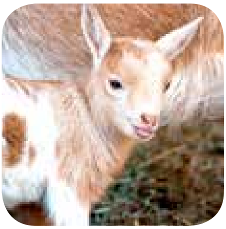
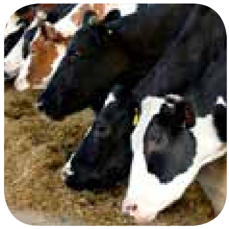
Fefana Publication

Amino Acids in Animal Nutrition



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Amino Acids in Animal Nutrition

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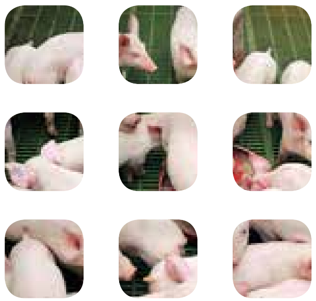
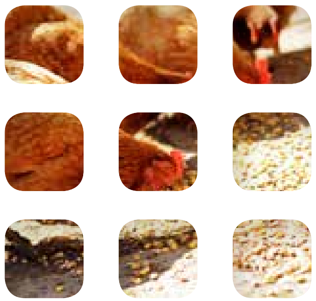


Preamble

FEFANA is the European Association of Specialty Feed Ingredients and their Mixtures. With over 100 member companies from 28 European countries, it represents business operators active throughout the feed chain, such as the specialty feed ingredient producers, premixtures manufacturers, users, importers and distributors.
Established in 1963, FEFANA has loyally represented and served the interests of its industry ever since 1963, and it is recognized as a representative partner to national and international authorities and fellow organizations in the feed and food chain.
Specialty Feed Ingredients and their Mixtures are essential ingredients in animal feed, ensuring balancing the nutritional quality of the feed and hence contributing to animal health and welfare. Quality and safety being two main cornerstones, we also focus on innovation and sustainability, which we believe to be present and future key features of our business.
With a unique framework, the association is able to draw on the exceptional knowledge of our membership, bringing together expertise and science. This is why we are able to provide you with such a valid publication.
The amino acids industry has been one of our core businesses since the establishment of the association and in this booklet we would like to provide relevant information about the role of the amino acids in the feed to food chain. Amino acids allow sustainable and efficient animal production providing safe nutritious animal protein for the growing human population.
We hope you find the information herein interesting and of relevance to your daily practice.

Didier Jans
FEFANA Secretary General
Vincent Hess
Chairperson FEFANA Working Group Amino Acids



1. INTRODUCTION

The need to feed a growing population leads to a trend for highly specialised units where animals of high genetic merit are raised in optimum conditions. Advances in husbandry techniques are also made in response to the needs for rearing high genetic merit livestock.
Improvements in feed efficiency (Figure 1) which have been gained over the last decades are a further example of the improvements which have been achieved from a combination of good breeding and good husbandry techniques. The nutrition of livestock has played a critical and essential role in these developments and is an element which needs to be continually updated as new scientific information becomes available.
Protein was recognised very early as an “organic body building substance” of strategic importance to an organism. With the development of feed nutrient analysis early in the nineteenth century, the first qualitative assessment of animal feeds was made possible. Yet, despite these developments, in practice, diets continued to be dominated by the concept that formulations were considered in terms of ingredients. New approaches are focusing on dietary nutrient supplying independently of the ingredients per se. It is well accepted that amino acids, as nutrients, are building blocks of protein and play an essential role in the nutritional composition of a feedstuff. Historically it was only the economic incentive which resulted in the use of supplemental amino acids in feed formulation. However there has been a gradual evolution and more emphasis is now being given to supplement amino acids in terms of sustainability and total nutrient supply.

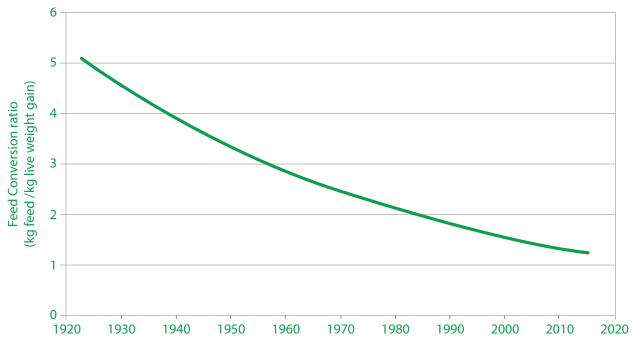


Figure 1 - *Improvements in the feed efficiency of broilers*

Only as a result of developments in analytical techniques has it been possible to demonstrate that an evaluation of protein to the point of the effectively utilised essential amino acids is required to optimise feed formulation and that this yields both practical and financial benefits. Over the last 2 decades crude protein concept has lost its overrated status which it had held since the start of nutrient evaluation.
The world population continues to increase, with over 9 billion people predicted in 2050. Correspondingly the requirement for protein to feed the increasingly affluent world population will rise. Thus as agricultural production becomes ever-more concentrated in specific regions it becomes even more important to use all natural resources as wisely and sparingly as possible. In this respect, the concept of sustainable agriculture is not solely related to animal production but also incorporates all aspects of animal feeding and the use of feeding regimes adjusted to demand.

Amino acids are essential nutrients which are an integral part of these feeding regimes. In most instances they may substitute feed protein sources, helping to save and spare protein, reduce nitrogen excretion and minimise greenhouse gas emissions. In the future, amino acids will become even more important to ensure that animal production systems are environmentally sustainable and make optimum use of the limited natural resources.

1.1 History of amino acid use in animal feed

Over the last 60 years the availability of supplemental amino acids has provided producers of feed and animal protein to maintain the affordable supply of food while society has greatly changed and grown its consumption.

Without amino acids it would have been very difficult, if not impossible, to produce the quantity of meat, milk, fish and eggs demanded by European consumers. The availability of amino acids has allowed feeds to be produced using smaller quantities of protein rich raw materials allowing these limited scarce resources to be used more sparingly.

Starting in the 1950's with methionine, amino acids have enabled fish meal to be eliminated from meat and egg production and at the same time allowed poultry producers to increase production based upon the use of vegetable protein sources. Availability of lysine in the 1960's has allowed these developments to progress further and be replicated in other species. Since then the amino acids threonine, tryptophan, valine, isoleucine, histidine, and arginine have all become available.

1.2 Amino acids and mixed feeds

Compound feeds used either as the sole component of a diet or as supplements to other feed materials, represent the major part of the total agricultural economic outlay and remains one of the most important concepts in agriculture.
The past decade has seen major increases in compound feed production coupled with a trend towards larger livestock units and higher performance levels. This has increased the importance attached to the utilisation of new findings in the areas of physiology of nutrition and in the design of new compound feed formulas. These developments place continuously higher demands on the formulator to produce feeds capable of meeting the requirements for high performance, cost-effective and environmentally friendly feeds. The following diagram shows the result of a very simple experiment with only

one small supplement of an amino acid to a pig feed (Figure 2).
Without using amino acids, lowering the protein content of the feed by 2% from 18 to 16% in the starter and 16 to 14% in the finishing diet, resulted in poorer growth (-2% crude protein no amino acids) compared with the controls (control no amino acids). However, after the addition of lysine to the low protein diets (-2% crude protein with amino acids) the level of performance was raised to that of the controls. Moreover, the pigs given the low protein diets with supplemental lysine excreted significantly less nitrogen compared with the controls. For many years, amino acids have provided the opportunity to achieve improved animal performance coupled with a saving in protein use and above all at a lower cost for animal production. In addition to these benefits, both animal producers and consumers have become increasingly more aware of the additional positive effects such as improved animal health and a reduced nitrogen load on the environment.

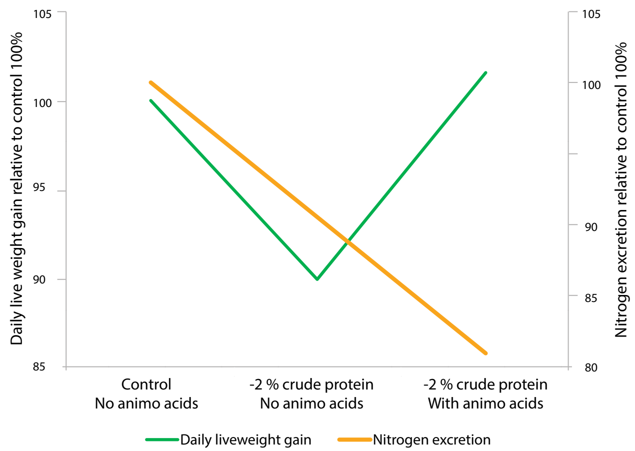


Figure 2 *The influence of amino acid supply on daily liveweight gain and N-excretion in pigs*

2 STRUCTURE AND CHEMISTRY OF PROTEIN AND AMINO ACIDS

2.1 Protein

2.1.1. Significance and composition

Protein (from the ancient Greek “protos”: the first or the most important) is the most important and quantitatively major component of all organisms and as such is a prerequisite of all life. In feed for animals, proteins cannot be replaced by any other component. Protein containing compounds are found in every cell and account for the major proportion of organisms. They are essential for maintenance, growth and reproduction of the whole organism. However they can only fulfil this role in association with other nutrients which provide energy, with vitamins, minerals, both macro and trace amounts and water.
The major elements in protein are carbon (C), oxygen (O) and hydrogen (H) the same as in fats and carbohydrates. In addition proteins contain nitrogen (N) and sometimes sulphur (S) and phosphorous (P) (Table 1).

Table 1 - *Proportion of elements in protein sources*

Carbon	51.0 – 55.0
Hydrogen	6.5 – 7.3
Oxygen	15.5 -18.0
Nitrogen	0.5 – 2.0
Sulphur	0.5 – 2.0
Phosphorous	0 – 1.5

The proportion of elements in protein sources is relatively constant (%).
Proteins are high molecular weight, modular compounds. The modular nature is part of the chemical structure since each protein

is made up of approximately 20 different amino acids. This unique structure which consists of amino acids in particular sequences gives to each protein a high degree of specificity with respect to the function which each individual protein performs. For example, enzymes are proteins with catalytic function whilst immune bodies display a defence function. Muscle protein enables animals to undertake physical work, and bone, skin and connective tissues have a supportive and protective function. However in animal production, the primary target is for the animal to produce protein which is the major essential component of meat, milk and eggs.
The series of amino acids within the protein molecule is genetically predetermined and referred to as the amino acid sequence.

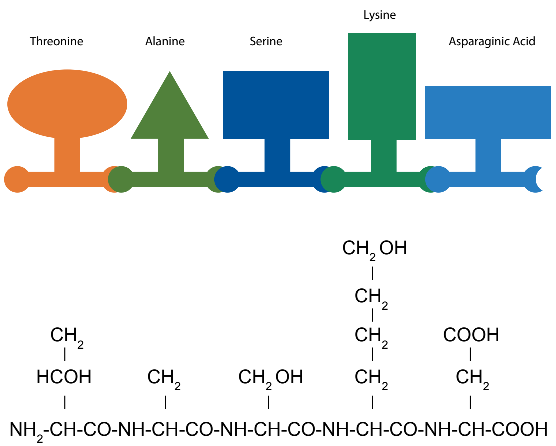


Figure 3 - *Amino acids as building blocks of proteins*

The amino acids are linked by peptide bonds between the carboxyl group of the one and the amino group of the next amino acid. A peptide chain can comprise up to several thousand amino acids and since there are only approximately 20 different amino acids, many will be repeated in the peptide chain.

2.1.2. Classification

Proteins can be classified into three groups according to their structure and solubility:

- **Sclero-proteins**; which have a fibrous structure and do not dissolve in water. They are comprised of a long linear chain as a supportive and paraplastic substance. Typical examples are collagen (connective tissue, cartilaginous substance) and keratin (skin, hair, wool, feathers).
- **Sphero-proteins**; which are essentially firmly intertwined and soluble in water or dilute saline. This group comprises albumins, globulins, histones, prolamines and glutelines.
- **Proteides**; which are made up of proteins but also contain a non-protein prosthetic group which tends to be firmly bound to the protein. Depending on the type of prosthetic group, proteides are:
    - Metalloproteins (e.g. haemoglobin)
    - Phosphoproteins (e.g. casein)
    - Lipoproteins (e.g. serum lipoproteins)
    - Nucleoproteins (e.g. nucleic acid + protein)
    - Glycoproteins (e.g. seromucoids)
    - Chromoproteins (e.g. myoglobin)

The type of binding between the protein portion and the prosthetic group differs according to the type of prosthetic group and each different type of proteide has a different function in the organism.
Apart from the proteins already mentioned, compounds containing nitrogen but with a non-protein nature are also found in animal tissues. These compounds include alkaloids, amides (asparagine, glutamine, and urea), betaine, choline and purines. They are grouped together as non-protein nitrogen (NPN) compounds.

From the point of view of the fundamentals of nutrition, amino acids are equivalent to proteins. It is primarily for this reason that the main emphasis in the nutrition of animals has shifted from a focus on protein as a whole to a focus on individual amino acids.

2.2. Amino acids

2.2.1. Chemical structure

Amino acids are characterised by the two characteristic functional groups in the molecule, as indicated by their nomenclature, by the amino group NH2 and the carboxyl group COOH.
Amino acids occur exclusively as structural protein units in which the amino group is bound to the α-position of the carboxylic acid group (carboxyl group). Chemically amino groups can bind in other positions, however only α-amino acids are relevant for animal nutrition.

Glycine is the simplest representative of an amino acid with two hydrogen atoms attached to the C atom (Figure 4). In all other amino acids which are found in protein, an aliphatic or aromatic substituent (=R) is attached to the carbon atom, in addition to the amino group.

There are two different types of amino acids, the so-called optically active isomers. Optically active isomers differ with respect to the spatial arrangement of the four bonds on the carbon atom. This gives rise to the two forms termed the L-form and D-form. These forms exist as mirror images of each other in the same way as the right hand is to the left hand (Figure 4). Apart from differences in the physiological efficacy in animal organisms, the chemical and physical characteristics of optical isomers are the same except for one property. They differ in the optical rotation of polarised light, hence the term optical isomers.

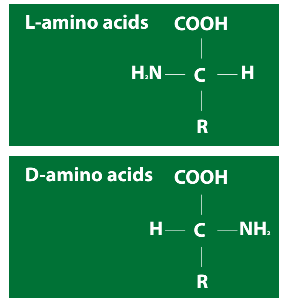
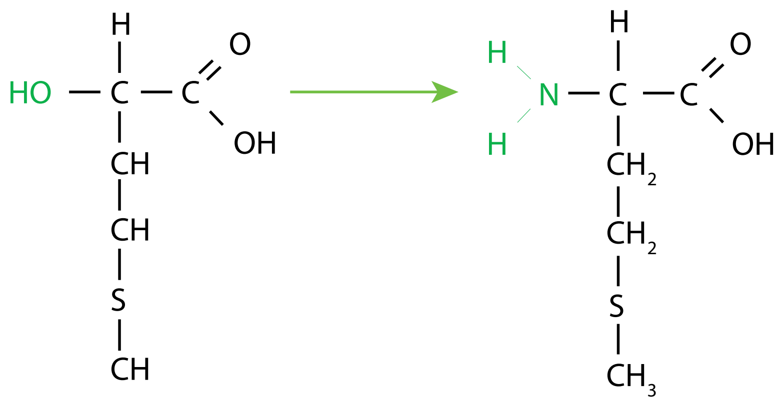


Figure 4 - *General structure of L- D- isomers*

Amino acids found in proteins belong to the L-series. If an animal is supplied with amino acids in both the D- and L-forms (that is a 50:50 mixture of L- and D-amino acids, also called a “racemic mixture”) then the D-form has to be converted into the L-amino acid before it can participate in metabolism. This can be achieved by deamination to the keto form and subsequent amination into L-amino acid. This conversion process is dependent on the animal species and takes place for individual amino acids with varying efficiency. In the case of Methionine, this conversion is so effective that from a nutritional point of view, separation of the D and L- isomers is not necessary.

2-hydroxy-4-(methylthio) butanoic acid (HMTBa) is a naturally occurring hydroxy analogue of methionine that can be converted into L-methionine in the tissues for use in protein synthesis. As a chemically synthesized feed supplement HMTBa occurs in a racemic mixture of D- and L- isomers, similar to synthetic methionine. The difference between HMTBa and methionine is that with HMTBa there is a hydroxyl group in place of the amino group at the second carbon (Figure 5). This difference allows classification of the former as an organic acid whereas the latter is an amino acid.



