Amino acids in animal feed: significance and determination techniques

To cite this article: M Sefer *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **854** 012082

Amino acids in animal feed: significance and determination techniques

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Abstract. Amino acids are fundamental for animal nutrition. Their presence is necessary to maintain the normal structure and function of the intestine, and they are key in regulating metabolic pathways for improving health, survival, growth, development, lactation, and reproduction. The animal feed industry invests great resources and efforts to obtain optimal formulations in which the composition of amino acids plays a key role. In support of these aspirations in recent decades, much attention has been paid to the development and improvement of analytical techniques for the reliable, rapid and accurate determination of amino acid content in animal feed. This paper outlines different methodologies for the analysis of amino acid content in animal feed. Various methods, based on different analytical techniques, are presented for determination of amino acids in feed for nutritional and regulatory purposes.

1. Introduction

Amino acids (AAs) are building blocks for proteins and must be present in cells for synthesis of polypeptides [1]. AAs such as histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp) and valine (Val) are not synthesized by animals, so they are known as essential amino acids (EAAs) and must be included in diet. They have an important role in maintaining homeostasis of the whole body. The AAs cysteine (Cys) and tyrosine (Tyr) do not belong to the group of EAA, because they can be synthesized from Met and Phe in the liver. However, some animals cannot form the carbon skeletons needed for Met and Phe. This leads to the prevention of *de novo* synthesis of Cys and Tyr in these animals. The presence of these two AAs is necessary to maintain the normal structure and function of the intestine.

AA balance is crucial for animal growth. The fact is that the quality of feed, in addition to the animal hybrid and gender, largely affects the quality of meat [2]. Between the 1960s and 1990s, nutritionists developed the ideal protein concept (optimal proportions and amounts of EAA for chicken and pork diets based on the belief that all non-EAAs were sufficiently synthesized in animals [3-5]. This concept is currently being used by the National Research Council (NRC) [6,7]. AA-based diets are necessary for animals to maintain and increase protein content. Based on a large number of reports and papers published in scientific and professional literature over the past few decades, the concept of functional AAs has been developed. It defines AAs as key in regulating metabolic pathways for improving health, survival, growth, development, lactation, and reproduction [8]. The addition of AAs glutamine (Gln) and arginine (Arg) to a conventional diet thought to provide adequate AA intake can maximise growth

potential in young animals and prevent diseases (e.g. obesity, diabetes, necrotizing enterocolitis, and intrauterine growth retardation) in both animals and humans [9]. Declaring the AA composition and content in animal feeds is mandatory in many countries. AAs are also monitored by means of official analysis [10].

In this paper, we will analyse the significance of AAs in the diet from the aspect of animal growth, health and meat quality, especially pork and poultry. The methods for determination of AAs in animal feed will be discussed, as well as their advantages.

2. Effects of amino acids on meat quality

Different kinds of AAs play an important role in meat flavour. Adding AAs to animal feed can improve meat flavour [13]. The following AAs play a key role especially for the pork meat flavour: Trp, Thr, Arg, Lys and Leu. L-Trp is regarded as the third most important AA additive in animal feed after Lys and Met. Studies have shown that Trp improves the quality of pork meat by decreasing stress [14]. Stress before slaughter can affect meat quality and result in pale and soft meat. Trp reduces stress by stimulating the secretion of serotonin in the brain, which could be beneficial for the improvement of the pork quality. Thr, another EAA, is used for muscle protein synthesis [15]. Supplementation of Thr in the feed significantly increased the growth and daily gain. Arg is an important AA for protein synthesis. Supplementation of 1% Arg increased the body weight gain by 6.5% and the carcass skeletal-muscle content by 5.5% while decreasing the carcass fat content by 11% [13]. Lys increases appetite in animals, increases the resistance of livestock to different diseases and participates in fat metabolism. Leu is an EAA that must be supplied by the diet. Leu regulates the intracellular signal pathways of muscle cells, thus enhancing protein synthesis in mammalian skeletal muscle [16]. Based on this knowledge, it can be concluded that AAs play a significant role in improving the quality of meat.

2.1. Amino acids in poultry nutrition

Just like most animals, chickens need AAs as a source of protein for growth of muscles and development. The most important AAs for poultry are Arg, Lys, Met, Cys, and Trp. These are an absolute must to be included in poultry feed and play a critical role in the health of the birds. Non-EAAs such as Gly, His, Leu, Ile, Phe, Thr, and Val are often included in feed by several poultry feed brands and are believed to be important for chicken production. All deficiencies of EAAs result in retarded growth or reduced egg size or egg production [17].

