Chapter 22
Requirement for and evaluation of dietary protein and amino acids

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This chapter:

- Describes the use of amino acids for maintenance and protein accretion
- Describes how protein quality can be assessed by in vitro and in vivo methods
- Defines the differnce between requirements and recommendations
- Describes the central elements of the Danish protein evaluation system
- Quantifies the absorption of individual amino acids

1. Introduction

The quality of protein as a feedstuff will depend on the efficiency with which it is converted into muscle deposited by the animal. The major determinants for this are the digestibility and the presence and ratio of essential amino acids in relation to the requirements of the animal. The protein quality of a feedstuff can be determined “in vitro” (Latin for “within the glass”, i.e. in a laboratory) or “in vivo” (Latin for “within the living”, i.e. using living animals). The benefit of in vitro compared to in vivo is that the evaluation is quicker and cheaper.

The main purpose of this chapter is to provide the reader with knowledge about the different in vivo and in vitro methods that are used in evaluating protein quality and requirements for protein and amino acids. Finally, the Danish system for protein evaluation is presented.

2. Amino acids in physiological processes

As it appears from Chapters 13 and 14, amino acids are a central part of the energy metabolism. However, in pigs fed a balanced diet and housed under proper conditions, oxidation of amino

acids for production of energy is not the main purpose of the amino acid supply. The fundamental function of amino acids is as building blocks in protein synthesis, where translation of the DNA-encoded mRNA dictates the order of amino acids making up the novel peptides and proteins. If an undersupply of energy takes place, amino acids are used as energy source. The need for a daily supply of amino acids varies among individual amino acids. As described in Chapter 9, some amino acids can be synthesized de novo and therefore do not need to be supplied in the feed. This is in contrast to the group of amino acids often called the essential or indispensable amino acids that must be supplied through dietary means. The latter group is by far the most important, since the essential amino acids are a main determinant of the level of production that can be obtained.

The qualitative and quantitative need for amino acids depends on which proteins need to be synthesized. When composing a diet, it is therefore important to address the specific physiological roles that should be satisfied in a given production. From the opening chapters of this textbook, it is evident that the major issues of pig production concern foetus development, milk production and growth, which are all processes where protein accretion is the central element. The physiological use of amino acids can be divided into two distinct, although dependent uses: amino acids for maintenance and amino acids for protein accretion.

2.1. Amino acids for maintenance

The amount of amino acids that should be supplied to maintain nitrogen equilibrium, i.e. to prevent loss of body proteins, is the maintenance requirement. This measure has no practical relevance since nitrogen for maintenance and nitrogen for growth are connected in growing animals. However, a distinction between the need for amino acids for maintenance and amino acids for growth illustrates the physiological importance of amino acids. The maintenance requirement covers losses of amino acids from the body surfaces such as skin and hair, basal endogenous loss from the gastro-intestinal tract as described in Chapter 9, use of amino acids to synthesize hormones, enzymes and non-protein nitrogen-containing compounds, and for cell renewal. Finally, an important aspect of maintenance includes the basal turnover of proteins.

There is a continuous turnover of proteins as illustrated in Figure 22.1. Proteins are broken down to amino acids contributing to an intracellular amino acid pool, from which new proteins are synthesized. The maintenance of proteins does therefore not by itself necessarily increase the demand for amino acids. The reutilization of body proteins to synthesize new proteins, however, is not completely efficient as some amino acids are irreversibly modified into e.g. hydroxyproline, hydroxylysine and 3-methyl histidine. The majority of the modified amino acids are excreted and some are reused to form other molecules.

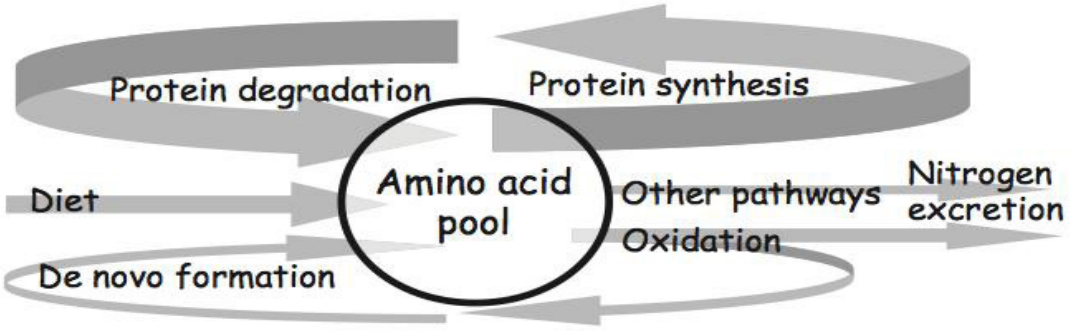

Figure 22.1.The amino acids used in protein synthesis may originate from proteins degraded during the continuous turnover of body proteins (Source: WHO technical report series no. 935, 2007).

Estimates of the protein requirements for maintenance were calculated for a growing pig. The requirement can be divided into different compartments in relation to the metabolic body weight ($kg^{0.75}$): surface loss, 0.10 g /$kg^{0.75}$ per day; protein turnover, 0.36 g /$kg^{0.75}$ per day; and basal endogenous loss from the gastro-intestinal tract, 0.64 g /$kg^{0.75}$ per day. These losses and other basal losses sum up to 1.73 g /$kg^{0.75}$ per day, which for a growing 60 kg pig corresponds to 37 g protein per day or approximately 10% of the total dietary intake of digestible crude protein. The protein turnover caused by the losses of specific endogenous protein is related to specific properties of the individual feedstuffs.

2.2. Amino acids for protein accretion

Early attempts to evaluate protein quality of feedstuffs included comparison of the amino acid composition of the feedstuff in question to egg white, milk and meat. The rationale of mimicking the amino acid composition of e.g. meat in diets for meat-producing animals is straight forward. However, several factors influence the utilization of the dietary protein and together with a complex amino acid metabolism, it is impossible to obtain maximal growth without adjusting the amino acid composition and quantity to the production type and level that the diet is meant to support. Consequently, the Danish amino acid recommendations for pigs include specific amino acid profiles for weaners, growers/finishers, gestating sows and lactating sows.