2 - hydroxy-4-(methylthio) butonic acid (HMTBa)

Methionine

Figure 5 - *HMTBa and Methionine*

2.2.2. Chemical characteristics

About 20 different amino acids have been identified following direct hydrolysis of common nutritional feed proteins. Based on chemical principles amino acids can be divided into three main categories, the neutral, acidic and basic amino acids (Table 2). This classification is based on the different types of substituents (R) which are present on the carbon atom in addition to the amino group. Acidic amino acids possess a second carboxyl group in the substituent R position, whilst the basic amino acids have an additional basic group.

Table 2 - *Classification of amino acids according to their chemical characteristics*

Acidic amino acids	Neutral amino acids	Basic amino acids
Asparaginic acid	Alanine	Arginine
Glutamic acid	Asparagine	Hystidine
	Cysteine / Cystine[1]	Lysine
	Glutamine	
	Hydroxyproline	
	Isoleucine	
	Leucine	
	Methionine	
	Phenylalanine	
	Proline	
	Serine	
	Tryptophan	
	Tyrosine	
	Valine	

[1] Two molecules of cysteine produce one molecule of cystine

3. PROTEINS IN AMINO ACIDS' NUTRITION

3.1. Digestion and absorption

Amino acids chemically bound in proteins must be separated from the parent protein unit, before they can pass from the lumen of the gut across the intestinal wall (absorption) into the blood. This separation occurs in the lumen of the gut with the help of proteolytic digestive enzymes (proteases). The activity of the proteolytic enzymes is aided by the secretion of dilute hydrochloric acid in the stomach. Presence of the acid acidifies the ingested feed in the stomach which results in denaturation of the protein. The process starts with denaturation of the protein and continues with the cleavage into individual amino acids or as pairs of amino acids (dipeptides), tripeptides and up to six amino acid units in length (oligopeptides).
The break-down of the peptide chains is carried out by endopeptidases (pepsin, trypsin, chymotrypsin) which cleave at the centre of a chain and exopeptidases which cleave from the terminal ends. The amino acids and oligopeptides are absorbed by mucosal cells which line the surface of the intestine and finally enter the bloodstream as free amino acids. Specific transport systems are responsible for the absorption of amino acids. The absorbed amino acids are transported via the portal vein into the liver, which is the principal organ for the metabolism of amino acids.

3.2. Protein metabolism and synthesis

The metabolism of protein is made up of two opposing processes which run in parallel. The accretion of proteins (anabolism = synthesis) and the breakdown of protein (catabolism = proteolysis) occur at one and the same time. Synthesis predominates in young growing animals and the protein is built into muscle whereas in mature animals a balance is reached between synthesis and proteolysis with no increase in the mass of the muscle but with continuous turnover.
The amino acid sequence of a protein is genetically predetermined and all the required amino acids must be present at the same time (synchronous synthesis). The organism is able to compensate for a

deficiency of non-essential amino acids within certain limits through auto-synthesis. However, protein synthesis comes to a stop if one of the essential amino acids is lacking because some amino acids (the essential ones) cannot be synthesised by the organism (see 3.4 essential amino acids). Amino acids which are not used to synthesise protein or that are released from protein during degradation must be broken down and excreted since the body has no mechanism to store them. The carbon skeletons of amino acids are metabolised to supply energy and the liberated ammonia which is derived from the nitrogenous component must be "detoxified" and removed from the body. This is achieved via the synthesis of urea in mammals, and uric acid in poultry, which is a process with a very high energy requirement.
During periods of severe energy deficiency, protein may be catabolised to supply energy for the upkeep of vital processes. However, compared to the metabolism of fats and carbohydrates, efficiency of the process is very low.

From the above it can be seen that:

- Protein and energy metabolism cannot be considered as unconnected. In feed formulation this is taken into account by considering the ratio of the limiting amino acids with respect to the metabolisable or net energy content of the feed.
- Matching of amino acids provided for metabolism with the actual requirement for metabolism must be as precise as possible in terms of both quantity and composition (see chapter 3.6 Ideal protein concept).

Apart from muscle growth only limited amounts of protein can be stored. Some storage occurs in the liver. Otherwise, the degradation of protein is relatively rapid and is expressed by the half-life. For example, digestive enzymes which have a short half-life are particularly affected and are thus highly susceptible to changing metabolic conditions with respect to amino acid supply. Hence a temporary deficiency in amino acids for the synthesis of the enzyme proteins can show up as a loss in performance.

Great importance is therefore attached to the concept of the continuous supply of free amino acids from the feed into the animal's metabolism (amino acid flux). This needs to be taken into account, when supplementing amino acids to mixtures of feed. In modern practical feeding systems, amino acid supplementation has been proven to be an effective method to continuously balance the amino acid supply at the site of protein synthesis.

3.3. Protein quality

Protein is not a nutrient. Animals require amino acids and not protein per se. The quality of protein supply is determined by its potential to cover the physiological requirements in terms of amino acids for maintenance and performance (growth, reproduction, production of milk and eggs). The quality of protein required is different depending on the animal species, age, genotype and sex as well as on the performance level. It follows therefore that there are two important factors with respect to protein quality, a) the amino acid profile which is the ratio of essential amino acids in the protein and b) their digestibility (see 3.7). Ingredients that contain excellent quality proteins are dried skimmed milk powder and complete egg protein. Other protein sources lack certain essential amino acids and thus are unable to closely match the requirement of the animal in terms of their amino acid composition.

Figure 6 demonstrates that soybean meal alone, with the exception of sulphur amino acids (methionine, cystine), can supply all essential amino acids necessary to satisfy the requirements of a pig (30 - 50 kg liveweight). However corn has an amino acid profile which meets the requirements for sulphur amino acids.

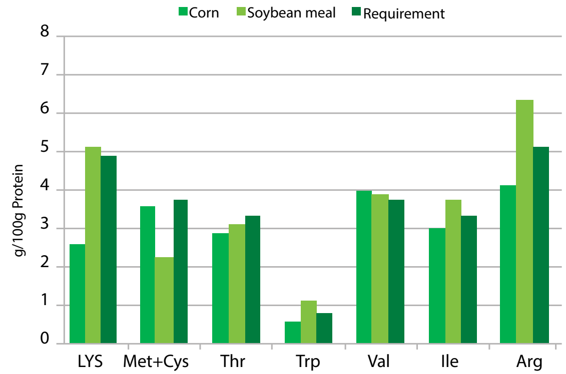


Figure 6 - *Amino acid composition of protein in soybean meal and corn compared to the requirements of broilers of 11-24 days of age*

By combining the two protein sources in the appropriate proportions it is possible to achieve the requirements in terms of limiting amino acids (Figure 7). The protein quality of this mix is greater than that of either of the two individual ingredients.

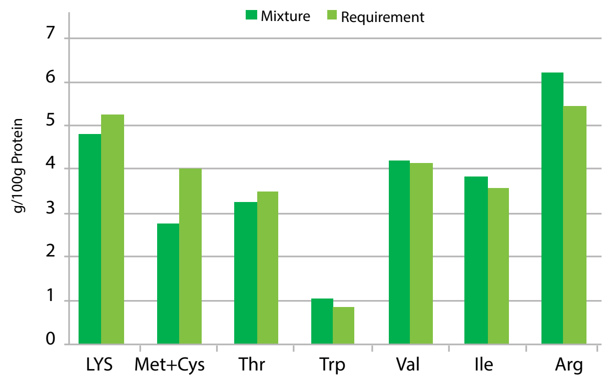


Figure 7 - *Amino acid content of a mixture of soybean meal and corn protein relative to the amino acid requirements of broilers of 11-24 days of age*

A protein quality must always refer to a specific situation with respect to protein use. In practice, standards of requirement for amino acids are usually given for age and performance levels for all of the major livestock species. With the aid of these standards the quality of a feed protein can be estimated as shown in the example in Figure 7.
For a particular feed ingredient or mixture of ingredients supplying protein, the amino acid which is in shortest supply compared with the requirement of the animal, is defined as the first-limiting amino acid. The first limiting amino acid limits the value of protein.
In the previous example the amino acid lysine is the first limiting amino acid in corn protein and methionine or the sum of sulphur containing amino acids in soybean protein.

Unfortunately, the total content of amino acids in a feed protein, which is determined by chemical analysis, is not fully digestible (see 3.7). A measure of the total amino acid content of a feed protein in comparison with a set of requirements can therefore only give a first approximation with respect to the quality.

The ruminant animals derive their amino acids supply jointly from dietary protein which escapes rumen degradation (by-pass protein) and microbial protein synthesized in the rumen. The dietary protein is extensively degraded in the rumen and is mainly used by rumen bacteria for their own protein synthesis. The microbial protein that reaches the intestine presents the most appropriate protein quality for ruminants since it has a similar amino acid profile as the animal requirements. The amino acid composition of the by-pass fraction usually is not in line with animal requirements therefore amino acids need to be supplied.

3.4. Essential amino acids

The animal itself is capable of synthesising about half of the amino acids. These amino acids are termed non-essential amino acids. However, about 10 amino acids (depending on the species) cannot be synthesised by the animal and a source must be supplied in the feed (see Table 3). For this reason they are termed the essential amino acids. A number of non-essential amino acids can only be synthesised from essential amino acids and are called semi-essential.

Table 3 - *Essentiality of amino acids in pigs and poultry*

Essential	Arginine(1), Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, Valine.
Semi-essential	Cystine (Cysteine)(2), Tyrosine
Non-essential	Alanine, Asparagine, Aspartic Acid, Glutamic Acid, Glutamine, Glycine, Proline, Serine.

(1) In swine, Arginine is essential only in young animals.
(2) Cystine = dimer of cysteine

The classification of amino acids into essential and non-essential should not be taken to imply that non-essential amino acids are not required for the synthesis of protein. The terminology non-essential simply indicates that the animal is able to synthesise these non-essential amino acids or convert them from one amino acid into another. To undertake such amino acid inter-conversions the animal requires sources of carbohydrates and suitable nitrogen compounds.

Sulphur containing amino acids

The two sulphur containing amino acids methionine and cysteine each contain a sulphur atom and they are present in animal and plant proteins in varying proportions. Methionine is an essential amino acid whereas cysteine is semi-essential. Depending on the species of animal cysteine may be responsible for up to 50% of the dietary methionine requirement. Scientific findings show that this proportion is less than 50% in high performance animals. In addition to its essential role as a protein building block and precursor of cysteine, methionine is also involved in a number of other biosynthetic pathways. Methionine is involved in metabolic pathways of compounds such as choline, creatine and adrenaline via the release of a methyl group and the formation of S-adenosyl-methionine in the methyl donor pathway. Thus methionine indirectly plays an important role in intermediate metabolism. In the organism, cysteine is produced from methionine via S-adenosyl-methionine-cystathionine. Cysteine is subsequently further metabolised into taurine or via a number of intermediate stages transformed into sulphate.

Basic and aromatic amino acids

Basic amino acids are listed in Table 2. Amongst those, lysine is one of the key essential amino acids, while arginine is essential only in very young animals.
Tryptophan, phenylalanine and tyrosine are aromatic amino acids. Besides being a constituent of body protein, tryptophan plays other important roles in metabolism. Tryptophan is involved in feed intake regulation, in the immune response and in coping with stress situations. Phenylalanine and tyrosine are interchangeable. The total requirement for phenylalanine and tyrosine can be met by phenylalanine alone but the converse does not occur for tyrosine. The limited evidence available indicates that tyrosine can only provide part of the requirement for phenylalanine.

Branched chain amino acids

Isoleucine, leucine and valine are the three branched chain amino acids. Their metabolism is unique, sharing the same catabolism pathway and therefore interfere with each other. Leucine is a strong regulator of the branched chain amino acid catabolism. As a consequence, minimum supply of valine and isoleucine must be ensured in the diet and an excess of leucine should be avoided.

3.5. Limiting amino acids

Protein synthesis is an indispensable process in the animal. For protein to be synthesised, the required essential and non-essential amino acids must be present at the site of synthesis according to the requirements of the animal. When the supply of one of this essential amino acid does not meet the animal requirements for this amino acid, it is said to be limiting.

Hence a limiting amino acid must be present in the diet in sufficient quantity to meet the total requirements of the animal. The first amino acid to limit protein synthesis is termed the first limiting amino acid. Once this amino acid has been supplied the next to limit synthesis is the second limiting amino acid and so on.
In typical diets offered to poultry and ruminants it is usually methionine which is first limiting, lysine usually being second limiting. In pigs lysine is the first limiting amino acid followed by threonine, methionine, tryptophan and valine. A sufficient quantity of the limiting amino acids in the diet to meet requirements also governs whether the other amino acids are efficiently utilised for protein synthesis.
This principle can be illustrated by the “Liebig barrel” where the level of fill in the barrel represents the capacity for protein synthesis of the animal (Figure 8). The capacity of the barrel is “limited” by the shortest stave (the first limiting amino acid). However, if the shortest stave is lengthened (dietary supplementation with the first limiting amino acid) then the capacity increases to the level of the “second-limiting” stave. This repeats for the next limiting amino acids.
In practical diets the “staves” can be lengthened through targeted supplementation of amino acids. This is demonstrated in Figure 8 for a typical European piglet feed with 5 limiting amino acids covered by supplementation.

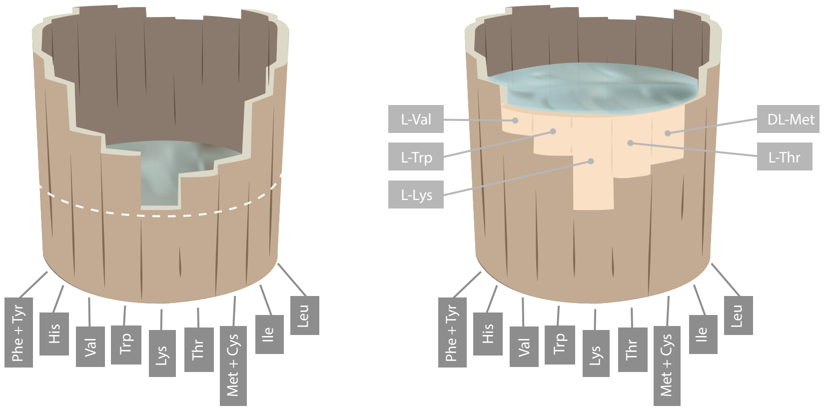


Figure 8 - *The "Liebig barrel" illustrating the concept of limiting of amino acids and the benefits of their supplementation in a current typical European piglet diet.*
The amino acid levels (length of the staves) are expressed in proportion of the animal requirements.

Building upon the concept of Liebig barrel, the idea developed that there is one ratio of amino acids that is "ideal". Since in pigs, lysine tended to be the first limiting amino acid it was decided that lysine would be taken as a reference value and the remaining essential amino acids would be referred to lysine, taking lysine as one hundred percent. For pigs and poultry, these ratios of essential amino acids compared to lysine are designated as 'the ideal protein' or the 'ideal amino acid profile' (see 3.6).

3.6. Ideal protein concept

The most recent data concerning ideal amino acid ratios are based on amino acids digested in the ileum in order to take into consideration the influence of amino acid losses during digestion and absorption. Further data for individual species can be found in chapter 8 "Amino acid responses and recommendations".
Since poultry have different requirements for some essential amino acids, namely the sulphur containing amino acids for feather growth, they have a different "ideal amino acid profile" to pigs. The requirement for essential amino acids for the basic maintenance of the animal compared with the requirement for growth changes with increased liveweight hence the ideal amino acid profile may change with increasing liveweight.
In the case of ruminant animals, especially dairy cow, lysine and methionine have been identified most frequently as first-limiting amino acids for both growth and milk protein production. The "ideal amino acid profile" or since protein provides amino acids, the "ideal protein" requirement, differs from one animal species to another and within an animal species differs depending on age and level of production. This is because the requirements for specific essential amino acids and hence the ratio, differs between species and differs according to the requirement in the body whether it be for maintenance, growth or reproduction. The concept of "ideal protein" helps considerably the task of the feed formulator. If the dietary requirements of an animal in terms of lysine are known and the ideal amino acid profile for that animal and stage of production is known, then the requirement for all the other amino acids can be estimated.

