limiting amino acid i.e. having a higher score can only be used in protein synthesis up to the level sustained by the limiting amino acid. The amount in excess will be deaminated and the carbon skeleton used as a source of energy. Consequently, the score for the limiting amino acid becomes the chemical score for the protein.

An example of the use of the ideal protein pattern to calculate chemical score of feeds is given in Table 3. For maize, lysine is the first limiting amino acid. For soybean meal, methionine +cystine (M+C) is first limiting.
Table 3. Calculation of the amino acid scores compared with the ideal pattern for chicks for maize meal, soybean meal, diet of maize-soya supplying optimum protein of 22.4%, and of this diet supplemented with 0.155% methionine to meet ideal balance for methionine plus cystine.

Table 3.

	Maize	Soya	Maize-Soya	Plus Met
Lysine	**55**	115	103	103
Methionine	107	**74**	**81**	116
M+C	111	**75**	**82**	**100**
Threonine	99	109	107	107
Tryptophan	82	149	136	136
Arginine	82	130	120	120
Histidine	176	160	163	163
Isoleucine	94	125	119	119
Leucine	208	132	147	147
Phenylalanine	164	176	173	173
P+T	154	160	159	159
Valine	114	112	112	112

Maize-soya: diet mix of 52.2% maize, 37.8% soybean meal, 10.0% fat/min/vit supplement

Plus Met: Maize-soya diet plus 0.155% methionine

When these two are mixed the surplus amino acids of one protein complement the deficiencies of the other. When the two are combined in a ratio to achieve the minimum crude protein needed by young chicks, currently estimated as 22.4% of a corn-soya diet, only the sulphur amino acids remain limiting. Supplementation with methionine will correct the deficiency. In this example the supply of lysine (CS 103) and threonine (CS 107) are also just met. The next amino acid in surplus is estimated as valine (CS 112), followed by isoleucine and arginine. This sequence of limiting amino acids has been demonstrated with growth trials in chicks (Fernandez et al., 1994). In theory it should be possible to decrease the diet crude protein by 10% to 20.2%, using less soya, but supplementing with methionine (0.221%) plus lysine (0.136%) and threonine (0.043%), all of which are now commercially available, to create the ideal protein balance with valine. Further reduction in the crude protein and Soya should be possible but only with more supplementation with methionine, lysine and threonine but also with valine, isoleucine and arginine, which are all closely similar in CS.

It is not necessary to meet the ideal balance for all amino acids. If one or more amino acids are limiting in the diet it is possible to increase the amount of protein to meet the needs of the limiting amino acids (Carpenter and De Muelenaere, 1965; Boorman, 1992). This can be important for areas where abundant cheap supplies of a poor quality protein are available. If complementary proteins and synthetic amino acids are not economically available then quantity can make up for quality. The disadvantage is the excess of the other amino acids is increased further and these need to be deaminated and excreted with reduction in the energy value of the diet and enhanced pollution consequences.

Figure 7a. Response of chicks to increase in protein with a constant amino acid composition supplied from a poor quality source (cereal-groundnut meal), or the poor quality source supplemented with methionine and lysine, or from a good quality source (cereal-herring meal).

Figure 7a illustrates the response of chicks to increase in the diet of either a good quality protein (i.e. close to the ideal pattern- cereal-fish meal) or a poor quality protein (cereal-groundnut meal) with one or more amino acids at less than optimum levels and with the poor quality protein supplemented with lysine and methionine in a constant proportion of the protein. Nearly equal growth can be achieved with much greater use of the poor quality protein (Wethli et al., 1975).

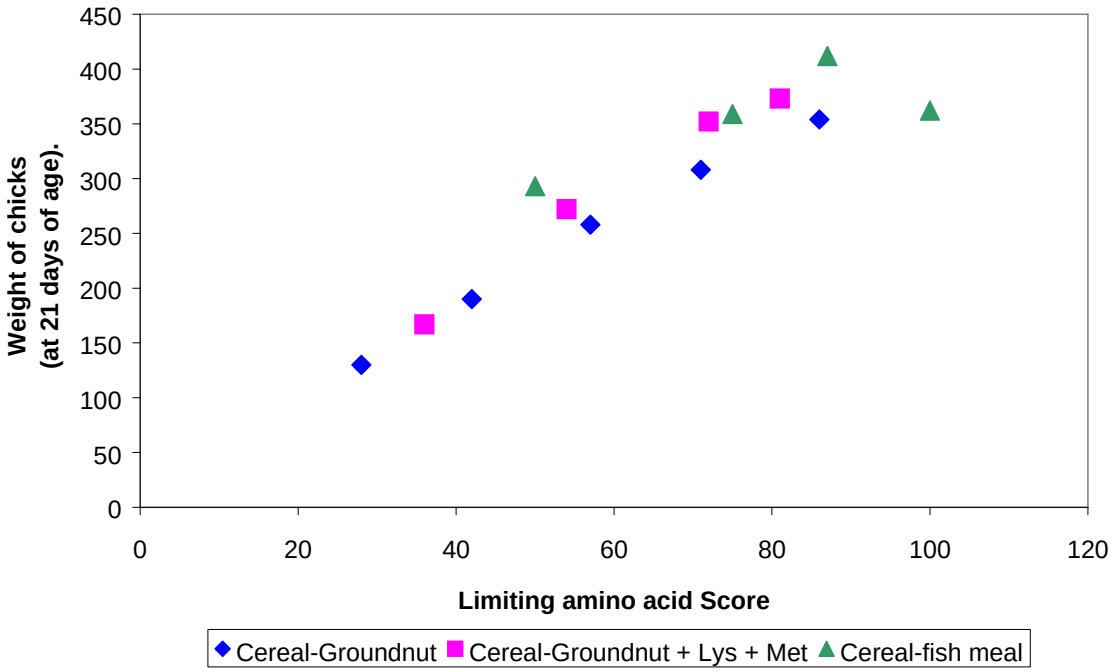

Fig 7a

Figure 7b. Response of chicks to a poor quality source (cereal-groundnut), or the poor quality supplemented with methionine and lysine, or from a good quality source (cereal-herring meal) expressed in terms of the score for the limiting amino acid calculated from the ideal protein ratio for chicks in Table 2 and a lysine requirement of 1.2% of the diet.

The growth response is plotted against the limiting amino acid score (using current estimates of amino acid requirements) in Figure 7b, confirming the over-riding factor is the supply of the limiting amino acid in the diet. The failure to achieve the same maximum growth reflects the reduced net energy value of the diet. Formulating a diet with constraints to limit the excess of one or more amino acids has the advantage of lowering dietary protein and reducing pollution but requires bringing in other proteins that have complementary patterns of amino acids, high in the limiting amino acids but low in those whose excesses are to be reduced. Such protein feeds are likely to cost more than the widely used feeds. Every time a new constraint is added to a best-cost diet matrix the cost of the diet is almost bound to increase and can never decrease.

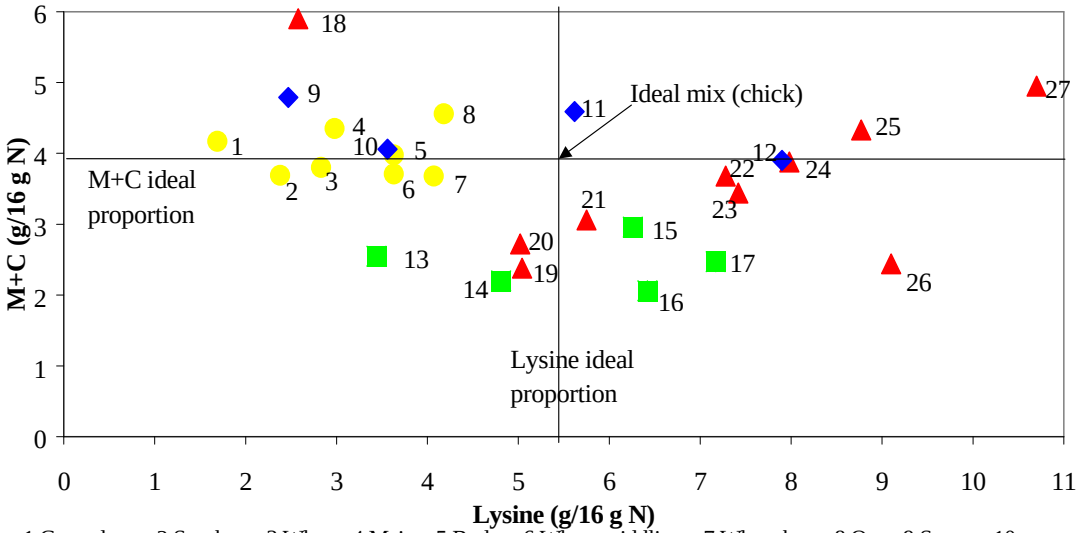

Fig 7b

1 Corn gluten; 2 Sorghum; 3 Wheat; 4 Maize; 5 Barley; 6 Wheat middlings; 7 Wheat bran; 8 Oats; 9 Sesame;10 Sunflower; 11 Rape seed; 12 Potato protein;13 Peanut; 14 Lupin; 15 Soya bean; 16 Field beans; 17 Peas; 18 Feather meal; 19 Meat&Bone 48%CP; 20 Meat meal 54%CP; 21 Poultry by-product; 22 Fish meal 56%CP; 23 Whey; 24 Fish meal Chile; 25 Spray-dried plasma; 26 Blood meal; 27 Whey protein concentrate. Yellow circles cereal; Green squares legumes; Blue diamonds vegetable protein concentrates; Red triangles animal proteins. Amino Acid data from Degussa

Figure 8a. Lysine and methionine plus cystine content of feed protein compared with the ideal protein balance of these two amino acids for chicks from 0-3 weeks of age.

