Editorial Board

Chief Editor
Dr. S.M. Durge, PhD

Associate Editors
Dr. B.P. Kamdi, PhD
Dr. Gopi M., PhD

Subject Editors:

Veterinary Sciences
Dr. J.J. Rokade, PhD
Dr. A.M. Ingale, PhD
Dr. G.S. Khillare, PhD
Dr. N.C. Dudhe, PhD
Dr. J. Raju, PhD
Dr. S.P. Uke

Agriculture Sciences
Dr. S.P. Landge, PhD
Dr. R.A. Patil, PhD
Dr. A.A. Hanumante, PhD

Fishery Science
Dr. S.S. Ghatge, PhD
Dr. S.P. Kamble, PhD

Dairy Science
G. Rathod
Dr. B.G. Nagrale, PhD
Dr. A.R. Madkar, PhD

Wildlife
Mary Gaduk
A.S. Khan
Dr. R.M. Sarode
Importance of Amino Acids in High Producing Dairy Cows
Manojkumar, V\textsuperscript{a}, Gopi, M\textsuperscript{b}, Mahesh kumar G.R\textsuperscript{a}, Saravanakumar, M\textsuperscript{a}, Dinesh, S\textsuperscript{a}.
\textsuperscript{a}M.V.Sc., Scholar, Division of Animal Nutrition, Indian Veterinary Research Institute, Izatnagar - 243122
\textsuperscript{b}Scientist, Central Avian Research Institute, Izatnagar – 243 122.

Abstract

Amino acids are very important for dairy cows particularly for milk production and milk protein synthesis. Unlike other animals amino acids are the required nutrients to the ruminants not the crude protein. Deficiency of amino acids causes reduction in feed intake and ultimately it affects the fertility, milk production and milk protein synthesis. The limited amino acids of corn based diet lysine and methionine can be provided via protected forms. NRC (2001) recommended 3:1 is the optimal ratio of lysine to methionine in metabolizable protein. Other than milk protein synthesis amino acids involved in synthesis of other amino acids by act as carbon skeleton and nitrogen donors, synthesis of lactose and increasing the nutrient perfusion to the mammary gland.

Milk is of supreme importance to survival, proper development, and dynamic growth of the neonate. Primary goal of the dairy industry is to maximize the milk production. In recent years, it shifted towards milk component like milk protein pricing structure and the health concerns of the public, on producing milk. In that, balancing for amino acids (AA) had a huge impact in dairy nutrition. Amino acids have enormous physiological importance as building blocks of proteins and synthesis of polypeptides that are the major component of animals’ muscles and tissues. In addition, amino acids play a significant role in various important biochemical and metabolic processes in the cells of animals. Hence, from growth to production and reproduction, amino acids play a large part in the productivity of farm animals and can contribute significantly to the profitability of a farm. It is important to monitor feed quality as the composition of amino acids in feeds is variable, to ensure that animals are consuming appropriate amounts of amino acids to maintain their health and productivity while maximizing profitability.

Amino acids supply

Generally proteins supplied to the cows can be divided into rumen degradable protein (RDP) and rumen degradable protein (RUP).
The cow absorbs and uses individual amino acids only not the protein. RDP is required by rumen microorganisms and amino acids (AA) are required by the cow. The rumen microbes, undegraded dietary amino acids and endogenous amino acids (from sloughed off cells and secretions in the digestive tract) all contribute to the amino acid pool available for absorption at the small intestine. Rumen microbial amino acids contribute about 50-75% of the total amino acids. The amino acid profile of microbial protein is very similar to the amino acid profile of milk which makes it a "high-quality" protein. About 70% of microbial protein is composed of amino acids (true protein (Korhoren et al., 2002). The amount of microbial amino acids produced in the rumen is dependent on mostly carbohydrate availability in the rumen, rumen pH, and the types of rumen microbes. The composition of amino acid in the microbial true protein is fairly constant (Chow et al., 1990) and doesn't change much with diet. Rumen undegraded protein (UIP) contributes a significant supply of amino acids to the small intestine. To increase milk protein yield the duodenal supply of individual AA should meet the requirement of AA for milk synthesis. For that we should maximize microbial amino acid production and then, supplement with undegraded amino acids. These both provide more volatile fatty acids for energy.

