Chapter 9
Dietary protein in pig nutrition

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This chapter will:

- Define and classify the nitrogenous compounds in pig diets
- Evaluate protein and amino acids in feedstuffs
- Explain why some amino acids are considered essential
- Quantify digestion of protein using different methods
- Describe the significance of endogenous nitrogen
- Describe and quantify how digestibility of protein is affected and can be improved

1. Introduction

Proteins are essential constituents of all living organisms and they are expressed in all cells in all phases of activity constituting the life of the cell. Different cell types express some cell specific proteins thus making up a large number of naturally occurring proteins. The variation in the type of expressed proteins among species is genetically encoded, and the variation among cell types within a species is determined by molecular modifications of the genetic material. However, all DNA encoded proteins are made up from the same 20 amino acids, some of which can be synthesized by the pig itself and some others needs to be supplied daily.

Protein or its constituent amino acids must be provided every day through the pig diet in amounts corresponding to the growth phase and production of the animal. The need for protein therefore varies during the life cycle, with the highest percentage of dietary protein in diets for young pigs and a declining dietary protein concentration in diets for older pigs. In sows, the percentage of dietary protein in the diet increases markedly during lactation in order to produce milk proteins to sustain piglet growth.

A correct supply of dietary amino acids is important for sustaining optimal protein accretion in growing pigs. If just one of the amino acids that cannot be synthesized *de novo* is deficient, the result is growth retardation. The importance of proteins in pig nutrition is not entirely related to animal performance. During the last three decades, a special focus on protein has developed upon

its nitrogenous compounds' impact on the environment. At present, the focus is on nitrogen leaching to the aquatic environment and drinking water, and on nitrogen fallout from evaporation from pig housing and slurry storage facilities. Supplementation of dietary amino acids in concentrations exceeding what can be utilized by the pig leads to increased output of nitrogen into the manure. There are several ways by which it is possible to influence protein digestion and consequently excretion of nutrients in the manure. Thus, knowledge about the digestion and absorption of protein in general and about individual amino acids, in particular, helps to reduce environmental impacts and optimize animal production.

2. Protein and amino acid terminology and classification

2.1. Dietary proteins

The protein sources used in Danish pig diets are primarily of plant origin and often from more than one species. Various products of soy beans are available of which hulled or dehulled toasted soybean meal are widely used in all types of pig diets. Peas and cake or meal from sunflower and rape seeds are also common in Danish pig diets although their inclusion is low or absent in diets for younger pigs. Potato protein concentrate and processed soybean protein concentrate are often included in diets for young pigs.

In green plant material, the protein fraction is 80-85%; the nucleotide fraction about 10%; and the soluble amino acid fraction about 5% of the total nitrogen content. In the growing plants, proteins are mainly enzyme proteins, but in the seeds (grain, rapeseed, soybean, sunflower) proteins are mainly storage proteins. Proteins in plants are found in the highest concentration in the seed, where the proteins are stored in membrane-enclosed protein bodies for germination purposes. The endosperm of grain contains some species-specific storage proteins of various sizes and physical and chemical structure.

The use of protein sources of animal origin in pig diets is restricted due to risk of infections by virus or prions. In fact, the only allowed protein products in EU of animal origin are currently blood plasma, milk, whey and various fish products.

The structure of protein determines its physical properties and thus affects its digestibility. The solubility of proteins varies from the insoluble keratin in e.g. hair and nail to the highly soluble albumins in e.g. blood plasma and milk. Protein can be precipitated from a solution and redissolved without affecting their properties. An example in nutrition where this is taken advantage of is the use of immunoglobulin-containing colostrum paste fed to weak newborn piglets unable to drink colostrum from the sow. Even though the colostrum has been up-concentrated by freeze drying, the immunoglobulins are still potent to stimulate the immune system of the newborn pig.

There are two groups to which proteins of both animal and plant origin can be classified: the simple proteins and the conjugated proteins. The simple proteins are hydrolyzed into only amino acids, whereas the conjugated proteins are hydrolyzed into amino acids and other non-nitrogenous compounds. The simple proteins consist of fibrous and globular proteins. Fibrous proteins are insoluble and very resistant to digestion by gastrointestinal enzymes. They are composed of elongated filamentous chains joined together by cross linkages to cell wall polysaccharides, often giving the fibrous proteins a structural role in cells and tissues of animals. Globular proteins are folded into a compact structure. They are often soluble, and examples of globular proteins are albumins and globulins in e.g. milk, egg and blood. The lipoproteins are examples of conjugated proteins and consist of proteins conjugated with lipids. They are central in the transportation of lipids within the body and are the main component of cell membranes. The polypeptide side-chains of proteins can be covalently attached with oligosaccharide chains by glycosylation to form glycoproteins, which is another example on a group of conjugated protein. The glycoproteins are often integral membrane

proteins having their extending extracellular segments glycosylated. The glycoprotein ovalbumin makes up the major part of the protein content found in egg white. Ovalbumin is of special interest in nutrition because the amino acid composition of egg white has contributed to defining an amino acid profile optimal for growth in animals (Chapter 22).

Denaturation of proteins is a modification of the physical and chemical properties by non-proteolytic processes leading to changes in their tertiary structure and thus loss of specific biological activities. In relation to animal nutrition, the most relevant agents leading to protein denaturation are high temperatures and acid-base properties. The effect of heat is of special interest because thermal treatment results in new linkages within and between peptide chains. These new linkages may result in impaired protein digestion since some of the new linkages resist hydrolysis by gastrointestinal tract proteases. Susceptibility of proteins to heat damage is increased in the presence of some carbohydrates. The Maillard reaction involves a condensation between the carbonyl group of a sugar with the free amino group of an amino acid, especially lysine. The darkening of overheated feedstuffs is symptomatic for the Maillard reaction. Heat treatment of feedstuffs and diets does not normally lead to damage of the lysine since the Maillard reaction happens noticeable above 120°C in dry feedstuffs (up to 14% water) and around 155°C in most other feedstuffs (above 15% water), which both are significantly warmer than the temperatures of 85-100°C reached during heat treatment and pelleting of commercial diets.