The lysine and M+C of some major feeds are compared with the ideal protein pattern for chicks in Figure 8a. This illustrates the cereals are low in lysine but have an excess of M+C. Legumes are richer in lysine but much poorer in M+C. Other vegetable protein concentrates can be very variable in lysine but generally have good M+C content. Animal proteins are usually of good quality with very high levels of lysine. Fish meal also meets the high requirement of chicks for M+C but meat and bone meal, and blood meal, are not such good sources. In diet formulation the aim is to meet the requirements for at least these first two limiting amino acids. This is readily accomplished by complementing the lysine deficient cereals with a lysine rich protein such as soya. But the deficiency of the protein concentrates in M+C means that the diets will still be deficient in M+C which is normally and most economically corrected by a small supplement of synthetic methionine.

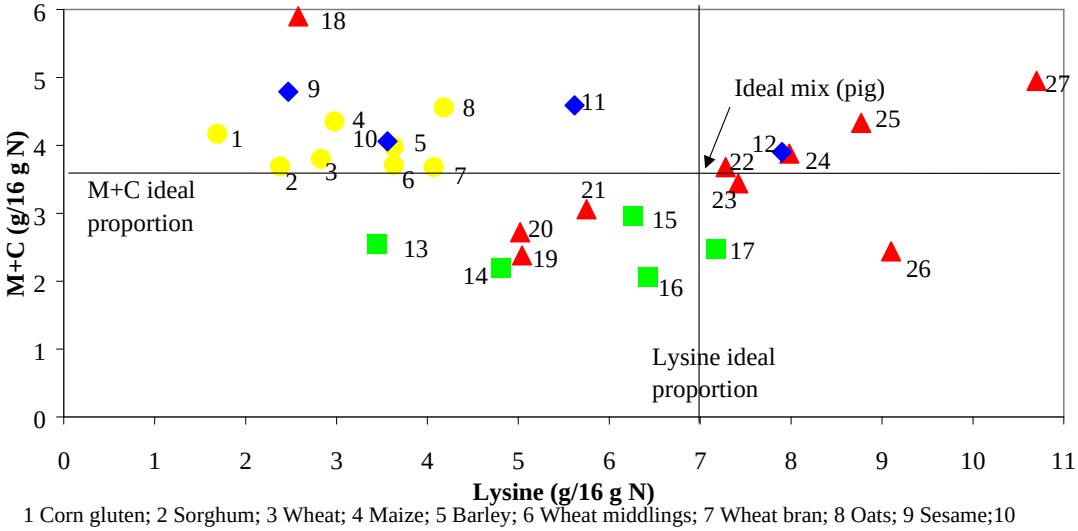

Fig 8.a.

1 Corn gluten; 2 Sorghum; 3 Wheat; 4 Maize; 5 Barley; 6 Wheat middlings; 7 Wheat bran; 8 Oats; 9 Sesame;10 Sunflower; 11 Rape seed; 12 Potato protein;13 Peanut; 14 Lupin; 15 Soya bean; 16 Field beans; 17 Peas; 18 Feather meal; 19 Meat&Bone 48%CP; 20 Meat meal 54%CP; 21 Poultry by-product; 22 Fish meal 56%CP; 23 Whey; 24 Fish meal Chile; 25 Spray-dried plasma; 26 Blood meal; 27 Whey protein concentrate. Yellow circles cereal; Green squares legumes; Blue diamonds vegetable protein concentrates; Red triangles animal proteins. Amino Acid data from Degussa

Figure 8b. Lysine and methionine plus cystine content of feed protein compared with the ideal protein balance of these two amino acids for pigs 30-60 kg live weight.

The target pattern for young pigs is compared with the same feed amino acids in Figure 8b. Here the ideal lysine is set much higher. Indeed so high that it would appear that only some of the animal proteins are rich enough in lysine to balance the cereals and achieve the ideal ratio. The difference in value compared with the chick is not real but more a reflection of the greater amount of research into the ideal protein pattern conducted with the pig, influenced by efforts to model growth of the pig and also to reduce environmental pollution by reducing dietary nitrogen to a minimum. As the ideal pattern is approached and excesses of indispensable amino acids are eliminated so dietary protein is reduced and the lysine as a proportion of that protein increases. Despite this concentration of the requirements the ideal M+C is still lower than for the chick. Consequently, the M+C requirement is easily met but lysine is normally the first limiting amino acid in practical diets for pigs.

Ileal digestible amino acids

In the past, most diet formulation has been based on the use of the chemically determined amino acid content of feeds, usually book values representative of the class rather than analyses of the individual batch. Some adjustment may be made using the determined crude protein content of the batch and published regression equations of amino acid content in relation to crude protein. The problem that not all the chemically determined amino acids are available to the animal at tissue level has been known for many years but lack of techniques to routinely determine amino acid availability has held back progress. Specific tests for available lysine, based on reaction of a free epsilon-amino group in lysine with reagents such as fluorodinitrobenzene (FDNB-available lysine), demonstrated the importance of the concept of availability of amino acids and the effects of processing in reducing amino acid availability (Carpenter and Booth, 1973).

Microbiological assays showed that heat processing also reduced the availability of other amino acids such as methionine, and even those without reactive groups such as leucine (Miller et al., 1965). While mild heating in the presence of reducing sugars can specifically reduce the availability of lysine, the major problem is a generalised reduction in the digestibility of the protein. Traditional methods of measuring digestibility by analysis of faecal residues is inappropriate for most mammalian and avian species. Extensive microbial fermentation in the hindgut (caeca or colon) ferments amino acid residues from undigested feed and replaces them with bacterial protein with a different amino acid profile. Digestion and absorption of amino acids is complete by the end of the small intestine. Analysis of amino acid residues reaching the terminal ileum enables the calculation of apparent ileal digestibility. However, part of the residues are not of feed origin but are of endogenous origin, shed mucosal cells, remains of digestive enzymes, secreted mucoproteins. These losses from normal metabolism are referred to as the basal endogenous loss. They are proportional to dry matter intake and not necessarily related to protein intake. Their contribution to the total ileal N will be greater when low protein feeds such as cereals are fed. Consequently, the apparent ileal digestibility of low protein feeds will be low, and apparent ileal digestible amino acids of feedstuffs are not additive, a property necessary for feed formulation.

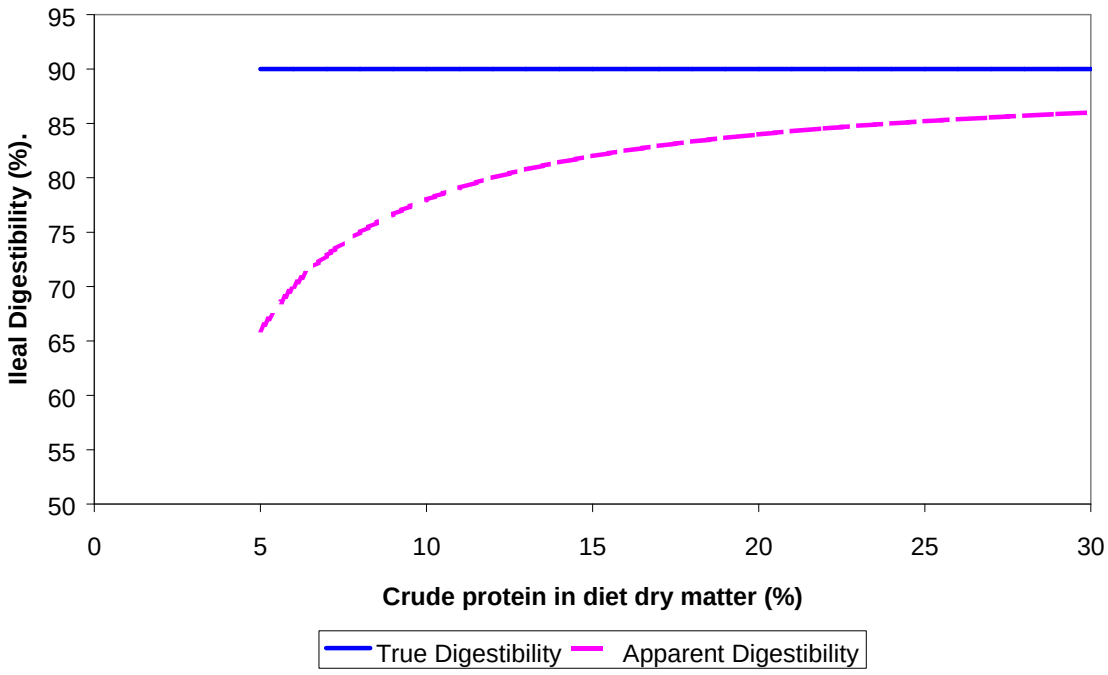

Fig 9

Figure 9. The effect of increasing the amount of a single feed protein in the test diet on true and apparent ileal digestibility of the protein. In this example, relevant to a pig, basal endogenous crude protein loss at the ileum is 1.2 g/100 g feed dry matter and the feed has 90% true ileal digestibility.

