HARNESSING THE VALUE OF RUMEN PROTECTED AMINO ACIDS TO ENHANCE ANIMAL PERFORMANCE – A REVIEW

Mitra Mazinani^{1*}, Erdogan Memili², Brian J. Rude²

¹Department of Animal, Veterinary and Food Sciences, Department of Agriculture, University of Idaho, Moscow, 83843 USA

²Department of Animal and Dairy Sciences, Faculty of Agriculture, Mississippi State University, Starkville, 39759 USA

*Corresponding author: mitram@uidaho.edu

Abstract

In general, higher mammals need nine amino acids (AA) in their diets as building blocks to synthesize proteins while ruminants can produce some of them through the synthesis of microbial proteins. Diet is utilized by ruminal microorganisms to synthesize microbial protein (MCP) which is digested in the small intestine (SI). Although protein and amino acid requirements in ruminants are subject to microbial protein synthesis, it is not enough for optimal daily production. Therefore, there is a current trend towards supplementing amino acids in ruminant diets. In the rumen, free amino acids can be degraded by rumen bacteria, therefore, the AAs need to be supplemented in a protected form to be stable in the rumen and absorbable post-ruminal for metabolic purposes. The main site of amino acid absorption is the small intestine (SI), and there is a need to keep AA from ruminal degradation and direct them to absorption sites. Several approaches have been suggested by feed scientists to decrease this problem such as defaunation and debacterization of the rumen against amino acid-fermenting fungi and bacteria, inhibitors or antagonists of vitamin B₆ enzymes, diet composition and also protecting AA from rumen degradation. A number of studies have evaluated the roles of amino acids concerning their effects on milk yield, growth, digestibility, feed intake and efficiency of nitrogen utilization of ruminants. The focus of this review was on experimental and research studies about AAs in feedstuff, metabolism, supplementing amino acids for ruminants and the current trends of using rumen protected amino acids.

Key words: essential amino acids, methionine, lysine, rumen protected amino acid, ruminants

Abbreviations: amino acids (AAs), microbial protein (MCP), lysine (Lys), methionine (Met), histidine (His), isoleucine (Ile), leucine (Leu), phenylalanine (Phn), threonine (Thr), tryptophan (Trp), valine (Val), essential amino acids (EAAs), rumen-protected amino acids (RPAAs), branched-chain amino acids (BCAA), growth hormone/insulin-like growth factor 1 (GH/IGF-1), tricarboxylic acid (TCA), small intestine (SI), methionine hydroxy analog (MHA), 2-hydroxy-4-methylthio butanoic acid (HMB), rumen protected lysine (RPL).

Protein is one of the major macromolecules that is made up of amino acids (AA). Amino acids are classified according to their structure, electrical charge of side chains, and essentiality in nutrition. In general, higher animals need nine essential amino acids in their diets as building blocks to synthesize proteins while ruminants can produce some of them through the synthesis of microbial proteins. Diet is utilized by ruminal microorganisms to synthesize microbial protein (MCP) which is digested in the small intestine. Microbial proteins are considered as the largest sources of amino acids and the proteins. Lysine (Lys), methionine (Met), histidine (His), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), and valine (Val) are essential amino acids (EAAs) for ruminants, and non-ruminants need them as well. Arginine is semi-essential during certain conditions including growth and some physiological stages such as early and late gestation and fetal development. Although protein and amino acid requirements in ruminants are subject to microbial protein synthesis, it is not enough for optimal production (Ayyat et al., 2019; Toledo et al., 2017; Zhao et al., 2019). Therefore, there is a current trend towards supplementing amino acids into the ruminant diets (Schwab and Broderick, 2017). On the other hand, supplementation of free AA in ruminants is useless because the amino acids are used in the rumen by microorganisms and they do not reach the small intestine where they are absorbed (Chen et al., 2020). Even though there are plenty of articles reporting results of studies on the uses of amino acids in ruminants' diet, most of them only researched a specific amino acid, and only few reviews have covered all amino acids and their metabolisms. Therefore, the focus of this review is on review and original research studies about AAs in feedstuff, metabolism and supplementing amino acids in ruminants and investigating current trends of using rumen protecting amino acids (RPAA) from different aspects (Figure 1). The objective was to highlight the major approaches in supplementing AA in ruminant nutrition as reported in the most relevant studies and their influence on feeding ruminants. This review is expected to be useful for researchers who focus on protein and AA needs of ruminants. Moreover, this information helps better understand the use of RPAA and their benefits as well as disadvantages. The emphasis is also given to the history of using amino acids in animal feeds, AAs metabolism, benefits of using different AAs and rumen-protected AA in animal growth, production, and reproduction.

