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SYNTHETIC AMINO ACIDS IN DAIRY CATTLE NUTRITION

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Abbreviations

AA: Amino acid/s
ADF: Acid Detergent Fiber
ARC: *Agriculture Research Council*
Arg: Arginine
ATP: Adenosine triphosphate
CNCPS: *The Cornell Net Carbohydrate and Protein System*
CP: Crude protein
DIM: Days in milk
DM: Dry matter
EAA: Essential amino acid/s
ECM: Energy-corrected milk
FM: Fresh matter
His: Histidine
Ile: Isoleucine
INRA: *Institut National de la Recherche Agronomique*
JDS: *Journal of Dairy Science*
Lys: Lysine
Lys:Met: Ratio of lysine to methionine
ME: Metabolizable energy
Met: Methionine
MHA (HMB)**:** Methionine hydroxy analogue
MP: Metabolizable protein
MUN: Milk urea nitrogen
N: Nitrogen
NDS: *Nutritional Dynamic system*
NDF: Neutral Detergent Fiber
NEAA: Non-essential amino acid/s
NPN: Non protein nitrogen
NRC: *National Research Council*
Phe: Phenylalanine
RDP: Rumen degraded protein
RP: Rumen-protected
RPAA: Rumen-protected amino acid/s
RPLys: Rumen-protected lysine
RPMet: Rumen-protected methionine
RUP: Rumen undegraded protein
SCC: Somatic cell count
Thr: Threonine
TMR: Total mixed ration
Trp: Tryptophan
Val: Valine
VLDL: Very low-density lipoproteins

ABSTRACT

English

All animals require amino acids (AA), which are the building blocks of proteins required for optimal growth, reproduction, lactation and maintenance. In ruminants, proteins and AA are first subjected to a microbial degradation in the rumen making it difficult to predict the quality and quantity of AA that will be absorbed by the animal. The absorbed AA are provided by the ruminally synthesized microbial protein, the ruminally undegraded protein and the endogenous crude protein. Microbial protein is insufficient to supply the adequate amounts of amino acids for optimal production (McDonald, 2011). The extensive degradation of valuable feed proteins in the rumen has lead research to develop the concept of protein protection from ruminal degradation with the principal objective of enhancing the supply of essential amino acids to the productive animal and reduction of nitrogen losses as urea in the urine. Methionine, lysine and histidine have been identified more often as the most-limiting AA for maximum dairy cattle production. In this review it was summarized the responses to dietary supplementation of these AA and the technics used to protect them from rumen degradation. With a more precise protein formulation in ruminant diets, nitrogen metabolism can be more efficient. Which means that milk production can be improved, feed costs can be decreased and the excessive nitrogen in feces can be reduced, a general concern regarding the environmental footprint of animal production.

Key words: nitrogen metabolism, rumen-protected amino acids, milk protein, dairy cows

RESUMEN

Español

Todos los animales necesitan aminoácidos (AA), que son las unidades estructurales de las proteínas, necesarios para un óptimo crecimiento, reproducción, lactación y mantenimiento. En los rumiantes, las proteínas y AA se someten primero a una degradación microbiana en el rumen, lo que hace difícil predecir la calidad y cantidad de AA que será absorbida por el animal. Los AA absorbidos son proporcionados por la proteína microbiana sintetizada en el rumen, la proteína no degradada en el rumen y la proteína cruda endógena. La proteína microbiana es insuficiente para cubrir las cantidades necesarias de aminoácidos para una producción óptima (McDonald, 2011). La extensa degradación de las proteínas de alto valor del alimento en el rumen ha llevado a desarrollar el concepto de la protección proteica contra la degradación ruminal con el objetivo principal de mejorar el suministro de aminoácidos esenciales al animal productivo y la reducción de las pérdidas de nitrógeno como urea en la orina. La metionina, la lisina y la histidina se han identificado más a menudo como los AA más limitantes para la máxima producción del ganado lechero. En esta revisión se han resumido las respuestas a la suplementación dietética de estos AA y las técnicas utilizadas para protegerlos de la degradación ruminal. Con una formulación proteica más precisa en las dietas de los rumiantes, el metabolismo del nitrógeno puede ser más eficiente. Lo que significa que se puede mejorar la producción de leche, reducir los costes de alimentación y reducir el exceso de nitrógeno en las deyecciones, una preocupación general con respecto a la huella ambiental de la producción animal.

Palabras clave: metabolismo del nitrógeno, aminoácidos protegidos en rumen, proteína de la leche, vacas de leche

RESUM

Català

Tots els animals necessiten aminoàcids (AA), que són les unitats estructurals de les proteïnes, necessaris per a un òptim creixement, reproducció, lactació i manteniment. En els remugants, les proteïnes i AA es sotmeten primer a una degradació microbiana al rumen, el que fa difícil predir la qualitat i quantitat d'AA que serà absorbida per l'animal. Els AA absorbits són proporcionats per la proteïna microbiana sintetitzada al rumen, la proteïna no degradada al rumen i la proteïna crua endògena. La proteïna microbiana és insuficient per cobrir les quantitats necessàries d'aminoàcids per a una producció òptima (McDonald, 2011). L'extensa degradació de les proteïnes d'alt valor de l'aliment al rumen ha portat a desenvolupar el concepte de la protecció proteica contra la degradació ruminal amb l'objectiu principal de millorar el subministrament d'aminoàcids essencials a l'animal productiu i la reducció de les pèrdues de nitrogen com urea en l'orina. La metionina, la lisina i la histidina s'han identificat més sovint com els AA més limitants per a la màxima producció del bestiar lleter. En aquesta revisió s'han resumit les respostes a la suplementació dietètica d'aquests AA i les tècniques utilitzades per protegir-los de la degradació ruminal. Amb una formulació proteica més precisa en les dietes dels remugants, el metabolisme del nitrogen pot ser més eficient. El que vol dir que es pot millorar la producció de llet, reduir els costos d'alimentació i reduir l'excés de nitrogen en les dejeccions, una preocupació general pel que fa a la petjada ambiental de la producció animal.