Correction for basal endogenous amino acids in the terminal ileum gives the true ileal digestibility. These values are independent of the level of protein in the feed (see Figure 9) and true ileal digestible amino acid values are additive. How the correction for endogenous losses is made is still a subject for debate (Boisen and Moughan, 1996; Sève and Hess, 2000). Endogenous losses measured with a N-free diet are too low. In the absence of dietary N the animal adapts its metabolism; food intake is depressed, proteolytic enzyme secretions are greatly reduced and consequently N loss at the ileum is reduced. Endogenous losses can be measured directly using isotopic labelling of either the feed protein or of endogenous proteins. Corrections based on this measurement have been termed real ileal digestibility. However, the feed itself may increase the endogenous loss. This may be a result of a high fibre content causing additional mucosal losses, a high viscosity preventing reabsorption of secreted proteins or antinutritional factors in the feed such as proteolytic inhibitors and lectins causing enhanced secretion of enzymes and increased mucosal cell turnover respectively. Such feed related losses are best regarded as a charge against the feed rather than an increase in the requirement of the animal. If the test protein is assayed at several levels of dietary inclusion then the basal endogenous loss can be determined by extrapolation to zero inclusion and the regression coefficient of the increase in ileal N or individual amino acid against N or amino acid intake is the true ileal digestibility and includes within it any increase in endogenous loss which is proportional to the test feed.

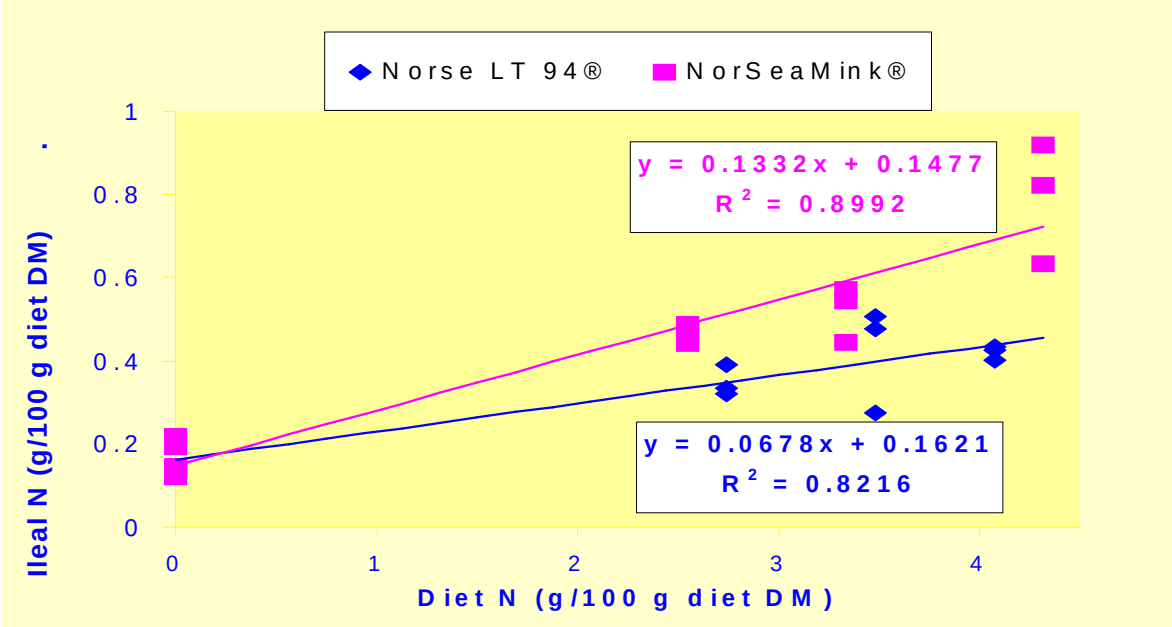

Fig 10

Figure 10.

Determination of ileal true digestibility of standard fish meal (NorseMink®) and of low temperature processed fish meal (Norse LT94®) in chicks by replacing enzymatically-hydrolysed casein with three levels of test protein. True ileal digestibility is 1-regression coefficient. The endogenous loss is given by the intercept value, which is reinforced by direct measurement of ileal N on the enzymically hydrolysed casein diet assumed to be 100% truly digested. True ileal digestibility is NorseMink® 86.7% ±1.24; Norse LT94® 93.2% ±0.88

Figure 10 illustrates the determination in chicks fed two commercial fish meals, one of standard quality and the other processed under low temperature conditions. In this study enzymically hydrolysed casein supplemented with amino acids to meet all amino acid needs was used to measure basal endogenous loss at the zero test protein level. Instead of determining the basal endogenous loss in every trial a mean value can be determined and used to correct apparent digestibility determined at a single level of inclusion of test protein. Such values have been termed "standardized" ileal digestibility (Boisen, 1997; Jansman et al., 1998; Rademacher et al. 1999a,b). Typical values for true and apparent ileal digestibility of protein in the pig for some protein concentrates are given in Table 4. Accounting for the basal endogenous loss substantially increases the value for true digestibility over apparent digestibility. Clearly there are substantial differences between proteins in the ileal true digestibility of the protein.

Table 4.

True Digestibility (TD) and Apparent Digestibility (AD) to the terminal ileum of pigs of crude protein (Nx6.25) of some protein concentrates.

	TD	AD
Fish meal 72% CP	88.4	84.4
Soya Hipro 48% CP	85.7	79.8
Sunflower seed meal 34% CP	81.2	74.9
Rapeseed meal	75.9	68.5
Peas	79.2	74.3
Beans	81.8	76.3
Meat meal high quality	79.3	75.3
Meat meal low quality	63.6	59.8

Data from Rhone-Poulenc.

On average these difference in N digestibility reflect individual amino acid digestibility. A first approximation to amino acid digestibility can be obtained from the product of N digestibility and amino acid content of the protein. A further refinement is to determine the digestibility of each amino acid. In general, the true digestibility of methionine is greater than that of N while that of cystine is lower, but for the majority of indispensable amino acids the ileal true digestibility differs very little from that of N. Typical values for standardized true ileal digestibility of N and key amino acids are given in Table 5.

Table 5. Standardised true ileal digestibility of feedstuffs for pigs (%)

	CP	Lysine	Methionine	M+C	Threonine	Tryptophan
Cereal grains						
Barley	80	76	82	81	80	77
Corn	83	76	87	84	80	76
Oats	76	81	84	78	75	77
Sorghum	92	90	93	93	94	98
Wheat	89	84	90	89	86	88
Cereal co-products						
Corn germ meal	70	65	81	69	72	66
Corn gluten feed	70	65	81	69	72	66
Corn gluten meal	87	87	97	93	90	86
Rice bran	64	62	71	62	62	75
Wheat bran (10% CF)	68	68	73	72	60	75
Wheat gluten feed	78	81	82	79	77	82
Wheat gluten meal	100	99	99	99	99	98
Wheat middlings (7%CF)	77	78	82	79	73	81
Legumes						
Beans, field	77	82	66	62	77	68
Lupin seeds	87	88	82	85	86	87
Peanut meal	85	81	85	81	83	86
Peas, field	79	81	74	70	76	70
Soybean meal, 44%CP	87	89	90	86	86	87
Soybean meal, 48%CP	87	89	90	86	86	87
Soybean, full fat	82	83	82	78	79	82
Other vegetable proteins						
Cottonseed meal	81	70	80	79	76	82
Linseed meal	75	82	85	85	79	84
Rapeseed meal	73	74	81	75	71	71
Sesame meal	84	82	84	84	79	84
Sunflower meal	81	79	88	83	80	83
Potato	90	90	91	82	86	80
Animal proteins						
Blood meal	88	94	88	88	89	91
Feather meal	67	49	58	63	69	56
Fish meal, 56%CP	85	89	89	85	88	86
Fish meal, 65%CP	85	89	89	85	88	86
Meat and bone meal, 42%CP	74	77	77	67	74	73
Meat and bone meal, 48%CP	74	77	77	67	74	73
Meat meal, 47%CP	77	77	84	79	78	78
Meat meal, 54%CP	77	77	84	79	78	78
Whey powder, delactosed	92	93	91	90	93	91

Data from Rademacher et al. (1999b).