A mixture of several AAs such as Cys, Gly, proline (Pro) and Glu, which are synthesized from pre-existing AAs (including EAA) by birds and had previously been thought to be non-EAA in chicken nutrition, was used in dietary formulations to yield better growth performance. There are many defined standards on the AA content of chicken diets during the first three weeks of their lives [11]. Reference values are given in the Dean and Scott Standard, the Huston and Scott Reference Standard, the modified Sasse and Baker Reference Standard, and Baker and Han's Ideal Chick Protein [12]. Common to all these standards that define the AAs in chicken diets are that their diet includes: (a) all EAA that are not synthesized by chickens; (b) several AAs (Cys, Glu, Gly, Pro, and Tyr) that are synthesized from either EAA or α-ketoglutarate plus ammonia by animals to various extents; and (c) no data on alanine (Ala), aspartate (Asp), asparagine (Asn), Gln, or serine (Ser) [1]. A few recent publications have challenged the NRC recommendations for AAs as being inadequate for current poultry strains. The Lys required each day by a white-egg-laying hen is 690 mg, or 0.69 g. Thus, the diet of a white-egg-laying layer eating 100 g of feed per day should have a Lys content of 0.69% [6].

Chickens have a high basal metabolism and require strictly balanced meals assembled from energy-rich foods [18]. Period of growth and gender can play an important role in defining the ratio of AAs in feed. Extensive studies with Lys, for example, have shown that males have higher requirements than females. Numerous studies have shown that only Lys is needed at a higher content in animal feed to achieve maximum weight gain in male poultry. That the Lys requirement is affected by gender but other AAs are not affected adds a complicating factor to use of ideal ratios for broiler chicks. Thus, for separate-sex feeding, female chicks having a 10% lower Lys requirement than male chicks means that

females would need to have ratios (to Lys) for all other indispensable AAs adjusted upwards by approximately 10%. The simplest solution to this gender difference in ratios is to use the male (gain:feed) requirement for Lys together with the male ideal ratios, i.e., for both sexes. The NRC model [6] shows these decreasing requirements in three growth periods: starter phase (0–3 weeks), grower phase (3–6 weeks) and finisher phase (6–8 weeks) [19]. Table 1 gives the AA requirements of poultry.

Table 1. Amino acids requirements of immature Leghorn-type chickens as percentages [6]

		White-Egg Laying Strains				Brown-Egg Laying Strains			
Amino acids	Unit	0 to 6 weeks	6 to 12 weeks	12 to 18 weeks	18 weeks to first egg	0 to 6 weeks	6 to 12 weeks	12 to 18 weeks	18 weeks to first egg
Lys	%	0.85	0.60	0.45	0.52	0.80	0.56	0.42	0.49
Met	%	0.30	0.25	0.20	0.22	0.28	0.23	0.19	0.21
Met +Cys	%	0.62	0.52	0.42	0.47	0.59	0.49	0.39	0.44
Thr	%	0.68	0.57	0.37	0.47	0.64	0.53	0.35	0.44
Val	%	0.62	0.52	0.41	0.46	0.59	0.49	0.38	0.43
Arg	%	1.00	0.83	0.67	0.75	0.94	0.78	0.62	0.72
Trp	%	0.17	0.14	0.11	0.12	0.16	0.13	0.10	0.11
Ile	%	0.60	0.50	0.40	0.45	0.57	0.47	0.37	0.42
Leu	%	1.10	0.85	0.70	0.80	1.00	0.80	0.65	0.75
His	%	0.26	0.22	0.17	0.20	0.25	0.21	0.16	0.18
Phe+Tyr	%	1.00	0.83	0.67	0.75	0.94	0.78	0.63	0.70
Gly+Ser	%	0.70	0.58	0.47	0.53	0.66	0.54	0.44	0.50
Phe	%	0.54	0.45	0.36	0.40	0.51	0.42	0.34	0.38

2.2. Amino acids in pig nutrition

Over the past two decades, there have been successful attempts to refine the patterns of some AAs in diets for lactating sows and pigs by addition of Arg, Gln, Glu, Pro, or Gly, or by determining mammary gland growth or changes of whole-body AA composition [1]. The AA composition in grower pig diet is listed in Table 2.

Table 2. Amino acid composition for grower pigs [6,7]

Amino acids	Content of AAs as a ratio of Lys (%)
Lys	100
Met	-
Cys	-
Thr	62
Val	68
Arg	42
Trp	19
Ile	54
Leu	102
His	32
Phe+Tyr	94
Met+Cys	57

AAs have been classified traditionally as nutritionally essential or nonessential based on growth or nitrogen (N) balance of animals. Nutritionally EAAs are those AAs with carbon skeletons that are not synthesized *de novo* and those AAs that usually are not synthesized in adequate amounts to meet the animal's needs and, therefore, must be provided in diets to sustain life [20].

Each body protein has its individual specific AA composition, turnover rate and maintenance needs, and the rate and priority of development of each proteinaceous tissue also changes, along with the other body tissues, as the animal grows. The AA composition of the whole body thus reflects the accumulated AA composition. The total quantity of AAs retained in all body components relates to a particular stage of development, but does or reflect the transfer between and within the various proteinaceous tissues or the pig's maintenance needs.

2.3. Amino acids in ruminant nutrition

Free AAs are not recommended as supplements in ruminant diets because they are degraded rapidly in the rumen. Balance must be achieved so that AAs protected from ruminal degradation are still available for intestinal absorption. These compounds should be stable, both when pelleted and when incorporated into silage-based total mixed rations in which the pH of corn silage can be as low as 3.6 [21].