Paraules clau: metabolisme del nitrogen, aminoàcids protegits en rumen, proteïna de la llet, vaques de llet

Introduction

Animal health, production and welfare are the pillars of animal husbandry. To optimize animal health and produce efficiently high quality livestock production, management of diet composition represents the most feasible strategy (McGrath et al., 2018).

Beef and dairy cattle industries have made significant advances in animal genetics, management, health and nutrition. However, intensive production systems might compromise cattle health and welfare (UN 2006).

Current climate change phenomenon represents an additional challenge for ruminant production as it may alter rangeland pastures and forages availability (Henry et al., 2012). Moreover, when animals are exposed to thermal stress, metabolic and digestive functions are often compromised.

The United Nations predicted that the global population could grow to over 9 billion by the year 2050 (UN 2008). It would increase demand of animal products and thus livestock industry should cover the increasing demand in a clean, green and ethical productive environment. Stakeholders involved in ruminant production have to reconsider the strategic use of resources and in such field nutrition plays a key to enhance ruminant health and production (UN 2006).

In the ruminant industry, feed contributes up to 70% of total production costs, therefore improving the efficiency of milk or meat production will have a significant impact on ruminant enterprises profitability (Bach, 2012).

The standard approach has always been to reduce feed costs and to increase feed efficiency so far. With this scenario, micronutrients are often misconsidered from the diet in the attempt to maintain profitability. This fact must be revised as they may be indispensable components of diet.

There is abundant evidence from scientific trials and practical experiences that indicate reliable and cost-effective ways of increasing profitability by rationing with the right nutrient profile including micronutrient additives (McGrath et al., 2018).

Production livestock systems need to consider a multidisciplinary approach focusing on the combination of essential nutrients, aiming to reduce carbon footprint together with the use of antibiotics therapies.

Protein or amino acids that reach the small intestine of dairy cattle are composed by microbial protein synthesized in the rumen, undegraded dietary protein and the

endogenous component. A diversity of factors affect the flow of each individual component and make very difficult to predict the quantity and profile of the amino acids that reach ruminants' small intestine.

It was over 150 years ago when crude protein (CP) became one of the essential nutrients for dairy cows at the *Weende Experiment Station* in Germany. Later, experimental evidences showed the shortcomings of CP as a single coefficient to define rationing in dairy cows and gradually, nutrition systems moved to digestible protein to define nutritional models that recognize that dietary protein requirements need to be defined in terms of rumen degraded protein (RDP) and rumen undegraded protein (RUP) to estimate metabolizable protein (MP) requirements and supply although recently the constancy and adequacy of MP-AA profile is questioned (Schwab & Boucher, 2007).

Dairy nutritionists now have three goals when balancing dairy rations for protein and AA. The first goal is to meet the RDP (ammonia, AA and peptides) requirements for maximum carbohydrate digestion and synthesis of microbial protein. The second goal is to meet the MP requirements of the cow for maintenance, growth, optimum health and reproduction, and desired levels of milk and milk protein production with minimal intake of RUP. And the last goal is to meet the protein (RDP and RUP) and AA requirements.

Methionine, lysine and histidine have been identified more often as the most-limiting AA for maximum dairy cattle production. In this review it was summarized the responses to dietary supplementation of these AA and the technics used to protect them against rumen degradation.

Finally, optimizing protein rationing in ruminant diets brings nitrogen (N) metabolism to be more efficient, production can be improved and N wasted through urine and feces can be reduced. The excretion of N, especially urinary, may become a potential source of water and air pollution, the latter as N_2O, a green-house gas, or as small particulate aerosol having a negative effect on air quality.

Aim of the study

The aim of this study was to review the protein metabolism in dairy cows and summarize the research done related to the dietary incorporation of synthetic amino acids.

1. Metabolism of dietary protein in rumen

Energetic requirements in ruminants are met by the volatile fatty acids coming from carbohydrates fermentation by rumen microbiota (McDonald, 2011). Feed fermentation provides to the microbiota the energy and N for maintenance and growth and its end-products, mainly volatile fatty acids and ammonia, contribute symbiotically to the host metabolism.

Protein content is significant in bacteria (on average, rumen bacteria contain in the order of 625 g of crude protein - N x 6,25 - per kg of DM), so the constant growth of microbial mass supposes a regular supply of microbial N available for the ruminants.

The N source for such synthesis is obtained from peptides, amino acids and mainly ammonia: all proceeding from the degradation of dietary protein plus an endogenous component coming from dead cells and urea synthesized in the liver from the absorbed ammonia, via saliva or by direct diffusion across the rumen wall (Figure 1).