2.2. Free amino acids

The free amino acids in pig diets have two origins: from free amino acids in plant materials and from supplemented crystalline amino acids.

In plant material, the concentration of soluble amino acids is dependent on the stage of maturation. In green plant material, about 5% of the total nitrogen is soluble amino acid nitrogen with higher concentrations in young growing plants and lower in mature plants. In grain of rye and wheat, the free amino acids make up 1-3% of the crude protein, with the highest concentration of free amino acids in the bran fraction and the lowest concentrations of free amino acids in the sifted flour fraction (endosperm).

Crystalline amino acids are sometimes referred to as industrial or synthetic amino acids. The commercially available crystalline amino acids are, at the moment, lysine, methionine, threonine, tryptophane and valine, and their use began with the introduction of lysine in the 1960's. These amino acids are present in low concentrations in many commonly used feedstuffs (i.e. cereals) relative to what is needed by the pig. By supplementing some of these amino acids to a diet, it is possible to reduce the use of protein sources because specific amino acids otherwise deficient in a diet can be added instead of increasing the inclusion of protein sources.

More than two hundred amino acids have been isolated from biological material, although only 20 amino acids are used for protein synthesis. Amino acids are characterized by having a basic amino group and an acidic carboxyl group adjacent to a side chain, which in the smallest amino acid glycine simply is a hydrogen atom and in other larger amino acids aromatic rings or branched alkanes. Except for glycine, free amino acids are optically active and should have an L-configuration in order to be incorporated into protein. Like methionine, crystalline amino acids can be manufactured in a racemic mixture containing half L-form and half D-form. The D-form can be deaminated and the resulting keto acid can be reaminated into L-form. However, when composing diets using crystalline amino acids, it is important to take the configuration into account since the conversion efficiency from D to L-form is unknown.

2.3. Non-amino acid nitrogenous compounds

There are several nitrogen-containing compounds in pig diets other than proteins and amino acids. Some of those are the nucleotides, amines, amides, nitrates and alkaloids, which collectively can be termed non-protein nitrogen or NPN.

Nucleic acid in DNA and RNA forms upon hydrolysis the nitrogen containing pyrimidines and purines. If not reused to produce nucleotides, the purines are, in the pig, metabolized into allantoin and excreted in urine, and the pyrimidines are metabolized to urea and also excreted in urine. The fraction of nucleotide nitrogen to total nitrogen content is about 10% in green plant material. However, the contribution of nucleotides to pig nutrition is not normally considered because they are an inevitable consequence of ingesting products originating from living organisms and because they can be synthesized from amino acids. There is currently debate about the beneficial effects for gut health and development of feeding nucleotides, e.g. feeding yeast or other single cell organisms with high nucleotide content, in the period after weaning characterized by rapid turnover of intestinal cells.

In general, amines, amides, nitrates and alkaloids are unwanted since they are often toxic, and feedstuffs with high concentrations should be avoided when formulating diets for pigs.

3. Measurements of protein and amino acids in feedstuffs

The proximate analysis of feedstuffs includes an analysis of the crude protein content. This measure and the composition of amino acids is used when composing pig diets. Two methods often used to determine crude protein are the Kjeldahl and Dumas methods. Both methods are accurate and measure the total content of nitrogen in the sample. The Kjeldahl method has for long been the standard method in analysis of crude protein, although the faster, more automated and non-waste producing Dumas method is the method preferred by commercial laboratories. The choice of method depends on the type of sample, i.e. liquid samples may be easier handled using the Kjeldahl method than using the Dumas method.

The Kjeldahl method starts by heating a substance with sulphuric acid, making the organic substance decompose by oxidation to liberate the reduced nitrogen as ammonium sulphate. The amount of ammonia is determined by back titration and the amount of nitrogen in the sample is calculated. The Dumas method consists of combusting a sample at high temperature in the presence of oxygen. After removal of water, COx and SOx, the nitrogen content is measured by a thermal conductivity detector. For both the Kjeldahl and the Dumas methods, the measured nitrogen concentration is related to protein concentration by multiplying the nitrogen concentration by the commonly used conversion factor 6.25, which dates back more than a century. The factor 6.25 of course depends on the actual amino acid composition of the protein, since amino acids vary in their content of nitrogen, ranging from 7.7% in tyrosine to 32% in arginine. Therefore, the natural variation among feedstuffs in the amino acid composition of the proteins, ideally should be corrected for by using factors specific for each feedstuff (Table 9.1). For example, if the nitrogen concentration in wheat is analysed to 1.75 g N/kg dry matter, it is reported as 10.9 g crude protein/100 g dry matter (conversion factor 6.25), whereas the actual content may be around 9.6 g crude protein/100 g dry matter (conversion factor 5.50) at an average fraction of 18.2% nitrogen in the protein. The conversion factor of 6.25 is used all over the world by both the food and feedstuff industries, even though everyone is aware that its prediction of true protein as total amino acid concentration is imprecise.