Not only have the different types of productions different demands for amino acids, but the types of proteins synthesized during growth are not constant for every level of dietary protein intake. When the supply of amino acids increases just above the level for maintenance, the N-balance becomes positive with especially accretion of visceral proteins and surface proteins. The accretion of muscle proteins, however, will not occur until the protein supply is sufficient to support a protein synthesis that is higher than the continuous protein degradation. Thus, although the amino acid composition of proteins is genetically encoded, the actual need for individual amino acids will change continuously according to protein intake and animal performance. The development of amino acid recommendations must therefore be based on experiments with animals of the same type and level of production that is also characteristic for the animals for which the recommendations are intended. This is the reason why recommendations change over time and may differ between breeds, housing systems, management regimens and countries.

The efficiency of amino acid utilization declines with increasing intake of dietary protein. This is primarily caused by two main factors. Firstly, the digestibility of nitrogen decreases with increasing feed intake, which is an issue concerning most other macro and micronutrients. Secondly, the utilization efficiency is dependent on the energy supply and thus intake of carbohydrate and fat. At lower levels of dietary protein intake, amino acids will be used for protein synthesis with high efficiency because protein is most limiting. As the protein intake increases due to higher concentration of protein in the feed, the protein synthesis increases as well, but the demand for energy for the anabolic processes increases too, and energy supply becomes most limiting. This results in increasing amino acid oxidation for production of energy to drive the processes, resulting in a lower proportion of dietary amino acids used for protein synthesis. It is therefore evident that sufficient energy supplied through carbohydrates and fat is a prerequisite for optimal utilization of dietary proteins. The Danish recommendations are based on the concept of having an appropriate balance between energy and protein by expressing the need for protein and amino acids on an energy basis, i.e. in grams per feed unit.

3. Evaluating protein quality

Chemical analyses of feedstuffs or complete diets can provide basic information about the quantity of nutrients in a particular feedstuff or diet (e.g. the concentration of fat, carbohydrates, minerals, vitamins or amino acids). The composition of amino acids in the protein to be evaluated can be determined by ion exchange chromatography after 23 hours of hydrolysis in HCl (see Chapter 9).

The protein quality can be determined partly by the quantity of essential and non-essential amino acids, and partly by the digestibility of the amino acids indicating the amount that can be absorbed from the gut and utilized, see Chapter 9.

Digestibility may be influenced by a number of factors not related to the feedstuff per se. Such factors are, e.g., 1) feed processing, 2) feeding regimen and form, e.g., dry, soaked or liquid diets, and 3) the animal eating the feedstuff. Furthermore, in diets including several feedstuffs, digestibility of single feedstuffs may be affected by other feedstuffs. Consequently, the method of determining the digestibility of feedstuffs and complete diets should consider all these factors. Therefore, in vitro methods that are rapid, reproducible and also able to support a reliable estimation of digestibility of the actual feed sample are of considerable importance in feed evaluation in the absence of less cost-effective in vivo results.

3.1. In vitro methods of evaluating protein digestibility

In pigs, several in vitro methods of assaying protein digestibility are available. The methods are based on incubation of feed to simulate digestion and have been refined over time. The official Danish in vitro method of estimating crude protein digestibility is called the ileal enzymatic digestible nitrogen assay, also known as “the Boisen Method” after its developer Sigurd Boisen of Research Centre Foulum.

The Danish in vitro method of crude protein digestibility involves two steps:

- Incubation of feedstuff /diet at pH 2 with pepsin,
- Incubation at pH 6.8 with pancreatin.

Following filtration, undigested crude protein is measured (N in residuals) and the proportion of digested crude protein (or crude protein digestibility) is calculated. It is assumed that all amino acids have the same digestibility as crude protein. Whilst this is not strictly correct, the evaluation of individual amino acid digestibility is currently too expensive in relation to the added value it can provide.

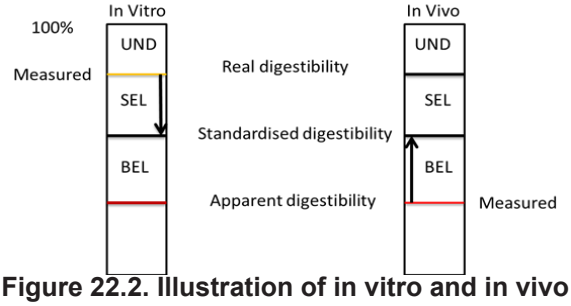

Figure 22.2. Illustration of in vitro and in vivo digestibilities of feed. UND = undigested crude protein; SEL = specific endogenous losses of protein; BEL = basal/unspecific endogenous losses of protein.

An important factor to keep in mind is that in vitro digestion does not take account of any endogenous losses, which is accounted for in vivo. Figure 22.2 illustrates differences between in vitro and in vivo digestibilities. Experimentally, the amount of undigested dry matter has been found to correlate with endogenous losses of protein. Therefore the amount of undigested dry matter (UDM) is used to calculate standardized ileal digestibility (SID), which is the value that is used for feed formulation and therefore the value that is essential for feed evaluation. Thus, during in vivo experiments, apparent digestibility is measured and a correction for the basal/unspecific endogenous loss of crude protein (fixed value, approx. 13 g N/kg dry matter) is made to achieve a value for standardized digestibility. During in vitro analysis, real digestibility is measured and corrected for the specific endogenous loss of crude protein (calculated from undigested DM) is made to achieve a value for standardized digestibility.

3.2. In vivo methods of evaluating protein quality

There are several options of evaluating protein quality using in vivo methods:

- digestibility studies
- N-balance
- performance trials (slope assay method)
- plasma urea nitrogen (PUN) or indicator amino acid oxidation.

Performance trials require more animals per experiment than any of the other methods discussed here, which may be perceived as a reason to pursue other methods of evaluation. The slope assay method is often used to establish both requirement for and availability of amino acids. The digestibility method of evaluating amino acids (and minerals) in feedstuffs is commonly used, whilst other methods that assess the availability of amino acids (and minerals) in feedstuffs are used less frequently today.