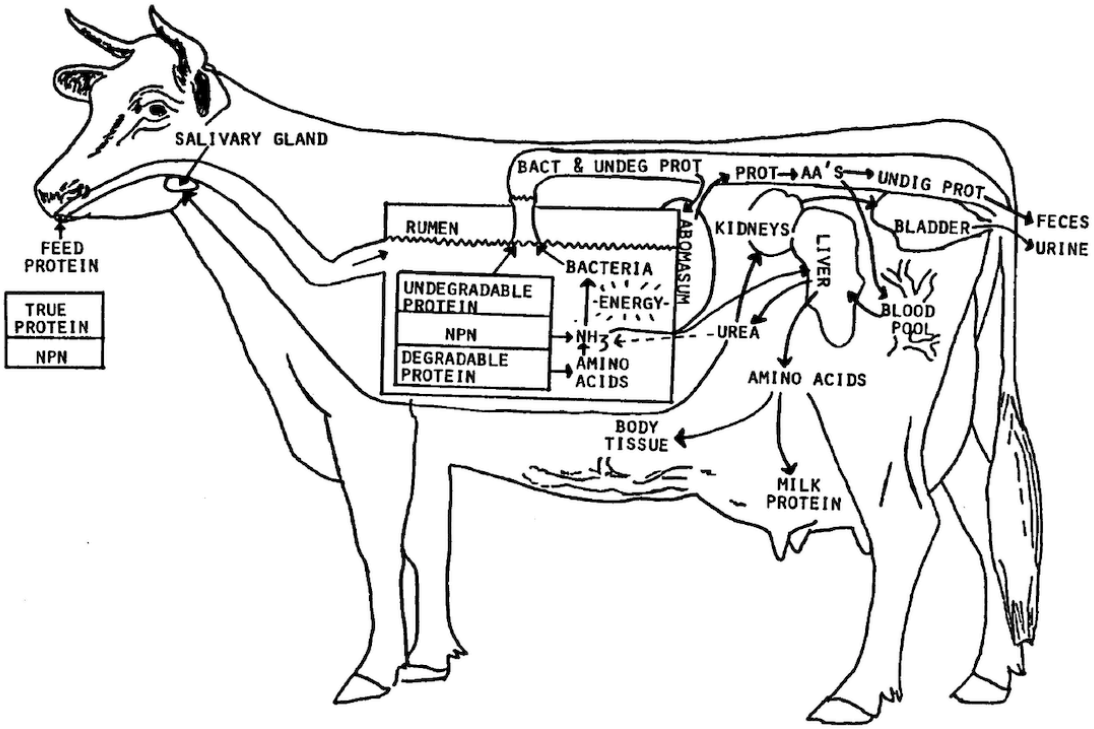

Figure 1. Schematic summary of protein metabolism in the lactating dairy cow (Stern & Mansfield, 1993).

Rumen bacteria use NH_3 as a main source of N. However, some species may need additional protein-N (intact protein or amino acids) to reach the maximum effective growth. Protozoa do not absorb N from NH_3 as fast as bacteria (Church, 1988).

Ammonia and/or amino acids (AA) utilization for rumen bacteria depends on the energy availability (ATP) from the carbohydrates fermentation. When energy is limiting, bacteria increase the fermentation of AA to NH_3, since bacteria have a limited capability to ferment fat. In high-starch diets, when highly ATP is available, dietary CP is used mostly for microbial protein synthesis (McDonald, 2011).

When ammonia exceeds the microbiota requirements, the excess leaves the rumen by absorption through the rumen wall, passing to portal vein and liver where is transformed into urea, and its eliminated by renal excretion. Although, some fraction could be recycled, via saliva or directly by diffusion through the rumen wall or the hindgut.

Urea recycling allows that, when animals receive rations containing less than 10% of CP, the amount of protein passing into the duodenum is usually greater than the amount of protein supplied with the food. When animals are fed diets with high CP, happens the contrary, there is a net loss of protein nitrogen between intake and duodenal flow (McDonald, 2011).

Dietary CP is partially degraded by bacteria by the action of proteases and extracellular peptidases linked to their cell wall, resulting in peptides and amino acids. The bacterial cells, where peptides are transformed into amino acids and most of these are deaminated, release ammonia, organic acids and CO_2. Soluble proteins get in contact with bacterial proteases by an adsorption process, while proteolysis of proteins contained in food particles requires the adhesion of bacteria to such particles. Protozoa also show high protease activity contributing to the degradation of non-soluble proteins.

Due to these processes, most of the dietary CP is degraded and its byproducts reused to synthetize microbial protein which generally constitutes the most important fraction (40-70%), however a significant fraction is able to resist and bypass rumen fermentation and reach unaltered the duodenum (20-50%) and the endogenous provenance (10-20%).

Synthesis and degradation processes that take place in the rumen determine the composition of the protein that reaches the duodenum: 1) microbial protein synthetized *de novo* in the rumen 2) Dietary CP or by-pass protein, 3) An small but significant fraction from endogenous origin.

2. Dietary protein composition

2.1 Factors affecting degradation of nitrogen compounds in rumen

2.1.1. Hydrolysis

Intensity of hydrolysis depends in turn on several factors, including enzyme concentration, accessibility, thermic treatments or feed conservation. Following, there will be discussed some of the most relevant:

2.1.1.1. Enzyme concentration and activity

More than 50% of rumen bacteria have proteolytic activity. Therefore, the proteolytic activity is more related to the quantity of bacteria than to the bacterial profile. As a consequence, the enzyme activity depends on the factors that determine microorganisms' growth. Protozoa growth is inhibited with pH values below 5,5 and thus, negatively affects to the degradation of non-soluble protein. Besides, the addition of antibiotic ionophores, such as monensin sodium (currently banned in the EU), significantly reduces the degradation of the protein in rumen and the deamination of amino acids.

2.1.1.2. Accessibility

Bacterial proteases require an intimate contact of bacteria cell wall with the food protein. Degradability is thus dependent on the content of food cell wall and its integrity, which can be modified by physical processing of the food.

2.1.1.3. Treatments

Heat treatment and formaldehyde application decrease protein degradability, Maillard reactions involves the degradation of sugars to phenolic compounds, the condensation of these with the amino acids and their subsequent polymerization, will result indigestible e.g. meals left after the oil has been extracted from oil-bearing seeds.