3.7. Amino acid digestibility

Proteins cannot be absorbed intact through the walls of the intestine, the molecules are too large. Thus for an amino acid contained in a feed protein to reach the site of protein synthesis, it must be released from its parent protein. Release from the parent protein occurs during digestion in the gut after which either the individual amino acids or

as di- and tri-peptides are absorbed through the intestinal wall (see chapter 3.1. on Digestion and Absorption). Hence factors which have an influence on digestion and absorption in the animal also play a key role in the animal nutrition (see 3.8).
The notion of digestibility has gradually developed on both a methodological and a conceptual level. Digestibility, which was initially measured at the fecal level, is now measured at the ileal level, due to changes caused by the microflora of the large intestine. On the one hand, undigested amino acids on the ileal level can be catabolised by the microflora of the large intestine or used for the synthesis of microbial protein. On the other hand, some amino acids are synthesized de novo by bacteria without being absorbed by the animal; these eventually end up in the faeces. Ileal digestibility therefore represents the absorption of amino acids in the digestive tract far better than fecal digestibility. However, the profile of amino acids at the end of the small intestine is not the only result of the absorption of dietary amino acids. The digestive process is also accompanied by secretions of digestive enzymes and cell desquamations, whose protein elements blend with the proteins of dietary origin in the intestinal lumen. These secretions are partially digested and reabsorbed, but some of them, escape this re-absorption. This fraction of endogenous origin can be measured by feeding the animal with a protein-free diet. Although this technique only enables basal endogenous or non-specific losses to be measured, it makes it possible to propose corrected apparent ileal digestibility coefficients which are called true or standardized, meaning corrected for the basal endogenous losses.
Furthermore, if the endogenous secretion of amino acids is estimated, then the standardized ileal digestibility may be calculated. Diet formulation based on ileal digestible amino acids gives a more accurate estimate of the amino acids available to the animal and is of greatest value with poor digestibility feedstuffs.
Differences in the digestibility of individual amino acids are an inherent characteristic of different raw materials. The digestibility coefficients of different amino acids in a range of raw materials for monogastrics are shown in Table 4. The percentages of digestible amino acids in ruminants for a range of feed materials are presented in Table 5.

The values have been drawn from a number of different sources and therefore represent a wide range of experimental techniques.
With respect to the nutritional evaluation of feedstuffs the information in Table 4 and Table 5 represents a major advance compared with the use of total amino acid content. If the evaluation of digestibility is carried out at the end section of the small intestine (terminal ileum) the effect of microbiological conversion which occurs in the large intestine is essentially eliminated.

Table 4 - *Digestibility coefficients (%) of amino acids measured in poultry[(1)] and pigs[(2)] of a range of feed materials*

	LYS		THR		MET		M+C		TRP		VAL	
	Poultry	Pig	Poultry	Pig	Poultry	Pig	Poultry	Pig	Poultry	Pig	Poultry	Pig
Cereals												
Barley	78	75	76	75	80	84	82	84	-	79	80	80
Maize	85	80	88	83	94	91	93	90	-	80	92	87
Oats	86	73	80	69	88	84	85	78	-	78	85	77
Sorghum	87	74	89	76	90	85	88	81	-	79	90	81
Wheat	84	81	83	83	90	89	91	90	-	88	88	86
Cereal by-products												
Corn gluten feed	71	66	75	70	84	84	73	76	-	66	82	75
Corn gluten meal	90	89	93	92	98	95	96	94	-	87	96	91
Wheat bran	74	68	75	65	74	76	75	74	-	76	75	72
Wheat middlings	81	75	79	72	80	82	75	79	-	80	82	78
Oilseeds and other protein sources												
Peas	79	83	81	76	-	80		75	-	73	82	77
Rapeseed meal	78	75	84	75	87	87	84	84	-	80	88	77
Rapeseed, full fat	87	78	80	71	86	81	80	80	-	73	85	70
Soybean meal, 48	91	90	89	87	91	92	88	89	-	89	91	88
Soybean, full fat, toasted	81	79	79	75	82	78	79	76	-	83	77	74
Sunflower meal, undecorticated	83	80	87	82	93	92	89	88	-	85	90	84
Other												
Fish meal , protein 65	89	93	91	92	92	93	89	91	-	89	92	92

[(1)] true digestible basis
[(2)] SID basis

Source: INRA – AFZ Tables of composition and nutritional values of feed materials, 2004

Table 5 - *Percentages of digestible amino acids in ruminants for a range of feed materials (AADI, expressed in %PDIE)*

	CP	PDIE	LYS	THR	MET	ILE	VAL	LEU
	%	g/Kg	%PDIE	%PDIE	%PDIE	%PDIE	%PDIE	%PDIE
Cereals								
Barley	10.1	87	6,8	5,0	1.9	5.2	5.8	8.1
Maize	8.1	84	5.7	4.8	1.9	4,9	5.5	10.2
Sorghum	9.4	87	5.4	4.7	1.8	5.1	5.7	11.0
Wheat	10.5	89	6.7	5.0	1.9	5.2	5.6	8,0
Cereal by-products								
Corn gluten feed	19.3	102	6.3	5.0	2.0	5.0	5.8	9.0
Corn gluten meal	60.6	460	3,5	4.2	2.0	4.6	4.9	13.8
Wheat bran	14.8	80	6,7	5,0	1,9	5.1	5.7	8.0
Wheat middlings	15.5	87	6,7	4.9	1.9	5.1	5.6	8.0
Oilseeds and other protein sources								
Peas	20.7	83	7.7	51.0	1.7	5.3	5.7	8.2
Rapeseed meal	33.7	138	6.8	5.1	2.0	5.1	5.7	8.1
Rapeseeds	19.1	61	7.4	5.3	2.1	5.2	5.8	8.0
Soybean meal, 48	45.3	2+29	6.9	4.7	1.5	5.2	5.4	8.2
Soybean, full fat, toasted	35.2	157	6.9	4.8	1.6	5.1	5.3	8.2
Sunflower meal, undecorticated	27.7	93	5.9	4.9	2.1	5.1	5.6	7.8
Forages								
Maize silage, 30% DM	84	66	6.90	5.21	1.97	5.39	5.95	9.06
Prennial rye-grass, 33% DM	11.7	64	7.17	5.33	1.93	5.53	6.09	8.47

PDIE: protein digestible in the small intestine, in g/kg when energy is the limiting factor for rumen microbial activity.
AADI: amino acids digestible in the small intestine.

Today the protected sources of methionine and lysine are increasingly used to balance the ratio in ruminants' diet.

3.8. Factors influencing amino acid digestibility

Technical processing is used to a great extent in feed production. Protein-containing feedstuffs and compound feeds are treated with steam and heat. For proteins damaged by heat during processing, the measurement of ileal digestible amino acids gives a better estimation of the amino acids available to the animal compared with total content or fecal digestibility. However the thermal damage which may occur to an amino acid during processing is not accurately measured by the ileal digestibility technique since although the amino acid can be absorbed it cannot contribute to metabolic reactions due to structural damage. This loss in ability to participate in metabolism can only be estimated by measuring the physiologically active amino acids.
Examples of different processing techniques and resulting reactions and effects that can restrict the availability of certain amino acids are shown in Table 6. The degree of heat used in the process is particularly important. Individual feeds are subjected to a number of different thermal treatments such as the toasting of soybeans and soybean meal, rapeseed products, peas and field beans; pasteurising of fish meal or drying of corn gluten and wet cereals. Compound feeds are subject to intense treatments (during pelleting up to 80°C; expanding up to 110°C; extruding up to 130°C) for technical, nutritional, physiological and hygienic reasons.
Components - such as reducing sugars - frequently present in compound feeds, favour the formation of Maillard products with lysine and reduce its availability under intensive feed processing conditions. At present the effect of these processing procedures on amino acid availability has not been extensively investigated for individual proteins and mixes, particularly with a view to precisely predicting the effects. However from the point of view of the feedstuff evaluation and formulation, the use of ileal digestible amino acids represents a real progress compared to using the total amino acid content and is a concept which should be more widely adopted by feed formulators.

By accounting for the different losses in the process of digestion/absorption, for example of lysine in cereals, a more accurate estimate of the requirement for supplementation can be made.
In ruminant nutrition, the reason behind the attempts to protect dietary protein is to avoid the degradation of high quality proteins in the raw materials by ruminal degradation. It is possible to protect proteins using several procedures such as heat treatment, chemical treatment or modification, and inhibition of proteolytic activity and identification of naturally protected protein. The use of these techniques in comparison to the usual sources of dietary proteins improves the supply of amino acids, resulting in a better performance by the animal and less N-emissions into the environment.

Table 6 - *Effects of processing and possible damage to amino acids*

Process	Reaction	Affected Amino Acids
Heating (drying, toasting)	Maillard Racemisation Degradation Cross linking	Lysine
Protein extraction	Protein-polyphenol reaction	Lysine, Methionine, Cysteine, Tryptophan
Alkaline treatment	Racemisation Degradation Cross linking	Lysine, Methionine, Cysteine, Phenylalanine, Histidine, Tryptophan
Storage (peroxide formation)	Oxidation products + amino acids	Lysine, Methionine, Cysteine, Tryptophan

3.9. Availability beyond digestibility

Frequently the terms digestibility and availability are interchanged. However, this can result in incorrect estimates of requirements since even digested and absorbed amino acids are not always completely available for protein synthesis. These can be estimated for limiting amino acids from N-balance trials or measurements of weight gain. Such measurements take into account the availability of amino acids at the metabolic level, a factor that is not considered by measuring

digestibility alone. Only at the metabolic level can the term availability be truly justified for an amino acid.
As shown in Table 4 the digestibility of amino acids can be estimated for various raw materials. In ruminants digestible amino acids are estimated as shown in Table 5. Apart from diet formulation based on the total amino acid content, research is in progress to determine requirements of key digestible amino acids. Work is also in progress to estimate the availability of amino acids at the physiological level but further research is required.

The availability of amino acids in cereals and protein-rich ingredients that have not been damaged by heat can be estimated by digestibility alone. For protein sources which have undergone thermal treatment in vitro availability tests are available.

The value of using standardized ileal digestible amino acids was demonstrated in trials with pigs. Although all diets were formulated with the same amount of digestible lysine, the efficiency of growth decreased with increasing intensity of the heat treatment. It has proven difficult to accurately estimate the degradation rate or catabolism of the essential amino acids in metabolic processes. In the future it will be even more important to be able to accurately measure amino acid catabolism in order to precisely estimate the requirement of utilisable amino acids, particularly for the limiting amino acids.

Future research must be aimed at developing adequate systems for the evaluation of amino acid availability, which are able to provide reliable predictions of availability under the conditions of modern feed processing. The limitations of the present system that considers only digestion and absorption will not be sufficient to satisfy future requirements.

4. ENVIRONMENTAL BENEFITS

In the past, increasing productivity has been the prime issue with respect to animal production. However in the late 1900s eutrophication became a prominent issue in Europe. In more recent times, the contribution of animal agriculture to environmental issues such as global warming, acidification, as well as land and water use have become increasingly in focus.

As far back as 1991, the Council of the European Union Community published the Nitrate Directive 91/676/EEC which was designed to reduce the pollution of waters due to nitrogen from farming. The Directive 96/61/EC (1996) put also into place an approach based on Integrated Pollution Prevention and Control (IPPC). According to this directive, large poultry and pig farms are authorized only if the polluting emissions in water and soil (including nitrates) and in the air (in particular ammonia) do not exceed maximum limits. Feeding measures including the use of lower protein diets through amino acid supplementation are officially recognised as BAT (Best Available Technique) in the BREF document published by the EU Commission JRC. Part of nitrogen excretion originating from animals can be avoided through dietary manipulation. These feeding measures include:

- Feed formulation based on digestible nutrient constraints. This system provides more digestible diets.
- Multiphase feeding. This involves splitting feeding programmes into shorter feeding periods. The nutrient supply is adjusted in accordance with the animal's requirement. Multiphase feeding is a series of diets with decreasing nitrogen contents.
- Reduced protein levels in the feed. These diets avoid an oversupply of non-essential amino acids and lead to reduced nitrogen excretion.

The principles are that the amino acids supplied in the feed in excess of the animal requirement are not used by the animal and are degraded into urea (mammals) and uric acid (poultry). These nitrogen compounds are excreted by the animals in the manure or in the litter bedding and generate ammonia (NH3), nitrous acid (NOx) and nitrous oxide (N2O); the latter is a powerful greenhouse gas with an effect about 300 times that of CO2.

Feeding animals with a diet supplying an optimal amino acid composition (i.e. without excessive amount of non-limiting amino acids) will improve the utilization of dietary protein by animals. It will reduce urea nitrogen output and subsequent emission of the greenhouse gas compounds (NH3, N2O…) in buildings and in the environment.
Reduction in the concentration of noxious gases such as ammonia in livestock houses can also have major benefits for animal welfare, as well as the health of farmers and employees who work in such environments.

Feed management using amino acid supplementation to lower dietary protein levels can potentially reduce nitrogen excretion up to 50% without detrimental effects on animal performance. Table 7 summarizes the beneficial effects on the environment of reduced protein diets in pigs and broiler.

Table 7 - *Typical reductions in emissions due to improved amino acid balance in pigs and broiler*

		Effect of 1 point reduction of dietary protein	Maximum potential effect using low protein diets[(1)]
Pigs	On total nitrogen excretion	-10%	-50%
	On ammonia content in the slurry	-10%	-50%
	On slurry pH		-1 point
	On ammonia emission in the air	-10%	-60%
	On water consumption	-2-3%	-28%
	On slurry volume	-3-5%	-30%
Broiler	On total nitrogen excretion	-10%	-30%
	On ammonia emission	-10%	-30%

[(1)] assuming availability of full range of essential amino acids

Additional potential to reduce emissions is achievable if supplementation with the next limiting amino acids (e.g. valine) is widely adopted. The use of amino acids in feed formulation results in the saving of natural resources. For instance, using methionine supplementation allows the saving of fish stock in the oceans. Together with lysine, these amino acids allow for very significant reduction of the import of protein-rich stuffs in the EU from third countries. These examples highlight the benefits of using supplemental amino acids to help reduce the competitive pressure on scarce natural resources.
In ruminant production, there are a number of differences compared to pig and poultry production. In dairy farming, large amounts of nitrogen enter farms every year as nitrogen fertiliser and as protein in purchased feedstuffs. Unfortunately much of this nitrogen remains on the farm rather than being incorporated into added value products such as milk, other animal products and crops sold off the farm. The overall low efficiency of utilization of dietary nitrogen in dairy farming is of particular concern with estimations of less than 20%. Consequently there is considerable interest in developing nutritional approaches to mitigate manure nitrogen output in ruminant productions.

The primary factor influencing excretion of nitrogen in animal manure is the nitrogen intake of the animal. Therefore the most effective method to reduce manure nitrogen output is to reduce dietary nitrogen intake which in practice implies the reduction of total crude protein in the diet.

Current understanding of amino acids nutrition in ruminants supports the concept that when essential amino acids are absorbed in the profile as required by the animal, the requirement for total amino acids are reduced and the efficiency of amino acids use for protein synthesis is maximized. In situations where the supply of one amino acid limits protein synthesis by the mammary gland, dietary supplementation of these amino acids in a reliable metabolisable form would improve the profile of absorbed amino acids, resulting in additional protein synthesis. Dairy nutrition based on amino acids allows for an improvement on efficiency of protein utilization and therefore, lowering crude protein levels in diets positively impact the environment.
A lot of research is currently under way investigating the full Life Cycle Assessment (LCA) of animal protein production from feed to food. Integrating the LCA across a broader range of factors starting with feed and the resulting animal performing through the retail chain to the end consumer will provide a more comprehensive picture of the issue. This provides the basis for future optimisation and improvement where supplemental amino acids will play a crucial role. Below Table 8 gives an example taken from a project conducted by IFIF and FEFANA highlighting the benefits of using amino acids in feeds upon environmental parameters.

The data below shows the positive contribution of feed production with improved amino acid balance and low protein diets on a range of environmental parameters.

Table 8 - *Evaluation of the relative environmental impacts using life cycle assessment on live weight production of pig or broiler in Europe with feeds supplemented with or without amino acids*

	Global warming potential	Global warming potential with emission from land use change	Acidification potential	Eutrophication potential
	CO2-Equivalent	CO2-Equivalent	SO2-Equivalent	Phosphate-Equivalent
Reference production without amino acid supplementation	100	100	100	100
Pig production with LYS, THR, MET and TRP supplementation	97	52	69	67
Broiler production with LYS, THR and MET supplementation	85	54	49	51

5. ECONOMICS OF USING AMINO ACIDS

5.1 Feed specification

A nutritionist must define a certain nutrient level of the feed he wants to produce. By reviewing recommendations from different sources, research reports in the scientific literature, and the perspectives gained by personal experience they can establish reasonable nutrient levels for feeds. These nutrient levels can be strongly influenced by production goals. These goals may be varied, from weight of production (live weight gain, egg mass, milk quality) to the efficiency of production or even per specific unit of output (breast meat yield, lean meat or milk constituent). Thus approaches must be chosen that allow nutritionists to optimally determine the correct level of amino acids in feed.