Table 9.1. Conversion factors from N to true protein based on actual amino acid composition in selected items (modified after [5]).	
Casein	6.15
Beef	5.48
Fish	5.58
Whole egg	5.70
Barley	5.45
Wheat	5.50
Corn	5.62
Soybean	5.50
Pea	5.36
Lupin	5.44

Another very important consequence of both the Kjeldahl and the Dumas methods is that estimation of crude protein concentration is based on measurements of total nitrogen, which does not necessarily have its origin from amino acids, peptides and proteins. As described above, feedstuffs also contain various amounts of non-amino acid nitrogenous compounds, and nitrogen from these compounds will be considered as representing true protein. Thus the estimate for crude protein is a rough measure covering true protein, nucleotides and even toxic compounds. However, the Kjeldahl method does not measure the nitrogen from nitrite and nitrate. An example underlining that crude protein should not be the only measure of quality for feedstuffs is the 2008 Chinese scandal of supplementing milk powder and products with nitrogen containing melamine in order to fake the nitrogen content and thus protein measured by the Kjeldahl method.

Amino acids can be determined by two methods for either free or total amino acids. The method for measuring total amino acids estimates both free and protein bound amino acids, and is the standard method used to determine amino acid content of feed, while the method for measurement of free amino acids can be used for premixes with high content of added crystalline amino acids. The major difference between the two methods is a 23-hour hydrolysis with HCl (EU standard) during which the protein is degraded. The amino acids - both free and protein-bound – are quantified by ion exchange chromatography.

From the above, it is obvious that crude protein also is a crude measure to use when composing diets for pigs. Ideally, the feedstuffs should be analysed for their content of all amino acids rather than crude protein. Naturally, the choice of measuring crude protein instead of 20 amino acids is dependent on analysis costs.

4. Amino acids and crude protein in feedstuffs

4.1.Essentiality of amino acids

Within the animal, 20 different amino acids are needed in the translation of mRNA to protein. All these amino acids are found in the feedstuffs, although in various concentrations. If an amino acid is in excess of what is needed, the amino acid can be deaminated and its amino group can be used for other purposes such as in urea formation and subsequent excretion in urine, or the amino group can be transferred to an α-keto-acid to form a new and different amino acid. This process is called transamination, and is an important mechanism to compensate for an intake of protein that contains an amino acid profile different to what is needed by the animal.

However, of the 20 amino acids making up the proteins some cannot be synthesized by the pig itself because the corresponding α-keto acids are not available. Consequently, these amino acids have to be supplied every day in the amounts needed. These amino acids are called the essential or indispensable amino acids, although in fact all 20 amino acids are essential for protein synthesis.

Table 9.2. Overview of essentiality of amino acids for pigs.

Essential	Semi-essential	Non-essential
Histidine	Arginine	Alanine
Isoleucine	Cysteine	Asparagine
Leucine	Tyrosine	Aspartic acid
Lysine		Glutamic acid
Methionine		Glutamine
Phenylalanine		Glycine
Threonine		Proline
Tryptophan		Serine
Valine		

There are nine essential amino acids and 3 semi-essential amino acids (Table 9.2). The essential are lysine, methionine, threonine, tryptophan, valine, isoleucine, leucine, histidine and phenylalanine. Some of the non-essential amino acids cannot be sufficiently synthesized by the pig in order to reach maximum growth and nitrogen retention. Arginine, cysteine, tyrosine and sometimes also glutamine and proline are considered to be semi-essential amino acids for pigs. In contrast to arginine, which is present in surplus amounts in common balanced diets, dietary cysteine and tyrosine concentrations can be low. These amino acids are considered semi-essential because they can only be synthesized from essential amino acids. Cysteine uses methionine (both sulphur containing amino acids) and tyrosine uses phenylalanine (both aromatic amino acids) as precursor. Therefore, the requirements are expressed as the sum of methionine and cysteine and of phenylalanine and tyrosine.

It is common to supplement pig diets with crystalline amino acids. The reason for doing so is to reduce the content of crude protein in the diet. Figure 9.1 shows the effect of supplementing crystalline amino acids to a grower diet, where soybean meal is gradually substituted with wheat. If no amino acids are supplemented, the example shows that the diet contains 20.4% crude protein and that lysine is the first-limiting amino acid. If the diet is supplemented with L-lysine, the crude protein concentration is reduced to 18.9% and threonine becomes the first-limiting amino acid. With use of all the currently available crystalline amino acids, the example shows that crude protein in the same diet can be reduced from 20.4 to 14.5%.

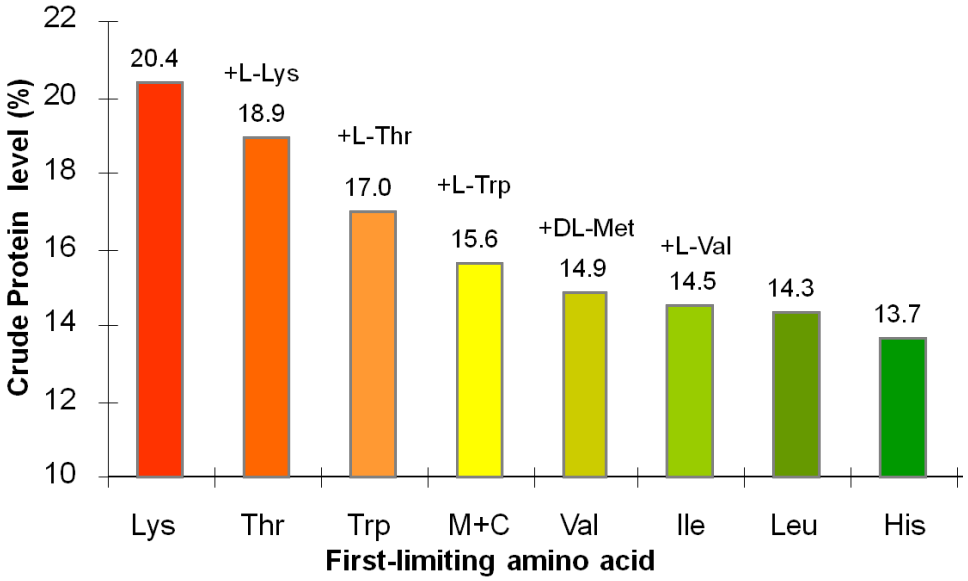

Figure 9.1. Changes in crude protein content in a diet supplemented with 5 crystalline amino acids (above columns) (Ajinomoto Eurolysine Information No. 37, 2011).