Although ileal digestible instead of total amino acids are now widely used in the commercial formulation of diets there is a paucity of evidence of the expected benefits (Sève, & Hess, 2000). If an existing diet (e.g corn-soya based) gives good production and the true ileal digestible amino acid supply will be in a similar proportion to the total supply as the ileal requirement to total requirement. Changing the basis of formulation will give no benefit. Substituting one feed with high digestibility with another of similar digestibility will also give similar performance by either system. However, synthetic amino acids are assumed to be 100% digested and a proper evaluation of their use requires the description of feed proteins in terms of ileal digestible amino acids. A major role of animal production is to use byproduct feeds arising from the processing of foods for human consumption. Where these byproducts have distinctly different availabilities then advantages for the use of ileal digestible instead of total amino acids can be demonstrated. Tanksley & Knabe (1984) demonstrated that 50% of soyabean meal could be replaced in a pig diet with meat and bone meal so long as the latter was supplemented with lysine and tryptophan to supply the same amount of digestible lysine and tryptophan. A number of studies have shown heat processing reduced growth in bioassays in a manner similar to the loss of ileal digestible amino acids while total amino acids remained little changed (Varnish & Carpenter, 1975; Parsons et al., 1992; Kim and Easter, 2001). In some studies ileal digestibility has not been able to account for differences in bioavailability (Batterham et al., 1990a,b; Beech et al., 1991; Batterham et al., 1993; Batterham et al., 1994; Moughan et al., 1991). The conclusion from this series of experiments has been expressed as ileal digestibility values of heat-processed meals are unsuitable for diet formulations as a proportion of the digested amino acids is in form(s) unavailable for tissue metabolism. These experiments all used apparent ileal digestibility values not true ileal digestibility. Moughan et al (1991) compared the determined growth of pigs fed a barley based grower diet with the response to lysine supplementation of a lysine deficient synthetic diet based on casein. The observed growth was 0.925 of the expected growth based on intake of apparently absorbed lysine. To achieve an equal ME intake 15.3% more dry matter was fed of the test diet than the synthetic diets. Thus the endogenous losses would be less on the synthetic diet leaving more of the supplementary lysine available to support growth. The series of experiments by Batterham et al. and Beech et al. all had the same form, a comparison of cottonseed meal, meat and bone meal and soybean meal as examples of feeds with low, medium and high ileal digestibility. The growth and N retention of pigs fed three diets formulated to supply the same limiting level of ileal lysine, methionine, threonine, tryptophan or isoleucine was measured. The main difference was observed between cottonseed meal and the other two meals, with smaller (lysine, threonine) and non significant differences (methionine, tryptophan), in N retention between meat and bone meal and soybean meal. Diets were fed on a scale to provide the same DE/ $W^{0.75}$ but the cottonseed diets had 8.3% less DE/kg than the soybean diets with the meat and bone meal diets intermediate. Consequently, the amount of dry matter fed differed and basal endogenous loss would be less for soybean than meat and bone meal or cottonseed meal allowing more of the absorbed limiting amino acid to be used for growth. The presence of gossypol and raffinose in cottonseed makes this protein particularly susceptible to heat damage by binding specifically with the epsilon-amino group of lysine (Martinez et al., 1961). This may make it unavailable without any major change in digestibility of the protein (see below). Cottonseed meal and products such as dried milk powders where reducing sugars are potentially present may be special cases where ileal digestibility fails to reflect the full loss of available lysine through early Maillard reactions. For the majority of protein concentrates this is unlikely to be a major factor. Indeed, the Batterham group in a study of isoleucine, where the meals used were cottonseed, lupin seed meal and soya bean meal, ileal digestibility correctly predicted growth performance (Batterham & Andersen, 1994). Correction for the known differences in ileal true digestibility must be an improvement over the use of chemically determined total amino acid content.

The intestinal tract in carnivorous fish is relatively short without any adaptation of the hind gut for microbial fermentation. Direct determination of apparent faecal digestibility is a reasonable indication of the net absorption of protein and amino acids. However, determination of digestibility in fish is difficult. Measurement of total intake of feed and of excretion of faeces is impossible and markers must be used. Soluble components can be lost from feed, especially in slow feeders, and from excreta collected from the water. The alternative of stripping digesta from the gut obviates this loss but may increase protein in the excreta by removing endogenous components that would be absorbed in the hind gut. The specialised facilities needed for maintenance of fish and determination of digestibility prevent the routine determination of digestibility. Instead, mink have been used as an alternative carnivore with little complication of hind gut fermentation of undigested residues. True faecal digestibility of protein in mink correlates with apparent digestibility in salmonids. In Norway all fish meals sold as LT meals have been tested to exceed 90% digestibility in the mink test. An alternative method is to determine the ileal digestibility in chicks. In a recent study, fish meals and fish feeds prepared under various conditions were assayed by both mink and chicks. The two assays ranked the materials similarly, led to the same conclusions as to effects of processing variables and showed a good absolute agreement (Figure 11). Mink digestibility of fish meals can also be accurately predicted by Near Infra-red Reflectance.

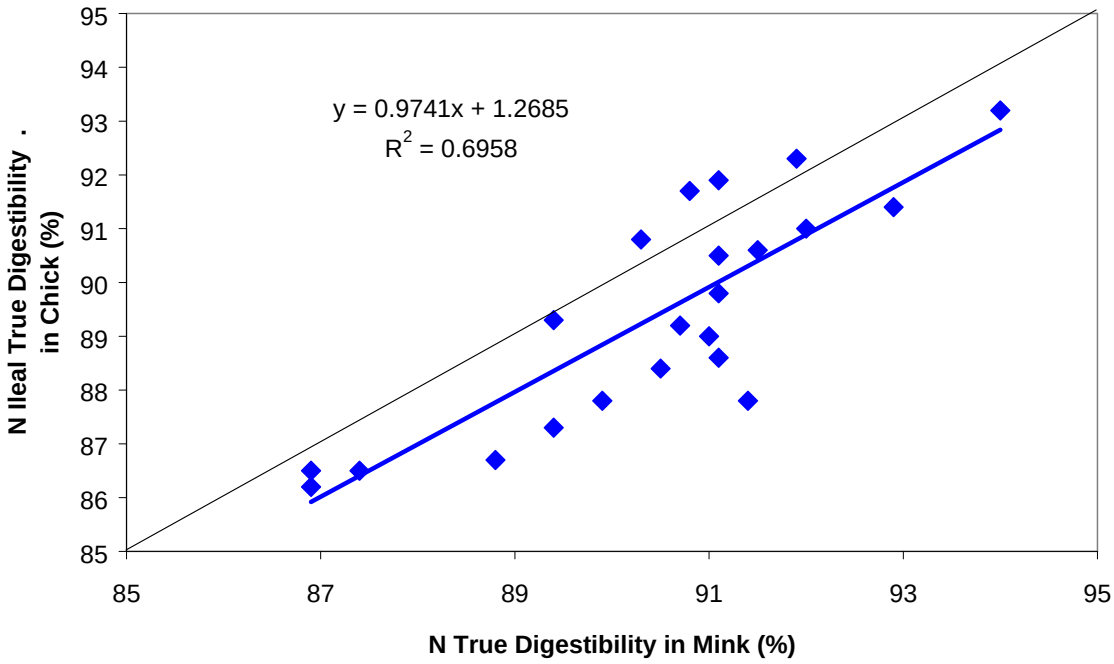

Fig 11

Figure 11. Comparison of determination of true N digestibility in fish meals and fish feeds using either faeces in mink or ileal digesta in chicks.

Effect of processing on protein quality for monogastric animals

Four distinct types of damage can occur. When proteins are heated under relatively mild conditions, even storage at 37°C, in the presence of reducing sugars or sucrose (which can hydrolyse to release reducing sugars) the epsilon-amino group of lysine reacts with the potential aldehyde group of the sugar to form early Maillard reaction products such as fructosyl-lysine. Fructosyl-lysine and formyl-lysine are absorbed but not metabolised. Reactive epsilon-amino groups can be conveniently measured with fluorodinitrobenzene (FDNB). Albumin heated under mild conditions with glucose had an ileal true N digestibility of 96% but the FDNB-available lysine was reduced to 69% of the control and availability of lysine by growth bioassay with chicks was also reduced to 69% of the control (Hurrell & Carpenter, 1978). Gossypol, in cottonseed, has a reactive aldehyde group which reacts similarly with lysine during processing to reduce the availability of lysine. It also contains about 10% of the non-reducing sugar raffinose but, as with sucrose, this must hydrolyse during heating to produce reducing sugars and results in loss of FDNB-available lysine (Martinez et al., 1961). With more severe heat in the presence of reducing sugars advanced Maillard reactions lead to a further fall in FDNB-available lysine but an even greater fall in digestible lysine and a general reduction in the digestibility of all the other amino acids in the protein (Miller et al., 1965).

In the absence of reducing sugars much higher temperatures, above 100°C for several hours, are required to bring about loss of FDNB-available lysine (Carpenter & Booth, 1973). Under these conditions cross links form between the epsilon-amino group of lysine and of the carboxyl group of aspartic acid and glutamic acid (or their amides) to form new peptide-like cross links (Hurrell et al., 1976). In addition cystine loses hydrogen sulphide to form a dehydroalanine residue plus a cysteine residue, the dehydroalanine and cysteine then recombine to form lanthionine creating a new C-S-C cross link between peptide chains. Dehydroalanine may also be formed by dehydration of serine. Under certain conditions, especially alkaline pH, the epsilon-amino group of lysine reacts with dehydroalanine to form a lysinoalanine cross link. These new cross links reduce the digestibility of the protein and hence the availability of all amino acids not just those directly involved. These conditions are not experienced during normal processing but have occurred when unstabilised fish meals have overheated through lipid oxidation during storage and transport. Even autoclaving at 133°C for 20 minutes at 3 bars as required for the treatment of meat and bone meal is estimated from these studies to cause only a 2 to 3% loss of FDNB-reactive lysine.

Heating protein in the absence of reducing sugars under much milder conditions (70 - 120°C for 20 minutes) brings about a loss of sulphydryl groups (cysteine residues) and an increase in disulphide bonds (cystine residues) with little loss of the total cysteine plus cystine (Opstvedt et al., 1984). Heating causes the formation of new S-S cross links and also the rearrangement of existing disulphide bonds during denaturation of the protein. These changes are associated with a 2 to 7% reduction in protein digestibility determined in trout of fish protein cooked at 95°C for 20 minutes. The digestibility of all amino acids is affected but that of cystine (16 - 26 % reduction) and aspartic acid (7 - 11 % reduction) were most affected (Opstvedt et al., 1984).