Calculating optimal dietary amino acid levels are based on economic key figures and can be modelled. This model not only considers changing feed cost with changing amino acids level, but also considers the effects of changing dietary amino acids on breast meat yield which in turn affects the income. Changing economic conditions will thus have an impact on the optimal dietary amino acid of a feed. The two most prominent definitions for profitability are “feed cost/kg weight gain or breast meat” and “gross margin” which means the income over feed cost per pig or per flock of birds.

Meta-analyses of performance data of broilers and pigs show that the chosen parameter has a strong impact on the determination of the nutrient level i.e. minimizing Feed cost/kg weight gain, or maximising weight gain and breast meat yield, or even minimising feed conversion ratio.

An example based on broiler experiments is given in Figure 9. Broilers were fed increasing levels of L-Threonine which were added to a threonine deficient basal diet from day 21 to 42. Dose-response data on weight gain and feed conversion ratio were analysed by exponential regression. Regression curves are showing feed cost/kg gain (i.e. combination of feed conversion ratio with feed cost), or gross margin (i.e. combination of weight gain and feed conversion ratio with price/kg gain and feed cost). Optimal dietary amino acid levels were higher for maximising gross margin compared to minimising feed cost/kg gain. Accordingly, gross margin maximised at 0.83 % dietary digestible threonine whereas feed cost/kg gain minimised at 0.75 %, respectively.

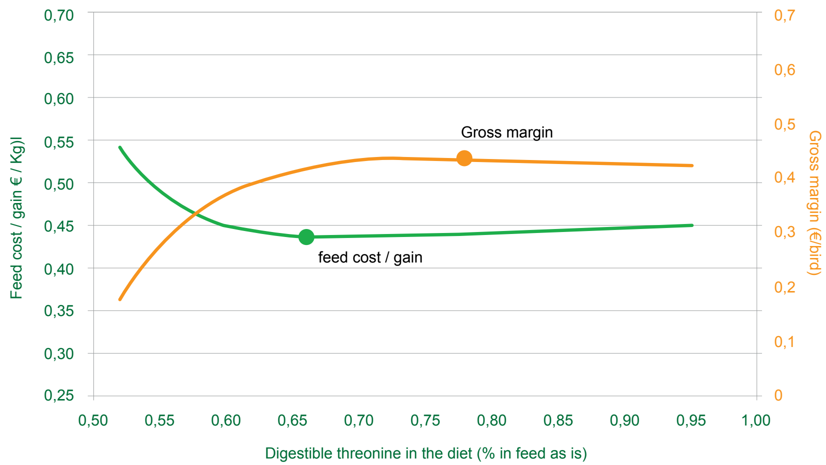


Figure 9 - *Effects of increasing levels of digestible dietary threonine on feed cost per kg weight gain and gross margin in 21-42 day-old broilers*

Nowadays, recommended amino acid levels allow for maximum technical or biological performance which is not necessarily the same as best economic performance. However, various ways of expressing profitability are available and comparison of feed cost/kg gain and gross margin suggest that gross margin should be preferred because it not only considers amino acid effects on feed conversion ratio but also those on growth. Moreover, it has been demonstrated that the goal of animal protein production drives the optimal dietary amino acid levels and consequently the lowest cost of feed typically does not provide the best economic returns.

5.2 Cost optimization

After having set the level of nutrients i.e. feed specification that achieves the desired performance, the nutritionist must find the right combination of ingredients at the lowest cost. To do so, a least cost formulation will be performed where available ingredients and their actual price will be combined to get the optimized feed.

This combination of feed specification as for example digestible lysine level and the price of the different ingredients like wheat, soybean meal and L-lysine commercial product will lead to the optimal incorporation rate of supplemental lysine in the feed. Therefore the incorporation rate of amino acids within a feed is strongly influenced by the price of the amino acids themselves but also by the other ingredients. As a consequence there is no fix supplementation rate of amino acids over time as nutritionist recalculate on regular basis the optimal feed mixture. Amino acids are considered as a powerful cost effective tool by the animal production industry.

6. ANALYSES OF AMINO ACIDS

The nitrogen content of different proteins is relatively constant and varies only slightly from an average value of 16%. The level of protein in a feedstuff is usually measured by determining the nitrogen content according to the conventional Kjeldahl or Dumas method; the protein level of the feed can then be estimated by multiplying the analysed N level by a factor of 6.25. Since NPN compounds are also included in the N-determination, the protein level estimated in this way is correctly referred to as crude protein. Other procedures can be employed to differentiate true and crude proteins (for example precipitation reactions).
The amount of crude protein in a feed provides a certain amount of information concerning its nutritional value. However, it must be used with care since it contains the NPN portion which cannot be utilised by monogastrics. Furthermore, protein level and protein quality are not correlated with each other (see chapter 3.3 Protein quality).

6.1. Protein-bound and free amino acids in raw materials and feeds

One of the most important prerequisites for the formulation of mixed feeds according to an animal's dietary requirements is precise knowledge of the quantitative amino acid composition of feed ingredients.

6.1.1. Total amino acids except tryptophan

The method of determination of total amino acids contents except tryptophan which is destroyed during acid hydrolysis, in raw materials and feeds is standardized (EN ISO 13903) and has been also published by the European Commission regulation N° 152/2009. The total amino acid determination includes the free amino acids and the protein bound amino acids.

The method is specific to amino acids based on the fact that amino acids are cations at pH 2.2 and give a specific coloured reaction with ninhydrin. It does not enable the determination of methionine hydroxy-analogue.

Determination of total amino acids (from proteins and free sources) is carried out after hydrolysis of the proteins under acidic conditions for 23 hours at 110° C. This hydrolysis makes it possible to obtain all the amino acids except methionine and cystine. Preliminary performic acid oxidation of the methionine (transformed into methionine sulfone) and of the cystine (transformed into cysteic acid) makes it possible to obtain all the amino acids except tyrosine which must be determined using hydrolysis without oxidation. The hydrolysates are then adjusted to a pH of 2.2 where the amino acids are in a cationic form for the resin exchange step. Amino acids are separated by ion exchange chromatography using an amino acid analyser or High Performance Liquid Chromatography (HPLC) with post column reaction (Figure 10).
Amino acid contents are determined, after reaction with ninhydrin at high temperature, using photometric detection at 440 nm for proline and 570 nm for all other amino acids (see chromatogram in Figure 11).

6.1.2. Free amino acids except tryptophan

EN ISO 13903 is the standardized method of determination of free amino acids contents except tryptophan. The amino acids are extracted using 0.1 mol/l hydrochloric acid. Co-extracted nitrogenous macromolecules are precipitated with sulfosalicylic acid and removed by filtration. The solution is filtered and adjusted at pH 2.2. The amino acids are separated on ion-exchange resins and dosed by photometric detection at 570 nm after reaction with ninhydrin in the same way as total amino acids.

6.1.3. Methionine hydroxy analogue

The methionine hydroxy analogues are extracted from the feeds with an aqueous extraction agent and determined by reverse-phase-HPLC with UV detection (method reference Z030.I06, approved in 2012 by EURL).

6.1.4. Total tryptophan

Tryptophan is destroyed by acidic hydrolysis and therefore requires a specific method of analysis. The principles are described in the standard method NF EN ISO 13904 and in the corresponding official method published by the European Commission Regulation N° 152/2009.
Determination of total tryptophan is carried out after basic hydrolysis of the proteins: The sample is hydrolysed under alkaline conditions with barium hydroxide. The hydrolysates are acidified with hydrochloric acid at pH 3.0 to perform the separation of the peak by chromatography.
The tryptophan from the hydrolysates is separated by reverse phase HPLC and does not need any colorimetric reaction. Indeed, tryptophan contains an indol functional group which gives spectroscopic properties of absorption and fluorescence in the UV spectrum.

6.1.5. Free tryptophan

The method is based upon - as above - NF EN ISO 13904 and European Commission Regulation N° 152/2009. Free tryptophan is extracted using 0.1 N hydrochloric acid. Tryptophan is separated by reverse phase HPLC and determined by fluorometric detection.

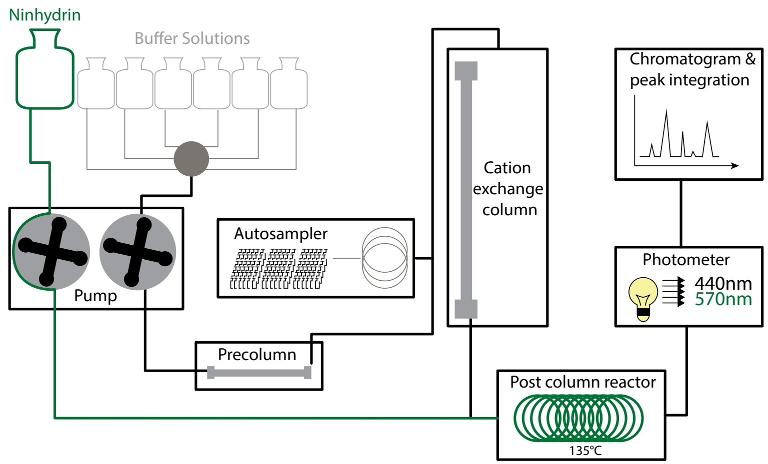


Figure 10 - *Diagram of an amino acid analyser*

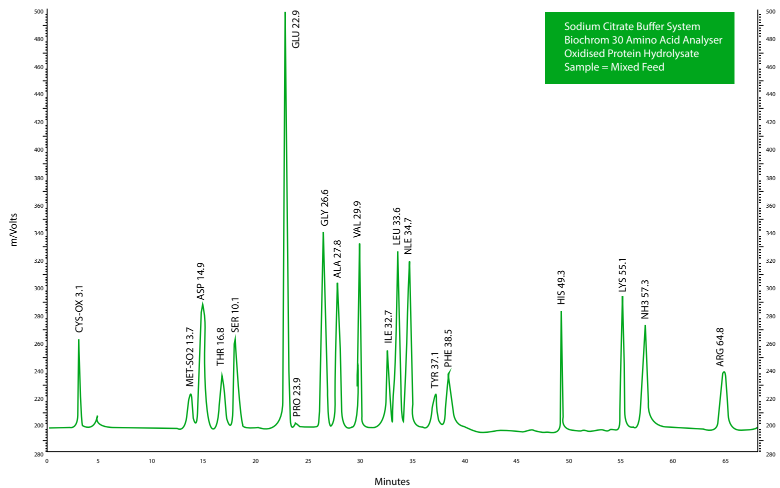


Figure 11 - *Chromatogram of an amino acid analysis*

6.2. Amino acids in pure products and premixes

6.2.1. Amino acids other than tryptophan

The method is standardised (EN ISO 17180 and AOAC 999.13) The amino acid trade products or premixes are dissolved in diluted hydrochloric acid and diluted with sodium citrate buffer. The solution is filtered. The amino acids are separated on ion-exchange resins and dosed by photometric detection at 570 nm after reaction with ninhydrin in the same way as total amino acids. Calibration must be done only with a reference solution containing the amino-acids to be determined.

6.2.2. Tryptophan

The analysis is similar to free tryptophan in feeds. The preparation of the sample takes into account the high content of supplemented tryptophan. Free tryptophan is extracted using 0.1 N chlorhydric acid. Tryptophan is separated by reverse phase HPLC and determined by fluorometric detection.

6.3. NIR Spectroscopy

The Near Infrared Reflectance (NIR) Spectroscopy technique can be used for fast evaluation of the nutritional content of feedstuffs. This method requires a sufficiently large number of chemically verified analytical values for instrument calibration. The population of samples used for calibration has to be representative in terms of type and composition of the type of feedstuffs to be tested.
The NIR method is based on the construction of a comprehensive calibration data set produced from samples that are analysed and their nutritive content evaluated by means of reference methods. NIR calibrations have been constructed for the amino acid composition of several different types of raw materials of both plant and animal origin (for example soy, wheat and fish meal). In addition, the

concentration of ileal digestible amino acids in raw materials can also be estimated by NIR. However, the analysis by NIR of mixed feeds for the determination of the concentration of total and supplemental amino acids has not been met with the same success so the amino acid analysis by NIR is not possible in mixed feeds. Nevertheless the technique is of great value for optimising the utilisation of raw material according to their actual amino acid content rather than calculated values.

7. AMINO ACID COMPOSITION OF FEEDSTUFFS

Different raw materials are characterised by their amino acid composition. In addition within a specific type of raw material but between different samples, differences in amino acid composition may occur as a result of cultivation and geographic influences. Therefore when mean tabular values of amino acid composition are used, consideration must be given to the fact that the actual levels of amino acids in the raw material may differ considerably from the value given in the tables.

Furthermore, when referring to the different sets of tables for the formulation of mixed feeds, particular attention must be paid to the manner in which the animal's requirements are expressed and how the tabular values are presented (total as is basis, apparent or true digestible amino acids basis).

Additional attention must be paid to the units:

- Amino acid content in the raw material expressed in % or in g/kg of fresh material (as is basis);
- Amino acid content expressed in % or in g/kg of the dry matter of the raw material;
- Amino acid content expressed in % of the crude protein.

If the crude protein content of the sample deviates from the tabular value, then the amino acid content shifts in the same direction. Thus, correlations between nitrogen and amino acid contents are used to set up predictive regressions. For each amino acids of a raw material, a slope (a) and intercept (b) can be determined with the equation Y (% of a given amino acid) = a X(N, % as fed basis) + b. Such equations are found in literature for a large range of feedstuffs. As an example, the amino acids composition of wheat can be estimated using the coefficients presented Table 9.

Table 9 - *Slopes (a) and intercepts (b) estimated to calculate the amino acid content of wheat using regression equations[1] (% as fed basis)*

	LYS	THR	MET	CYS	TRP	VAL	ILE	LEU	ARG	PHE	TYR	HIS	SER
a	129	156	85	117	61	229	204	385	245	297	162	137	278
b	86	36	16	26	36	55	0	34	82	-42	0	0	0

a and b have been multiplied by 1000 for better reading

[1] Amino acid content (% of the feed) = a × N (% as fed basis) + b

The values presented in Table 10 are expressed on as is basis, meaning the content in g per kg of fresh material. Alterations in dry matter content need to be considered when formulating a diet.

It is now recommended to calculate the amino acid composition of a diet using standardized ileal digestible amino acid levels to ensure the amino acid composition of the diet meets the requirement of the animal. Thus in the following section related to the amino acids requirements for pigs and poultry, the standardized/true amino acids digestible coefficients in the ileum are presented for the same selection of raw materials in Tables 11 and 12.