One way to reduce nitrogen loss from pig production would be to compose diets consisting of very low crude protein and supplemented with the essential amino acids in amounts sufficient to cover their own requirements and for de novo synthesis of the semi-essential and non-essential amino acids. However, the use of essential amino acids for synthesis of non-essential amino acids is inefficient and, therefore, the ratio among essential and non-essential amino acids is of interest. An optimal ratio for nitrogen utilization at normal crude protein level is at approx. 50:50, but it is possible to have ratios up to 70:30 between essential amino acids and non-essential amino acids in low-crude protein diets without significant negative effects on nitrogen utilization. This would require use of great amounts of crystalline amino acids, which of course would make the diets more expensive and less relevant for use under commercial conditions.

4.2. Composition of feedstuffs

The concentration of crude protein in the different feedstuffs varies greatly, see Figure 9.2. The feedstuffs contributing with the most dietary protein belong to the group of feedstuffs called protein sources, and as described before, the most commonly used protein sources are hulled or dehulled toasted soy bean meal, cake or meal from sunflower and rape seeds, peas, and potato and soy protein concentrate.

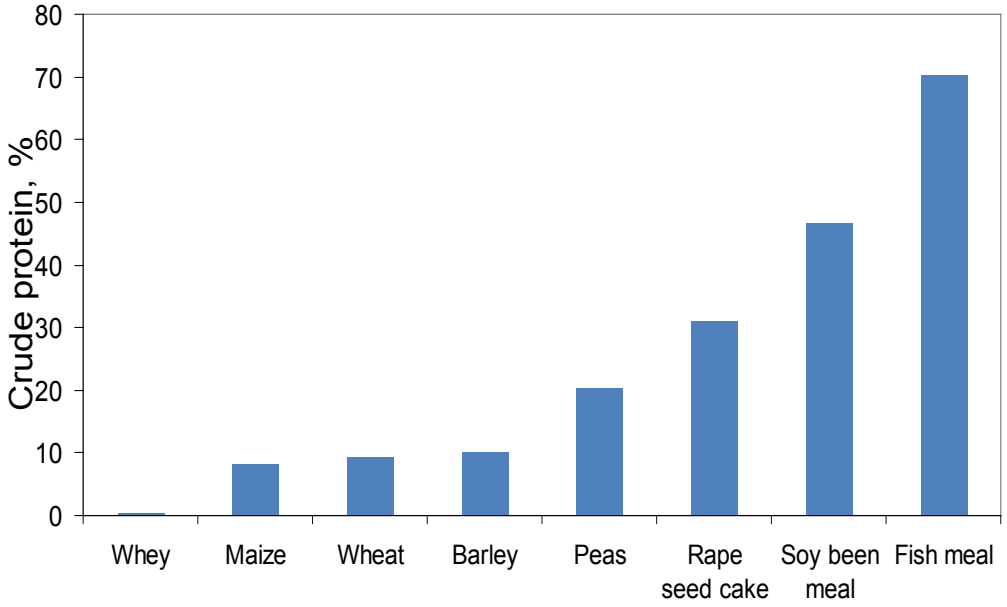

Figure 9.2. Variation in crude protein concentration in commonly used feedstuffs (as-fed basis).

All feedstuffs contain all 20 amino acids and the natural variation in the individual amino acid concentration is dictated by the genomic characteristics of the species. In relation to the requirement of the animals, some of the essential amino acids are in deficit. The primary limiting amino acids in diets for pigs are typically lysine, methionine, threonine and tryptophan, which all are commercially available and commonly used when formulating diets low in crude protein. Valine is often the next in the order of limiting amino acids in typical pig diets. The concentration of lysine and valine of some common feedstuffs are shown in Figure 9.3, which also indicates that the combination of several feedstuffs in a diet will complement and add up towards fulfilling the nutritional recommendations of individual amino acids.

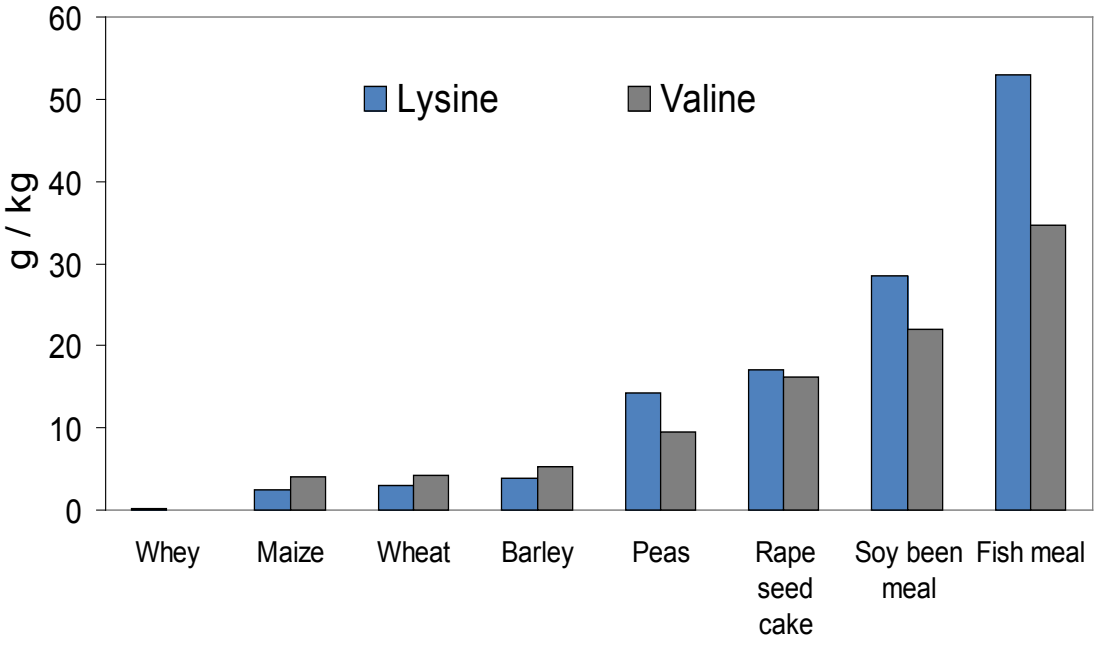

Figure 9.3. Lysine and valine concentrations in some common feedstuffs (as-fed basis).