In response to these findings the Norwegian fish meal industry developed low temperature processed fish meal where the temperature is not allowed to exceed about 70°C at any stage. This material has about 5% units better digestibility determined with mink than regularly processed fish meal where the temperature may exceed 100°C for an hour or more. Heating also induces racemisation of amino acids, particularly aspartic acid. D-aspartic acid can be detected in regular fish meals and its formation has been demonstrated in fish processed under various conditions with temperatures in the range 95 - 127 °C but not at 70°C (Luzzana et al., 1996, 1999).

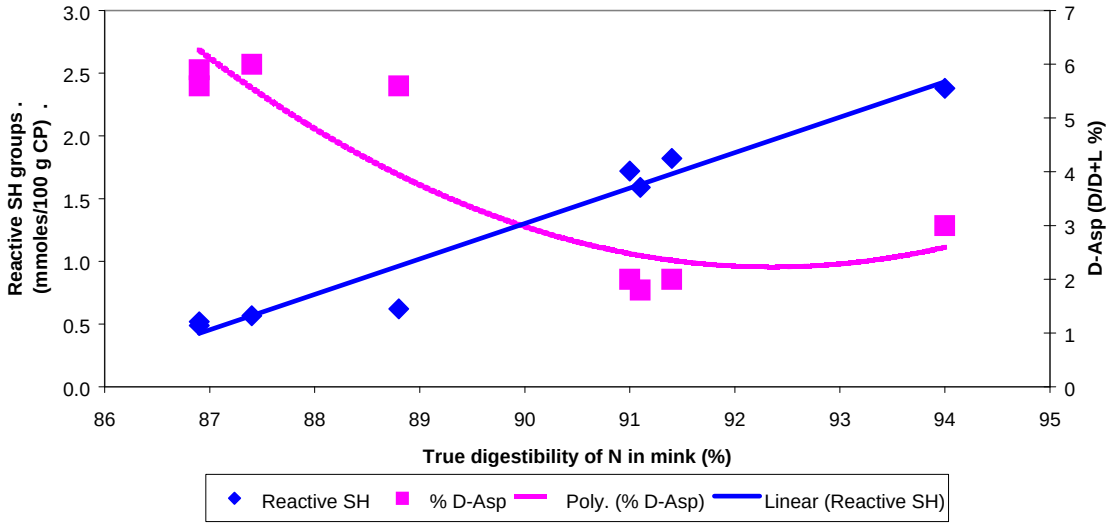

Fig 12

Figure 12. Mink digestibility of N, reactive sulphydryl groups and D aspartic acid content of standard fish meal (NorseMink®) and of low temperature processed fish meal (Norse LT94®) and fish feeds made from the meals under different conditions of severity of extrusion of the feed.

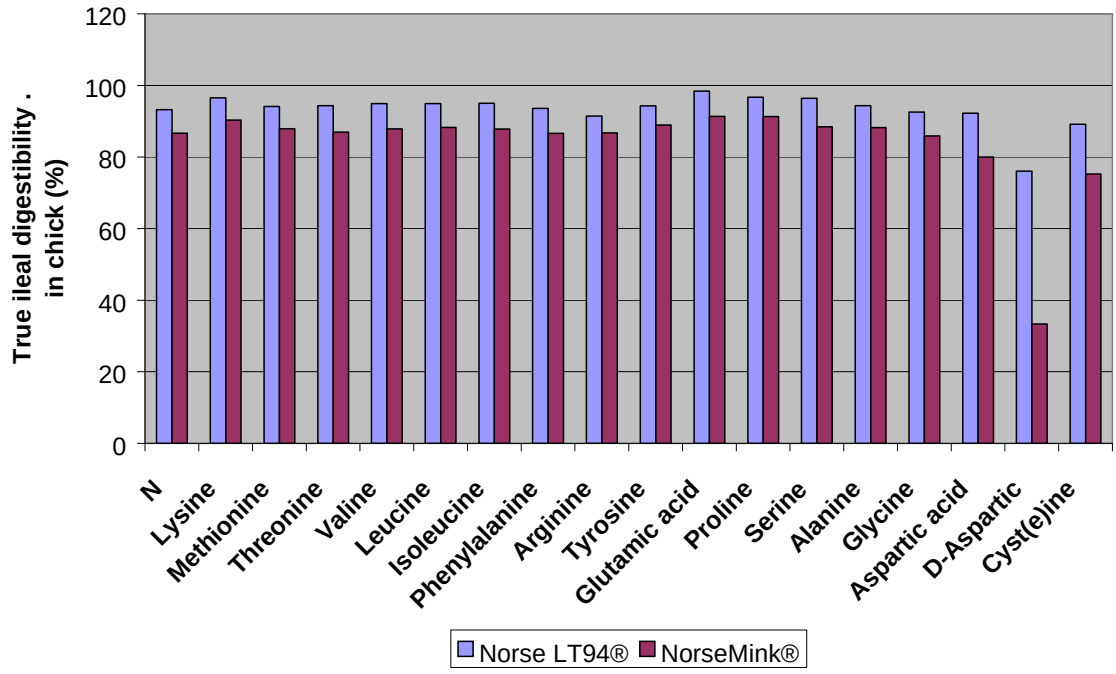

Fig 13

Figure 13. True ileal digestibility of amino acids of commercial fish meals prepared under low temperature or regular processing conditions.

In a recent collaborative study the kinetics of loss of sulphydryl groups and the formation of D-aspartic acid has been studied and the changes have been related to reduction of digestibility of the protein in mink (Figure 12) and to the ileal digestibility of individual amino acid in the chick (Figure 13). Low temperature processing increased the digestibility of all amino acids but the effects were greatest for cyst(e)ine and aspartic acid. D-aspartic acid was very poorly digested (Miller et al., 2001). The presence of D-amino acids in the peptide chain prevents the action of proteolytic enzymes.

Protein quality for ruminants

In the ruminant feed is fermented in the rumen, volatile fatty acids are absorbed from the rumen and omasum and provide the major part of the metabolisable energy to the animal. The fermented digesta leave the rumen along with the microbial biomass and are subjected to further digestion in the abomasum (true stomach) and intestines much as in the monogastric animal. Microbial protein is digested and absorbed in the small intestine and supplies the major part of the absorbed amino acids. The amino acid balance of microbial protein is good with methionine determined as the first and lysine as the second limiting amino acid for growing sheep (Storm & Ørskov, 1983, 1984). The amino acid needs of the animal can be met at maintenance level by microbial protein alone. With increase in energy supply above maintenance there is extra microbial protein produced and a low level of production can be sustained.

Figure 14. Calculated requirements of a dairy cow for metaboliseable protein (MP) per unit of fermentable metaboliseable energy (FME) and the contribution that can be expected from rumen microbial organisms (RMO). Calculated from Alderman & Cottrill (1993).

The microbial protein yield is limited by the fermented energy supply. For moderate and high levels of production the

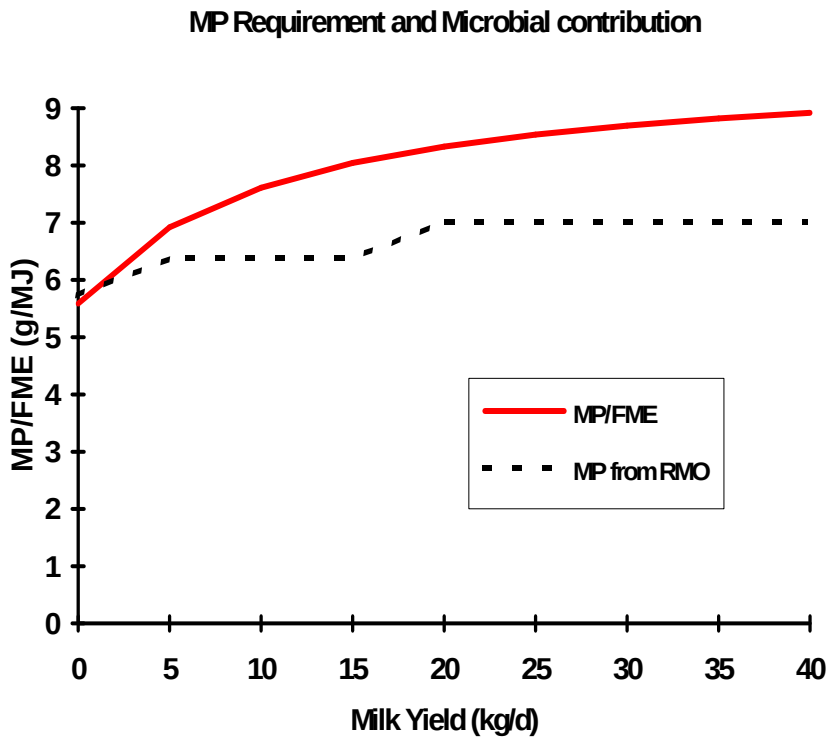

Fig 14

microbial amino acid supply needs to be supplemented with dietary sources of protein or protected amino acids that escape degradation in the rumen (Figure 14). The rate of digestion of feed, particularly roughage feed, and the rate of passage of residues from the rumen, are important determinants of voluntary feed intake and productivity. Consequently, considerable emphasis is placed on maintaining optimal conditions in the rumen to maximise microbial growth and digestion.