3.2.1. Slope assay method

With the use of the slope assay method, the protein source to be evaluated is included in the diet to replace the basal diet at increasing rates. The response parameters (e.g. growth) are compared to a standard curve. The standard curve is made from the basal diet with increasing concentrations of the first limiting nutrient (e.g. lysine). The slope of the test product relative to the standard curve is defined as availability. However, only one amino acid can be evaluated at a time which is in contrast to the digestibility method with which the digestibility of all amino acids can be measured at the same time.

3.2.2. Digestibility

In 1973, T. Zebrowska was the first to show that crude protein (nitrogen) that was absorbed (disappeared) in the hindgut had no biological value. The implication is that amino acids that disappear in the hindgut, and any form of nitrogen absorbed in the hindgut, will not contribute to the supply of essential amino acids. A consequence of this is that protein (amino acid) digestibility has to be measured at ileal level and not at total tract level to reflect the level of amino acids that are absorbed and therefore can be used for metabolic processes.

Ileal digestibility of amino acids in pigs can be measured either using the slaughter method or by using cannulated pigs. With either method, it is possible to measure the digestibility of all amino acids at the same time. Markers (undigestible substances added to feed) are used if only random sampling (typical for digesta) is applied as opposed to total collection. The equation of calculating the apparent ileal digestibility (AID) is:

$$\text{Apparent ileal digestibility (AID), \%} = [1 - (AA_{digesta}/AA_{diet}) \times (Marker_{diet}/Marker_{digesta})] \times 100$$

where the amino acid concentration in the feed (AA_{diet}) and ileal ($AA_{digesta}$) samples are related to the marker concentration in the feed ($Marker_{diet}$) and ileal ($Marker_{digesta}$) samples. With the slaughter method, the experimental pigs are slaughtered and the last quarter of the small intestine is tied off and its' content collected. The content is analysed for amino acids and marker content (same procedure for cannulated pigs). The cannulation method involves surgical fitting of a cannula approximately 15 cm anterior to the ileal-caecal junction. Ileal collection takes place by opening the cannula and collecting digesta into a bag attached to the open cannula. Collection is typically done over two days between morning feeding and afternoon feeding. Before collecting ileal samples, an adaptation period of 4-5 days (minimum) is needed to introduce the experimental feedstuff. It has been found that the digestibility of nutrients does not differ between day and night. Different cannulas can be used, but the most commonly used cannula is the simple T-cannula. Comparison of different cannulas has not revealed any significant differences in results once the basal endogenous losses are taken into account. It has been proven experimentally that the slaughter method requires more animals to achieve the same level of accuracy as the simple T-cannula method.

The measurement of ileal digestibility is defined as the apparent ileal digestibility. The loss of amino acids at the end of the small intestine is made up of three different fractions:

- non-digested amino acids
- basal endogenous losses
- specific endogenous losses

For details, see Chapter 9 and Figure 22.2. Both non-digested amino acids and specific endogenous losses are feedstuff specific whilst basal endogenous losses are animal specific. The standardized ileal digestibility is calculated by correcting the apparent ileal digestibility for basal endogenous losses. Standardized ileal digestibilities are additive, whilst apparent ileal digestibilities are not, and therefore standardized ileal digestibilities are used for feed formulation.

3.2.3. Reactive lysine in heat-damaged feed

Measurement of the digestibility of reactive lysine instead of total lysine is a method that recently became wide-spread for evaluation of lysine digestibility. The theory behind this methodology is that during feed processing, there is a risk that lysine will undergo Maillard reaction (see Chapter 9), which will make part of the lysine unavailable for metabolic processes. Measurement of total lysine will therefore be misleading as it includes the unavailable fraction. The unavailable fraction can be calculated from the content of furosine, which can be measured at the same time as total lysine. It is far more common to determine the reactive lysine content by letting true lysine react to homoarginine and measure the homoarginine content, which is done when measuring other amino acids. When method with the reactive lysine is applied, only the true lysine is measured as reactive lysine. There are some problems with this method that need to be solved before it can become a standard procedure, but it definitely has potential. In a feedstuff such as distillers dried grains with solubles (DDGS), the reactive lysine method has been found to underestimate the content of digestible lysine.

3.2.4. N-balance

With the N-balance method, the difference between N intake and N excreted in faeces and urine is measured. Whilst N excreted in faeces gives an estimate of apparent N digestibility, the N loss in urine represents the N loss either due to physiological processes, surplus N or imbalanced amino acid composition. To measure N-balance, pigs need to be housed in metabolic cages so that faeces and urine can be collected separately. For a typical N-balance trial, an adaptation period of 5-10 days is used, followed by a collection period of 3-7 days. In general, it can be assumed that a longer collection period will result in more accurate measurement. Adding an undigestible colour marker to the feed (e.g. iron-oxide) to colour the faeces at the start of the collection period will allow individual start collection times to be determined. Similarly, the marker is again added at the end of the collection period, so individual end collection times can be determined. A variation of up to 48 hours in collection end time is not uncommon for a group of 12 animals. Faeces and urine are collected twice daily and stored in a fridge or freezer during the collection period. Once the collection period is completed, either all the collected faeces is dried or, more commonly, a sub-sample is dried (oven- or freeze-dried). The nutrient content in urine samples is typically measured directly; alternatively the urine can be freeze-dried. Freeze-drying is typically conducted before measuring gross energy (GE). Often the same animal will be fed different diets during the experimental period, which ensures that the animal becomes its own control. N-balance studies can be conducted with pigs or model animals such as rats. N-balance studies are often used in relation to environmental research where the output of N is an important evaluation factor.

3.2.5. Performance

Performance studies can be conducted for comparison of protein quality and are often used to compare different batches of the same feedstuff. In a performance study, average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR) are recorded. These studies will typically run over a few weeks. Animals are often housed in groups and each group is fed one experimental diet for the duration of the experiment. Performance studies can be conducted with either pigs or model animals such as rats.