2.1.1.4. Forage conservation

Haymaking tends to decrease protein degradability due to the loss of soluble nitrogen and leaves, whereas ensiling increases degradability since the fermentation process increases the content of non-protein nitrogen.

2.1.2. Rumen retention time

Retention time in the rumen increases protein exposition to proteinases; this brings on an increase in degradability.

Time of permanence in the rumen depends in turn on:

2.1.2.1. Intake level

An increase in the intake level leads to a reduction in the mean retention time of the feed.

2.1.2.2. Forage/Concentrate ratio

Retention time increases with a higher concentrate portion in the diet, it may be explained by a reduction in rumination and salivation. However, this effect is compensated and in many cases reversed by the negative effect of including high proportions of concentrate on pH and protein degradability.

2.1.2.3. Particle size

A small particle size of the forages (less than 2 mm) favors the passage through the omasal reticulum hole and therefore reduces retention time of the forage in the rumen. When forages are supplemented with concentrates, retention time is increased due to a lesser rumination and salivation.

2.1.2.4. Physiological phase

At the end of gestation, growth of the fetus/es limits the rumen space and as a consequence, rumen mean retention time tends to decrease.

2.1.2.5. Environment

Low environmental temperatures have a positive effect on digestive transit, leading to a reduction in the time of food staying in the rumen.

3. Duodenal microbial protein

3.1. Factors affecting synthesis of microbial protein in the rumen

Rumen is an anaerobic compartment and thus microbial synthesis is limited and directly related with the available energy for the microorganisms and the available nitrogen in form of ammonia, amino acids and peptides. For a maximum growth of the microorganisms it is also necessary the adequate supply of sulphur (for the synthesis of sulphured amino acids) and phosphorous (part of nucleic acids molecules) and other macro and micro minerals.

Rumen has an anaerobic environment, therefore microbiota can only use a small fraction (10-20%) of the fermented energy. The rest is source of energy for the host animal as volatile fatty acids or eliminated as gas methane. Available energy comes mostly from dietary carbohydrates, a minor fraction comes from protein and microorganisms cannot ferment fat.

The efficiency of utilization of energy by microbes varies considerably. It has been calculated that for every 1000 g of organic matter fermented in the rumen about 40 g of microbial nitrogen can be produced under optimal conditions, but only a minor value is really used: 32 g of microbial N (McDonald, 2011) with a huge variability (from 9 to 50 g of MN/kg DOM fermented in the rumen. (ARC 1984)

The major factors are listed below:

3.1.1. Turnover rate of the rumen content

The efficiency of microbial synthesis decreases as the rumen turnover rate decreases. This rate is positively related with the bacterial fraction placed at the growing phase, if bacteria are not in the exponential phase of growth it takes longer and there is an increment of energy expenditure for maintenance functions at the expense of growth. The rate of ruminal renewal is subjected mostly to feed intake level.

3.1.2. Type of carbohydrate

It has been observed experimentally that the efficiency of microbial synthesis tends to increase with a higher proportion of concentrate in mixed diets until this proportion reaches 50-60%, and decreasing again when the proportions of concentrate are above this percentage. The source of carbohydrate, which determines the rate of fermentation and consequently the available energy for rumen microorganisms, also affects the efficiency of microbial synthesis. When it is in the shape of volatile fatty acids (like in silages) or with high content of fat, microbial protein synthesis is reduced since these sources are not usable for microorganisms.

3.1.3. Nitrogen availability

Microorganisms use ammonia, AA and peptides from the degradation of nitrogen compounds (true protein and NNP) as a source of nitrogen that reach the rumen through food and recycled urea.

It is considered that microorganisms capture nitrogen from slow degradable fraction of the true protein with a 100% of efficiency, whereas nitrogen from NNP and the soluble or immediately degradable fraction of the protein is used with a lower efficiency, close to 80% (McDonald, 2011).

Although the most important source of N is ammonia, which can entirely come from NNP (for example urea), in order to achieve the maximum efficiency of microbial synthesis, at least 30% of the nitrogen available must be in the form of amino acids or peptides.

It is estimated that for a maximum efficiency of microbial synthesis, the concentration of ammonium N in rumen should be above 50 mg/L (Satter and Slyter, 1974). Although this point is open to discussion, some authors assume that there is not a threshold level and ammonia N requirements depend of energy availability (Song and Kennelly, 1990). Moreover, other authors found that ammonia-N requirement may vary to attain the maximum level of bacterial growth or DM degradation (Balcells, 1993).

3.1.4. Synchronization in energy and protein availability

A higher fermentation rate of carbohydrates than protein implies a lower efficiency of synthesis, due to the deficit of available nitrogen that would result in the first hours post-ingestion. If the imbalance is due to a lower rate of fermentation of the carbohydrates rather than from the source of nitrogen (which is easy to happen if this is constituted mostly by NNP), there would be an initial accumulation of ammonia in rumen and an increase in its absorption and elimination in urine in the first hours, and a possible deficit in the later hours, resulting in a decrease in efficiency. In addition, the urea excretion involves energy consumption and excessive absorption of ammonia can lead to alkalosis and toxicity problems.