The N requirements of the ruminant are two fold:

1. A source of degradable N to meet the needs of the rumen micro-organisms. This can be largely met by non-protein N sources such as urea which are converted to ammonia in the rumen but growth of bacteria are stimulated by the supply of peptides. Supplying degradable protein instead of ammonia stimulates growth of amylolytic bacteria by up to 18.7% (Russell et al., 1992). Digestion of fibrous feeds is also increased by the provision of preformed dietary protein (Carro & Miller, 1999). The supply of degradable N should be at a rate commensurate with the release of energy during fermentation. Too rapid supply of ammonia, from high levels of urea or from rapidly degradable diet proteins such as grass, leads to high rumen ammonia levels. This is absorbed from the rumen converted to urea in the liver and largely excreted in the urine. If the capacity of the liver to convert the ammonia is exceeded, ammonia increases in the blood to reach toxic levels.
2. A source of undegradable N that is digested in the small intestine and provides amino acids to complement the microbial amino acids and meet tissue needs.

As with the monogastric animal, energy is the main driving force of metabolism. In addition to setting the limits for tissue growth or milk production, the fermentable energy supply is the main determinant of microbial amino acid supply.

For dietary protein the main characteristic is the rate and extent of degradation of the protein in the rumen. This not only describes the contribution to ammonia and peptide needs of the microbes but also the supply of amino acids to meet tissue needs. Considerable variation exists between feedstuffs in the rate of protein degradation. This is normally measured by the disappearance of feed N from bags of synthetic material with defined small pore aperture that prevents the loss of undegraded feed particles but does not impede the ingress of microbes (Figure 15). The rate of loss can be described as : N loss = $a + b(1- e^{-ct})$ where a = loss at time zero and represents soluble N easily washed from the bag and assumed 100% degraded, b = insoluble but potentially degradable N, c = rate constant describing the fraction of the remaining pool b degraded in unit time.

Figure 15. The rate of loss of feed nitrogen from polyester bags suspended in the rumen.

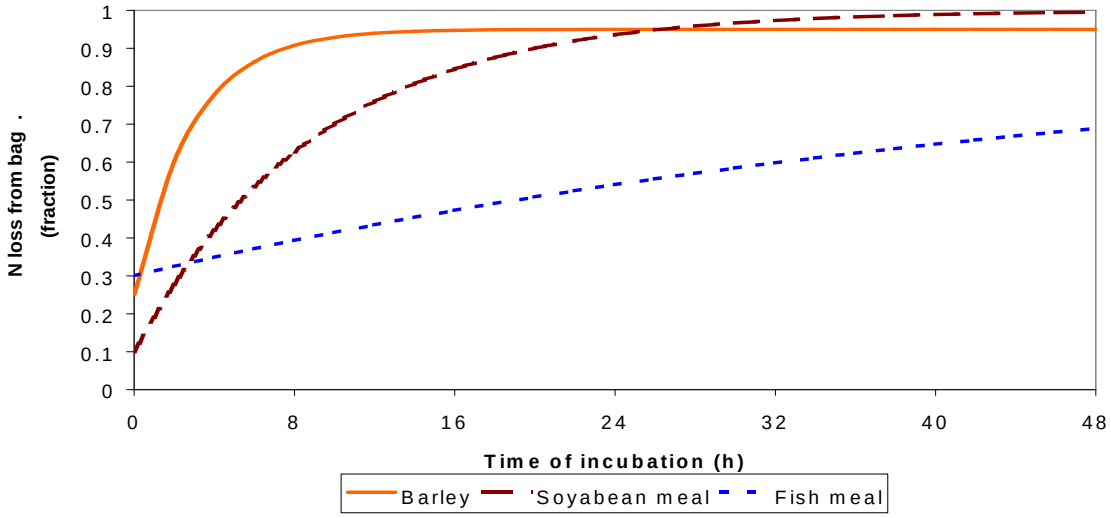

Fig 15

Figure 16. Representation of three outflow rates on the proportion of small feed particles (<1.0 mm) remaining in the rumen

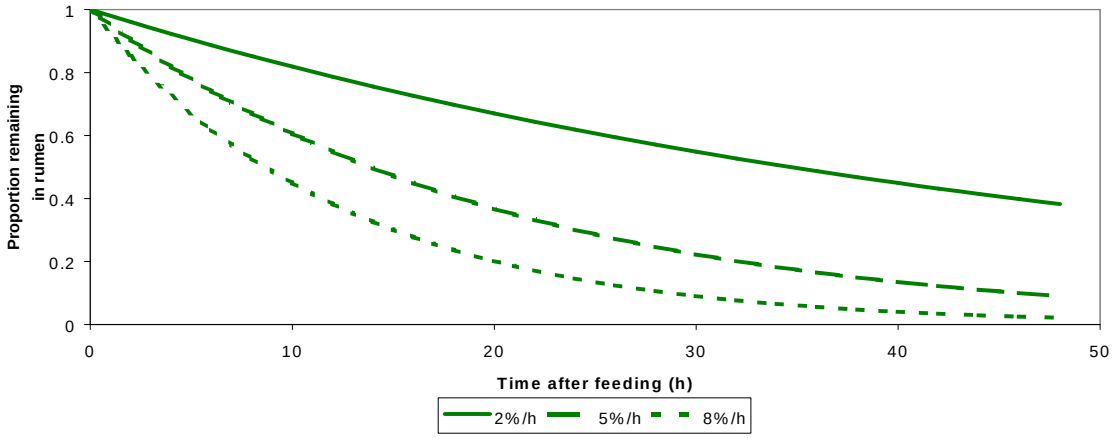

Fig 16

The feed residues in the synthetic bag cannot leave the rumen, but feed residues do leave the rumen at rates that are determined by the character of the diet and the level of feeding. Particles of concentrate feeds are small enough to leave the rumen immediately after ingestion. Their rate of leaving also follows an exponential pattern with a rate constant r (Figure 16). The equation describing the rate of degradation within the bag is combined with the rate of passage to give the effective degradation over the summed time for which the feed is subjected to fermentation.

Effective degradability = a + (bc/ c+r).

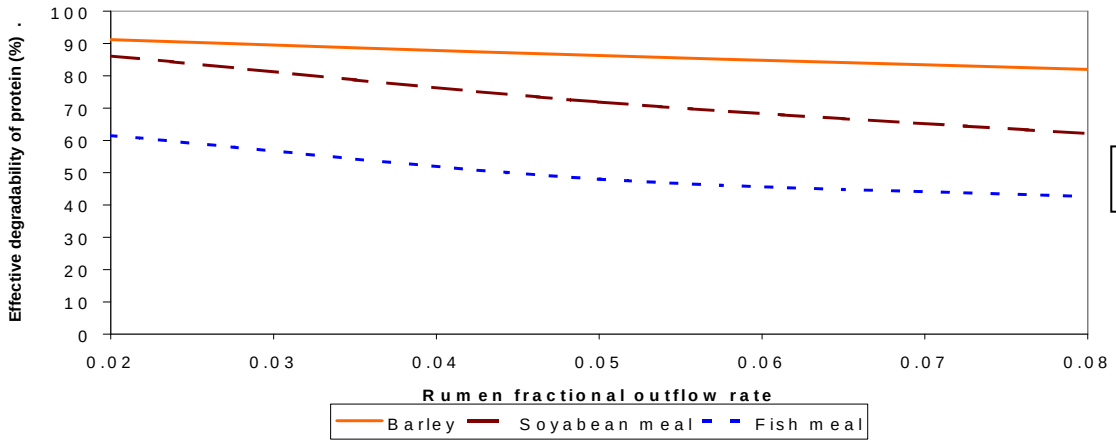

Fig 17

Figure 17. Effect of rumen fractional outflow rate on effective protein degradability. Proteins of intermediate degradability with a large potentially degradable pool (b) but an intermediate degradation rate constant (c) are the most affected.

The effect of rumen outflow rate on effective protein degradability is shown in Figure 17. Example values of feedstuff protein degradabilies for three rumen outflow rates of 2, 5 and 8 % per hour, representing maintenance feeding, a moderate level of feeding at about twice maintenance as in beef cattle, or a high level of feeding at over three times maintenance as in high yielding dairy cows and lactating ewes are given in Table 6.

Table 6. Descriptive parameters of N loss from synthetic fibre bags suspended in the rumen and calculated effective degradability (%) at three rumen outflow rates. (Data from Alderman & Cottrill (1993)).

