AMINO ACIDS IN
FARM ANIMAL
NUTRITION

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Edited by

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Preface

The past two or three decades have been marked by significant advances in our knowledge of the amino acid nutrition of farm animals. These developments have undoubtedly been the result of market-led activities, in particular the industrial manufacture and commercial exploitation of pure methionine and lysine. In Western Europe, the momentum for the extended use of amino acids is gathering pace with the realization that these supplements may have an important role to play in the amelioration of nitrogen pollution from animal wastes. In addition, as global fish stocks decline and with increasing consumer apprehension over the use of animal protein concentrates in livestock nutrition, the prospects for alternative products are being considered. Against this background, a number of companies are now poised to launch into the animal feed industry at least two other amino acids, namely threonine and tryptophan. Further impetus for the exploitation of pure amino acids has recently emerged from the establishment of a link between the incidence of immune hypersensitivity in neonatal animals and dietary protein source and level. Judicious use of amino acid supplements together with overall reductions in antigen loads from specific protein sources are options available for the alleviation of post-weaning stress in calves and piglets.

It is opportune, therefore, to consider recent advances and future developments which may determine the outlook for amino acids in farm animal nutrition. It is hoped that this book will provide the basis for such a reflection. To this end, I have enlisted the expertise of a team of authors internationally recognized for their innovative contributions in the field of amino acid metabolism and requirements of farm animals.

A recurring theme in this book relates to methodological advances, which have been quite striking during the past 25 years. Over this period, the primacy of ion-exchange chromatography has steadily been eroded by the emergence of high performance liquid chromatography. Ileal digestibility of amino acids has now become the routine technique for measuring bioavailability. An inescapable feature of several investigations has been the consistent demonstration that cereal grains are not only poorly endowed with the critical indispensable amino acids, but this deficit is compounded by reduced ileal digestibility of these amino acids. Other studies have suggested that for a number of heat-processed feedstuffs, amino acids may be absorbed in forms that are inefficiently utilized for growth and optimum feed efficiency. For some raw materials therefore, the fundamental assumption that ileal digestibility reflects availability is being questioned.

The adoption of a new system based on metabolizable protein has renewed focus on amino acid utilization in ruminant animals. However, the proposed framework still lacks the appropriate methodology for calculating requirements and responses in terms of individual amino acids. In addition, any future refinement of the system would need to recognize the role of low-molecular weight peptides, absorbed from the gut, in the supply of tissue nitrogen.

Innumerable studies have been conducted to determine the responses of non-ruminant farm animals to individual amino acids. In the early investigations, progress was thwarted by the preoccupation with determining 'requirements'. Attitudes are now changing, albeit very slowly, as nutritionists realize that it is the response of an animal to graded inputs of an amino acid that is important, rather than a statement of immutable requirements. By and large amino acid responses have been obtained with the graded supplementation technique but an alternative method based on diet dilution has also been proposed. Recent studies with the latter procedure have shown conclusively that in the broiler chick, amino acids are more efficiently utilized when provided as pure supplements rather than as components of intact protein, particularly if poor-quality protein sources are used. Such demonstrations of superior utilization of pure amino acids are, in the main, confined to studies with animals fed ad lib. In the case of the pig, feeding frequency may determine efficiency of utilization.

Continuing dissatisfaction with empiricism has meant that much effort is now being expended in the 'systems approach' involving the development of dynamic computerized models. There is growing recognition that the commercial need for accurate nutritional prediction in animal production cannot be fulfilled solely by empirical methods. The role of models in this respect is no longer a matter for debate. In addition, however, mechanistic models may also assist in identifying important limitations in current knowledge so that rapidly declining resources can be employed more effectively to generate a sound database for the future.

The methodology for dietary protein evaluation is now firmly directed at the development of amino acid patterns in perfect balance as embodied in the 'ideal protein' concept. This approach is now finding wide application, even in the new metabolizable protein system for ruminants. Intragastric infusion of amino acids provides a novel technique for establishing the ideal balance for ruminant animals. General, non-specific amino acid imbalances regularly occur in farm animal nutrition, precipitating adverse effects on food intake and growth. However, recent studies confirm that the efficiency of utilization of limiting amino acids remains unimpaired by such imbalances.