4. Essential *vs* nonessential amino acids

Of the twenty primary AA that are present in proteins, ten are usually classified as being ‘‘essential’’ (or indispensable). These include arginine (Arg), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp) and valine (Val). Amino acids termed essential (EAA) cannot be synthesized by animal tissues or if they can (Arg and His), not at rates sufficient to meet requirements, particularly during the early stages of growth or for high levels of production. It is understood that when EAA are absorbed in the profile as required by the animal, the requirements for total EAA are reduced and their efficiency of use for protein synthesis is maximized (NRC, 2001). Amino acids classified as ‘‘nonessential’’ (NEAA) are those which are readily synthesized from metabolites of intermediary metabolism and amino groups from surplus AA. Unlike the EAA, there remains little evidence that the profile of absorbed NEAA is important for the efficiency of use of absorbed AA for protein synthesis. However, research is currently limited to accept the fact that a selected NEAA, if provided in amounts greater than provided by the diet, may have some benefit to the animal in certain situations (Schwab & Boucher, 2007).

4.1. Limiting essential amino acids

The term limiting AA is used to identify the EAA that are in shortest supply relative to requirements. There is a limiting AA theory, called by many authors as the “Central dogma of animal protein nutrition”, and it is described with the barrel and stave example (Schwab & Boucher, 2007; Picture 1).

Each stave of the barrel has a different height, relative to the full length of the barrel, so the length of the shortest stave will determine the volume of liquid that the barrel can hold. The shortest stave determines the capacity or volume of the barrel, because it is the most limiting. In the same way, the efficiency of use of absorbed AA is determined by the supply of the first limiting AA.

Picture 1. Barrel and stave example of the AA limiting theory (Schwab & Boucher, 2007).

In the barrel that is shown in picture 1, Met is the first limiting AA. If the supply of Met (i.e., length of the stave), relative to requirements, is increased such that it is co-limiting with Lys, then Met and Lys will be co-limiting AA. Increasing the supply of Met such that it is equally limiting with Lys will also rise the efficiency of use of absorbed AA (i.e., the barrel can hold more liquid).

4.2. Requirements of essential amino acids

According to the *National Research Council* (NRC, 2001) studies, total NEAA requirements are met for the most limiting EAA. Then, the efficiency of use of MP for protein synthesis will be determined by how well the profile of EAA in MP matches the profile required by the animal and by the amount of total EAA in MP.

This principle led nutritionists to identify those limiting EAA when dairy cows are fed conventional rations differing in ingredients composition. Table 1 shows EAA composition of body tissue, milk, microbes and common feeds.

Lys and Met have been identified most frequently as first-limiting EAA in MP of dairy cows. By infusing individual AA or combinations of EAA into the abomasum or duodenum, there has been evidence of effects on N retention and milk protein production.

Further confirmation has been obtained by feeding EAA-protected Met (RPMet) and Lys (RPLys) and determining their effects on weight gains in both growing cattle and milk protein yields.

Lys and Met were firstly identified as co-limiting when lactating cows were fed diets without (Schwab et al, 1976) or with minimal protein supplementation (Rulquin, 1987). Later, (Richardson & Hatfield, 1978), determined Met as the first limiting EAA and Lys as the second limiting EAA. Later Lundquist et al. (1983) concluded EAA limitation would be related to dietary protein supplement, basically RUP when animals were fed soybean sources Met was the first limiting AA whereas in corn-based rations Lys was the first limiting AA. In most dairy rations soybean and corn are included so this means that Lys and Met are nearly always co-limiting. In this sense, Schwab et al. (1976) concluded after several EAA-infusion trials that the limiting sequence of Lys and Met for lactating cows will be determined by the ingredient composition of the diet.

Other EAA have also been evaluated for their possible limitation after Lys and Met supplementation. Particular attention has been given to His and the branched-chain AA (BCAA); isoleucine, leucine and valine in part because some models predict them as more limiting than other AA. However, results with His are much more positive than with BCAA.

Using the total intra-gastric nutrition technique, Fraser et al. (1991) concluded that His was limiting after Met and Lys for lactating cows when casein was the infused protein. However, Vanhatalo et al. (1999) observed that His was the first-limiting EAA only when post-peak lactating Finnish Aryshire cows were fed a grass silage-based diet with any protein supplementation. As the NRC (2001) reviewed, the factors that probably contributed to His being first limiting in the study by Vanhatalo et al. (1999) were: i) the low content of RUP in dietary DM, ii) the low content of His in microbial protein as compared to feed proteins (Table 1) and iii) the low content of His in barley and oats as compared to corn (Table 1).

In the Ferraretto et al. 2016 meta-analysis, where several dietary concentration of AA were evaluated, Val was positively correlated with energy-corrected milk (ECM) but negatively with milk protein yield. Thr was negatively correlated with milk fat content and yield. Ile was negatively correlated to ECM and milk protein yields. Trp was negatively correlated to ECM, milk fat content and milk fat yield. Moreover, Lean et al. (2018) demonstrated that Leu availability was associated with increase in milk protein.

In this aspect it is necessary to remark that feed ingredients commonly used in dairy (Table 1) have an AA composition that could be lower than the microbial protein and then increased in duodenal RUP. This may increase the EAA-imbalance (Schwab and Ordway 2001).

The NRC (2001) protein model indicates the optimal use of MP for milk protein production when Lys in MP is 7.2% and Met 2.4%. The optimum ratio for Lys to Met in MP is 3:1 using this model. Schwab and Ordway (2001) indicated that it is not possible to achieve the thresholds of 7.2% Lys and 2.4% Met (as recommended by the NRC model) by using conventional feedstuffs, and so dairy diets have to be formulated with bypass proteins or pure AA to meet animal's requirements.

Feed sources with an AA profile that complements that of the ruminal microbes are blood meal, fish meal, meat and bone meal, of which inclusion levels or their use is strictly controlled or prohibited entirely for use in ruminant feeds in many countries.