	Fractional N loss in rumen Parameters			Effective degradability (%) Fractional rumen outflow rate /hr		
	a	b	c	0.02	0.05	0.08
Cereals						
Barley	0.25	0.70	0.35	91	86	82
Maize	0.26	0.69	0.01	49	38	34
Wheat	0.45	0.51	0.38	93	90	87
Cereal co-products						
Dried brewers grains	0.05	0.65	0.05	51	38	30
Distillers grains, maize	0.32	0.46	0.05	65	55	50
Maize gluten feed	0.61	0.36	0.09	90	84	80
Maize gluten meal	0.08	0.76	0.03	54	37	29
Rice bran	0.29	0.60	0.06	74	62	55
Wheet feed	0.34	0.57	0.11	82	73	67
Legume seeds						
Beans, V. faba	0.42	0.56	0.16	92	85	79
Lupins	0.26	0.73	0.13	89	79	71
Peas	0.56	0.44	0.09	92	84	79
Oilseed meals						
Cottonseed meal	0.33	0.60	0.06	78	66	59
Groundnut meal	0.27	0.77	0.09	90	77	68
Linseed meal	0.38	0.60	0.10	88	78	71
Palmkernel meal	0.24	0.70	0.07	78	65	57
Rapeseed meal	0.32	0.61	0.16	86	78	73
Soyabean meal	0.10	0.90	0.11	86	72	62
Sunflower seed meal	0.30	0.65	0.17	88	80	74
Animal products						
Feather meal	0.13	0.77	0.01	39	26	22
Fish meal	0.30	0.63	0.02	62	48	43

Degradability alone is not sufficient to describe the value of feed protein. Undegraded protein leaving the rumen must also be digested in the small intestine. Microbial protein has a true digestibility of 85% in the small intestine. Feed proteins are generally well digested but values can range from 50 to 90%. The amino acid composition of the digested protein is as important as for the monogastric animal. In the main the amino acid composition of the undegraded protein is similar to that of the feed protein (Rulquin & Vérité, 1993). Reasonable estimates of individual amino absorption from the intestine can be obtained by multiplying the true N digested by the amino acid/ N ratio of the original feed. Ruminant grade fish meal is a protein shown to give beneficial response in ruminants in many situations. The ruminant grade material is prepared using fresh raw material so there has been little autolysis and consequently the soluble N content (parameter a) is reasonably low. It is processed under regular heat conditions that reduce the extent of degradation of the insoluble fraction and give high levels of undegraded protein. This is well digested in the small intestine and the amino acid composition, rich in methionine and lysine , complements the first two limiting amino acids of microbial protein. Fish meal has been used in many trials as a positive control to test the efficacy of other, possibly cheaper, proteins.

Many processes have been studied to reduce the rate and extent of degradation of proteins in the rumen. The aim is to reduce the amount of excess production of ammonia in the rumen and to increase the supply of amino acids to the intestine. This process is mainly of advantage for proteins of good amino acid balance. There is no point in possibly reducing the production of good quality microbial protein by restriction of degradable N in order to provide an unbalanced source of undegradable feed protein. Heat treatment of feedstuffs decreases effective degradability and increases the supply of amino acids to the intestine. The formation of new S-S crosslinks is one factor. Splitting the S-S crosslink with reducing agents increases degradability. Soyabeans are normally heated to reduce trypsin inhibitors and consequently the normally processed meal already has a slow rate of degradation. Rapeseed meal is not normally subjected to the same degree of heat treatment in extraction of the oil. Additional heat treatment of rapeseed meal markedly reduces the degradability. Treatment with aldehydes such as formaldehyde and glutaraldehyde also reduce degradation in the rumen but by cross linking between lysine residues can also lead to reduced intestinal digestibility of the undegraded protein. Reaction with sugars, particularly xylose present in lignosulphonate binders, under mild conditions to form earlier Maillard compounds also reduces degradation at the expense of some lysine but without too much loss of intestinal digestibility (Wallace & Falconer, 1992; Harstad & Prestløken, 2000).

Amino acid supplements are rapidly broken down in the rumen and very little survives to reach the rumen, even at high rumen outflow rates. Methionine, has been successfully protected by a number of coating techniques, principally using lipids or pH-sensitive polymers but these techniques are more difficult to apply with the more polar lysine. The first two limiting amino acids in practical ruminant diets are methionine and lysine. The initial estimates of the requirements for these two amino acids for dairy cows are 2.5% and 7.3% of intestinally digested protein respectively (Rulquin & Vérité, 1993) values that are comparable to 1.8 and 7.0 of the ideal protein pattern for pigs. Trials in which diets have been supplemented to these levels have shown marked response in milk production and protein content of the milk (Sloan, 1997). This level of lysine can be achieved by maximising microbial protein (8.1 % lysine in total amino acids, Storm & Ørskov, 1983) and supplementation with a lysine rich protein concentrate such as soyabean meal (6.3 % lysine in total amino acids). However, if maize grain is used as a major energy or maize gluten meal as a protein supplement the high undegradable protein with low lysine contribution from these feeds makes lysine limiting. Similarly feather meal, although of low degradability, has a very low lysine content. The methionine requirement is not so easily reached. Rumen bacteria are also a good source of methionine (2.5 % methionine and 1.0% cystine in total amino acids, Storm & Ørskov, 1983) but soyabean meal (1.5 % methionine in total amino acids) and most other vegetable protein concentrates are low in methionine. Only by inclusion of a portion of fish meal (3.1 % methionine and 1.0% cystine in total amino acids) or protected methionine can the ideal balance of intestinally absorbed methionine be achieved.

Immunological effects of dietary proteins

Protein concentrates should not be thought solely in terms of their supply of indispensable amino acids and a source of dispensible amino acid N. Proteins have effects on the immune system. Studies of protein-energy malnutrition in children emphasised the role of protein deficiency in impairing cell mediated immune responses. Animal studies confirmed that protein deficiency reduces immune status. The role of individual amino acids is less clear. Phenylalanine and tyrosine restricted diets actually enhance cytotoxic immunity in tumour-bearing animals, reducing tumour growth and spread (metastasis). In contrast, excess arginine depressed tumour growth by 50%. Deficiency of branched chain amino acids and of arginine + lysine increased splenocyte proliferation but sulphur amino acid deficiency decreased splenocyte and lymphocyte proliferation. Increasing dietary methionine increases the lymphocyte response to stimulation with phytohaemagglutinin with the maximum response at a greater level than needed for maximum growth rate. Supplemental cysteine was also effective, having approximately 70% of the response to methionine (Tsiagbe et al., 1987; Austic et al., 1991; Konashi et al., 2000).

More recent studies have examined the effects of glutamine and arginine in enhancing the immune system. Glutamine is preferentially metabolised by the intestinal mucosa and by lymphocytes. By maintaining mucosal cells it improves the gut barrier function against bacterial infection. As a precursor for glutathione (GSH) it helps maintain the antioxidant status of cells, especially the intestinal mucosa and lymphocytes. Inhibition of glutathione synthesis leads to degeneration of mitochondria and structural damage to many tissues including skeletal muscle and lung but especially to fast turning over tissues such as intestinal mucosal cells (Mårtensson et al., 1990). The GSH level in lymphocytes is a very critical, decreasing with oxidative stress in a number of disease situations with loss of immunocompetence (Dröge & Breitkreutz, 2000; Grimble, 2001). The best studied case of GSH deficiency is human immunodeficiency virus (HIV). Not only is the extent of GSH depletion prognostic of the onset of AIDS but supplementation with N-acetyl cysteine restores GSH levels and prevents progression of the disease (Herzenberg et al., 1997). Giving whey protein isolate to HIV+ patients also increased GSH levels and improved body weight (Bounos et al., 1993; Micke et al., 2001). Whey protein isolate is particularly rich in methionine (2.5 g/16 g N) and cyst(e)ine (2.7 g/16 g N) and presumable acts to provide the necessary precursors for GSH synthesis. The importance of maintaining GSH levels is now being demonstrated in farm animals. Steers fed a diet supplying only 60% of maintenance requirement had liver GSH levels reduced to 26% of the control values (Sansinanea et al., 2000). Protein deficient pigs had erythrocyte GSH reduced to 80% of the controls. An inflammatory stimulus further depleted GSH in the protein deficient pigs but was without effect in the protein replete pigs (Jahoor et al., 1995). Nutritional strategies to increase GSH levels are not likely to be beneficial to the immune system in healthy animals but deserve investigation in cases where disease and oxidative stress are compromising the immune response and causing decreased GSH levels. For example, there is evidence of reduced immune response at the onset of lactation in high yielding cows, when body protein reserves are being rapidly mobilised to meet amino acids needs for milk protein secretion, and an increase in susceptibility to mastitis at this time (Piccinini et al., 1999; Mehrzad et al., 2001).

Arginine can affect the immune system by increasing growth hormone with consequent effects on thymus weight and responsiveness and as the substrate for nitric oxide (NO) synthesis. Nitric oxide is important as a local messenger, for example as endothelial relaxation factor involved in the maintenance of blood vessel dilatation and blood pressure control. It is also produced in much greater quantities by activated macrophages. NO is converted by reaction with superoxide radical to peroxynitrite ($ONOO^-$), and during decomposition to the formation of the even more reactive hydroxyl radical ($OH^·$), as the final bacterial killing agents. However, excess peroxynitrite production is also damaging to local tissues, causing nitrosation of proteins and destruction of antioxidants such as GSH. Immune modulating diets including arginine have been shown to be clinically beneficial in humans subjected to traumatic stress enhancing protein synthesis and wound healing. On the other hand excessive NO production contributes to increased gut mucosal permeability and bacterial translocation across the mucosa (Suchner et al., 2002). Animal studies have also given conflicting results. Channel catfish (*Ictalurus punctatus*) fed either a purified diet containing 2% arginine or a practical diet with 1.3% arginine were challenged with a virulent strain of *Edwardsiella ictaluri*. The arginine enriched diet reduced mortality (Buentello & Gatlin, 2001). Coccidiosis in poultry increases plasma nitrite + nitrate (measure of NO production) and reduces plasma arginine but dosing with additional arginine did not reverse the growth depression, did not increase plasma nitrite + nitrate and did not reduce lesion scores (Allen, 1999; Allen & Fetterer, 2000).