Comparative issues are given some prominence in this book. Traditionally, the amino acid nutrition of farm animals has been considered separately for ruminants and non-ruminants. Such a situation can no longer be justified. If this book helps to improve exchange and integration of information across the species barrier, then it will have served an important function.

Many excellent reviews on various aspects of amino acid metabolism and nutrition have appeared over the past few years but their publication in different journals and texts is perceived as a disadvantage. It is hoped that this book will appeal to final year honours undergraduates and postgraduate students as a coherent synthesis of the existing literature. Authors were asked to provide comprehensive reviews with a critical appraisal of current knowledge. Attempts have, therefore, been made to support chapters with a comprehensive bibliography, wherever possible.

I am grateful to all contributing authors for their cooperation and forbearance with an impatient editor. Any success that this book may enjoy will be entirely due to their efforts and expertise. Finally, I feel that it is incumbent upon me to thank Mr T. Hardwick of CAB INTERNATIONAL for his encouragement during the preparation of this book.

Note in proof: It is with the deepest regret that I record here the death of Dr E.S. Batterham, one of my contributing authors. His distinctive and innovative approach to amino acid research is well recognized. There is no doubt in my mind that his legacy of ideas will continue to inspire further research, and not just in the field of non-ruminant nutrition.

J.P.F. D'Mello
Editor

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1

Amino Acid Metabolism in Farm Animals: An Overview

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Introduction

Dietary protein supply is one of the major factors influencing the productivity of farm animals. Supplementation of the diets of animals with amino acids to enhance the quality of the dietary protein is common practice, particularly in the poultry and pig industries. The importance of amino acids to the industry has been one of the reasons that many aspects of amino acid metabolism in farm animals have been extensively studied.

As is well known there are 20 amino acids commonly found in proteins. In addition there are many more amino acids which are rarely found in proteins. All of the protein-bound amino acids, with the exception of glycine, have an optically active carbon atom to which the amino group and the carboxyl group are attached. In the vast majority of cases it is the L-isomer which is of metabolic and nutritional significance. The main role of amino acids in all living organisms is as the monomeric unit from which proteins are synthesized. In addition amino acids also serve as important sources of energy, especially glucose. Some amino acids are also the precursors of other biologically important compounds, for example adrenaline and some of the bile salts.

Biosynthesis of Amino Acids in Animal Tissues

The metabolism of amino acids has been reviewed many times but several aspects require emphasis in order to understand some of the variations in nutritional requirements for and nutritional responses to amino acids between species.

Table 1.1. Generalization of amino acid requirements of animals.

Not generally synthesized	Synthesized from essential amino acids	Usually non-essential but some animals have a requirement
Lysine	Tyrosine (from phenylalanine)	Arginine if urea cycle absent
Histidine	Cystine (from methionine)	Glycine in uricotelic species
Leucine		Proline if conversion from glutamate deficient
Isoleucine		
Valine		
Methionine		
Threonine		
Tryptophan		
Phenylalanine		

The so-called non-essential amino acids are largely synthesized by transamination of intermediates of glycolysis and the tricarboxylic acid cycle. However, for their synthesis an adequate supply of amino groups must be available. It is possible to feed diets to animals where the supply of amino groups is limiting and as a consequence their growth rate can be increased by supplying non-protein nitrogen in the diet.

Examination of the qualitative requirements of a variety of species of animals shows a remarkable consistency (Table 1.1). Variations between species can usually be traced to minor differences in their metabolism. For example, birds do not have an active urea cycle and therefore require arginine in the diet (see Chapter 10). Rapidly growing mammals will also respond to arginine in the diet not because they do not have an active urea cycle but because the vast majority of the arginine that is synthesized is catabolized in the liver by the active arginase associated with the urea cycle. Consequently not enough leaves the liver to support the rapid growth of the extra-hepatic tissues. Another example of qualitative differences between species is the need for cats to have arginine in the diet. Cats given a mixture of amino acids deficient in arginine rapidly become comatosed as a result of ammonia poisoning. This is readily reversed on supplying arginine or ornithine in the diet; the cat only has a limited capacity to synthesize ornithine from glutamate (Baker and Czarnecki-Maulden, 1991). Poultry often need proline in the diet due to their limited capacity to synthesize it from glutamic acid (Boorman and Lewis, 1971).