5. Quantitative aspects of protein and amino acid digestion and absorption

When dietary protein is ingested it must be hydrolyzed into amino acids in order to fulfill their role in protein synthesis in the pig body. The proportion of absorbed amino acids to ingested crude protein defines an important measure of protein quality, and this is measured in digestibility experiments. The digestion and absorption processes are described in Chapter 5. Figure 9.4 shows the utilization of crude protein in a diet containing 17.3% crude protein for growing pigs composed of 56% barley, 23% wheat and 18% soybean meal as main ingredients, and supplemented with L-lysine, DL-methionine and L-threonine. Even though the diet was optimized to fulfill the amino acid recommendations, only 38% of the crude protein was retained in the animal, leaving 62% of the supplemented crude protein as lost to the slurry. The crude protein lost with urine can be reduced by combining different feedstuffs including crystalline amino acids, making the profile and the concentration of the dietary amino acids close to the requirements of the animals. Looking at individual feedstuffs as well as the diet as such, the digestibility is an important factor for protein utilization, giving rise to a major impact on the protein value of a feedstuff.

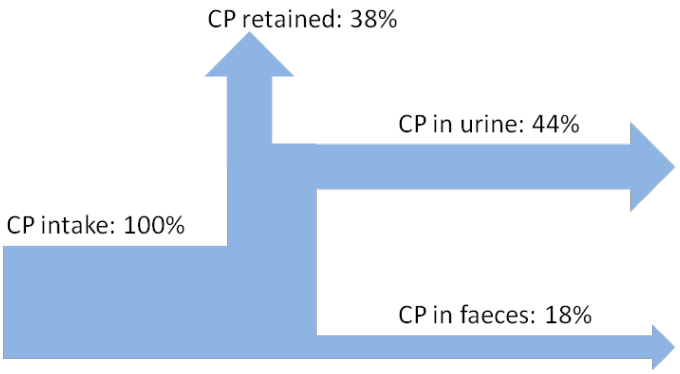

Figure 9.4. Utilization of crude protein in a diet for growing pigs measured by collecting faeces and urine from 6 pigs in balance cages for 7 days.

Amino acid digestibility reflects enzymatic hydrolysis and microbial fermentation of ingested protein, peptides and amino acids and absorption of amino acids and peptides. These processes involve proteins and other nitrogen containing compounds that are of dietary origin or from endogenous secretions. The digestibility of a nutrient is defined as the difference between its amount in the ingested feed and the amount in the faeces.

5.1. Endogenous nitrogen

When evaluating the digestibility of crude protein it is often important to consider the contribution of endogenous nitrogen. This is simply nitrogen that has been digested, absorbed and excreted back into the gastrointestinal tract and is present there as non-feed nitrogen. The form of its entry can be as saliva, gastric, pancreatic and intestinal secretions, bile, sloughed intestinal epithelial cells, and parts of the intestinal microorganisms are of endogenous origin. In the small intestine, about 70% of the endogenous protein is reabsorbed thus contributing to the normal protein metabolism. In literature, it is most common to look at the net endogenous losses after reabsorption, and in the following text endogenous loss refers to net loss. The proportion of lost endogenous nitrogen is not constant. The loss can be divided into two pools, one fraction only related to the quantity of dry matter passing through the gastrointestinal tract and unrelated to the quality and the quantity of the protein in the diet. The other fraction is a loss related to the diet ingredient composition. The two fractions are also called unspecific and feed specific endogenous loss of nitrogen. The reported amino acid composition of the endogenous protein is not consistent among different experiments.

The quantity of dietary protein contributes only little to the loss of endogenous nitrogen at the ileal level. The primary contributing factors are the non-protein components of the diet. Increased intake of dry matter and dietary fibre, and especially the amount of undigested dry matter, correlates well with the endogenous loss of nitrogen and results in a corresponding linear increase in the loss of endogenous nitrogen. Antinutritional factors are compounds present in most feedstuffs, although in various concentrations, decreasing the nutritional value of the feed. For instance, the use of legumes in pig diets is restricted because of antinutritional factors. Examples are trypsin inhibitors resulting in increased pancreatic secretions and low protein digestibility, and tannins, which are bitter plant phenols that either bind and precipitate or modify protein and amino acids, resulting in high secretions and losses of endogenous enzymes and a general poor digestibility of feed proteins.

There are several methods to estimate the loss of endogenous nitrogen. The total and feed specific endogenous losses can be estimated by using a tracer technique, where endogenous proteins can be distinguished from dietary protein by labeling the amino acids in the feed or the body protein with stable isotopes like ^{15}N. Values for the specific ileal loss of endogenous proteins of course vary greatly among feedstuffs, but the gross loss of crude protein is often in the range from 25 to 35 g per kg dry matter intake. Classical methods for estimation of the unspecific or basal loss include measuring ileal nitrogen loss after feeding a nitrogen-free diet, or extrapolating ileal nitrogen loss to zero g protein in experiments feeding different levels of dietary protein. Results on ileal loss of nitrogen vary to a great extent. The majority of the reported values are in the range from 10 to 20 g protein per kg dry matter intake. In the Danish protein evaluation system, the net loss of endogenous protein at the ileal level is estimated at 13 g/kg dry matter of basal loss (not feed specific) and the feed specific loss is calculated from the amount of undigested dry matter. In this system, the total net endogenous loss is estimated at 20-30 g/kg dry matter for most feedstuffs. This amount should be seen in the context of a typical pig diet containing 170-220 g of crude protein per kg.