Spray-dried blood plasma is used in USA in diets for early-weaned pigs. Numerous trials have shown increased feed intake and growth compared with other protein sources during the first two weeks after weaning but little response thereafter (Coffey & Cromwell, 2001). The improved growth is largely brought about by improved feed intake in the critical transition period. The reasons for this are not fully known. Weaning at three weeks of age is at a time when plasma immunoglobulin levels are at their lowest. Colostrum antibodies have declined but production of immunoglobulins by the piglet is only just beginning. The response to blood plasma is greatest in commercial environments and less in clean experimental conditions suggesting the involvement of antigenic challenge to an immature immune system. The response has been shown to be associated with the immunoglobulin fraction leading to the current hypothesis that immunoglobulins, especially IgG, bind with viruses and bacteria in the intestinal lumen, prevent adherence to the mucosal cells, prevent damage to the mucosal cells, shortening of villi and loss of absorptive surface and maintain digestive enzyme activities.

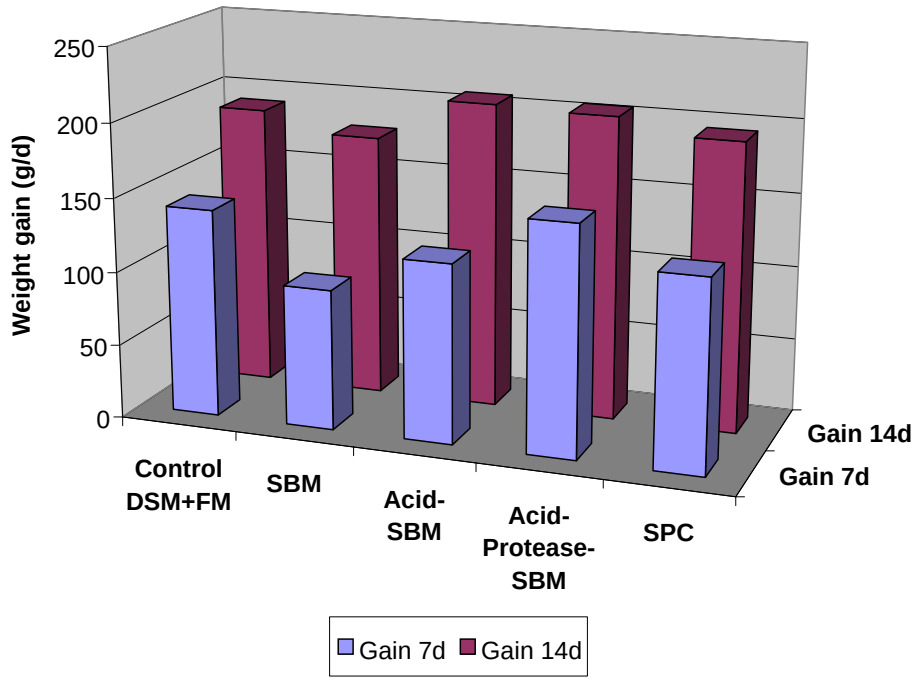

Figure 18. (Above)

Figure 18.
Growth of piglets in the first week and first two weeks after weaning onto diets containing dried skim milk + fish meal (Control DSM+FM), commercial heat-treated soya (SBM), SBM treated with acid (Acid-SBM), SBM treated with acid and protease from Aspergilus species (Acid-Protease-SBM) or soy protein concentrate (SPC; Danpro A-02). Data from Rooke et al., (1998).

Dietary proteins, particularly the legume proteins, also have antigenic properties. These may have adverse effects on intestinal morphology, on intestinal myoelectric activity affecting the rate of passage of digesta and growth of calves fed milk replacers (Lallès, 1993). In the newly weaned pig they have transient effects, reducing growth in the first week post weaning, until tolerance is developed (Miller et al., 1994; Rooke et al., 1998; Figure 18). Similarly, soyabean meal and an alcohol extract of soya bean meal (soybean molasses) caused inflammatory responses in the distal intestine of salmon (Krogdahl et al., 2000). These reactions may predispose to infections. In both calves and pigs these adverse reactions may be accompanied by diarrhoea and death. The allergenic proteins, glycinin and β-conglycinin, in soya are resistant to normal proteolytic digestion either in the abomasum or intestine. Both can be detected immunologically in the duodenum. β-conglycinin is most resistant to acid digestion in the stomach whereas glycinin is more resistant in the intestine and can still be found in ileal digesta (Sissons & Thurston, 1984; Lallès et al., 1999). Apparent N digestibility in the calf of commercial soya preparations varies greatly and is best predicted by the concentration of immunoreactive β-conglycinin (Lallès et al., 1996). The antigenicity of these storage proteins is not removed by normal solvent extraction, heating and steam desolventisation. They can be denatured by hot aqueous ethanol or by partial acid or enzymic hydrolysis.

Protein-rich feeds as sources of nutrients other than amino acids.

Protein concentrates are also a source of many other nutrients that should be taken into account when formulating diets. These include the major minerals, Ca, P, Na, K, Cl, vitamins, including B_{12}, choline and vitamin D, and essential fatty acids. Consideration should be given to these nutrients because they may be either beneficial or in some cases can be at such high levels as to be detrimental and limit the inclusion level.

Fish meal and meat and bone meal are good sources of calcium and phosphorus, in an ideal ratio of 2:1 and these are of high availability when included in diets for mammals or birds. Plant protein concentrates have much lower levels, especially of calcium, with a ratio more in the region of 1:2. Furthermore, the phosphorus is mainly present bound as phytate so the total phosphorus is about 1/3 available for poultry and fish. The deficiency of calcium in both cereals and plant protein concentrates is readily and economically corrected with limestone but supplementary phosphorus sources are expensive. The high level of phytate P also leads to high faecal P output and environmental pollution. Phosphorus is the main cause of eutrophication in aquaculture. In many countries legislation limits the amount of P that can be disposed in manure on land. This has given added impetus to the development of phytase enzyme that can be added to the diet to hydrolyse phytic acid and improve the availability. Consequently, dietary P levels can be reduced and less P is excreted. In aquaculture much of the calcium requirements are obtained by uptake from the water but P must be supplied from the diet.

The digestibility in fish of P from fish meal is surprisingly low and variable and appears also to be inversely related to the ash content (NRC 1993). Replacing a small part of the fish meal (51.8% reduced to 41.0% of diet) with 20% soyabean meal, or canola meal or peanut meal increased diet true digestibility of P from 21.5% to 40.6 - 43.4%. The replacement reduced total P from 1.74% to 1.5 -1.6%. If the same amount of P was absorbed this reduction in intake would account for an increase in true digestibility to 24.6%. The much larger increase in digestibility reflects a 70.7% increase in the amount absorbed despite replacing fish meal P with mainly phytate P of zero digestibility (Riche & Brown, 1999). The reduction in calcium supply by substitution of the part of the fish meal is the most likely cause of the improved digestion of P.

Appetite or voluntary feed intake is important in all species but especially so in aquaculture where feeds must first attract fish or crustaceans and then be palatable to be accepted. Amino acids, betaine and inosine appear to act as attractants. Glycine, proline, taurine and valine appear to be preferred by carnivorous fish while aspartic and glutamic acids are preferred by omvivorous fish (NRC, 1993). Trimethylamine and its oxidation products as well as highly oxidised oil are deterents for salmonids. Thus freshness of fish used in the preparation of fish meal and stability of the oil through use of antioxidants are important factors for quality meals.

Protein-rich feeds as sources of anti-nutritional factors

The legume proteins contain protease inhibitors, lectins, tannins, phytates, antigenic proteins flatulence factors (oligosaccharides), and oestrogens (Huisman & Jansman, 1991). To this list can be added high fibre (non-starch polysaccharides) levels, which limit the inclusion levels in many situations, and contamination with mycotoxins. The brassicas contain glucosinolates, tannins, phytate and have high fibre levels. The relevance of the different factors varies with animal species. Processing is available to deal with several of these problems - dehulling, heating, solvent extraction, addition of enzymes as is appropriate for the target animal species. Plant breeding, as in production of double zero rapeseed meal or canola meal, is another avenue. Reference has been made previously to the different susceptibility of calves, fish and early-weaned piglets to antigenic proteins. For both calves and fish the general principal is the greater the degree of processing of vegetable proteins, with increase in protein content from meal to protein concentrate to protein isolate, the better the performance but also the greater the feed cost. The improvement may be due to removal of a number of the factors but the exact reason is not known. Even using soya protein concentrate with 68% protein content of high digestibility, growth of turbot and salmonids is significantly reduced when more than 50% of the fish meal protein is replaced (Day & Plascencia Gonzalez 2000; Sveier et al., 2001). Studies of digestibility of canola meal for trout also suggest that high levels of fibre, either alone or with phytate, result in poorer digestibility of protein (Mwachireya et al., 1999). Insoluble fibre increases the rate of passage through the intestinal tract while soluble fibre increases the viscosity of the digesta, reduces the diffusion of nutrients to the absorptive mucosa. Pea fibre has been shown to increase the flow of water, mucus and endogenous N to the ileum of pigs. The endogenous N loss was best described as a function of the water holding capacity of the diet (Leterme et al., 1998). Antigenic proteins may also enhance the turnover of intestinal mucosal proteins. Desquamated epithelial cells and mucus in turn encourage the growth of bacteria in the intestine. Bacterial degradation of this protein may result in production of ammonia, which is absorbed and lost via urine. True endogenous faecal N loss is then underestimated and digestibility overestimated. In addition, and possibly of greater concern, is the additional energetic costs of enhanced intestinal protein turnover.

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