Often methionine is the amino acid which limits growth. A significant proportion of dietary methionine is used for the biosynthesis of cysteine via the trans-sulfuration pathway. Because of this pathway dietary recommendations often link cysteine and methionine together. It is however very important to realize that the pathway is not reversible and that it is essential to supply methionine in the diet. Conversely it is often said that because of the presence of this pathway there is no need to supply cysteine in the diet. Baker (1989) illustrates the importance of a detailed consideration of this interconversion in determining the requirements of cysteine and methionine. For example, the methionine requirement for the growing chick can range from 0.27 to 6.0 g kg^{-1} depending on the cysteine content of the diet. He also illustrates the need, when considering the total requirement in g kg^{-1} diet for a pair of interconvertible amino acids, to remember that the molecular weight of the product (e.g. cysteine) and the precursor (e.g. methionine) is rarely the same. Other classic examples in amino acid nutrition where product-precursor relationships need to be taken into account include phenylalanine/tyrosine, cysteine/tyrosine and carnosine/histidine, and these are referred to in several other chapters in this volume.

Utilization of Isomers of Amino Acids

All amino acids used in protein synthesis must be in the L-form but D-amino acids are found naturally, for example in bacterial cell walls. Commercial production of amino acids often involves chemical synthesis although the use of stereospecific fermentative processes is becoming of increasing importance. Often commercial diets are supplemented with crystalline amino acids and it is therefore of importance to be able to predict the utilization of racemic mixtures. Some racemic mixtures are almost as effective as the L-isomer. The differences that do occur (see Table 1.2, which presents data for the chick) can be explained by differences in the metabolism of individual amino acids.

D-amino acids can be catabolized by D-amino acid oxidase to yield keto acids. These keto acids can normally be transaminated to yield the L-isomer of the amino acid. There are no transaminases for lysine and threonine in animal tissues, hence the D-isomers of these amino acids are not nutritionally active. Sometimes the keto acid is readily catabolized hence reducing its potential to be transaminated to form the L-isomer. Presumably this competition between irreversible oxidation and transamination of the keto acid is the reason why some other D-amino acids are not readily interconverted into the L-isomer. This topic is considered further in Chapter 3 of this volume. Inclusion of D-isomers in the diet will, unless they are utilized with the same efficiency as the L-isomer, result in an increase in nitrogen excretion by the animal and potentially an increase in pollution of the environment.

Table 1.2. Utilization of dietary D-amino acids by the growing chick. (From Boorman and Lewis, 1971. Reproduced by permission of Academic Press.)

Amino acid	Nutritional value
Methionine	Almost equivalent to the L-isomer
Phenylalanine	
Leucine	
Proline	
Valine	Approximately half as potent as the L-isomer
Tryptophan	Of little or no nutritional value
Histidine	
Isoleucine	
Lysine	
Threonine	
Arginine	