Limiting amino acids

Amino acids can be classified into essential and non-essential amino acids. Essential amino acids are amino acids that the animal cannot make by itself and must be provided through dietary intake to ensure normal function of the animal. They include phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, histidine, arginine, leucine and lysine. Non-essential amino acids are amino acids which can be synthesized within the animal's body, usually from other amino acids or other compounds which includes alanine, aspartic acid, cysteine, cystine, glutamic acid, glycine, hydroxyproline, proline, serine and tyrosine. These categories were initially established for growth, in fact rapid growth (Bequette and Nelson, 2006). If EAA are deficient in diets, ruminal microorganisms can support low levels of growth and production performance. Regardless of whether an amino acid is termed essential or non-essential, animals need sufficient amounts of all amino acids to meet all their metabolic needs, whether they need to produce milk, build muscle and tissues or reproduce. The requirement for certain amino acids will vary depending on the species, gender, diet and stage of life of the animal. Protein production by the cow is limited by the particular amino acid that is in shortest supply. That amino acid is called the as the "first-limiting" amino acid.
in the diet. It depends upon the amino acid profile of the ingredients in the ration. For dairy cows lysine and methionine are typical first-limiting amino acids. Arginine is also a concern. Three different branched-chain amino acids can also become limiting (valine, isoleucine, and leucine). Apart from essential amino acids some non-essential amino acids (NEAA), particularly glutamic acid and proline might limit synthesis of milk proteins (Meijer et al. 1993). Histidine is important when lower RUP diets are fed. Histidine is the first limiting AA for milk and milk protein yields when high forage, grass silage diets, supplemented with barley and oats, with or without feather meal (Vanhatalo et al., 1999).

**Protection of amino acids**

Because the amino acid composition of microbial protein is relatively constant, undegradable protein can have a major effect on the balance of amino acids available to the animal and synthesis of milk protein. But the undegradable protein may not always provide optimal quantities of the specific amino acids required for milk protein synthesis. Methionine and lysine have been shown to possess low uptake-to-output ratios in the mammary gland, suggesting that they are limiting to milk protein synthesis in the corn based diet (Chow et al., 1990). We can supply these amino acids in a protected form to increase milk protein. These amino acids can be coated with a pH-sensitive copolymer of styrene and 2-methyl-5-vinylpyridine which resist breakdown at rumen pH level, gets breakdown in the abomasum and releases the amino acids for protein synthesis (Chow et al., 1990). The drawback of providing excess methionine by rumen protection is that it is one of the most toxic AA. It can be synthesized via remethylation of homocysteine but this also does not represent new or net synthesis of methionine because the only source of homosysteine in the body is from methionine catabolism. However, the synthetic hydroxyl-analogue of methionine, 4-thiomethyl-2-hydroxybutanoic acid (HMtBA), has been used effectively as a source of methionine in pig, poultry and dairy cow diets. The advantage with HMtBA is that is not toxic, as is free methionine (Bequette and Nelson, 2006). Conversion of HMtBA to methionine in mammary gland accounts 20% of milk protein methionine derived from the supplemented HMtBA given to dairy cows.

### Table 1: Sources of limiting amino acids

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Good Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methionine</td>
<td>Fish meal, Corn Gluten Meal, Rumen Bypass Methionine</td>
</tr>
<tr>
<td>Lysine</td>
<td>Blood Meal, Fishmeal, Processed Soybean, Rumen Bypass Lysine</td>
</tr>
<tr>
<td>Arginine</td>
<td>Feather Meal, Fishmeal, Processed Soybean</td>
</tr>
</tbody>
</table>
Challenges to Predicting Amino Acid Requirements

Milk protein yield increases in a curvilinear manner in response to post-ruminal supplementation with protein (Guinard and Rulquin, 1994). The profile of AA delivered to the mammary gland for milk protein synthesis does not reflect that which leaves the rumen and which is available for absorption. Therefore, it is necessary to find out the exact pattern of AA required to maximize production and efficiency. For that we need to consider the potential transformations of the AA supply, beginning at the level of the small intestines, traversing through the gut tissues and how these are divided between the mammary gland and other tissues. Lastly, we will need to consider mammary metabolism of AA, and the factors and mechanisms that regulate or limit transport and incorporation of AA into milk protein.