3.2.6. PUN

Plasma urea nitrogen (PUN) measures urea content in the plasma. The urea concentration is of significance as it indicates a protein imbalance or a surplus in supplied protein. If the pig is fed a diet that is unbalanced in amino acids and the first limiting amino acid is supplied, the concentration of urea will decrease because all other amino acids are more efficiently utilized for protein accretion and thus not catabolized. Measuring PUN to indicate efficiency of protein utilization is performed in many types of performance experiments on protein and amino acids. An alternative approach using PUN measurement is based on the short time for reaching a steady state in PUN concentrations, which takes less than 48 h to achieve, meaning that pigs can be fed a new diet and the response to it can be seen 48 h later. If, for example, five different diets and five pigs are used, it takes only ten days to conduct a trial using a Latin square design, which can be repeated with the same animals and diets, and in that way additionally investigate if requirements change with age and weight.

3.2.7. Indicator amino acids oxidation method

The indicator amino acid oxidation method is a further development of the PUN concept; achieving a steady state (48 h) followed by the measurement of the oxidation response of one amino acid. This is the amino acid that is supplied in excess of protein synthesis and therefore oxidized

by the body. Furthermore, when an amino acid is provided at a deficient dietary level, most of this amino acid will be used efficiently, and protein synthesis and oxidation of the limiting amino acid will remain low and constant. As the supply of the amino acid increases above the requirement for protein synthesis, increased catabolism of the amino acid ensues. The oxidation is measured using an isotope-labeled amino acid (e.g. ^{15}N or ^{13}C labeling) because this provides a sensitive means of estimating the partition of that amino acid between oxidative and non-oxidative (protein synthesis) disposal. The inflection (break point) in the oxidation curve has been suggested to represent the physiological requirement for the test amino acid.

3.3. Ideal protein concept

In 1981, the ideal protein concept was introduced by ARC (Agricultural Research Council, UK) as a method to determine protein requirements. The ideal protein concept is based on the theory that the ratio of amino acids required is constant. Therefore it is only necessary to test the requirement for one amino acid to calculate the requirements of all amino acids. Lysine is used as the baseline (typically the first limiting amino acid in a cereal based diet) and the requirement for other amino acids are expressed relative to the requirement for lysine. The ideal profile has been reviewed several times and different profiles have been reported. The profiles used in different countries differ slightly due to different experiments that are taken into account to determine the ideal protein profile, but also genotype, production goals, amino acid analyses and other circumstances differ between countries.

Nitrogen metabolism in pigs is illustrated in Figure 22.3. Optimizing protein intake aims at reducing the excretion of nitrogen in faeces and urine by feeding diets with highly digestible protein, a protein profile close to ideal protein and no more protein than the animal's requirement. Additionally, feeding feedstuffs that will result in low specific endogenous protein losses reduces the N output in faeces and urine with obvious environmental and economic advantages.

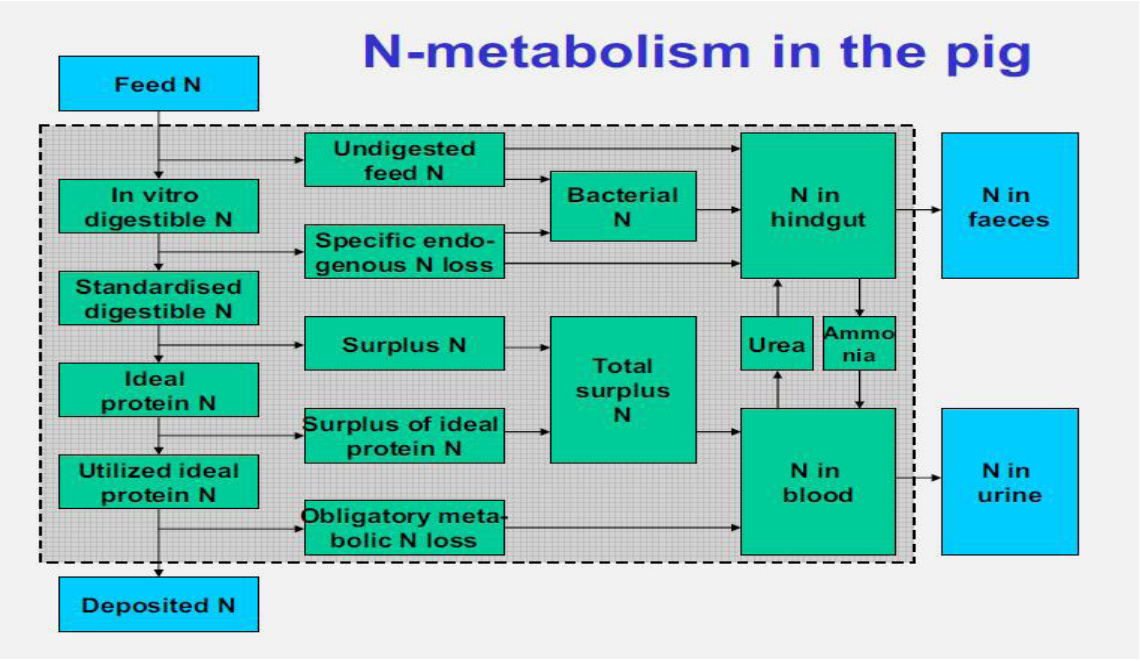

Figure 22.3. Nitrogen metabolism in pigs.

The amino acid profile of sow milk was initially considered as the optimal profile for piglets, and was therefore used as basis for the first recommendations. There is, however, a difference between the amino acid profile of sow milk and ideal protein for growth. The initial assumption was

based on the fact that the profile is very constant and apparently not influenced by the dietary feed intake of the sow. In Table 22.1, the amino acid composition of sow milk, whole body, deposited protein during growth, ileal endogenous losses and hair is reported.

Table 22.1. Amino acid composition (g amino acid/160 g N (approx. 1 kg crude protein)) of sow milk compared with the composition in whole body, deposited protein during growth, ileal endogenous losses, hair and ideal protein.