5.2. Digestibility of protein and amino acids

Absorption of amino acids and small peptides takes place in the small intestine (Chapter 5). After passing the small intestine, protein and amino acids are more or less lost to the animal, meaning that they can only indirectly contribute to the nutrition of the animal by supporting the fermentative processes by the microflora population of the large intestine. The caudal part of the small intestine is called the ileum. Ileal digestibility of amino acids reflects therefore a more correct measure than faecal digestibility because it indicates the proportion of amino acids that is absorbed and potentially can be utilized by the animal. These concerns give rise to defining some commonly used types of digestibility:

- apparent faecal digestibility (AFD),
- apparent ileal digestibility (AID),
- true ileal amino acid digestibility (TID), and
- standardised ileal amino acid digestibility (SID).

5.2.1. Apparent faecal digestibility

The apparent faecal digestibility (AFD) of a nutrient is defined as the difference between the amount in the ingested feed and the amount in the faeces:

$$AFD, \% = ((Protein_{intake} - Protein_{faeces}) / Protein_{intake}) \times 100$$

The digested amount is called apparently digested because the faeces contains endogenous proteins, which have already been digested before they were secreted back into the gastrointestinal tract. The use of the apparent or apparent faecal digestibility should be restricted to evaluation of protein as such and not individual amino acids, because the amino acid profile in the faecal

proteins is modified by microorganisms. Furthermore, when the endogenous proteins are not considered, the difference between apparent and true digestibility (defined below) is greater in diets low in protein and high in e.g. antinutritional factors leading to increased ileal loss of endogenous proteins.

5.2.2. Apparent ileal digestibility

When considering digestibility of amino acids it is, as previously discussed, important to distinguish between the small intestine and large intestine. Inserting a T-cannula by surgery 5-10 cm anterior to the ileum-caecum valve makes it possible to collect ileal outflow of digesta, see Figure 9.5. This technique is the most widely used for ileal digesta collection. The challenge in using the T-cannula is that only a subsample is taken and therefore an indigestible marker needs to be included in the feed to make it possible to estimate actual amounts (see Chapter 5). Furthermore, precautions should be made to obtain representative digesta samples in relation to particle separation, sampling frequency and time from feeding. The apparent ileal digestibility (AID) of protein and amino acids (AA) is the difference between the diet and ileal digesta:

$$AID, \% = ((AA_{intake} - Ileal\ AA_{outflow}) / AA_{intake}) \times 100$$

As for the apparent faecal digestibility, the ileal digestibility does not consider the amount of endogenous protein.

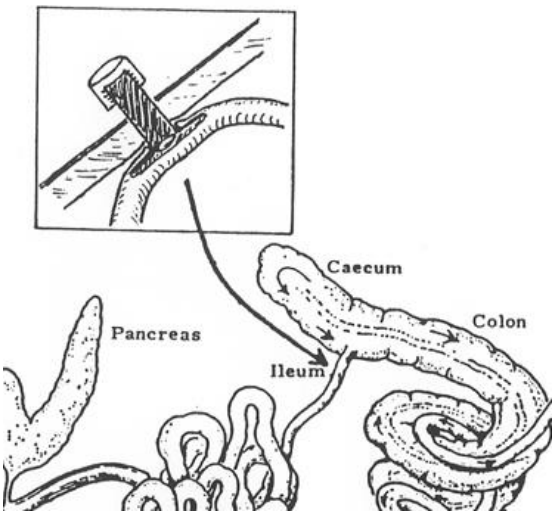

Figure 9.5. T-cannula inserted in the ileum and exteriorized through the skin.

5.2.3. True ileal digestibility

The true ileal digestibility (TID) is calculated by correcting the ileal outflow for the total ileal loss of endogenous protein and amino acids:

$$TID, \% = ((AA_{intake} - (Ileal\ AA_{outflow} - total\ ileal\ AA_{endo})) / AA_{intake}) \times 100$$

This coefficient is a measure of the proportion of dietary amino acid intake that was actually absorbed from the small intestine. In relation to diet formulation, the true ileal amino acid digestibility is not very relevant. The reason is that the true digestibility does not provide a measure of the amount of amino acids available to the pig for physiological processes, and that the feedstuff-specific endogenous loss of amino acids is not considered although it can be significant for some ingredients. Furthermore, data on the feedstuff-specific and total loss of endogenous amino acids

for individual feedstuffs are difficult and expensive to obtain as implied in the section above on endogenous nitrogen.

5.2.4. Standardised ileal digestibility

The feedstuff-specific endogenous loss of protein and amino acids is included in the standardised ileal digestibility (SID), which is calculated by correcting the ileal outflow for the basal (unspecific) endogenous loss:

$$SID, \% = ((AA_{intake} - (Ileal\ AA_{outflow} - unspecific\ ileal\ AA_{endo})) / AA_{intake}) \times 100$$

The correction for the unspecific ileal loss of endogenous amino acids makes it possible to correct the experimental results for the influence of the nitrogen-free part of the experimental diet. Using this method of a uniform standardization among feedstuffs results in a measure that reflects the proportion of amino acids sustaining growth and basal metabolism. The strength in using standardised ileal digestibilities is that the values from different feedstuffs are additive, i.e. concentrations in several feedstuffs can be summed up, which is an important issue when formulating diets. If amino acid recommendations are expressed on a standardised ileal digestibility basis, then they include amino acids for both growth and basal metabolism (maintenance), and the recommendations can be expressed in g per kg diet.