Mammary uptake of amino acids

The mammary gland is the major site of nitrogen utilization in lactating ruminants. Amino acid uptake depends on three factors, arterial concentrations of amino acids, rate of mammary blood flow, (quantity of each amino acid reaching the glands per unit time) and the extraction process by which carrier systems transfer blood amino acids across basal membranes of the secretory cells. Once inside the cell, amino acids may undergo RNA directed polymerization to form milk proteins subsequently secreted by exocytosis, they can be retained in the cells in the form of structural proteins or enzymes, they can enter into metabolic reactions yielding CO2, urea, polyamines, and nonessential amino acids (NEAA) and they can pass unchanged into milk, blood, or lymph. The first one is the major route but not for all essential amino acids and second and fourth are the minor routes (Mepham, 1982). The amino acid transporters expressed by mammary tissue do not appear to be unique to the organ (Baumrucker, 1984). Under normal physiological conditions, transportation of AA can be limited by substrate concentration and transporter capacity that is, a change in either the number of transporters or in the concentration of AA at the cell surface. An energy source is required to establish and maintain the AA concentration gradient. Several transporters use Na+ as an exchange molecule. As each transporter generally has affinity for more than one AA, there is potential for antagonism among AA wherein elevated concentrations of one AA inhibit transport of other AA. However, most AA can be transported by more than one transporter (Shennan, 1998), so this potential antagonism is probably partially negated.

Uptake and output

Some amino acids are taken up by the mammary gland more than it puts out in milk. With some non-
essential amino acids, the mammary gland takes up fewer than are found in the milk produced. Therefore the pattern of amino acids needed for milk production is different than the amino acid pattern of milk. The arteriovenous net balance technique has been used to monitor mammary uptake of AA. Based on this comparison, valine, leucine, isoleucine, arginine, lysine and threonine are not limiting since their extractions generally exceed milk protein outputs. By contrast, the extraction of phenylalanine, methionine and histidine are observed to be less than milk protein outputs (Guinard and Rulquin, 1994) (Bequette et al., 1998). The extraction rate of glutamic acid is the highest of all amino acids and output in milk is 244%, of mammary arterial uptake (Derrig et al., 1974). Glutamine and its counterpart, glutamic acid, together make up 18% to 23% of milk protein-bound amino acids (Kaufmann and Hagemeister, 1987) and quantitatively they are the most abundant in milk protein. In addition to direct incorporation into milk protein, some absorbed amino acids are an energy source and carbon precursors of other amino acids synthesized in mammary cells. Branched chain amino acids like valine, leucine and iso leucine and ornithine which is derived partly from absorbed arginine, they all act as nitrogen precursors for other AA synthesis.

**Balancing amino acids**

Current feeding schemes subdivide EAA requirements into net requirements for maintenance functions as the first priority, for growth, lactation and/or reproduction. The requirement of metabolizable protein (MeP) determined from the AA composition of endogenous protein losses, and that needed for net synthesis of muscle, milk and fetal tissue proteins. Hence this approach is satisfactory for balancing amino acids. Milk protein content and yield can be increased by improving the profile of AA in MeP, by reducing the amount of surplus pro-

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Total Uptake</th>
<th>Mammary Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>8.53</td>
<td>3.40</td>
</tr>
<tr>
<td>Histidine</td>
<td>3.29</td>
<td>2.74</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>8.80</td>
<td>5.79</td>
</tr>
<tr>
<td>Leucine</td>
<td>13.04</td>
<td>9.18</td>
</tr>
<tr>
<td>Lysine</td>
<td>9.14</td>
<td>3.40</td>
</tr>
<tr>
<td>Methionine</td>
<td>2.82</td>
<td>2.71</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4.51</td>
<td>4.75</td>
</tr>
<tr>
<td>Threonine</td>
<td>4.76</td>
<td>3.72</td>
</tr>
<tr>
<td>Valine</td>
<td>10.01</td>
<td>5.89</td>
</tr>
</tbody>
</table>

Table 2: Uptake of amino acids by the mammary gland versus output of amino acids in milk (g/100 g amino acids)
tein in the diet, and by increasing the amount of fermentable carbohydrate in the diet. Unlike in monogastric species (Fuller et al., 1989), it is more difficult to determine the accuracy the amino acid requirements of ruminants for growth and milk production. The NRC (2001) publication concluded that 2.2% of the metabolizable amino acids should be methionine and 7.2% should be lysine for optimum milk volume and milk protein production. The ratio of 3:1 is regarded as the optimal ratio of lysine to methionine in MeP. Without supplementation of individual amino acids, we cannot obtain this ratio. When rations are balanced for Lys and Met, due to the improved efficiency of use of MeP, less energy is needed to eliminate surplus amino acid-N as urea, allowing energy to be put to a more productive use.

**Amino acid deficiency**

One of the first and most important signs of an amino acid imbalance in the feed is a reduction in feed intake. Initially animals will eat more food to compensate for the deficiency, after a few days they decrease their food intake substantially. In contributes to low body weight and a general reduction in muscle development. In ruminants, under-nutrition of amino acids can have a negative effect on fertility, especially during early ovulation and reduced milk production. It can be prevented through dietary manipulations, such as adjusting the types and quantities of the various common feeds.