Amino acid	Sow milk	Whole body	Deposited	Endogenous losses	Hair	Ideal protein for growth, piglet feed (SID)
Lysine	71	66	69	30	33	73
Threonine	39	39	38	45	59	44
Methionine	18	19	19	10	4	23
Cystine	13	11	10	16	134	16
Tryptophan	12	8	n.d.[1]	12	n.d.	15 (16)[2]
Isoleucine	41	35	40	25	35	39
Leucine	81	72	77	40	77	74
Valine	54	48	51	35	60	49
Histidine	25	29	32	15	11	23
Phenylalanine	39	39	37	30	23	41
Tyrosine	42	27	28	20	9	39

1) n.d., not determined. (Boisen, 2007)
2) 20% SID Trp:Lys: 15 ; 22% SID Trp:Lys: 16

4. Protein and amino acid recommendations

Formulation of a complete feed for animals requires two elements: the digestibility of amino acids and other nutrients (including energy content) of each feed ingredient, and the requirement of the animal. Amino acid requirements can vary greatly between animals of the same weight. This variation has been estimated to be more than 15%, which partly explains variations in slaughter weight and age at slaughter for pigs housed under the same management system.

4.1.Requirement vs recommendations

Recommendations are based on requirements of the animals within a group. The requirements are estimated based on experimental data obtained in e.g. performance trials, PUN, N-balance and oxidation experiments. Performance trials are, however, the most commonly used method. It is important that recommendations are made for groups of animals rather than individuals, since feed formulation for individuals is not possible under practical conditions. Recommendations are generally based on the average of the group to achieve the most economic results or best animal performance.

Sometimes recommendations are purposely lower than requirements for reasons of animal health, economics or environmental concerns. For example, in herds where problems with diarrhoea in weaned piglets are common, low protein diets have been found to successfully reduce the frequency and intensity of diarrhoea. This is part of a recognized strategy to reduce antibiotic use in Danish pig production. Recommendations are sometimes made to achieve the economic optimum rather than maximum level of performance. For example, the Danish recommendation for tryptophan (20% of lysine level) is below the requirement for maximum growth (21-22% of lysine level) as the price of tryptophan so far has been too high to support a recommendation that would result in maximum production.

4.2. Estimating amino acid requirements

Estimations of amino acid requirements have been made for decades, and it is a research area that will continue to be explored in the future, as breeding progress and changes in genotype are likely to lead to changes in amino acid metabolism. Furthermore, the commercial availability of single amino acids can be expected to increase. There are currently five single amino acids available for commercial pig production on the market; L-lysine, L-threonine, DL-methionine, L-tryptophan and L-valine. These amino acids are typically the most limiting amino acids in diets for young pigs. The production cost of other essential amino acids is currently not economically viable for commercial pig production.

In Denmark, amino acid recommendations are evaluated regularly for several reasons:

- new knowledge suggesting that the recommendations should be changed
- changes in feed or commercial amino acid product prices
- new commercial essential amino acids becoming available
- environmental restrictions
- breeding progress may have caused requirements to change.

To establish the optimal dose of an essential amino acid relative to lysine (typically the first limiting amino acid), the amino acid in question is fed at different inclusion rates in dose-response experiments. It is important that lysine is fed at a level slightly below the recommendation, so that lysine itself is a limiting factor. This is because the requirements for amino acids are expressed relative to lysine. If the lysine level is too low compared to the recommendation, it might lead to overestimation of requirement for the investigated amino acid as per cent of lysine, and, contrary, if the lysine level is above requirement, the requirement for the investigated amino acid as per cent of lysine will be underestimated. For example, if the optimum Val:Lys ratio is estimated to 70% in an experiment where the lysine concentration was very low (e.g. 9 g SID lysine/feed unit), the corresponding Val:Lys ratio may be 61% in pigs fed sufficient lysine. This deviation is caused by a greater efficiency of lysine utilization in the pigs fed the lower lysine concentration.

Commonly, the tested amino acid is fed at a minimum of five different doses with levels below and above the expected requirement. Once the trial is completed, different statistical models are used for describing the results and for estimating the ratio between the test amino acid and lysine that leads to maximum performance. Of the statistical models, the broken-line and polynomial models are frequently used. The broken-line approach models the results into two straight lines, the first showing the increase in the response parameter with increasing dose, and the second a horizontal line indicating the maximum response as a plateau. The intersection of the two lines in the broken-line model estimates the optimum test amino acid to lysine (AA:Lys) ratio. The polynomial models describe the results by a smooth curve that can have a horizontal plateau or a decreasing curve at greater doses. The optimum test amino acid to lysine ratio is often defined as 95% or 100% of the dose modeled to result in greatest response. The choice of statistical model affects the conclusions to a large degree. Therefore, it is often seen that experiments are evaluated using several statistical approaches, see Figure 22.4. This means that estimation of the optimal ratio is often not one number, but a range, which should ideally form the basis for a recommendation for the entire population.