Table 10 - *The total amino acid content of selected raw materials on an “as is basis” (g/kg)*

Feedstuff name	DM%	CP%	LYS	THR	MET	CYS	M+C	TRP	ILE	VAL	LEU	PHE	TYR	P+T	HIS	ARG	ALA	ASP	GLU	GLY	SER	PRO
Cereals																						
Barley	86.7	10.1	3.8	3.5	1.7	2.3	4.1	1.3	3.6	5.2	6.9	4.9	2.8	7.8	2.2	4.8	4.1	6.0	23.1	4.0	4.2	10.9
Maize	86.4	8.1	2.4	3.0	1.7	2.0	3.7	0.5	3.0	4.1	10.2	4.0	3.4	7.4	2.4	3.8	6.1	5.3	15.4	3.1	4.1	7.5
Oats	88.1	9.8	4.1	3.4	1.8	3.2	5.0	1.2	3.7	5.2	7.2	4.9	3.5	8.4	2.1	6.6	4.7	8.5	16.8	5.0	4.8	6.0
Rye	87.3	9.0	3.5	3.1	1.4	2.0	3.5	0.9	3.1	4.3	5.4	4.0	2.3	6.3	1.9	4.5	3.9	6.9	20.8	4.0	4.0	9.1
Sorgum	86.5	9.4	2.2	3.1	1.5	1.8	3.3	1.0	4.0	5.1	12.8	5.0	3.8	8.9	2.1	3.8	8.7	1.8	19.7	2.9	4.3	8.0
Wheat	86.8	10.5	3.1	3.2	1.7	2.6	4.2	1.3	3.8	4.7	7.1	4.9	3.3	7.8	2.4	5.3	3.9	5.5	30.0	4.4	5.2	10.4
Cereal by-products																						
Corn DDGS	88.2	24.6	6.2	8.6	4.3	4.8	9.0	1.7	8.1	11.6	24.5	10.3	6.2	16.5	6.7	10.1	15.1	13.9	38.3	10.0	10.6	21.1
Corn gluten feed	88.0	19.3	5.8	6.6	3.3	3.8	7.0	1.2	5.9	8.9	15.6	6.8	4.6	11.4	5.6	8.9	12.4	10.7	27.4	8.1	8.0	16.2
Corn gluten meal	89.5	60.6	10.7	20.3	14.6	10.8	25.4	2.8	24.8	27.9	96.6	37.6	30.3	67.8	12.5	19.4	52.9	36.1	125.6	16.5	30.8	53.2
Wheat bran	87.1	14.8	5.8	4.7	2.3	3.1	5.4	1.9	4.7	6.7	9.1	5.8	3.6	9.4	3.8	9.1	6.3	9.4	29.8	7.0	6.4	9.8
Wheat middlings	88.1	15.5	6.2	4.9	2.4	3.1	5.5	2.0	5.0	7.0	9.5	6.1	4.1	10.2	4.0	9.7	6.7	10.1	31.1	7.4	9.5	10.3
Oilseeds and other protein sources																						
Alfalfa. dehydrated	90.6	16.7	7.7	6.8	2.5	2.3	4.7	2.4	6.5	9.2	11.6	7.5	5.2	12.7	3.6	7.4	9.0	19.4	15.8	7.5	7.1	8.2
Peas	86.4	20.7	15.0	7.8	2.0	2.8	4.8	1.8	8.6	9.7	14.7	9.7	6.4	16.1	5.2	17.8	9.0	24.1	33.8	9.1	9.6	8.6
Rape seed meal	88.7	33.7	18.0	14.5	6.9	8.2	15.1	4.1	13.6	17.0	22.6	13.1	9.8	20.3	8.8	20.3	14.7	23.9	56.8	16.7	14.9	20.7
Rape seed full fat	92.2	19.1	11.9	9.1	4.2	4.7	8.9	2.5	7.7	10.0	12.2	7.2	5.5	12.8	5.1	11.4	8.6	13.5	28.6	8.9	8.5	12.4
Soybean meal profat 44	86,7	43,3	26,6	17,0	6,2	6,5	12,7	5,6	19,9	20,8	31,9	21,7	14,6	36,3	11,5	32,0	19,0	49,0	77,1	18,1	21,8	21,6
Soybean meal, profat 48	87,8	45,3	27,8	17,7	6,4	6,7	13,1	5,9	20,9	21,8	33,4	22,8	15,1	37,9	12,0	33,6	19,9	51,4	80,8	19,0	22,8	22,4
Soybean full fat, toasted	88,6	35,2	21,8	14,2	5,3	5,7	11,0	4,5	16,2	16,8	26,2	17,6	12,4	30,0	9,4	26,0	14,3	39,3	62,4	14,9	17,9	18,5
Sunflozer meal, undecorticated	88,7	27,7	10,0	10,0	6,4	4,8	11,2	3,4	11,3	13,5	17,0	12,1	6,5	18,6	6,7	22,6	12,3	24,4	52,7	15,9	12,0	12,1
Other																						
Fish meal. protein 65	91.7	65.3	48.9	27.0	17.9	5.4	23.3	6.5	27.3	32.4	46.8	25.5	19.8	45.3	16.0	38.8	41.2	60.2	82.6	42.9	25.4	26.5
Skimmed milk. powder	94.7	34.1	26.8	14.9	9.7	2.7	12.4	4.4	18.6	21.0	31.9	16.4	14.5	30.9	9.4	12.5	11.3	26.1	70.2	6.6	18.6	33.9
Whey powder. acid	98.0	9.7	7.2	5.4	1.5	1.7	3.2	1.2	5.2	4.8	7.7	2.7	1.4	4.1	1.9	2.1	3.8	8.4	14.6	1.8	4.1	3.5

Source: INRA – AFZ Tables of composition and nutritional values of feed materials. 2004

Table 11 - *The true digestible amino acid content of selected raw materials for poultry on an "as is basis" (g/kg)*

Feedstuff name	LYS	THR	MET	CYS	M+C	ILE	VAL	LEU	PHE	TYR	P+T	HIS	ARG	ALA	ASP	GLU	GLY	SER	PRO
Cereals																			
Barley	3.0	2.7	1.4	1.9	3.4	2.9	4.2	5.7	4.1	2.3	6.5	1.8	4.0	3.0	4.5	20.6	3.5	3.3	9.5
Maize	2.0	2.6	1.6	1.9	3.4	2.8	3.8	9.8	3.8	3.2	7.0	2.2	3.6	5.7	4.8	14.8	2.8	3.8	7.2
Oats	3.5	2.7	1.6	2.7	4.3	3.2	4.4	6.3	4.4	3.0	7.4	1.9	6.0	3.9	7.3	15.6	-	3.9	5.4
Sorgum	1.9	2.8	1.4	1.6	2.9	3.7	4.6	12.2	4.9	3.6	8.5	1.9	3.6	8.3	1.6	18.9	-	4.0	7.7
Wheat	2.6	2.7	1.5	2.4	3.8	3.4	4.1	6.5	4.5	2.9	7.0	2.2	4.6	3.3	4.7	28.8	3.6	4.7	10.0
Cereal by-products																			
Corn gluten feed	4.1	5.0	2.8	2.4	5.1	4.8	7.3	13.9	5.8	0.0	0.0	4.6	7.8	-	-	-	-	-	-
Corn gluten meal	9.6	18.9	14.3	10.2	24.4	23.8	26.8	94.7	36.8	29.4	66.4	12.0	18.8	51.8	34.3	123.1	14.7	29.6	51.6
Wheat bran	4.3	3.5	1.7	2.3	4.1	3.6	5.0	7.2	4.6	-	-	3.1	7.6	-	-	-	-	-	-
Wheat middlings	5.0	3.9	1.9	2.2	4.1	4.1	5.7	8.1	5.2	-	-	3.4	8.4	-	-	-	-	-	-
Oilseeds and other protein sources																			
Alfalfa. dehydrated	4.6	4.7	1.9	0.9	2.7	4.8	6.7	9.0	5.8	3.2	9.0	2.5	5.9	-	-	-	-	-	-
Peas	11.9	6.3	-	-	-	7.2	8.0	12.8	8.0	-	-	4.5	-	7.8	-	-	-	-	-
Rape seed meal	14.0	12.2	6.0	6.7	12.7	11.8	15.0	20.3	11.9	8.9	20.9	7.8	18.1	13.1	20.3	52.3	13.9	13.1	18.4
Rape seed full fat	10.4	7.3	3.6	3.5	7.1	6.5	8.5	10.9	6.5	4.6	11.1	4.3	10.5	-	-	-	-	7.1	-
Soybean meal. profat 48	25.3	15.8	5.8	5.8	11.5	19.2	19.8	30.7	24.2	14.0	35.2	11.2	30.9	17.7	46.8	76.0	16.2	21.0	20.8
Soybean. full fat. toasted	17.7	11.2	4.3	4.3	8.7	12.8	12.9	21.0	14.1	10.0	24.0	8.1	22.1	-	-	-	11.2	14.3	-
Sunflower meal. undecorticated	8.3	8.7	6.0	4.0	10.0	10.3	12.2	15.3	11.1	5.8	16.9	6.0	21.0	10.6	21.7	49.5	13.0	10.6	10.9
Other																			
Fish meal. protein 65	43.5	24.6	16.5	4.3	20.7	25.4	29.8	43.5	23.5	16.8	40.3	14.2	36.1	-	49.4	74.3	-	20.8	-

Source: INRA – AFZ Tables of composition and nutritional values of feed materials, 2004

Table 12 - *The standardized ileal digestible amino acid content of selected raw materials for pigs on an "as is basis" (g/kg)*

Feedstuff name	LYS	THR	MET	CYS	M+C	TRP	ILE	VAL	LEU	PHE	TYR	P+T	HIS	ARG	ALA	ASP	GLU	GLY	SER	PRO
Cereals																				
Barley	2.9	2.6	1.4	1.9	3.4	1.0	2.9	4.2	5.7	4.1	2.3	6.5	1.8	4.0	2.9	4.6	20.3	3.0	3.4	9.2
Maize	1.9	2.5	1.5	1.8	3.3	0.4	2.6	3.6	9.5	3.6	3.1	6.7	2.1	3.5	5.4	4.6	14.3	2.5	3.6	6.7
Oats	3.0	2.3	1.5	2.4	3.9	0.9	2.9	4.0	5.8	4.1	2.8	6.9	1.7	5.8	3.2	6.5	14.4	3.5	3.6	4.3
Rye	2.5	2.2	1.1	1.7	2.9	0.7	2.4	3.2	4.2	3.3	1.8	5.0	1.5	3.6	2.7	5.3	18.5	2.9	3.1	8.2
Sorgum	1.6	5.4	1.3	1.4	2.7	0.8	3.3	4.1	11.0	4.3	3.2	7.6	1.6	3.1	7.0	1.5	16.9	1.9	3.5	4.0
Wheat	2.5	2.7	1.5	2.4	3.8	1.1	3.4	4.0	6.4	4.5	3.0	7.1	2.2	4.7	3.1	4.6	28.5	3.8	4.6	9.9
Cereal by-products																				
Corn DDGS	3.6	5.3	3.3	2.8	6.0	0.5	5.8	7.7	19.1	8.1	4.7	12.9	4.0	7.7	10.1	8.2	26.4	4.6	7.0	-
Corn gluten feed	3.8	4.6	2.8	2.6	5.3	0.8	4.6	6.7	13.1	5.7	3.8	9.5	3.9	7.7	9.9	7.5	21.6	5.3	6.2	12.2
Corn gluten meal	9.5	18.7	13.9	9.9	23.9	2.4	22.8	25.4	91.8	35.3	28.5	63.7	11.5	18.4	49.2	33.6	116.8	13.9	29.3	42.0
Wheat bran	3.9	3.1	1.7	2.2	4.0	1.4	3.5	4.8	6.8	4.6	2.9	7.4	3.0	7.6	4.2	6.7	25.6	4.6	4.7	7.9
Wheat middlings	4.7	3.5	2.0	2.4	4.3	1.6	4.0	5.5	7.7	5.1	3.4	8.6	3.4	8.5	4.8	7.7	27.7	5.4	7.6	-
Oilseeds and other protein sources																				
Peas	12.5	5.9	1.6	2.0	3.6	1.3	6.8	7.5	11.8	7.8	5.2	12.9	4.4	15.8	6.8	19.8	28.4	7.1	7.6	6.7
Rape seed meal	13.5	10.9	6.0	6.6	12.7	3.3	10.6	13.1	18.5	10.9	7.8	18.9	7.4	17.7	11.8	18.2	49.4	13.0	11.6	16.1
Rape seed full fat	9.3	6.5	3.4	3.8	7.1	1.8	5.2	7.0	8.7	5.3	4.1	9.3	3.7	9.2	6.2	9.7	23.5	6.4	6.0	10.2
Soybean meal. protein 44	23.9	14.6	5.6	5.6	11.3	5.0	17.7	18.3	28.4	19.5	13.3	33.0	10.5	30.1	16.3	43.6	69.4	15.2	19.4	-
Soybean meal. profat 48	25.0	15.4	5.9	5.8	11.7	5.3	18.8	19.2	29.7	20.7	13.9	34.5	10.9	31.6	17.1	46.3	72.7	16.2	20.3	20.2
Soybean full fat. toasted	17.2	10.7	4.1	4.3	8.4	3.7	12.0	12.4	19.9	13.6	9.7	23.4	7.6	21.6	10.6	30.7	48.7	11.0	14.0	13.5
Sunflower	8.0	8.2	5.9	3.9	9.9	2.9	9.7	11.3	14.8	10.9	6.0	16.9	5.8	24.5	10.2	20.7	48.0	11.4	9.8	10.5
Other																				
Fish meal. protein 65	45.5	24.8	16.6	4.6	21.2	5.8	25.4	29.8	44.0	23.5	18.2	41.7	14.2	36.5	37.9	53.0	77.6	38.2	23.1	24.6
Skimmed milk. powder	26.0	13.6	9.4	2.3	11.7	-	16.4	18.7	30.6	16.1	14.1	30.0	8.9	12.0	10.1	24.3	61.1	5.5	14.7	32.5
Whey powder. acid	6.4	4.6	1.4	1.5	2.9	1.0	4.6	4.2	7.0	2.4	1.3	3.7	1.7	1.8	3.2	7.5	13.3	7.4	3.3	3.0

Source: INRA – AFZ Tables of composition and nutritional values of feed materials, 2004

8. AMINO ACIDS RESPONSES AND RECOMMENDATIONS

The assessment of amino acid requirement is an ongoing topic with overwhelming importance for the livestock industry. Dietary supply of nutrients like amino acids to meet animals` requirements directly impacts on the economic bottom line of operations. It is well known that imbalances in dietary amino acids profiles result in reduced growth rate and feed efficiency. In addition, amino acid deficiencies could impair the animals` immune function. Diets formulated with balanced amino acid profile will help to reduce nitrogen losses in animal manure and result in environmental benefits. Amino acids requirements are influenced by many factors, e.g. animal species and breed, age, gender, physical activity, body protein accretion level, health status and others. In general, requirements can be assessed in-vivo with various trial techniques, measuring animal responses to changes in dietary amino acid supply. Differences in techniques and statistical data evaluation may lead to different requirement figures. Amino acid requirements are listed for major animal species in requirement tables, which are published by different scientific bodies like NRC (USA), CVB (The Netherlands), GfE (Germany), INRA (France) or FEDNA (Spain). These tables are kept to meet the respective current scientific level.

The indication of amino acid requirements, where possible on a digestible basis, is mostly expressed as dietary concentration, and in some cases as per animal and per day. Requirement data for minor but essential amino acids are not always available across the covered species. In such cases, the figures have been calculated based on ideal amino acid profiles. However, ideal profiles are not available for all species and in such cases requirement figures are given for selected amino acids only.

In summary, considering all the options available as alternative nutritional strategies, amino acid supplementation offers the following benefits:

- Cost efficient amino acid supply
- Reduction of dietary crude protein content
- Decrease of N-excretion and environmental N-load
- Prevention of digestive disorders
- Improved energy utilization
- Providing higher amino acid availability compared with protein bound amino acids
- Balancing of amino acid variations in feed ingredients
- Allowing for high dietary nutrient density

Therefore using amino acids in compound feed formulation ensures optimum weight gain associated with high feed efficiency and low production cost. Amino acid supplementation allows realization of the full genetic growth potential
This booklet sums up amino acid requirement information based on literature and industry research and is meant as recommendation for nutritionists or other interested parties.

8.1. Broilers

Many diverse factors are considered when estimating nutrient requirements. Researchers and national councils in different countries have stipulated different optimum levels of energy contents for broiler finishing diets. Furthermore, the composition of feeds used in the growing stages and their period of application are influenced by availability of raw materials in different countries. Finally the weight when the bird is slaughtered is influenced by variation in consumer demand.
Ration formulation must also account for the changing voluntary intake of the bird as intake is reduced in response to the high energy concentration of poultry finishing diets. The amino acid concentration of the diet must be increased in order that the absolute intake of amino acids does not fall. The recommended amino acid profile changes with the age of the bird, which is generally divided into three

phases, as given in Table 13. Based on this profile, recommendations for the true digestible amino acid content for broilers' diet is given in Table 14 for standard levels of energy.

Only the proportion of amino acids that can be digested and absorbed from feed protein is made available for metabolism, the remainder is eliminated as part of the faeces. The digestibility of amino acids can differ between different raw materials even though the ingredients may have the same amino acid content. Thus ingredients may differ markedly in their "value" as an amino acid supplier to the organism. In order to account for these differences in amino acid digestibility there has been a tendency for recommendations of amino acid requirements to be made on the basis of digestible amino acids which increases the precision of formulation of the amino acid requirements of the bird.