**Amino acids benefits**

As building blocks of proteins AA regulate metabolic pathways for whole body homeostasis. They are the most abundant organic nutrients in the milk of farm animals, present in both free and peptide-bound forms (Rezaei et al., 2016). The intake of methionine and lysine have a strong effect throughout the fertility cycle. These two amino acids are particularly important for embryonic development and deficiency negatively impact fertility. Supplementation of AA is important in the development of the mammary gland. Lactating mammals require large amounts of AA to support milk synthesis by mammary glands during lactation. Uptake of AA from the arterial blood is the ultimate source of proteins and bioactive nitrogenous metabolites in milk. Balancing rations for individual amino acids improves feed efficiency in dairy cows. Branched-chain AA (BCAA; leucine, isoleucine and valine) and arginine are extensively degraded, whereas glutamate, glutamine, alanine, aspartate, asparagine, proline, and polyamines (key regulators of gene transcription and translation) are actively synthesized by lactating mammary tissue. BCAA are catabolized extensively in lactating mammary tissue to provide amino groups for biosynthesis of other amino acids, such as glutamate and glutamine, which are necessary for neonatal growth and digestive tract maturation. Glutamate is either amidated to form glutamine or transa-
minated with pyruvate (or oxaloacetate) to produce alanine or aspartate (Rezaei et al., 2016). Leucine increases protein synthesis in bovine mammary epithelial cells (MEC). Arginine is markedly deficient in the milk of sows, cows, humans, and many mammals due to the extensive catabolism. As mammary tissue does not contain pyrroline-5-carboxylate dehydrogenase or proline oxidase activity it cannot convert arginine, ornithine, or proline into glutamate or glutamine (O'Quinn et al., 2002). This explains the high abundance of proline in milk protein. Nitric oxide, a major endothelium-dependent relaxing factor is a metabolite of arginine catabolism and it enhances blood flow and, therefore, the uptake of nutrients by the lactating mammary gland (Lacasse et al., 1996).

**Other roles of amino acids**

Although lysine is limiting on most corn based dairy rations, it is almost always taken up in excess by the udder. Like leucine, all the excess lysine taken up is oxidized and levels of oxidation are higher in late than in early lactation (Mabjeesh et al., 2000). Along with glutamine and arginine, it is a major nitrogen carrier in the body. Catabolism of lysine in the mammary gland yields two nitrogens which are transferred to glutamic acid ((Bequette and Nelson, 2006). Methionine is involved in the synthesis of phospholipids, carnitine, creatine and polyamines. It provides methyl groups for transmethylation reactions and it contributes to the synthesis of choline, an important component of cell membranes. It provides sulfur for cysteine synthesis (Bequette and Nelson, 2006). Glutamine is essential in situations of metabolic stress and is a precursor for nucleotides for cell replication (McKeehan, 1982). Catabolic pathways of branched chain AAs in the udder are very similar, to pathways that occur in other tissues. Leucine, valine, and isoleucine are catabolized by mammary cells to yield amino-groups, CO2 and organic acids, and amino-groups used for alanine, glutamate and glutamine synthesis. When insulin levels or tissue sensitivity are high the catabolism is inhibited (Bequette and Nelson, 2006). Studies in dairy cows indicate that only ~50-70% of lactose derives from plasma glucose (Bickerstaffe et al., 1974). Glycerol and EAA are prime candidates as precursors for lactose synthesis, which requires involvement of multiple regulated enzyme like NAD-dependent malate dehydrogenase and PEPCK. So EAA involved in the synthesis of substantial amounts of non-EAAs and they provide carbon skeletons for lactose synthesis also.

**Conclusion**

Thus ruminants only need amino acids for efficient growth and milk production not the crude protein. So it is prime to provide enough amino acids in an adequate manner for absorption in small intestine in order to attain optimum growth and milk yield. Balancing the ration for amino acids improves feed efficiency which
leads to higher milk production and protein yield. It is important to know the amino acids available for absorption, mammary uptake and output of amino acids for predicting the amino acid requirement. The major limiting amino acids lysine and methionine can be provided as protected amino acids and by improving the profile of AA in metabolizable protein we can increase the milk protein content and milk yield.

REFERENCES


of histidine alone or in combinations with methionine and lysine. J. Dairy. Sci. 82 (12): 2674-2685.