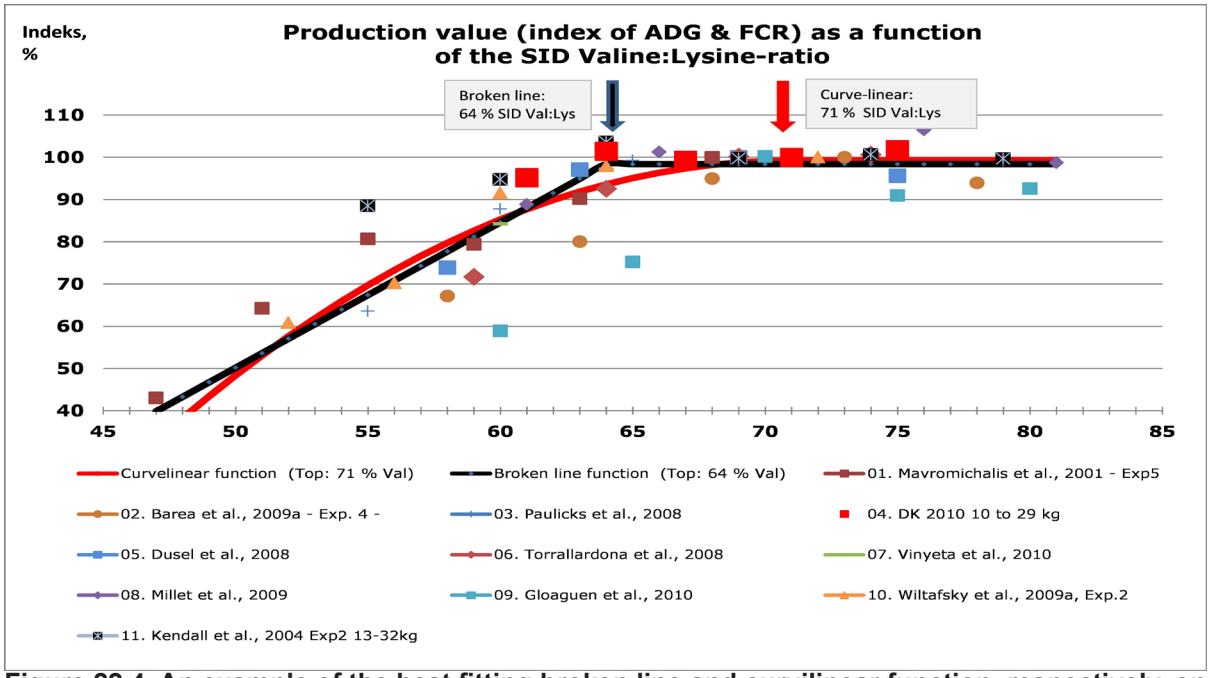

Figure 22.4. An example of the best fitting broken line and curvilinear function, respectively, on a meta-analysis of SID valine:lysine.

Care should be taken when different studies regarding an amino acid are compared in e.g. meta-analyses: An experiment with well documented feed analyses should ideally rank higher than an experiment with less or no effort done in this respect. Fewer feed analyses can give misleading conclusions due to normal variance at laboratories and between laboratories. Due to the inevitable differences between laboratories (5% differences in lysine level between major European labs were documented several times), the choice of lab used is documented to potentially alter the estimated SID AA:Lys requirement 4-5%.

A review of literature reveals a number of factors that have been identified to influence amino acid requirements. Several attempts have been made with some success to build models that encompass some or all of these factors. Although such a model is not currently used in Denmark, other countries (e.g. The Netherlands) do use such models.

5. The Danish protein evaluation system

Up to 2002, protein for Danish pigs was evaluated on the basis of digestibilities measured as apparent faecal protein digestibility. Each feedstuff was assigned one faecal digestibility value, and the faecal digestibility for protein was used for all amino acids. Research has, however, demonstrated that infusion of protein or essential amino acids in the caecum or large intestine does not contribute to the pigs' supply of essential amino acids since these amino acids are not absorbed in the caecum and large intestine. Thus, in 2002 a new system for protein evaluation based on standardised ileal amino acid digestibility was introduced together with a new energy evaluation system.

The Danish protein evaluation system is based on standardised ileal digestibility with a digestibility per amino acid per feedstuff. The practical solution is to express the digestibility of the individual amino acids as per cent of protein digestibility, e.g. the digestibility of lysine is 94% of the crude

protein digestibility in barley. This means that if crude protein digestibility changes, the digestibility of all amino acids automatically changes.

The digestibility of individual amino acids as percentage of protein digestibility are presented as table values, and for the most feedstuffs, the protein digestibility is also presented as a table value. For cereals and cereal by-products, the actual crude protein digestibility is calculated on the basis of the analysis of crude protein and in vitro digestibility.

The recommendations for amino acids are given as standardised ileal digestible (SID) amino acids, and these are, in Denmark, given as gram per energy unit of feed (g/feed unit).

5.1. Calculating availability of protein and amino acids

Standardised digestibilities are preferred over apparent digestibilities as they are independent of trial factors (e.g. inclusion rates in diets, etc.) and reflect the basal properties of the feedstuffs. It can therefore be assumed that standardised digestibilities are additive. Standardised digestibilities for crude protein and amino acids can be calculated in multiple ways. Below, two methods of two types of feedstuffs are described that are based partly on in vitro determination and partly on digestibility trials with pigs (in vivo determination).

5.1.1. Cereals and cereal by-products (method 1)

This method is based on the in vitro-in vivo difference method in which the in vitro assay called real protein digestibility at ileal level (RPDi) is used to measure and subsequently correct for feedstuff specific endogenous protein losses. Standardised crude protein digestibility (std. dig. CP) can then be calculated according to the equation below:

$$\text{Std. dig. CP} = \frac{CP\ [g/kg\ DM] \times \frac{RDPi}{100} \div 0.066\ [g/g\ UDMi] \times UDMi\ [g/kg\ DM]}{CP\ [g/kg\ DM]} \times 100 \qquad (1)$$

where CP is crude protein [g/kg DM] × RDPi/100 = real digested crude protein, and 0.066 [g/g UDMi] × UDMi [g/kg DM] = feedstuff specific endogenous loss. UDMi is an abbreviation for undigested dry matter at ileal level and is calculated on the basis of feedstuff analyses (see Chapter 21). The equation can be rewritten with units in g/kg DM:

$$\text{Std. dig. CP} = \frac{real\ digested\ CP - feedstuff\ specific\ endogenou\ s\ CP\ loss}{CP} \times 100 \qquad (1a)$$

In principle, the method of calculating the standardised digestibility of an amino acid is identical to the method of calculating the standardised crude protein digestibility. However, it is assumed that the real digestibility of the amino acids equals RPDi and that the amino acid profile of the feedstuff specific endogenous protein is constant. Consequently, there is one constant per amino acid; for instance the feedstuff specific loss of lysine is 2.11 mg per g UDMi (see Table 22.2 for details).