3. Analytical techniques for the determination of amino acid composition

Due to their numerous implications in biological processes, AAs have been studied for decades. Before the appearance of mass spectrometry, numerous efforts were made to achieve the best possible resolution of AAs in complex matrixes using derivative reagents. Except for Tyr, Trp, and Phe, which are all AAs with an aromatic ring that allows them to be determined by UV spectroscopy in their native form, most of the AAs do not possess chromophore groups. Derivatization is, therefore, required for these compounds to convert them into a form suitable for UV or fluorescence detection. Since the discovery of the ninhydrin reaction for AA derivatization some decades ago, significant method developments have been achieved using a plethora of derivatization reagents. Briefly, the derivatization procedure can be performed using three main approaches: (i) the post-separation derivatization mode, commonly used in liquid chromatography (LC), (ii) the pre-separation mode used in (LC), gas chromatography (GC) and capillary electrophoresis (CE), and (iii) the in-capillary mode, restricted to CE [22].

3.1. Capillary electrophoresis

CE is a microanalytic technique for separating AAs. Its advantages over the high performance liquid chromatography method (HPLC) are its speed of application and the fact that gradient elution is unnecessary [23]. CE using indirect UV detection was applied for the analysis of nine AAs (Asp, Glu, Cys, Tyr, Asn, Pro, Gln, Leu and Try). AAs were prepared by dilution in distilled water from their powder forms, to a concentration of 150 μg/ml, for method development and reproducibility studies [24].

3.2. NMR spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy uses the magnetic properties of the nucleus of the atoms for analysis and is one of the most powerful analytical methods for determining organic and inorganic compounds [25]. The complexity of the equipment and the price of the instrument mean this method is rarely applied for routine determination.

3.3. Gas chromatography

Different methods based on GC were employed for determination of AAs in heterogeneous samples, as well as in animal feed. GC-MS is one of the commonly used methods in AA analysis. The advantages of GC-MS over other chromatographic methods are high resolution and excellent productiveness. The main limitation of this method is that AAs are not volatile compounds, so they have to be transformed by chemical derivatives into vaporous compounds before analysis [26].

3.4. Liquid chromatography

High performance liquid chromatography (HPLC) and ultra-performance liquid chromatography (UPLC) with ultraviolet (UV) and fluorescence (FL) detection are commonly used for determination of

AAs [27-29]. UPLC is a suitable method for determining 17 AAs in low and high protein feed. UPLC is increasingly being utilized due to its rapid separation of AAs in approximately 35 minutes compared with 2 hours for a typical ion exchange chromatography (IEC) analysis. As mobile phases, they used mixtures of trifluoroacetic acid and water in different relationships, a mixture of methanol and water, acetic acid and acetonitrile. The derivative agents most commonly used are *ortho*-phthalaldehyde (OPA), (9H-fluoren-9-yl)methyl chloroformate (FMOC), 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) and phenyl isothiocyanate (PITC) [30]. Alternative methods have been developed to increase the selectivity. Within the last 15 years, LC-electrospray-tandem mass spectrometry (LC-ESI-MS/MS) was developed for AA analysis. LC-ESI-MS/MS methods have been published for the analyses of underivatized or derivatized AAs. LC-ESI-MS/MS methods compare well with established AA analytical methods, although there are limitations with the measurement of some target analytes. Zhang et al [31] indicate that three methods, HPLC post column, UPLC and LC/MS/MS give similar performance (100% recovery) for most AAs.

3.5. Amino analyser

Amino analyser has become a widely accepted analytical technique for determining the AA composition in various research domains. The AAs are separated by IEC with a visible wavelength detector (IEC-VIS) and determined by reaction with ninhydrin using spectrophotometric detection at 570 nm (440 nm for proline). This method has been the most widely performed method for several decades and is still commonly used. This method is official by Commission Regulation (EU) No 152/2009. IEC requires derivatization of AAs, which can be performed by post-column derivatization using ninhydrin. Post-column ninhydrin derivatization has been the preferred method for many years by a majority of laboratories. Most systems are capable of resolving and quantifying roughly 40 AA peaks in a typical sample [32]. It is very important that the analysis of AA composition is simple and rapid. The quantitation of AA in matrices should be performed in relation to known reference or calibration standards. IEC-VIS can determine all AAs, with the exception of Trp, which is determined by HPLC with fluorometric detection.

4. Conclusion

The requirements for AAs in animal feeds are well defined in various sets of recommendations such as those of NRC. Requirements vary depending on the species and age of animals. AAs should be supplied either in the form of protein or crystalline AAs in feed to meet animals' requirements.

On the other hand, AAs affect not only the quality of meat and animal products as valuable foods, but they also contribute to food's colour, flavour and aroma. The content of AAs and quality of proteins in the meat of animals largely depends on AA content and ratios in feed. Also, the optimal content and ratio of AAs in animal feed depends on various factors, for example of the type, age and sex of the animals. Considering the significance of the animal feed industry, and the growing need for healthy and protein-rich food, it is necessary to focus all available resources in the development of reliable and accurate analytical methods for assessing the quality of food for nutritional and regulatory purposes.

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