Table 13 - *True ideal digestible amino acids ratios for broilers (ideal protein)*

	Starter	Grower	Finisher
LYS	100%	100%	100%
MET	39%	41%	43%
M+C	73%	75%	77%
THR	65%	67%	68%
TRP	16%	17%	17%
ARG	103%	105%	107%
ILE	67%	67%	67%
LEU	105%	105%	105%
VAL	79%	80%	81%

Table 14 - R*ecommendations for true digestible amino acid content of complete diets for broilers (in % in feed as is)*

	Starter	Grower 1	Grower 2	Finisher 1	Finisher 2
LYS	1.23	1.17	0.98	0.83	0.61
MET	0.48	0.48	0.40	0.36	0.26
M+C	0.91	0.88	0.74	0.64	0.47
THR	0.80	0.78	0.66	0.56	0.41
TRP	0.20	0.20	0.17	0.14	0.10
ARG	1.27	1.23	1.03	0.89	0.65
ILE	0.82	0.78	0.66	0.56	0.41
LEU	1.29	1.23	1.03	0.87	0.64
VAL	0.97	0.94	0.78	0.67	0.49

8.2. Laying hens (including rearing)

At peak lay, under commercial conditions, the most advanced hybrid birds produce eggs with an average laying efficiency of 95%. However in such flocks the performance of individual hens varies widely. Therefore sub-optimal nutrient supply will primarily penalise the performance of the best individual birds. Thus feed formulations are designed to contain all essential ingredients and predominantly amino acids to meet the requirements of birds with the highest performance level during peak lay over weeks 20 to 42 (Table 15).

Table 15 - *Recommendations for true digestible amino acids for layers (in % in feed as is)*

	Rearing diets			Laying diets	
	Chick starter	Pullet 1	Pullet 2	Light hens[1]	Heavy hens[2]
LYS	0.73	0.60	0.51	0.73	0.66
MET	0.36	0.31	0.28	0.38	0.35
CYS	0.30	0.26	0.22	0.24	0.22
THR	0.51	0.41	0.35	0.52	0.48
TRP	0.14	0.11	0.10	0.15	0.14
ARG	0.78	0.64	0.55	0.78	0.71
ILE	0.58	0.47	0.40	0.58	0.52
VAL	0.68	0.56	0.47	0.68	0.61

(1) Feed intake of 105 g/hen/day
(2) Feed intake of 115 g/hen/day

8.3. Turkey

As already mentioned with broilers many diverse factors are considered when estimating Turkey nutrient requirements. The nutritional requirements of turkeys during the growing period differ according to the sex of the bird and whether a heavy or light type is finished. Hens are finished over a shorter period compared with toms and the final finishing weight differs (Table 16).

Table 16 - *Recommendations for digestible amino acids for male heavy turkeys (in % in feed as is)*

	Week 1 – 2	Week 3 – 5	Week 6 – 9	Week 10 - 13	Week 14 - 17	Week 18 - 22
LYS	1.55	1.41	1.31	1.14	1.01	0.91
MET	0.61	0.57	0.55	0.48	0.44	0.40
M+C	1.12	1.05	1.00	0.89	0.79	0.72
THR	0.98	0.91	0.85	0.76	0.68	0.61
TRP	0.24	0.23	0.21	0.19	0.17	0.15
ARG	1.59	1.46	1.38	1.21	1.09	0.98
ILE	1.05	0.97	0.93	0.82	0.74	0.66
LEU	1.05	0.97	0.93	0.82	0.74	0.66
VAL	1.22	1.13	1.05	0.92	0.83	0.75

8.4. Ducks

There are only limited literature references with respect to the amino acid requirements of ducks and geese. These groups of animals are of particular relevance in Asia and Eastern European countries where large numbers of these birds are found. A selection of the amino acid requirements of waterfowl is presented in Table 17.

Table 17 - *Recommendations for the total amino acid content of complete diets for ducks (Muscovy) (in % in feed as is)*

	Starter (0-3 weeks)	Grower (4-7 weeks)	Finisher (8-12 weeks)
Crude protein	19-22	17-19	15-18
LYS	0.95	0.85	0.75
MET	0.45	0.40	0.30
M+C	0.85	0.65	0.60
THR	0.75	0.60	0.50
TRP	0.23	0.16	0.16
ARG	0.77	0.69	0.61
VAL	0.56	0.43	0.40
HIS	0.16	0.14	0.11

8.5. Pigs

In modern animal production, nutrition is of primary importance to optimize pig performance. Diets have to meet the animal nutritional requirements. For each nutrient, requirement of the pig is generally assume equal to the sum of two compartments: requirements for maintenance and requirements for production.

Maintenance requirements for energy are generally grouped as the heat production, the animal activity and the energetic cost of the maintenance protein production. For amino acids, maintenance requirements are represented by desquamation, protein turnover, and basal endogenous losses. Maintenance requirements are not constant and are in particular dependant on the live weight and the housing conditions. For instance, when the temperature is low, pigs will need more energy to maintain body heat, or when the health conditions are degraded, pigs will need more nutrients for specific protein production (immunoglobulins, mucins, etc).

Production requirements represent the needs for growth or other production (foetus, milk, etc.) These requirements depend, on the one hand, upon the composition of the protein and fat deposited and on the other hand, upon the utilization yield of nutrients (energy and amino acids).

To express the amino acids and energy requirements of pigs, nutritionists have to estimate the availability or digestibility of nutrients supplied by the feed. Researchers have worked since a long time on these topics to propose concepts which estimate as close as possible the animal utilization of the nutrients. It exists various ways to express the requirement. However, for energy and amino acids utilization by the pig, the Net Energy (NE) system and the Standardized Ileal Digestibility (SID) of amino acids are the most advanced system to express the requirements. Both NE and SID amino acids systems are much better correlated with the animal response than any other systems (metabolisable energy or total amino acids) and are therefore the best predictors of animal performance. NE system takes into account the losses of energy by the faeces, urine and gas and by the heat increment (Figure 12). Since in the metabolisable energy system, diets formulated with reduced levels of protein tend to be undervalued from the point of view of energy, it is advantageous to formulate diets on the basis of NE. The SID values of amino acids come from the apparent digestibility of amino acids corrected by the basal endogenous losses of the pigs. The coefficients of SID of individual amino acids in a selection of raw materials are listed in Table 4, Chapter 3.8.

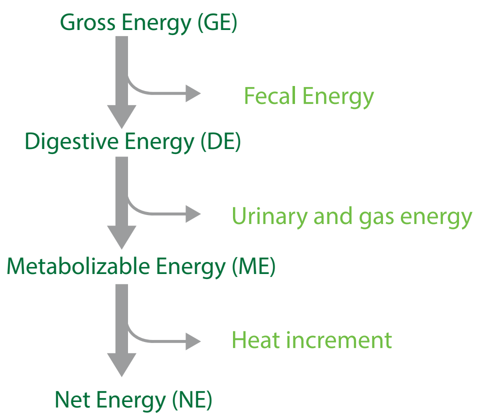


Figure 12 - *Breakdown energy utilisation in pigs*

During the growth of pigs from birth to slaughter weight, the daily energy and nutrient requirements as well as the daily feed intake, change considerably. In growing pigs, the amino acids requirements are dependent on the targeted level of performance. Thus the nutrient contents of the feed for pigs must be adjusted according to:

- the target daily live-weight gain
- the daily feed intake or degree of dietary restriction

Moreover, diets should be well balanced between nutrients (i.e. NE and SID lysine and between amino acids). If one of the essential amino acids is not supplied at an adequate level, growing performance will be limited. The concept of an optimal balance between all the essential amino acids in feeds is commonly called the ideal protein concept.

Lysine is the first limiting amino acid in pig production. The amount of ingested lysine, on a daily basis, has a direct influence on animal growth performance. The ideal protein is thus represented by a profile in which the supply of each essential amino acid is expressed as a percentage of the dietary lysine content. Each of these ratios (Table 18) can thus be directly introduced as a constraint in feed formulation.

Table 18 - *Standardised ideal digestible amino acid ratios for pigs (ideal protein)*

	Piglet	Pigs Grower	Finisher
LYS	100%	100%	100%
M+C	60%	60%	60%
THR	65%	67%	68%
TRP	22%	20%	19%
ARG	42%	42%	42%
ILE	53%	53%	53%
LEU	100%	100%	100%
VAL	70%	65%	65%
HIS	32%	32%	32%
PHE + TYR	95%	95%	95%

In practice, different types of diet are applied over time periods of different length and for different weight ranges according to the genotype of pig (different growth). In general, crude protein and SID lysine content of the diet decline during the course of the growing period in response to the increased feed intake of the growing pig and the reduction in the rate of protein deposition and increase in fat deposition. Recommendations for the levels of NE and SID lysine in complete diets for pigs are shown in Table 18. The values presented are good indications for use in general practise, however adjustments should be made according to the genotype of the pig, the potential for protein deposition and environmental factors. Three levels of average daily gain (ADG) are given in Table 19 for growing pigs. The minimum dietary levels of all the other essential amino acids are set by applying the ideal profile showed in table 18. As a result, the recommended content of each amino acid in pig feed is given in Table 19.

Table 19 - *Energy and digestible lysine requirements of growing pigs (barrows and gilts are not separated)*

Phase/weight	Piglet		Grower			Finisher		
	<10kg	20kg						
ADG g/day)	280	600	675	750	1000	750	950	1150
NE (MJ/day)	5.1	9.6	75	16.7	22.2	17.8	22.6	27.3
Lysine SID (g/day)	6.4	11.4	12.8	14.3	19.0	14.3	18.1	21,9
ADFI (kg/day)	0.45	0.9	1.6	1.6	2.0	2.1	2.1	2.6
NE (MJ/kg Feed)	11.4	10,7	9.4	10.4	11.1	8.5	10.7	10.5
Lysine SID (g/kg feed)	14.3	12.7	8.0	8.9	9.5	6.8	8.6	8.4

To notice: for entire males, NE and SID lysine requirements are higher than above mentioned figures (about 10% more for SID g lysine / MJ NE) due to better growth capacity

In the past, a single diet has often been fed to pigs during the entire growing period. However such a concept does not correspond to the changing nutrient requirements of the pig and is no longer appropriate with the desire to optimise nutrient use and reduce nitrogen excretion. In addition when protein prices are high, this feeding strategy results in a waste of protein and is therefore not economically viable. The alternative to a single diet strategy is phase feeding, where the nutrient supply is adjusted according to the nutrient requirements of the pig during different growth phases. By adjusting the nitrogen supply according to the amino acid demands of the pig, the feed is utilised more efficiently and a reduction of 20% in N-excretion can be achieved. If in addition, the crude protein content of the diet is further reduced by accurately matching the amino acid requirements of the pig and feeding essentially an ideal protein a further reduction of 25% in N-excretion can be achieved. In that respect, the reduction of crude protein in feeds has been limited by a lack of information on the exact levels of branched chain amino acids (Val, Ile, Leu) for optimum growth. Recent publications have confirmed the levels showed in Table 18. The availability of L-Valine gives now the nutritionist the opportunity to implement further decrease in dietary protein content.

Moreover, when maintenance requirements are higher than usual, pigs will need more nutrients to achieve the same performance. This is the case when health conditions are deteriorated (infections, dirty rooms, etc). Two amino acids are particularly impacted and deserve more attention under such conditions: threonine as it is used for immunoglobulin (immune response) and mucin production (gut protection) and tryptophan as its metabolism is increased with the activation of the immune function and the production of acute phase protein (protein rich in tryptophan).

Table 20 - *Recommendations for digestible amino acids content in feed for pigs (% in feed as is)*

	Piglet (<10Kg)	Pigs (20Kg)	Grower pig (DWG 675 g/day)	Grower pig (DWG 750 g/day)	Grower pig (DWG 1000 g/day)	Grower pig (DWG 750 g/day)	Grower pig (DWG 950g/day)	Grower pig (DWG 1150g/day)
LYS	1.43	1.27	0.80	0.89	0.95	0.68	0.86	0.84
M+C	0.86	0.76	0.48	0.53	0.57	0.41	0.52	0.50
THR	0.93	0.83	0.54	0.60	0.64	0.46	0.58	0.57
TRP	0.31	0.28	0.16	0.18	0.19	0.13	0.16	0.16
ARG	0.60	0.53	0.34	0.37	0.40	0.29	0.36	0.35
ILE	0.76	0.67	0.42	0.47	0.50	0.36	0.46	0.45
LEU	1.43	1.27	0.80	0.89	0.95	0.68	0.86	0.84
VAL	1.00	0.89	0.52	0.58	0.62	0.44	0.56	0.55
HIS	0.46	0.41	0.26	0.28	0.30	0.22	0.28	0.27
PHE + TYR	1.36	1.21	0.76	0.85	0.90	0.65	0.82	0.80

8.6. Veal calves and heifer replacement calves

Veal calves are fed Calf Milk Replacer (CMR) until slaughter and are offered rolled barley for animal welfare reasons. Often the composition of the CMR changes 6 weeks after weaning from grower to finisher formula.
For heifer replacement calves, from three weeks of age in addition to CMR, the calves are offered a calf starter or calf rearing diet plus good quality hay in order to stimulate good rumen development and function. Also corn silage is often fed. This initiates the development of a viable microbial population that supplies microbial protein and contributes an increasing proportion of the total protein requirements. The gradual transition from a pure milk or milk replacer based diet to one more suited to adult ruminants allows the use of milk substitute feeds of various quality and price. The sooner weaning is achieved from the milk substitute feed (early weaning), the sooner the requirements for high quality protein decline.
Traditional CMRs contain only milk protein, mainly skim milk powder that meet the amino acids requirement of calves. In recent years, alternative protein sources are used as replacement of skim milk powder in CMR formulation. This calls for evaluation of the CMRs amino acids supply to meet the calves` requirements. In the first weeks of life a milk substitute rich in casein is advantageous; however from five weeks of age onwards a milk substitute feed in which the amino acids are predominantly derived from whey and soy protein can be adopted. Milk substitute feeds containing high levels of non-milk proteins and a low proportion of skimmed milk may need to be enriched with lysine, methionine and other essential amino acids. The amino acids supplementation level is related to protein sources used in milk replacers. Highly processed plant protein sources can be used as milk substitute, such as soy protein concentrate (SPC) and wheat gluten. Using wheat gluten as milk substitute will require higher lysine supplementation compared to soy protein concentrate, as wheat gluten is deficient in lysine. A combination of different protein sources will improve amino acids balance. When milk protein is substituted by plant protein sources, it is essential to evaluate the amino acid supply of the milk replacer.
During weeks 1 - 8 of life the average daily weight gain of growing calves is approximately 1200 g and in weeks 9 - 16 approximately 1400 g. The nutrient composition of CMR for heifer replacement calves and veal grower is similar (20 – 22% protein). The veal finisher drops in crude protein and amino acids concentration. Clearly, the essential amino acids concentration in CMR is closely related to feed intake and performance levels.
Table 21 shows the amino acid ratios for calves from 60 to 220 kg body weight, related to lysine on a total amino acids basis.

Table 21 - *Recommended amino acid ratios in % of lysine for calves (60 - 220 kg LW)*

LYS	100
MET	29
M+C	55
THR	67
TRP	16
ARG	106
ILE	47
LEU	111
VAL	69
HIS	39

Table 22 shows the estimated daily amino acids requirement per calf in gram. As for other species, the daily amino acid requirement strongly depends on performance levels. The estimated lysine requirement is based on the minimum amount of amino acids needed to stimulate maximum protein deposition rate of calves.

Table 22 - *Amino acid requirements (g/d) in calves (60 - 220 kg LW)*

Daily gain (g)	900	1100	1400
LYS	16.3	24.7	27.1
M+C	8.9	13.6	14.9
MET	4.7	7.2	7.9
THR	10.9	16.5	18.1
TRP	2.6	4.0	4.3
ARG	17.2	26.2	28.7
ILE	7.7	11.6	12.7
LEU	18	27.4	30.0
VAL	11.2	17.0	18.7
HIS	6.3	9.6	10.6

8.7. Dairy cows

In ruminants, protein supply at intestinal level from the rumen-microbial population must be estimated in addition to the supply from dietary protein that is not degraded by the ruminal bacteria. The latest fraction is known as the non-degradable protein fraction or by-pass protein.

Feeding systems such as INRA (France) and NRC (USA) focus on defining the amino acid composition of the microbial fraction, the quantity of microbial protein produced in the rumen and the amino acid requirements of dairy cows. Such data has allowed dairy nutritionists to formulate and design feeds based on metabolisable protein and on digestible amino acids. Thus the amino acid requirements for milk production can be precisely estimated and diets for dairy cows are increasingly supplemented with amino acids.