Toxicity of Amino Acids

The balance of a mixture of amino acids in the diet is very unlikely to exactly meet the requirements of each of the animal's tissues. A deficiency of an amino acid is likely to cause a reduction in performance. Excesses of amino acids can also be deleterious. Even small excesses of some amino acids, for example methionine, can cause problems. In addition, an excess of one amino acid, while not toxic in itself, can induce an apparent deficiency of another amino acid. For example a relatively small excess of leucine can cause an apparent deficiency of isoleucine. The susceptibility of an animal to imbalances and excesses of amino acids is influenced by the overall protein supply. Animals fed relatively high levels of protein are more tolerant. Ruminants appear to be particularly sensitive to methionine toxicity, presumably because they do not naturally experience wide variations in the amino acid composition of the digesta entering the intestine. However, a wide range of non-protein amino acids occurs naturally in certain crop plants and particularly in tropical legumes. For example, the aromatic amino acid, mimosine, occurs in *Leucaena leucocephala*, a ubiquitous species yielding timber and palatable forage for ruminants. A structural analogue of arginine, canavanine, is widely distributed in various tropical legumes, including *Canavalia ensiformis*, *Gliricidia sepium* and *Indigofera spicata*. Both amino acids are endowed with potent toxic properties. In temperate regions, the factor causing haemolytic anaemia in cattle and sheep consuming forage brassicas has been identified as a ruminal metabolite of *S*-methylcysteine sulfoxide, an analogue of methionine, distributed throughout the plant. The toxicity of amino acids is discussed in detail in Chapter 4 of this volume.

Arginine Metabolism

As indicated previously, the primary direction of arginine metabolism in mammals is via the urea cycle, enabling the disposal of excess amino acid nitrogen. However, the secondary metabolism of arginine is also of considerable biochemical and physiological significance. Thus, the action of arginine decarboxylase permits many organisms to synthesize putrescine and other polyamines. In addition, putrescine may be synthesized by the action of ornithine decarboxylase (ODC). In animals, however, only the latter pathway is functional. Although the specific functions of polyamines await elucidation, recent studies suggest that these compounds are essential for normal growth and development in all living organisms, and may regulate RNA synthesis and stabilize membrane structures. Polyamine production appears to be an indispensable feature of all tissues actively engaged in protein synthesis. In Chapter 15, Oldham points out that arginine uptake by the mammary gland from the blood supply substantially exceeds the quantities of this amino acid secreted in milk. This is attributed to the need to synthesize non-essential amino acids, particularly proline, within the gland. However, the excess uptake of arginine may also reflect the need for polyamine synthesis by tissues actively synthesizing proteins in the mammary gland. Polyamine synthesis is an important focal point for the action of anti-nutritional factors. Thus in lectin-induced hyperplastic growth of the small intestine, levels of putrescine, spermidine, spermine and cadaverine are markedly enchanced (Pusztai *et al.*, 1993). On the other hand the growth-retarding effect in chicks fed *Canavalia ensiformis* has been attributed to inhibition of polyamine synthesis (Chapter 4). The non-protein amino acid, canavanine, which this legume contains, is metabolized to canaline, a potent inhibitor of ODC (D'Mello, 1993).

A striking feature of arginine metabolism, elucidated recently, relates to the synthesis of nitric oxide (NO). The biosynthesis of NO involves the oxidation of arginine by NADPH and O_2 via the action of NO-synthases with the intermediate production of N^{ω}-hydroxy-arginine. It is now known that NO plays a key role in vasorelaxation, neurotransmission, immunocompetence, male reproductive performance and gut motility (Moncada *et al.*, 1991). It is suggested in Chapter 4 that canavanine may inhibit NO synthesis through its structural antagonism with arginine. Enneking *et al.* (1993) arrived at a similar conclusion from their studies on canavanine-induced feed intake inhibition in pigs.

Catabolism of Amino Acids

The K_m values of the enzymes involved the catabolism of amino acids are higher than those of the amino acid-activating enzymes; for example the K_m for threonine by serine-threonine dehydratase is $8.4–13 \times 10^{-3}$ M and the K_m for the threonyl tRNA synthetase is 4.3×10^{-6} M (Kang-Lee and Harper, 1978). When the supply of an amino acid is low it is used relatively efficiently for protein synthesis. As the supply of amino acid is in excess of that required for protein synthesis amino acid oxidation increases. This phenomenon has been observed for the majority of the so-called essential amino acids. This ensures that when amino acids are in short supply they are preferentially used for body protein synthesis. The activity of most amino acid degradative enzymes increases with the dietary supply of protein. This can occur via an increase in the amount of enzyme present; for example rat liver histidase increases seven-fold when rats are changed from a 180 g kg^{-1} diet to one containing 800 g kg^{-1}. Some amino acid catabolizing enzymes exist in active and inactive forms. Phenylalanine hydroxylase, the first and rate-limiting step in the main route of catabolism of phenylalanine, is such an enzyme; it can be activated by substrate and by glucagon. Glucagon increases phosphorylation of the enzyme protein. Phenylalanine increases the activity of the enzyme by allosteric activation.