In any case, the NRC recommendations are still based on studies from 2001, and hence, the dairy industry is waiting for a new release. At this moment, there are two principal systems used to formulate diets on an amino acid basis that regularly update the requirements of the dairy cow. The first is the factorial system used in the *Cornell Net Carbohydrate and Protein System* (CNCPS) initially developed by O'Connor, J.D from

the Cornell University. The second is the ideal protein system initially developed by Schwab, C.G from the University of New Hampshire and Rulquin, H. from *Institut National de la Recherche Agronomique* (INRA) in France.

On the latest release of the CNCPS (v6.55), Burhans (2016) suggests 6.77% of Lys in MP and 2.85% of Met, or ~2.38 ratio Lys:Met in MP. New equations have been introduced in the system so that basically the amino acids result to have a higher efficacy of utilization. Burhans concludes that the levels of inclusion required to optimize milk protein will usually require the use of ruminally protected amino acids.

Table 1. A comparison of the EAA profiles of body tissue and milk with that of ruminal bacteria and protozoa and common feeds (NRC, 2001)*.

Item	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	EAA
	% of total EAA										(%CP)
Animal products											
Lean tissue	16.8	6.3	7.1	17.0	16.3	5.1	8.9	9.9	2.5	10.1	-
Milk	7.2	5.5	11.4	19.5	16.0	5.5	10.0	8.9	3.0	13.0	-
Rumen microbes											
Bacteria	10.2	4.0	11.5	16.3	15.8	5.2	10.2	11.7	2.7	12.5	-
Protozoa	9.3	3.6	12.7	15.8	20.6	4.2	10.7	10.5	2.8	9.7	-
Forages											
Alfalfa hay	12.5	4.7	10.3	17.9	12.4	3.8	11.6	10.6	3.6	12.7	41.2
Alfalfa silage	10.9	4.7	11.1	17.9	12.1	3.8	11.7	10.7	2.7	14.1	35.6
Corn silage	6.2	5.7	10.6	27.2	7.9	4.8	12.1	10.1	1.4	14.1	31.6
Grass hay	11.7	4.9	10.0	18.8	10.5	13.9	11.8	10.9	3.7	13.6	33.1
Grass silage	9.4	5.1	10.9	18.8	10.1	3.7	13.4	10.2	3.3	15.0	32.6
Grains											
Barley	13.4	6.1	9.2	18.5	9.6	4.5	13.5	9.1	3.1	13.0	37.7
Corn grain, cracked	11.5	7.8	8.9	27.9	7.1	5.3	11.5	8.8	1.8	10.0	40.1
Corn gluten feed	10.9	8.3	8.8	25.4	7.7	4.5	10.4	9.8	1.6	12.6	35.4
Oats	16.6	5.9	9.1	17.7	10.1	4.2	12.5	8.4	2.9	12.6	41.2
Sorghum	9.4	5.7	9.3	31.9	5.4	4.2	12.3	7.8	2.5	11.6	42.8
Wheat	13.6	7.1	9.6	19.3	8.1	4.7	13.3	8.4	3.5	12.3	34.4
Plant proteins											
Brewers grains, dry	14.7	5.1	9.8	20.0	10.4	4.3	11.7	9.1	2.5	12.1	39.2
Canola meal	16.5	6.6	9.0	15.9	13.2	4.4	9.5	10.4	3.4	11.1	42.6
Corn DDG w/sol.	10.7	6.6	9.8	25.4	5.4	4.8	12.9	9.1	2.3	12.4	13.8
Corn gluten meal	7.1	4.7	9.1	37.2	3.7	5.9	14.1	7.5	1.2	10.3	45.2
Cottonseed meal	26.0	6.6	7.3	13.8	9.7	3.7	12.5	7.6	2.8	10.0	42.6
Lindseed meal	20.9	4.8	11.0	14.5	8.7	4.2	11.1	8.9	3.7	12.3	42.2
Soybean meal	16.2	6.1	10.1	17.2	13.9	3.2	11.6	8.7	2.8	10.2	45.3
Sunflower meal	20.8	6.2	9.9	15.2	8.0	5.6	11.0	8.7	2.9	11.7	42.2
Animal protein											
Blood mea	7.8	11.3	2.2	22.7	15.9	2.1	12.1	7.7	2.8	15.4	56.4
Feather meal	16.2	2.7	11.4	19.9	6.0	1.8	11.6	11.1	1.7	17.6	42.7
Fish meal, menhaden	13.1	6.4	9.2	16.2	17.2	6.3	9.0	9.4	2.4	10.8	44.5
Meat and bone meal	19.5	5.3	7.7	17.2	14.5	3.9	9.4	9.1	1.6	11.8	35.7
Whey, dry	5.0	4.5	12.1	21.2	17.6	3.3	7.0	14.1	3.5	11.7	42.2

*All numbers are average values from different literature reports reviewed in the NRC 2001.

5. Imbalance between dietary/microbial protein input and requirements

The microbial protein contribution to the total protein that reaches the duodenum has a constant amino acid content (McDonald, 2011) and a relatively high biological value, ensuring a continuous and relatively balanced amino acids supply to the ruminant intestine, despite the quality of the diet. This is an undoubtedly advantage when available resources are poor or unbalanced in EAA. Instead, when the quality of the protein consumed is high, its degradation in the rumen is wasteful, and considerably limits the possibilities to increase the supply of EAA to meet the demand of high levels of production.

Microbial protein may be enough to meet the host's needs at maintenance, at the end of the growth phase or at the beginning of gestation, as long as the energy input is adequate.