Methionine or lysine have been identified either as the first limiting or co-limiting amino acids in the dairy cow feeds given a range of standard diets:

- In terms of amino acid composition, microbial protein is well suited to the qualitative amino acid requirements of dairy cows. However, compared with microbial protein, most dietary proteins have lower levels of methionine and lysine relative to the total requirements for essential amino acids. Moreover, lysine and cystine often have lower intestinal digestibility than other amino acids in rumen undegraded dietary protein (UDP).
- The contribution of lysine to the total amino acids in UDP is often lower than in the same feeds before exposure to ruminal fermentation and lysine and methionine are the first/ co- limiting amino acids in ruminally synthesised protein for cattle.

To date, the information with respect to the amino acid requirements of ruminants and dairy cows is not as complete as that which has been accumulated for pigs and poultry. The data presented in Table 23 are estimates of methionine and lysine requirements for dairy cows. For instance a dairy cow of 650 kg live weight and producing 30 kg milk/head/day with a composition of 4% fat and 3.4% protein, the daily duodenal lysine and methionine requirements are approximately 130 g and 41g respectively.

Table 23 - *Methionine and lysine requirements (Lys DI and MetDI, g per day, digestible in the intestine) for dairy cows at 600 and at 650 kg live weight in relation to daily milk production*

Milk production	600 kg LW			650 kg LW		
FCM[1], kg/day	PDI2	LysDI	MetDI	PDI[2]	LysDI	MetDI
		g per day	g per day		g per day	g per day
35	2 075	145	46	2 100	147	46
40	2 315	162	51	2 340	164	52
45	2 555	179	56	2 580	181	57

[1] Fat Corrected Milk
[2] Protein Digestible in the Small Intestine

In practice, one should pay attention to LysDI and MetDI shortages when supplies are below the critical levels set at 6.8 % LysDI / PDIE and 2.1 % MetDI / PDIE.

The amino acid profile of microbial protein is being of high biological value for dairy cows. However from experiments carried out with high-yielding cows it has been shown that the methionine and lysine content of microbial protein is insufficient to meet optimal performance. Such deficiencies can be corrected via supplementation with methionine and/or lysine in forms that are stable in the rumen and available at the small intestine. Use of such technologies allows performance in milk and the total amount of milk protein produced to be optimised. Whilst supplementation with rumen protected amino acids produces highly beneficial responses in terms of milk production there are also benefits to be gained in terms of energy metabolism and hepatic function of dairy cows. Strategic supplementation with a highly bio-available source of methionine can contribute to the reduction in the level of blood ketone bodies (beta-hydroxy butyrate and acetone) in cows. Hence rumen stable methionine can make an important contribution to the reduction of the syndrome of ketosis which is prevalent during the first third of lactation in high-yielding dairy cows.
Positive results have also been obtained when sheep and goats have

been given supplements of rumen-stable methionine. The amino acid profile in the protein of wool is characterised by a high proportion of sulphur containing amino acids. Supplementation with rumen-stable methionine has resulted in increased wool growth and improvements in wool quality, as well as improved milk and protein performance like in dairy cows.
In ruminants feed protein is primarily broken down by deamination in the rumen where ammonia is released and used as a source of nitrogen by the ruminal bacteria for their own protein synthesis. However, if the ammonia production from deamination of feed protein or non-protein nitrogen compounds is in excess of that which can be metabolised into microbial protein, the ammonia concentration in the rumen rises and ammonia passes into the blood stream of the cow. Excess ammonia in the systemic circulation must then be metabolised in the liver with the formation of urea and for this reason excess protein supply is a burden to high producing dairy cows. The supply of rumen-stable amino acids is a highly efficient mean of closing the gap between the requirements in terms of protein without exceeding the total nitrogen available for degradation in the rumen.

8.8. Farmed aquatic species

Edible protein supply of animal origin for mankind is covered increasingly by aquaculture. More and more aquatic species are proving as being able to be farmed. What started with salmon and trout has extended to other cold and warm water species. Fish have the capacity to exhibit extremely high growth rates and very high feed conversion efficiency. Therefore high dietary levels of amino acids are required to exploit the high performance potential. In fish, amino acids are a major source of energy for all metabolic processes.
Despite the importance of protein in fish nutrition, fish have amino acid requirements like any terrestrial species. Global research is aiming for solutions to replace fishmeal by plant proteins. Plant proteins are often deficient in some essential amino acids and therefore those are added to the aquatic diets in supplemental form to maintain dietary amino acid balance.

This also increased the knowledge level of amino acid requirements of fish in relation to species, age and performance. Young fish have a higher requirement for nutrient density compared with older fish, therefore crude protein and amino acid levels need to be accordingly higher. However, also the aquaculture sector that is enjoying still considerable growth rates is aware of environmental constraints in terms of water pollution, in particular with nitrogen and phosphorus. In this context, feed formulation based on minimum protein supply and covering essential amino acid needs becomes a prevailing paradigm in the aquaculture industry.

Table 24 - *Typical amino acid content in diets of growing fish and crustacean*

	Amino Acids (in % of CP)		
Species	LYS	M+C	THR
Trout	5.1	2.4	3.8
Salmon	5.0	2.4	3.8
Seabream	5.0	2.4	3.7
Seabass	4.8	2.2	3.6
Halibut	5.0	2.4	3.7
Cod	5.0	2.4	3.7
Tilapia	4.6	2.1	3.8
Catfish	5.0	2.3	3.6
Carp	6.0	3.5	4.2
Shrimps	5.3	2.5	3.6

Without supplemental amino acids, the amino acid requirements of the wide range of different aquatic species can only be met by significantly raising dietary crude protein level. In such scenario the requirement of the first limiting amino acid can only be met from the amino acids contained in feed ingredients, leading to a major excess of dietary protein.
At the same time use of amino acids is totally compliant with the concept of sustainable aquaculture with the reductions in environmental nitrogen load. These factors are increasingly important.

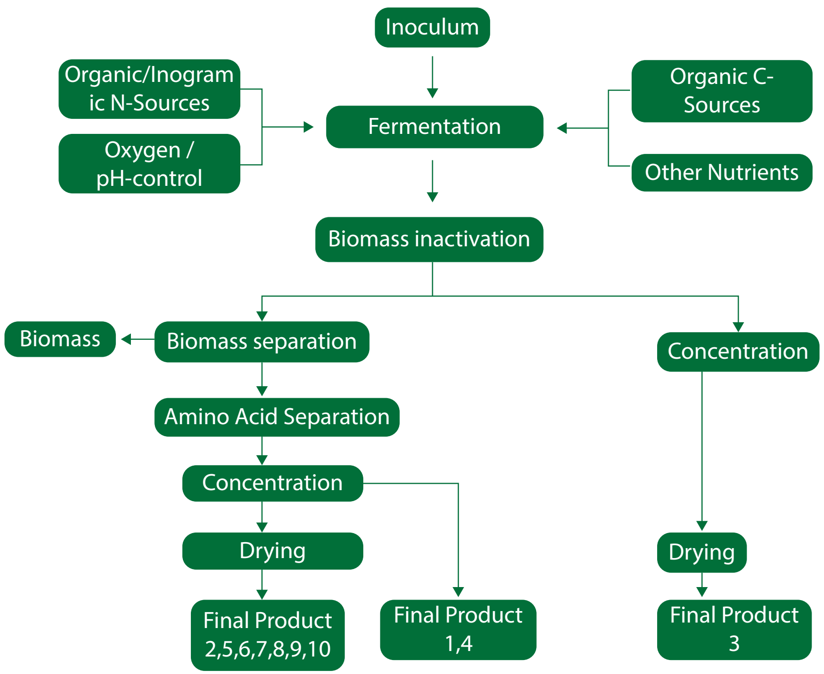
9. MANUFACTURE AMINO ACIDS

Amino acids are either produced by fermentation or chemical synthesis. The products produced by chemical synthesis are produced as racemic mixtures, the D- and L- form of the amino acids. Today in commercial practice all amino acids except sulphur-containing amino acids (methionine) sources are produced by fermentation. This is due to the fact that fermentation only produces the L-isomeric form that is utilizable within animal organisms. For methionine sources the D-isomer is also utilizable and thus DL-methionine sources are produced by chemical synthesis.

9.1. Fermentation

The generalised flow diagram for the production of all amino acids by fermentation is shown below. Advanced modern biotechnology has allowed rapid progress to be made in the selection of specialised microorganisms that transform carbohydrates such as starch and sugar, through fermentation to amino acids in a highly efficient and sustainable manner. Sufficient quantities of nitrogen and a range of micro nutrients must also be supplied during the process. Hygiene and control of the conditions with the fermenter are critical; they are continuously monitored to ensure optimal production and product quality. After fermentation, the microorganisms are inactivated and further processing steps take place to produce the various end products (Figure 13). A detailed list of the main amino acids products available on the market in the EU is provided in chapter 12.
See Figure 13 General Fermentation Manufacturing-Process on the following page.

FERMENTATION MANUFACTURING PROCESS

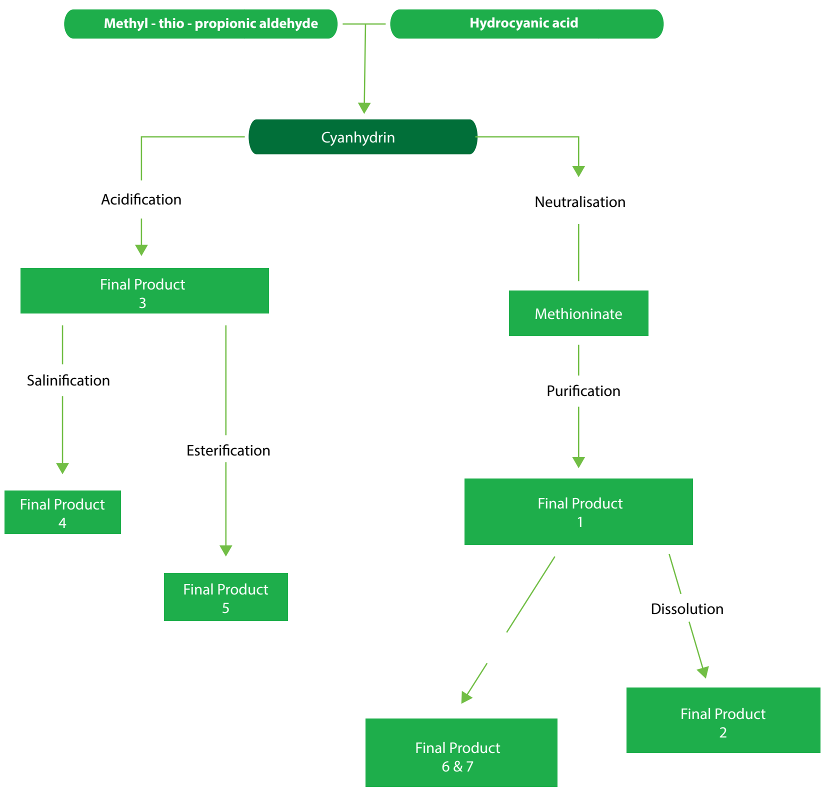


1. Lysine / Concentrated liquid L-lysine (base)
2. Lysine / L-lysine monhydrochloride technically pure
3. Lysine / L-lysine produced by fermentation with Corynebacterium glutamicum
4. Lysine / Concentrated liquid L-lysine monohydrochloride
5. Threonine / L-threonine technically pure
6. Tryptophan / L-tryptophan technically pure
7. L-Arginine 98% produced by Corynebacterium glutamicum (ATCC 13870)
8. Histidine / L-histidine monohydrochloride monohydrate 98% produced by Escherichia coli (ATCC 9637)
9. L-valine with a purity of at least 98% (on dry matter) produced by Escherichia coli (K-12 AG314) FERM ABP 10640
10. L-isoleucine with a purity of at least 93.4% (on dry matter) produced by Escherichia coli (FERM ABP 10641)

Figure 13 - *General fermentation manufacturing-process*

9.2. Chemical Synthesis

Starting from petrochemical derived raw materials a complex multi-step chemical process transforms these small molecules into methionine or its analogue. This process is performed in large scale facilities under optimally controlled reaction conditions, ensuring a highly efficient process. These products can be in dry or liquid form and can be further reacted or coated to produce additional products (Figure 14).



1. DL-methionine technically pure
2. Concentrated liquid sodium DL-methionine technically pure
3. Hydroxy analogue of methionine
4. Calcium salt of hydroxy analogue of methionine
5. Isopropyl ester of hydroxylated analogue of methionine
6. DL-methionine technically pure protected with copolymer vinylpyridine/styrene
7. DL-Methionine technically pure protected with ethyl cellulose

Figure 14 - *Methionine, its salts and analogues: general production process*

10. TECHNOLOGY OF USE IN FEEDS

10.1. Transport, storage dosing and mixing

Transport

Supplemental amino acids in powder form are transported in bags on pallets, in bigbags (i.e. Flexible Intermediate Bag Containers) on pallets or in bulk trucks. In the case of bulk transport, unloading is executed mainly with blowers.
Liquid amino acids are transported in containers or in liquid bulk trucks.

Storage

Storage of bags and bigbags is usually in the warehouse at the feed plant under ambient temperature conditions. Bulk products are stored in silos.
Liquid amino acids are stored in the container at the plant, or in liquid tanks. Depending on the geographical region, tanks should be insulated and equipped with a heating device.

Dosing and Mixing

Dosing depends on the physical nature of the product. Dry products are dosed directly or via premixture into the main mixer (Figure 15)

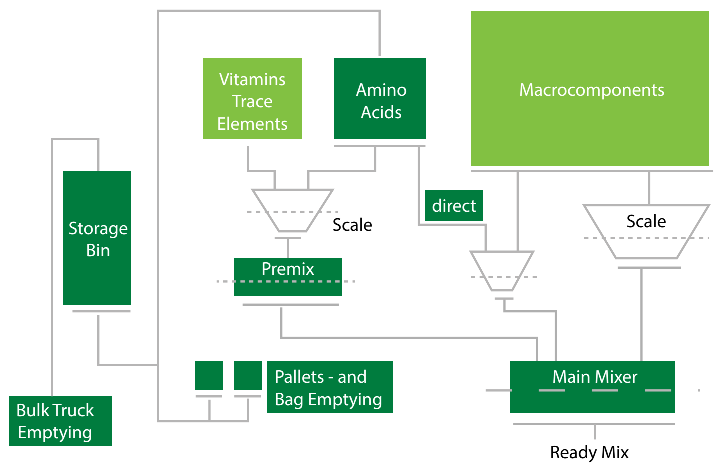


Figure 15 - *Addition of powder amino acids in feed plant*

Liquid amino acids are added into the main mixer with pumps and spraying nozzles (Figure 16).

Prior to addition into the main mixer, liquid amino acids should not be mixed with each other due to different pH-values causing exothermic reactions. Also, mixing with other liquid feed ingredients and additives is not recommended.

An example of a recommended sequence of addition of liquids into the main mixer is as follows:

1. Methionine Hydroxy Analogue
2. Choline Chloride
3. Organic Acids
4. Liquid Lysine
5. Molasses
6. Fat(s) or Oil(s)

The supplier of the liquid product should always be consulted in case of doubt of mixability with other products.

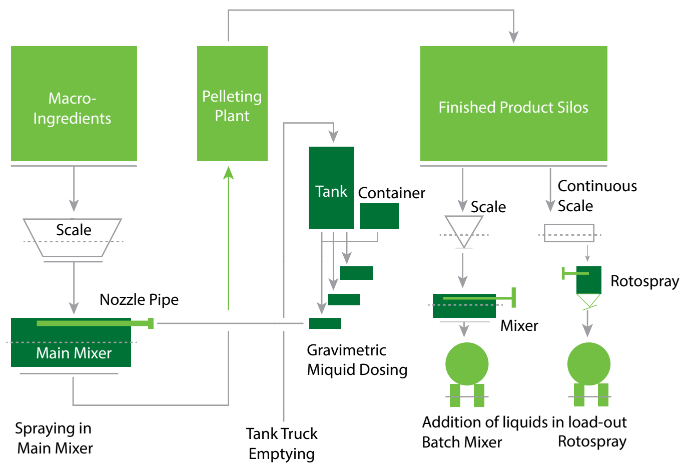


Figure 16 - Addition of powder amino acids in feed plants

10.2. Stability in feed processing

The EU registration process for feed additives requires that the stability of the products is tested prior to approval. Supplemental amino acids are inert substances with long term stability. Feed processing techniques such as pelleting, expansion or extrusion do not impact on stability of supplemented amino acids. However, similarly to most feed ingredients and additives, excessive temperatures should be avoided.