The standardised amino acid digestibility (Std. dig. AA) is expressed with the following equation:

$$\text{Std. dig. AA} = \frac{AA\ content\ \ [g/kg\ DM] \times \frac{RDPi}{100} \div AA\ loss\ [g/g\ UDMi] \times UDMi\ [g/kg\ DM]}{AA\ content\ \ [g/kg\ DM]} \times 100 \quad (2)$$

where AA content [g/kg DM] × RDPi/100 = real digested g per kg dry matter of amino acid in question, and AA loss [g/g UDMi] × UDMi,[g/kg DM] = feedstuff specific endogenous loss in gram per kg dry matter of the amino acid in question (see Table 22.2 for amino acid constants). However, before substitution in the equation, all constants in mg in Table 22.2 must be converted to gram. Thus, the equation (2) can be rewritten as below with all units in g/kg DM:

$$\text{Std. dig. AA} = \frac{real\ digested\ \ AA - feedstuff\ \ specific\ \ endogenous\ \ loss\ of\ AA}{AA\ content} \times 100 \quad (2a)$$

The in vitro method provides an estimate of the amino acid availability of a given feedstuff in a batch. Only a chemical analysis and an in vitro analysis are required to determine the value of the feedstuff in question.

The drawback of the in vitro method is that the parameters in equation 2 are assumed to apply to feedstuffs/diets in general. Well-documented knowledge about anti-nutritional factors and their effect on endogenous protein secretion is therefore partly ignored. In some cases (such as rye, peas, untreated soybean meal etc.), the endogenous loss is underestimated in the general equation. Accordingly, real digestibilities of individual amino acids may differ slightly if, for instance, some amino acids are overrepresented in a crude protein fraction that is difficult to digest. In cereal, for instance, the lysine content is higher in the hull crude protein than in the average crude protein fraction. This is the reason why lysine from cereal is not digested as well as crude protein on average.

5.1.2. Feedstuffs other than cereals and cereal by-products (method 2)

This method is based on a meta-analysis of published values for in vivo protein and amino acid digestibilities of different feedstuffs and on basal endogenous losses of protein and amino acids. The meta-analysis makes it possible to correct for different cannulation techniques (reentrant, ileorectal anastomosis, T cannula, steered ileo-caecal valve cannula, and post valve T caecum) and experimental designs (nitrogen-free, casein and regression) employed in the different studies. Adjusted averages, based on the meta-analysis, are made for in vivo digestibilities of the feedstuffs and basal endogenous protein and amino acid losses are estimated. Based on the meta-analysis, standardised digestibilities can be calculated according to the equations below:

$$\text{Std. dig. CP} = \frac{CP\ [g/kg\ DM] \times \frac{in\ vivo\ \ digestibility}{100} + basal\ \ CP\ loss\ [g/kg\ DM]}{CP\ [g/kg\ DM]} \times 100 \quad (3)$$

$$\text{Std. dig. AA} = \frac{AA\ content\ \ [g/kg\ DM] \times \frac{in\ vivo\ \ digestibility}{100} + basal\ \ AA\ loss\ [g/kg\ DM]}{AA\ content\ \ [g/kg\ DM]} \times 100 \quad (4)$$

The basal endogenous crude protein loss (basal crude protein) and the basal endogenous amino acid loss (basal AA) are defined as the minimum loss of protein and the individual amino acids for digestion of one kg dry matter (see Figure 22.2).

This method is based on comprehensive data material: 79 scientific publications and 203 in vivo digestibilities of different feedstuffs. The basal endogenous protein loss was estimated on the basis of 45 observations in 36 publications, while the amino acid profile was based on 10-31 observations depending on the amino acid in question.

A comparison of the two methods is presented in Table 22.2. It is clear that the estimates for the basal endogenous protein losses are nearly identical in both methods; 13.2 g vs 11.7 g per kg dietary dry matter for cereal (method 1) and non-cereal (method 2) products, respectively.

Table 22.2. Amino acid profile of endogeous protein and basal and feedstuff specific protein/amino acid losses with two methods.

		Cereals and cereal by-products (method 1)		Non-cereals (method 2)
	Amino acid profile (g/160 g N)[1]	**Basal (g/kg DM)**	**Feedstuff specific (mg/g UDMi)[2]**	**Basal (g/kg DM)**
Crude protein	1000	13.2	66.0	11.7
Lysine	32	0.42	2.11	0.37
Threonine	45	0.59	2.97	0.53
Methionine	10	0.13	0.66	0.12
Cystine	16	0.21	1.06	0.19
Tryptophan	12	0.16	0.79	0.14
Isoleucine	28	0.37	1.85	0.33
Leucine	44	0.58	2.90	0.51
Histidine	15	0.20	0.99	0.18
Phenylalanine	30	0.40	1.98	0.35
Tyrosine	25	0.33	1.65	0.29
Valine	39	0.51	2.57	0.46

1) 160 g N is sometimes used instead of writing 1000 g crude protein.
2) UDMi, undigested dry matter at ileal level.

Assuming a constant composition of the amino acid profile of the endogenous protein, it is possible to calculate the endogenous losses of essential amino acids. The basal endogenous loss of an amino acid can therefore be estimated as: basal endogenous loss of protein × content of the amino acid in endogenous protein. With method 2, for instance, the basal endogenous loss is estimated to:

11.7 g crude protein/kg DM × 32/1000 g lysine/g crude protein = 0.37 g lysine/ kg DM

The feedstuff specific endogenous loss of a certain amino acid (per g UDMi) is found by multiplying the protein loss per g UDMi by the content of the amino acid in question in the endogenous protein. For threonine, for instance, the calculation is:

0.066 g crude protein/g UDMi × 45/1000 g threonine/g crude protein = 0.003 g threonine/g UDMi

The values of the feedstuff specific endogenous amino acid loss per g UDMi can be used for calculating standardised amino acid digestibilities according to equation 2.