Some examples of standardised ileal digestibility of crude protein are: maize dry distillers grain with solubles (DDGS), 73%; barley, 76%; rape seed cake, 76%; wheat, 85%; toasted soybean meal, 88% and fish meal 92%. The difference in digestibility among these feedstuffs reflects their different concentration in fibre and antinutritional factors.

5.3. Crystalline amino acids

The use of crystalline amino acids gives rise to some concerns regarding the efficiency of utilization of the dietary crystalline amino acids. When formulating diets, the standardised ileal digestibility (SID) of crystalline amino acids is considered to be 100 per cent. There is, however, a potential microbial metabolism of crystalline amino acids in the gastrointestinal tract. The level of crystalline amino acid metabolism will depend on the composition of the microbial population and the substrates available for microbial metabolism. When considering the metabolism of amino acids (see Chapters 13 and 14), it can be speculated that the readily absorbable crystalline amino acids are absorbed more rapidly than sugars from complex carbohydrates and amino acids of protein origin. During the early absorptive phase after ingesting a meal, this time lag may lead to an increased utilization of the crystalline amino acids for oxidation and gluconeogenesis compared to the amino acids of protein origin. The consequence is that full utilization of free amino acids that are added requires frequent feeding, and significant impaired utilization of free amino acids has been shown with only one meal per day. The practical importance of microbial utilization of free amino acids is not known. The consequence could, however, be slightly higher experimentally determined estimates of amino acid requirements, when experiments are based on high percentages of free amino acids compared to experiments based on mostly protein-bound amino acids.

6. Factors influencing the quantitative digestion and absorption of protein and amino acids

6.1. Anti-nutritional factors

As mentioned above, the loss of endogenous protein is increased by antinutritional factors such as insoluble fibre, lignin, enzyme inhibitors, tannins, and lectins. Pea, bean and soybean are known for containing trypsin and chymotrypsin inhibitors and lectins, and all these factors increase

the specific ileal loss of endogenous protein. However, heat treatment or toasting is a common procedure to eliminate these antinutritional factors, thus improving the protein digestibility and reducing the risk of diarrhoea in young pigs. Furthermore, through plant breeding, varieties have been developed, containing lower levels of trypsin and chymotrypsin inhibitors and lectins.

Indigestible fibre can be regarded as an antinutritional factor since it increases the ileal loss of endogenous proteins. For instance, dehulled barley meal results in a loss of 16 g/kg dry matter, whole barley results in a loss of approx. 23 g/kg dry matter, and barley hulls in a loss of 31 g/kg dry matter.

6.2. Heat treatment

To control contamination with potential toxic bacteria or fungi and to alleviate the unwanted effects of antinutritional factors, it is normal procedure to heat treat feedstuffs. Commercial manufacturing of diets often includes a final pelleting process developing heat, and if there is any risk of Salmonella contamination, diets need, according to Danish legislation on Salmonella control, to include a final heat treatment step where the temperature of the leaving product is minimum 81°C. At this temperature, microorganisms are destroyed without denaturing proteins naturally occurring in the feedstuffs. However, the natural content of phytase in cereals is known to lose some of its activity by heat treatment and pelleting (see Chapter 11), and this may indicate that some other of the naturally occurring intrinsic proteases lose some activity as well. The level of intrinsic proteases is not considered to have practical measurable effect on protein digestibility in normal dry pig diets.

Feeding unprocessed soybeans results in growth depression, but by heat treatment or toasting of soybean meal at 100-115°C for 20 minutes, the trypsin inhibitors causing low protein digestibility are eliminated. The standardised ileal digestibility of lysine in DDGS is approx. 14 percentage units lower than in the originating maize or wheat, and this may in part be due to processing steps involving heat and partly the higher level of fibre inducing feed specific endogenous loses. As discussed previously, overheating may result in reduced lysine availability as a consequence of the Maillard reaction. However, in well-functioning manufacturing processes, temperatures are not normally high enough to mediate this reaction.

6.3. Liquid feeding

When hydrated, the kernels activate a battery of preformed hydrolytic enzymes degrading the pools of lipids, carbohydrates, proteins, organic bound phosphate and mineral chelating and sequestering compounds. When ungerminated seeds are used as feed, these preformed enzymes constitute all plant-derived hydrolytic activities. Recent findings show that soaking the feed improves the digestibility of protein from 80 to 83% in a cereal-based diet without any added exogenous enzymes. The increase in protein digestibility by soaking is thought to occur through the activation of different enzyme systems naturally present in the kernel, contributing to the degradation of the chemical complexes in the kernel and release of peptides suitable for absorption.

Fermented liquid feed has several benefits when feeding pigs, including improved gut health and nutrient digestibility. When applying this feeding method, the typical strategy is to ferment the cereals and/or soybean meal before adding the remaining ingredients. A successful fermentation results in a final pH between 3.5 and 4 and an elimination of the population of enterobacteria. The production of acids is mainly due to fast proliferating lactic acid bacteria fermenting carbohydrates in particular sugars. Increased proteolysis due to an increase in the number of proteolytic bacteria, lower pH, and breakdown of carbohydrate structures allowing endogenous enzymes better access to structural proteins, leads to improvements in dry matter and crude protein digestibility in the range 3 to 8%. After fermenting (non heat treated) soybean meal and bean, improvements in crude protein digestibility have been observed in the range 12 to 35%. This notable improvement may be due to breakdown of antinutritional factors such as trypsin inhibitors.