11. REGULATORY ASPECTS INCLUDING LABELLING IN THE EUROPEAN UNION

This book is published under the framework of FEFANA Working Group Amino Acids, therefore EU legislation is covered in this chapter. In other parts of the world different regulatory frameworks exist and should be followed.
The regulation and labelling of feed additives is comprehensively managed by the EU under the European Commission approach starting in 2002 'Food safety - from farm to fork', an umbrella was created for all feed and food legislation.
The general food law - set up in Regulation No. 178/2002 - aims at ensuring a high level of protection of human life and health, taking into account the protection of animal health and welfare, plant health and the environment. A number of individual pieces of legislation have been introduced since 2002; those most relevant to amino acids are briefly discussed in the following sections.

11.1. Feed hygiene legislation

The Feed Hygiene legislation – Regulation No. 183/2005 - was another milestone in the EU feed regulation, laying down the basis for improved control on product and operators active in the feed chain. Traceability along the whole food chain, from manufacturer to final user, as well as HACCP are two major measures introduced with this legislation.

11.2. Feed additive legislation

Feed additives are products used in animal nutrition for purposes of improving the quality of feed and the quality of food from animal origin, or to improve the animals' performance and health, e.g. providing enhanced digestibility of the feed materials. Feed additives may not be put on the market unless authorisation has been given following a scientific evaluation demonstrating that the additive has no harmful effects, on human and animal health and on the environment. Furthermore data

are required to demonstrate the efficacy of the additive in its intended use before the marketing authorisation is granted. Authorisations are granted for specific animal species, specific conditions of use and for ten years periods after which they have to be renewed.

The European Food Safety Authority (EFSA) is responsible for conducting the evaluation of the data submitted when requesting authorisation. After a favourable opinion of the EFSA, the Commission prepares a draft Regulation to grant authorisation, following the procedure involving Member States within the relevant Standing Committee.

Additives are currently classified into the following categories:

- Technological additives (e.g. preservatives, antioxidants, emulsifiers, stabilising agents, acidity regulators, silage additives)
- Substances for reduction of the contamination of feed by mycotoxins
- Sensory additives (e.g. flavouring compounds, colorants)
- Nutritional additives (e.g. vitamins, minerals, amino acids, trace elements)
- Zootechnical additives (e.g. digestibility enhancers, gut flora stabilizers)
- Coccidiostats and histomonostats

Amino acids fall under the scope of feed additive legislation and are categorised as nutritional additives.

A Community Register of feed additives has been established, which lists all EU approved feed additives, as well as the conditions of use for which the authorizations have been granted. It is publicly available and regularly updated by the European Commission.
Maximum residue limits (MRLs) and post-market monitoring plan may be established for a feed additive if deemed necessary; however, this is not the case for nutritional additives including amino acids.
The legislation contains additional provisions, including provisions re-

garding the labelling and packaging of feed additives and procedures for supervision, modification, suspension, revocation and renewal of authorisations, and about confidentiality and data protection.

11.3. Labelling requirements for amino acids

In the European Union labelling of both feed additives and premixtures are regulated by Regulation 1831/2003, Article 16, and its annexes.
In addition, based upon Regulation No. 1831/2003, there exists extensive guidance in the form of a Code of Practice on Labelling, which is the result of collaboration among key feed sector interest groups, i.e. FEFANA, FEFAC and EMFEMA respectively.
All feed additive labels must bear the approval number which the company responsible for placing product on the EU market received from its national health authorities, as foreseen by Feed Hygiene Regulation (EC) N° 183/2005.
Additionally Regulation (EC) N° 767/2009 on the placing on the market of feed, lays down provisions for amino acid declaration on compound feed labels.

12. PRODUCT INFORMATION FOR SUPPLEMENTAL AMINO ACIDS USED IN ANIMAL FEEDS

This publication focuses on the use of amino acids for their nutritional benefits as building blocks of protein. Amino acids have also been registered within the EU for use as flavouring substances or for the manufacturing of chelated trace minerals: these applications are not covered in this chapter nor treated as nutritional additives either under Regulation 1831/2003.

The full and updated list of amino acid products registered within the EU can be found in the register of feed additives on the EU website. At the time of publication the following products are listed in the EU register:

- Lysine / L-lysine monhydrochloride technically pure
- Lysine / Concentrated liquid L-lysine (base)
- Lysine / L-lysine produced by fermentation with Corynebacterium glutamicum
- Lysine / Concentrated liquid L-lysine monohydrochloride
- Methionine / DL-methionine technically pure
- Methionine / Concentrated liquid sodium DL-methionine technically pure
- Methionine / Hydroxy analogue of methionine
- Methionine / Calcium salt of hydroxy analogue of methionine
- Methionine / Isoproply ester of hydroxylated analyogue of methionine
- Methionine / DL-methionine technically pure protected with copolymer vinylpyridine/styrene
- Methionine / DL-Methionine technically pure protected with ethyl cellulose in ruminants
- Threonine / L-threonine technical pure
- Tryptophan / L-tryptophan technically pure

- L- Arginine 98% produced by Corynebacterium glutamicum (ATCC 13870)
- Histidine / L-histidine monohydrochloride monohydrate 98% produced by Escherichia coli (ATCC 9637)
- L-valine with a purity of at least 98% (on dry matter) produced by Escherichia coli (K-12 AG314) FERM ABP 10640
- L-isoleucine with a purity of at least 93.4% (on dry matter) produced by Escherichia coli (FERM ABP 10641)
- L-cystine
- L-tyrosine

Table 25 highlights the main characteristics of the products.

Table 25 - *Table of amino acids products in the EU Community Register of Feed Additives*

		Lysine / L-lysine monhydrochloride technically pure	Lysine / Concentrated liquid L-lysine (base)	Lysine / L-lysine produced by fermentation with Corynebacterium glutamicum	Lysine / Concentrated liquid L-lysine monohydrochloride
EU Community Register of Feed Additives Entry		3.2.3	3.2.2	3.2.5	3.2.4
Production		Produced via fermentation by micro-organisms	Produced via fermentation by micro-organisms	Produced via fermentation by micro-organisms	
Specification		Minimum of 78% L-Lysine	Minimum of 50% L-Lysine	Minimum of 40% L-Lysine	Minimum of 22.4% L-Lysine
Chemical name (where appropriate)		L-diamino n-caproic acid monohydrochloride	L-diamino n-caproic acid	L-diamino n-caproic acid sulphate	L-diamino n-caproic acid monohydrochloride
Chemical formula		C6H15N2O2Cl	C6H14N2O2	C12H30N4O8S	C6H15N2O2Cl
Technical data	Molecular weight	182.7	146.2	200.9	182.7
	Solubility	64.2g in 100ml water at 20°C	Freely soluble in water	Partially soluble in water	Freely soluble in water
	Stability	Stable in premixes and mixed feeds	Stable in premixes and mixed feeds	Stable in premixes and mixed feeds	Stable in premixes and mixed feeds
	Maximum inclusion level	No upper limit	No upper limit	No upper limit	No upper limit
	Other characteristics	Completely absorbable	Completely absorbable	Completely absorbable	Completely absorbable
	Product form at 20°C	Liquid	Powder	Liquid	Liquid
Registered for		All animal species	All animal species	All animal species	All animal species
Recommended Matrix values for feed formulation as is basis	Content of active substance	78%	50%	*	22.4%
	Nitrogen	15.3%	10.2%	*	5.0%
	ME (pig), MJ/Kg	18.4	11.8	*	5.3
	NE (pig), MJ/Kg	14.4	9.2	*	4.1
	ME (poultry), MJ/Kg	17.2	11.0	*	4.9
	Chloride	19.1%	0%	0%	5.5%

*Differences in concentration exist between the products offered on the market; EU legislation sets the minimum legal requirements. Values for individual products are available in specific literature and from suppliers.

		Methionine / DL-methionine technically pure	Methionine / Concentrated liquid sodium DL-methionine technically pure	Methionine / Hydroxy analogue of methionine	Methionine / Calcium salt of hydroxy analogue of methionine	Methionine / Isopropyl ester of hydroxy analogue of methionine	Methionine / DL-methionine technically pure protected with copolymer vinylpyridine/ styrene	Methionine / DL-Methionine techinically pure protected with ethyl cellulose
EU Community Register of Feed Additives Entry		3c301	3c302	3c307	3c308	3c309	3c303	3c304
Production		Produced via chemical synthesis	Produced via chemical synthesis	Produced via chemical synthesis	Produced via chemical synthesis	Produced via chemical synthesis	Produced via chemical synthesis	Produced via chemical synthesis
Specification		minimum of 99% DL-Methionine	minimum of 40% DL-Methionine	minimum of 88% acid	minimum of 84% acid	minimum 95% ester	minimum of 74% of DL-Methionine	minimum of 85% DL-Methionine
Chemical name (where appropriate)		DL-amino-methyl mercapto butyric acid	DL-amino-methyl mercapto butyric acid, sodium salt	DL-hydroxy-methyl mercapto butyric acid	DL-hydroxy-methyl mercapto butyric acid	DL-hydroxy-methylmercapto butyric acid isopropyl ester	DL-hydroxy-methyl mercapto butyric acid	DL-hydroxy-methyl mercapto butyric acid
Chemical formula		C5H11NO2S	C5H10NO2SNa	C5H10O3S	C10H18O6S2Ca	C8H16O3S	C5H11NO2S	C5H11NO2S
Technical data	Molecular weight	149.2	171.18	150.2	338.4	192.3	149.2	149.2
	Solubility	4.8 g /100 ml water at 20°C	Freely soluble in water	Freely soluble in water	7.4g /100 ml water at 25°C	2.5g/100ml water at 30°C	Partially insoluble in water	Nearly insoluble
	Stability	Stable in premixes and mixed feeds	Stable in premixes and mixed feeds	Stable in premixes and mixed feeds	Stable in premixes and mixed feeds	Stable in premixes and mixed feeds	Stable in premixes and mixed feeds	Stable in premixes and mixed feeds
	Maximum inclusion level	No upper limit	No upper limit	No upper limit	No upper limit	No upper limit	No upper limit	No upper limit
	Other characteristics	Completely absorbable	Completely absorbable	Completely absorbable	Completely absorbablea	Completely absorbable	Completely absorbable	Completely absorbable
	Product form at 20°C	Powder	Liquid	Liquid	Powder	Liquid	Beadlets	Beadlets
Registered for		All animal species	All animal species	All animal species	All animal species	Ruminants	Ruminants	Ruminants
Recommended Matrix values for feed formulation as is basis	Content of active substance	99%	40%	**	**	95%	77%	65%
	Nitrogen	9.4%	3.8%	0%	0%	0%	8.3%	8.0%
	ME (pig), MJ/Kg	22.0	8. 9	17.6	16.8	N/A	N/A	N/A
	NE (pig), MJ/Kg	17.3	5. 5	15.3	14.1	N/A	N/A	N/A
	ME (poultry), MJ/Kg	21	8.5	16.8	16.8	N/A	N/A	N/A
	Chloride	0%	0%	0%	0%	0%	0%	0%

**EU legislation sets the minimum legal requirements. Values for individual products are available in specific literature and from suppliers.

		Threonine / L-threonine technical pure	Tryptophan / L-tryptophan technically pure	L- Arginine 98% produced by Corynebacterium glutamicum (ATCC 13870)	Histidine / L-histidine monohydrochloride monohydrate 98% produced by Escherichia coli (ATCC 9637)	L-valine with a purity of at least 98% (on dry matter) produced by Escherichia coli (K-12 AG314) FERM ABP 10640	L-isoleucine with a purity of at least 93.4% (on dry matter) produced by Escherichia coli (FERM ABP 10641)
EU Community Register of Feed Additives Entry		3.3.1	3.4.1	3c3.6.1	3c3.5.1	3c3.7.1	3c3.8.1t
Production		Produced via fermentation by micro-organisms	Produced via fermentation by micro-organisms	Produced via fermentation by micro-organisms	Produced via fermentation by micro-organisms	Produced via fermentation by micro-organisms	Produced via fermentation by micro-organisms
Specification		minimum of 98% L-Threonine	minimum of 98% L-Tryptophan	minimum of 98% L-Arginine on dry matter basis	minimum of 98% histidine HCl*H20	minimum of 98% L-valine on a dry matter basis	minimum of 93.4% L-isoleucine on a dry matter basis
Chemical name (where appropriate)		L- amino-hydroxy butyric acid	L-amino indolyl propanoic acid	2-amino-5-guanidinopentanoic acid	2-amino-3-(1H-imidazol-4-yl) propanoic acid monohydrochloride	2-amino-3-methylbutanoic acid	2-Amino-3-methylpentanoic acid
Chemical formula		C4H9NO3	C11H12N2O2	C6H14N4O2	C6H10N3O2Cl	C5H11NO2	C6H13NO2
Technical data	Molecular weight	119.1	204.2	174.2	191.55	117.15	131.17
	Solubility	9 g/100 ml water at 20°C	1 g /100ml water at 20°C	75g / 100ml water at 20°C	16g / 100ml water at 20°C	8.9 g/100ml water at 20°C	4 g/100ml water at 20°C
	Stability	Stable in premixes and mixed feeds	Stable in premixes and mixed feeds	Stable in premixes and mixed feeds	Stable in premixes and mixed feeds	Stable in premixes and mixed feeds	Stable in premixes and mixed feeds
	Maximum inclusion level	No upper limit	No upper limit	No upper limit	No upper limit	No upper limit	No upper limit
	Other characteristics	Completely absorbable	Completely absorbable	Completely absorbable	Completely absorbable	Completely absorbable	Completely absorbable
	Product form at 20°C	Powder	Powder	Powder	Powder	Powder	Powder
Registered for		All animal species	All animal species	All animal species	All animal species	All animal species	All animal species
Recommended Matrix values for feed formulation as is basis	Content of active substance	98.0%	98%	80%	74.0%	96%	92%
	Nitrogen	11.8%	13.7%	25.7%	16.2%	11.9%	9.8%
	ME (pig), MJ/Kg	16.0	26.4	17.2	N/A	22.9	20.8
	NE (pig), MJ/Kg	12.4	20.3	13.3	N/A	17.7	16.1
	ME (poultry), MJ/Kg	14.6	23.9	N/A	N/A	22	20.0
	Chloride	0%	0%	0%	0%	0%	0%

Acronyms and Abbreviations

AA = Amino Acid
AADI = amino acids digestible in the small intestine
ALA = Alanine
ARG = Arginine
ASN = Asparagine
ASP = Aspartic acid
BAT = Best Available Technique
BREF = Best available technique REFerence
CMR = Calf Milk Replacer
CP = Crude Protein
CVB = Centraal Veevoeder Bureau (the feed central office in The Netherlands)
CYS = Cystine
DE = Digestible Energy
DM = Dry Matter
EFSA = European Food Safety Authority
EURL = European Union Reference Laboratory
FEDNA = Fundación Española para el Desarrollo de la Nutrición Animal (Spanish foundation for the development of animal nutrition)
GE = Gross Energy
GfE = Gesellschaft für Ernährung (society for nutrition in Germany)
GLN = Glutamine
GLU = Glutamic acid
GLY = Glycine
HACCP = Hazard Analysis Critical Control Point
HIS = Histidine
HPLC = High Performance Liquid Chromatography
IFIF = International Feed Industry Federation
ILE = Isoleucine

INRA = Institut National de la Recherche Agronomique (the French national institute of agronomic research)
IPPC = Integrated Pollution Prevention and Control
JRC = Joint Research Centre (European Commission)
LEU = Leucine
LYS = Lysine
M+C = Methionine + Cystine
ME = Metabolizable Energy
MET = Methionine
MRLs = Maximum Residue Limits
NE = Net Energy
NIR = Near Infrared Reflectance
NPN = Non-Protein Nitrogen
NRC = National Research Council
P+T = Phenylalanine + Tyrosine
PDIE = Protein digestible in the small intestine
PHE = Phenylalanine
PRO = Proline
SER = Serine
SID = Standardized Ileal Digestibility
THR =Threonine
TRP =Tryptophan
TYR = Tyrosine
UDP = UnDegraded Protein
VAL = Valine
VLDL = Very Low Density Lipoproteins

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This booklet is intended to provide the best-of-our-knowledge basic information to anyone interested to get a better understanding about Amino Acids in Animal Nutrition. However, FEFANA does not take any responsibility for whatever use of the information provided herewith, by either the general public or any actor in the food and feed chain. For more detailed information on specific use please refer to the technical documentation and safety data sheets as provided by supplier.

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