5.2. Relative amino acid digestibility

The standardised amino acid digestibility can be expressed relative to the standardised crude protein digestibility determined with method 2. The relative amino acid digestibility is expressed with equation (5):

$$\text{Relative AA dig.} = \frac{Std.dig.AA}{Std.dig\ CP} \times 100 \qquad (5)$$

If only table values are used for crude protein digestibility, it is of no importance whether absolute or relative amino acid digestibilities are used. A calculation model with relative values was chosen because it was decided to calculate the standardised crude protein digestibility for a range of cereal varieties and by-products of cereal on the basis of current analyses. That way it is possible to take variation between batches of cereal in crude protein content as well as enzymatic digestible organic matter (EDOM) into account. EDOM is an in vitro assay to simulate digestion in the full gastro-intestinal tract. With the model selected, amino acid digestibilities automatically change if crude protein digestibilities change.

If the relative amino acid digestibility is lower than 100%, the amino acid is not as available as the crude protein, which is the case for, for instance, barley (94%).

5.3. In vitro digestibilities vs fixed table values

In order to illustrate the flexibility and consequence of selecting in vitro as opposed to table values, barley is used as an example (see Table 22.3). It is well known that crude protein content in barley varies with nitrogen fertilization, which is the reason why foreign batches of barley generally have higher crude protein content than barley grown in Denmark at lower levels of N fertilization. Foreign table values for in vivo digestibilities may therefore overestimate digestibility under Danish conditions.

The calculations can be based on “barley 9%” (9% crude protein in the feedstuff), which corresponds to Danish barley, whereas foreign barley has a crude protein content corresponding to “barley 11.5%” or “barley 14%”. It is then assumed that starch, with a digestibility of 100%, is substituted by crude protein with a digestibility of 90%. The lower digestibility of crude protein in the EDOM analyses compared with starch is why EDOM values are slightly decreasing with increasing crude protein content. Consequently, UDMi is marginally increased with increasing crude protein content and the endogenous protein loss will also be affected marginally. Standardised digestible crude protein and lysine were calculated according to equations 1, 2 and 5. It is clear that crude protein and amino acid digestibilities increase with the crude protein content in barley.

Table 22.3. Effect of crude protein content in barley on availability of crude protein and lysine.			
	Barley 9%	**Barley 11.5%**	**Barley 14%**
Crude protein, % of DM	10.8	14.7	16.5
EDOM, %	83.8	83.5	83.2
EDOMi[1], %	79.0	78.7	78.4
RPDi - standard, %	90	90	90
UDMi, % of DM	23.2	23.6	23.8
St.dig.crude protein, %	75.8	78.5	80.5
Lysine[2], % of crude protein	3.8	3.4	2.9
St.dig.lysine[3], %	71.3	73.8	75.6

1) EDOMi, enzymatic digestible ileal organic matter. Simulates digestion in the stomach and small intestine.
2) Lysine content calculated with the equation: 5.41-0.15*crude protein % of DM.
3) Lysine has a relative digestibility of 94% of that of crude protein.

Compared to low protein feedstuffs (i.e., cereals, cereal by-products) the endogenous protein and amino acid losses have less influence on the crude protein and amino acid digestibility in feedstuffs high in crude protein. One can therefore expect that table values for crude protein feedstuffs provide the best estimate as a significant change in crude protein content only marginally affects crude protein digestibility due to an already high content of crude protein.

Most feedstuffs have a fixed table value based on in vivo experiments (method 2) as this is currently considered the best estimate of crude protein and amino acid digestibilities.

For cereals used in pig feed (barley, wheat, oats and triticale) and by-products of these, method 1 is used for determining standardised crude protein digestibilities. This method is the most appropriate for Danish conditions and takes into account the significant variations in cereals in terms of crude protein content and in UDMi, which determine the feedstuff specific endogenous loss. In practice, table values are used for RPDi as RPDi is extremely constant within feedstuffs, while UDMi is calculated according to the EDOM analyses.

5.4. Summary of the Danish protein evaluation system

The standardised digestible crude protein and amino acid in feedstuffs are calculated according to these criteria:

- Chemical analyses of crude protein content and, for the majority of the feedstuffs, table values for amino acid content in per cent of crude protein. For barley and wheat, regression equations describing the amino acid content in per cent of crude protein as a function of the crude protein, are used instead of table values.
- Table values are used for standardised crude protein digestibility of most feedstuffs.
- The standardised crude protein digestibility is calculated for cereals and cereal by-products with the general equation (1) where table values are used for RPDi, while UDMi is calculated on the basis of EDOM analyses.
- For amino acids, table values for relative amino acid digestibilities are used for all feedstuffs expressing the standardised amino acid digestibility relative to the standardised crude protein

digestibility. These are determined on the basis of published values for in vivo digestibilities by correcting for basal endogenous protein and amino acid losses.

- The content of standardised digestible amino acid is calculated with the following equation: content of amino acid × standardised crude protein digestibility × relative amino acid digestibility.

5.5. International differences

The French values for SID of amino acids in feedstuffs are based on values obtained with ileorectal anastomosis (large intestine removed) from three different laboratories in France. The results are used to create one complete list of table of values for all feedstuffs. The table values used in Denmark and The Netherlands are based on literature values for AID and recalculated to SID values. For basal endogenous losses, the French use basal endogenous loss values obtained at individual laboratories. The Dutch use an average literature value for estimated basal endogenous losses without taking into account differences in cannulation techniques. Different cannulation techniques have a significant effect and are therefore taken into account in the Danish table values. In addition to literature values for AID, table values from other countries were also considered in the production of Danish table values for SID. There is some overlap between the three tables, but the differences for most feedstuffs are minor. In Denmark, estimates for SID for barley and wheat are routinely tested in vitro and used to update the table values for SID.

6. Concluding remarks

It is evident from the final part of this chapter that the Danish protein evaluation system is very complex. Despite its complexity, any evaluation system fully depends on the data used for estimating the equations in the underlying models. The chemical composition of the feedstuffs changes over time and new feedstuffs are introduced. This makes it necessary to continuously test the nutritional properties by use of in vitro and in vivo methods. Along with providing more precise data on the feedstuffs, the protein and amino acid requirements of the pigs should also be better defined, allowing improved utilization of the nutrients. The in vitro and in vivo methods are not perfect and the methodologies should continuously be developed for providing better and more precise documentation of feedstuffs' nutritional value as well as the requirements of the animals.

7. Key references

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