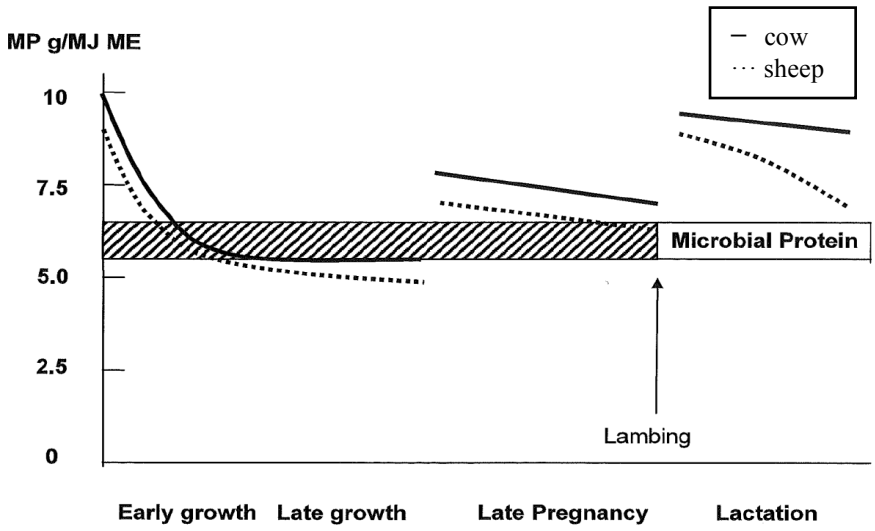

Figure 2. Evolution of metabolizable protein needs in the sheep and cow productive cycles (McDonald, 2011).

However, as can be observed in Figure 2, microbial protein covers more than 60% but does not meet the full MP requirements in early growth, late pregnancy and lactation. Therefore it is necessary to provide RUP protein, in addition to degradable protein. (McDonald, 2011). In high producing dairy cows, the RUP fraction should provide a larger proportion of the total absorbable AA's.

6. Essential amino acids supplementation

The approach to cover differences between the microbial protein supply and EAA requirements in MP involves: i) the optimization of microbial yield without overfeeding of RUP, ii) to include feedstuffs with high Lys (e.g. fishmeal, blood meal, and soybean products), and iii) the inclusion of rumen-protected AA (RPAA).

Due to the variable AA composition of feed protein sources, supplementation of EAA has been common in dairy diets formulation.

6.1. Methionine and Lysine

As reviewed in the NRC (2001), the response of supplementing dairy cows diets with an optimum ratio for Lys:Met of 3:1 in MP includes variable increments in content and yield of protein in milk, milk yield and feed intake.

Rulquin and Delaby, 1997 and other studies indicate that:

8. Content of protein in milk is more responsive than milk yield to supplemental Lys and Met, particularly in post-peak lactation cows.
8. Increases in milk protein percentage are independent of milk yield.
8. Casein is the most influenced milk protein fraction.
8. Increases in milk protein production to increased supplies of either Lys or Met in MP are the most predictable when the resulting predicted supply of the other AA in MP is near or at estimated requirements.
8. Milk yield responses to Lys and Met are more common in cows during early lactation than in mid or late lactation cows.
8. Production responses to increased supplies of Lys and Met in MP typically are greater when CP in diet DM approximates normal levels (14 to 18%) than when it is lower or higher.

In addition to the effects on milk protein production, there are also reports of increased percentages of fat in milk with increased amounts of Met or Met plus Lys in MP. The increments in milk fat generally have been observed in association with increases in milk protein but increases also have been observed without increases in milk protein.

It is not clear why the Met and Lys supply in MP may increase fat content of milk. One reason may involve a positive effect of Met on "*de novo*" synthesis of short- and medium-chain fatty acids in the mammary gland (Pisulewski et al.1 996) although not all authors have observed such effect (Rulquin and Delaby, 1997). A second potential reason may involucrate choline requirement for milk fat synthesis (Sharma and Erdman, 1988) in this sense the NRC (2001) indicates that Met is a methyl donor for choline synthesis, and this is a required substrate for very low-density lipoproteins (VLDL).

Regarding to the milk urea nitrogen (MUN), Donkin et al. (1989) reported a 10% increase in MUN with supplementation of Met and Lys to a control diet. It can be hypothesized that the increased MUN concentration for RPAA supplementation may be due to excess of Met, Lys, or both combined that are deaminated into urea and carbon backbones within the liver. The carbon backbones would be used for gluconeogenesis or energy, whereas the newly formed urea would be excreted into milk or urine.

6.2. Histidine

Recent studies have suggested that His plays a role as a co-limiting AA in dairy cow diets. Giallongo et al. (2016) evaluated the effects of supplementing rumen-protected (RP) Met, Lys, and His in MP-deficient diets on performance of dairy cows. The diet deficient in MP supplemented exclusively with rumen-protected His increased milk protein content, and when supplemented with rumen-protected Met, Lys, and His together, it further increased yields of milk fat, protein, ECM and ECM feed efficiency compared to the diet deficient in MP without RPAA supplementation. In the same direction, Lean et al. (2018) observed that His elicited a positive response in milk yield despite the small difference between treatments; control and treatment groups were supplied with 2.57% and 2.61% of His in MP, respectively.

His maybe important when lower RUP diets are fed. His 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 as primary source of supplemental RUP are fed.

7. Rumen protection for synthetic amino acids

Microencapsulation is designed to increase the amount of a nutrient that passes through the rumen without degradation by the microorganisms, thereby resulting in the delivery of a larger portion of that nutrient to the duodenum.

Several methods have been tested to protect protein (or other compounds) against rumen fermentation; most of them are based on the application of heat, chemical agents, or a combination of both that alter the characteristics of the protein and increase its resistance to proteolytic enzymes.