6.4. Exogenous enzymes

The supplementation with the exogenous enzymes phytase (phytate degrading/phosphorus liberating) and xylanase (carbohydrate degrading) has become a standard in diets, and some commercial piglet diets also contain the protease subtilisin. Subtilisin is a bacteria-derived serine endopeptidase commonly used as an active component in washing detergents. When included in pig diets, it is intended to increase protein digestibility although documentation of significant effect is difficult to find at present.

It is possible to improve protein digestibility by the use of carbohydrate degrading enzymes. Studies on diet supplementation with xylanase, glucanase, cellulase or amylase have shown variable results on nitrogen metabolism, but most studies show some improvements in protein digestibility and nitrogen balance. The exact mechanisms are unknown, but it may partly be due to degradation of the structural carbohydrates of the cereals allowing improved access to the embedded proteins for the endogenous and naturally occurring proteases. Furthermore, a stimulating effect on the secretion of endogenous enzymes has been observed after glucanase and xylanase supplementation.

7. Case study on reducing crude protein

To illustrate the significance of supplementing crystalline amino acids to diet for young pigs especially, two examples of diets can be used; one diet without crystalline amino acids and one diet including crystalline amino acids (Table 9.3). If a diet for 10-30 kg weaners (Table 7, Chapter 2) is formulated without crystalline amino acids, it will contain 290 g crude protein/kg corresponding to 252 g standardised digestible crude protein/kg diet. When supplemented with L-lysine, DL-methionine, L-threonine and L-tryptophan, the corresponding levels of crude protein are 202 and 173 g/kg, mainly because the inclusion of soy bean meal is reduced from 52% to 26%.

The diets were optimized using commercial optimization software. It is seen from Table 9.3 that L-valine was not included in the diet. The reason is that diets are formulated on a least-cost principle, where the inclusion of a feedstuff is determined by the price of the feedstuff and its effect on the dietary formulation to fulfil the minimum requirements set by the user. Thus, L-valine was in this example too expensive to use. However, if maximum crude protein content was set at 156 g/feed unit, L-valine would be included in the diet.

The two diets are optimized to supply the same level of essential amino acids and are expected to result in the same level of growth. Thus, the potential in reducing nitrogen excretion from pig production by including crystalline amino acids is obvious. By supplementing the 4 crystalline amino acids, nitrogen excretion is reduced from 1160 g N/pig to 590 g N/pig from 7-30 kg. This corresponds to a reduction in nitrogen excretion by 50%. The utilization of the dietary protein into gain was increased from 33% to 51%. However, more benefits can be expected, since it is well known that the frequency of diarrhoea increases with the level of dietary crude protein, and that feeding very high levels of certain protein sources, such as 52% soy bean meal in the diet without crystalline amino acids, is not possible. The maximum recommendations of 162 g digestible crude protein/feed unit for 9-30 kg pigs should therefore be respected when optimizing diets, both due to animal health concerns as well as environmental concerns.

Table 9.3. Formulations of 10-30 kg diets either without (w/o) or with (w) supplementation of crystalline amino acids and composition of standardised ileal digestible (SID) amino acids and crude protein in g SID/kg as-fed or in g SID/feed unit for growing pigs (FUgp).

g/kg as fed	**w/o**		**w**		
Wheat	159		434		
Barley	250		250		
Soybean meal	523		260		
Fat	39		17		
Limestone, 38% calcium	16		16		
Mono calcium phosphate	5.1		9.3		
Salt	3.9		3.7		
Vitamin/mineral premix	4.0		4.0		
L-lysinehydrochloride	-		3.800		
DL-Methionine	-		1.184		
L-Threonine	-		1.122		
L-Tryptophan	-		0.013		
L-Valine	-		0.000		
g SID	**g/kg**	**g/FUgp**	**g/kg**	**g/FUgp**	**Recommendation (9-30 kg), g SID/FUgp**
Lysine	14.7	13.3	11.4	10.4	10.4
Methionine	3.6	3.3*	3.6	3.3	3.3
Met + Cystine	7.5	6.8	6.5	5.9	5.6
Threonine	9.4	8.6	6.9	6.3	6.3
Tryptophan	3.44	3.12	2.23	2.03	2.03
Valine	12.0	10.9	7.7	7.0*	7.0
Isoleucine	10.9	9.9	6.8	6.2	6.0
Leucine	19.0	17.3	12.2	11.1	10.6
Histidine	6.6	6.0	4.2	3.8	3.5
Phenylalanine	12.8	11.6	8.3	7.5	5.9
Crude protein	252	229	172	157	Max. 162 / Min. 150
Crude protein, g total	290	264	201	183	-

* The first-limiting amino acid (the content in diet equals the recommendation)

8. Concluding remarks

The focus on crude protein in evaluating feedstuffs and diets should not stand alone as several parameters can make this measure differ from the content of true protein. Instead, attention should be on the far more important characteristics; amino acid profile, amino acid concentration and digestibility.

Since only approximately one-third of the supplied protein is retained in the growing pigs under typical commercial conditions, the potential for improvements seems obvious. The choice of protein source should be highly digestible feedstuffs, with a low content of antinutritional factors. Subsequently, a variety of protein sources and crystalline amino acids must be included in the diet, since no particular feedstuff can supply the animal with the amino acid composition/profile needed to fulfill the requirement of the animals. Finally, the manufacturing processes of the protein sources and the feed processing, including the perspectives of supplementing exogenous enzymes, should be considered for optimal utilization of dietary protein and amino acids.

The importance of improving protein utilization is apparently still growing even though it has received much attention during the last three decades. The primary driving force for future improvements of protein utilization will remain the same; to reduce the effect of nitrogen in eutrophication of natural ecosystems. However, also the pig and eventually the pig farmer will experience benefits of improved protein utilization, through improved animal health, better growth and feed conversion ratios.

9. Key references

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