The increase in oxidation as amino acid supply exceeds the requirements for protein anabolism has often been used to assess the requirement of an animal for an individual amino acid. With some amino acids the inflection point is not always distinct. Methionine is such an amino acid. Kim *et al.* (1983) were able to determine the methionine requirement of pigs using ^{14}C-phenylalanine as an indicator amino acid. At methionine concentrations below those required for maintenance protein is degraded to supplement the deficient amino acid supply and other amino acids such as phenylalanine are in excess. As the methionine supply increases protein anabolism increases and the excess of phenylalanine is reduced and thus phenylalanine oxidation is reduced. Thus by monitoring the oxidation of the indicator amino acid the supply of methionine which exceeds the requirement for protein synthesis can be determined. The use of such techniques has played a major role in determining the amino acid requirements of farm animals, especially the larger species. The results of such studies are referred to many times in this volume. Amino acid catabolism and protein synthesis would appear to be linked processes. The anabolic drive hypothesis of Millward and Rivers (1988) suggests that before their oxidation excesses of amino acids exert a transient anabolic effect on protein deposition. These authors suggest that 'there are advantages in consuming levels of indispensable amino acids in excess of the level required to match identifiable needs'. Although the hypothesis was developed in relation to a more accurate estimation of human amino acid requirements it does suggest that an animal exhibiting a rapid rate of protein synthesis will have an enhanced rate of amino acid oxidation and hence nitrogen excretion. The exact link between amino acid oxidation and protein synthesis is difficult to demonstrate experimentally but if enhanced protein synthesis is by necessity linked with an enhanced catabolism of amino acids then this has major implications in attempts to reduce nitrogen pollution from highly productive farm animals.

Amino Acid Nutrition and Nitrogen Pollution

Increasingly the animal industry is obliged to reduce the effects of intensive production systems on the environment. Until recently consideration of the response of animals to amino acid supply was confined to maximizing the efficiency of outputs like meat and milk but little or no attention was paid to reducing the output of nitrogenous materials in the faeces and the urine. Output in the faeces can be minimized by increasing the digestibility of feeds; for example, anti-nutritional factors in leguminous seeds, such as protease inhibitors, can be removed by heat treatment and potentially by enzyme treatment. Endogenous protein losses also contribute significantly to faecal nitrogen. Enzyme treatment of feedstuffs also appears to be a promising way to reduce the indigestible fibre content of the diet and hence loss of endogenous faecal secretions (Tamminga and Verstegen, 1992).

Reduction in the loss of nitrogen in the urine can be achieved by carefully balancing the dietary supply of amino acids to the requirements of the animal for the desired production response and, as is indicated many times in this volume, these requirements vary with age and physiological status. In addition it is necessary to consider carefully the efficiency with which these amino acids are used. Dietary protein (amino acids) is not used with a constant efficiency; as maximum production is approached the efficiency with which amino acids are used falls and hence the proportion of dietary nitrogen that is excreted increases. This needs to be considered when evaluating the level of production to use in an animal farming enterprise.

Part of the inefficiency with which amino acids are utilized is a consequence of the turnover of proteins. The extent of protein synthesis and degradation per unit of metabolic size in mammals at maintenance is relatively constant, approximately $16\,\mathrm{g\,kg^{-0.75}}$ (Millward and Garlick, 1977); even in rapidly growing mammals and birds the ratio of protein deposition to protein synthesis rarely exceeds 1:3. This turnover of protein is an essential component of metabolic control but it is energetically expensive, contributing in some cases to 20% of basal heat production (Buttery, 1978). The reutilization of amino acids released on protein catabolism is