Heat treatment causes the denaturation of proteins, by alteration of its three-dimensional structure, without rupture of peptide bonds. This entails a reduction of its solubility and accessibility with concomitant reduction of its rumen degradability. However, an excessive heating increases Maillard condensation reactions with melanoidinic compounds formation, which also involves sugars degradation to phenolic compounds. Resulting a reduction in intestinal protein digestibility (Van Soest, 1994).

Protein treatments with chemical agents have the objective to decrease degradability creating a reversible modification on them depending on the pH. Which inhibits their degradation in the rumen-reticulum compartment (where the pH is close to neutral or moderately acidic) but not in the abomasum and the proximal duodenum where the pH is much lower (Tamminga, 1979).

Nowadays, the microencapsulation technique has a widespread application in the agricultural, food and pharmaceutical industries. This technique is also applicable to the ruminant feed industry, as it protects nutrients from degradation in the rumen, making possible to increase the bioavailability of the core ingredient in the small intestine. Microencapsulation is defined as a process in which particles of solids or droplets of liquids or gases at micron sizes are surrounded by a coating material or embedded in a homogeneous or heterogeneous matrix to create small capsules with many useful properties (Thies, 1996).

Sýkora et al. (2007) concluded that materials to be chosen as a coating matrix should have these specific properties to protect the core nutrient/feed from ruminal degradation:

1. Be insoluble in the rumen (<6 pH).
2. Be soluble in the more acidic juice (pH 1.5 –2) of the abomasum.
3. Be resistant to microbial attack.
4. Possess mechanical properties to withstand breakage (e.g. flexibility and strength).

The encapsulated product should also contain a high amount of the core/active ingredient, have a smooth surface and appropriate specific gravity. The capsules must be sufficiently dense to ensure that they do not remain floating at the top layer of the rumen content for an unlimited time.

It is important to note that the different RPAA products differ significantly in the post-ruminal delivery of AA due to differences in ruminal stability, mode of protection and AA inclusion level.

7.1. Lipid encapsulation

Microbes cannot use the lipids because they are not able to ferment; this capability is used to protect some compound against rumen fermentation. Lipid-protected products maintain the integrity of the protective coat in the rumen but are easily digested by the intestinal enzymes in the duodenum, where the active core components are released. When a fat-coated product is designed, an active AA is embedded in a lipid matrix forming small spheres and then coated with lipid material. In general, coating fats consist of a network of calcium salts and fatty acids with a melting point of ≥40ºC and having at least 14 carbon atoms.

Lipid coating has the advantage of using relatively low-cost food-grade materials compared to formulated polymeric coatings (Jenkins & Bridges, 2007). In addition, fats and fatty acids are commonly used in dairy rations, which further justifies the idea of using the same ingredient as a coating material. This protection method includes low payloads of the active material and its limited post-ruminal release and absorption. The latter is generally inversely related to the degree of rumen protection (Gadeyne et al. 2017)

7.2. Surface coated

These products consist of a core of AA and starch coated with several thin layers of ethyl cellulose and stearic acid. The technology used combines coating materials and applications that allow a large payload of AA. Because enzymatic digestion of the ethyl cellulose is minimal, the degradation of the product occurs primarily through physical action and abrasion. The result is a product with a slow degradation in the rumen and a slow release of Met in the intestine (Schwab and Ordway 2003).

7.3. Use of synthetic polymers

In this method, the active ingredient is coated with multiple layers including an inner coating of zein or caseinate and an outer layer consisting of a delayed-release material such as gum arabic, gelatin, ethylcellulose, or hydroxypropyl methylcellulose (Gadeyne et al. 2017).

The 2-layer copolymers are insoluble at high ruminal pH levels and soluble at a low pH level (abomasum), which allows a rapid release. This technology purely relies on the difference in pH levels between the rumen and small intestine and is not affected by enzyme function.

7.4. Methionine-hydroxyl analogues

Adding a chemical blocking group to the α- amino group of Met or removing the acyl group is how the amino acid analogues have been created (Bester, 2012). These Met derivatives like isopropyl-DL-Met, *t*-butyl-DL-Met, N-stearoyl-DL-Met, N-oleyl-DL-Met and capryl-caprolytic-DL-Met have shown some resistance to ruminal degradation.

Schwab and Ordway (2001) evaluated a Met hydroxy analogue (MHA-DL-α-hydroxy-γ-mercaptobutyrate), commonly called HMB, and showed to have a good replacement value for absorbed Met. The HMB is absorbed over the rumen and omasum wall via passive diffusion.

A 2-hydroxy-4-(methylthio)butanoic acid has also been shown to be converted to Met after absorption across the ruminal and omasal epithelium (Belasco, 1972). Therefore, 2-hydroxy-4-(methylthio)butanoic acid can be used as a substitute for RPMet.

However, other studies found no or minimum effect on blood Met concentrations questioning the use of HMB to substitute RPMet in achieving the desired Met level in MP.

8. Take-home message

Balancing for AA offers the opportunity to decrease the amount of protein fed to the cow while maintaining or improving performance. Increases in milk protein and fat concentrations have been regularly obtained. Increases in milk are less frequent and are more commonly observed in early lactation cows.

Lipid encapsulation, surface coating, synthetic polymers and AA analogues are the research lines to keep improving the AA supply to the small intestine.

Moreover, balancing AA during the transition period has the potential to optimize health and production, nonetheless more research must be conducted to determine individual AA requirements more accurately.

Although the use of rumen-protected AA has been primarily in geographical areas where milk protein is well remunerated within the milk pricing scheme, a closer analysis of the response behind AA balancing encourages the use, irrespective of whether there is a premium for milk protein or not.

The use of RPAA products not only includes a reduction of production costs, it also decreases the N excretion in the feces, a general concern regarding the environmental footprint of animal production.

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