Understanding the importance of amino acid for ruminant nutrition

Defining essential amino acids for ruminants has faced some difficulties stemming from the use of different feedstuff and the AA effects on rumen microorganisms. It was around the 1970s that researchers were conducting some studies to ascertain the effects of using amino acids in dairy cow nutrition; although they used free amino acids that can be degraded by rumen bacteria, most of the results did not show positive effects. Therefore, the researchers concluded that as free AAs are mostly degraded in the rumen, they need to be supplemented in a protected form to be stable in the rumen and absorbable post-ruminal for metabolic purposes. These observations have led to several studies aimed at supplementing rumen-protected AAs. Results indicated that feeding rumen protected AAs may increase milk yield and milk protein (Robinson et al., 1998). Following these findings, several studies

identified that Met and Lys are the most limiting AAs for lactating dairy cows because they are most limited amino acids in the common feedstuffs. Results of later experiments indicated that not only dairy cows may benefit from AAs supplementation by increasing feed intake, milk yield, and milk protein but also transition cows and growing calves may have better responses to environmental changes (Mazinani et al., 2020; Zhou et al., 2016). The other effects of supplementing AAs were improvements in immuno-metabolic status and general health (Zhou et al., 2016).

Figure 1. Graphical abstract of amino acid supplementation to enhance animal performance

Amino acids in feedstuffs

The term “essential amino acids” refers to those that play several important roles in metabolism, reproduction, animal performance and other functions, which are not synthesized in animal cells or at least not at the rate needed; therefore, they should be supplemented through diet. Feedstuffs that are used as protein sources come from animal origin (e.g., meat meal, blood meal, meat and bone meal, fish meal, feather meal, etc.), usually have greater crude protein contents from plant origin (soybean meal, canola meal, peanut meal, etc.) and among them, soybean meal is the most common protein source in the US dairy farms. All these protein sources have different amino acid profiles and most of them are deficient in one or more AA. This imbalance can affect animal performance in growth or production metrics (Li et al., 2011). Schwab and Broderick (2017) suggested that microbial proteins are the best available sources according to their indexes for milk synthesis, soybean meal and fish meal were ranked as the second and third best sources for their AA profiles. Schwab (1994) suggested that the ideal ratio of Lys to Met in duodenal digesta is 15:5 in total EAA in diet.

Table 1. EAA composition in common animal feed sources

	% of total EAA										(% CP)
	Lys	Met	Arg	His	Ile	Leu	Phe	Thr	Trp	Val	EAA
Grains											
barley	9.6	4.5	13.4	6.1	9.2	18.5	13.5	9.1	3.1	13.0	37.7
corn grain, cracked	7.1	5.3	11.5	7.8	8.9	27.9	11.5	8.8	1.8	10.0	40.1
corn gluten feed	7.7	4.5	10.9	8.3	8.8	25.4	10.4	9.8	1.6	12.6	35.4
oats	10.1	4.2	16.6	5.9	9.1	17.7	12.5	8.4	2.9	12.6	41.2
sorghum	5.4	4.2	9.4	5.7	9.3	31.9	12.3	7.8	2.5	11.6	42.8
wheat forages	8.1	4.7	13.6	7.1	9.6	19.3	13.3	8.4	3.5	12.3	34.4
alfalfa hay	12.4	3.8	12.5	4.7	10.3	17.9	11.6	10.6	3.6	12.7	41.2
alfalfa silage	12.1	3.8	10.9	4.7	11.1	17.9	11.7	10.7	2.7	14.1	35.6
corn silage	7.9	4.8	6.2	5.7	10.6	27.2	12.1	10.1	1.4	14.1	31.6
grass hay	10.1	3.7	11.7	4.9	10.0	18.8	13.4	10.2	3.7	13.6	33.1
grass silage	10.1	3.7	9.4	5.1	10.9	18.8	13.4	10.2	3.3	15.0	32.6
Plant proteins											
brewers grains, dry	10.4	4.3	14.7	5.1	9.8	20.0	11.7	9.1	2.5	12.1	39.2
canola meal	13.2	4.4	16.5	6.6	9.0	15.9	9.5	10.4	3.4	11.1	42.6
corn DDG*	5.4	4.8	10.7	6.6	9.8	25.4	12.9	9.1	2.3	12.4	13.8
corn gluten meal	3.7	5.9	7.1	4.7	9.1	37.2	14.1	7.5	1.2	10.3	45.2
cottonseed meal	9.7	3.7	26.0	6.6	7.3	13.8	12.5	7.6	2.8	10.0	42.6
linseed meal	8.7	4.2	20.9	4.8	11.0	14.5	11.1	8.9	3.7	12.3	42.2
soybean meal	13.9	3.2	16.2	6.1	10.1	17.2	11.6	8.7	2.8	10.2	45.3
sunflower meal	8.0	5.6	20.8	6.2	9.9	15.2	11.0	8.7	2.9	11.7	42.2
Animal protein											
blood meal	15.9	2.1	7.8	11.3	2.2	22.7	12.1	7.7	2.8	15.4	56.4
feather meal	6.0	1.8	16.2	2.7	11.4	19.9	11.6	11.1	1.7	17.6	42.7
fish meal, menhaden	17.2	6.3	13.1	6.4	9.2	16.2	9.0	9.4	2.4	10.8	44.5
meat and bone meal	14.5	3.9	19.5	5.3	7.7	17.2	9.4	9.1	1.6	11.8	35.7
whey, dry	17.6	3.3	5.0	4.5	12.1	21.2	7.0	14.1	3.5	11.7	42.2
Animal products											
lean tissue	16.3	5.1	16.8	6.3	7.1	17.0	8.9	9.9	2.5	10.1	–
milk	16.0	5.5	7.2	5.5	11.4	19.5	10.0	8.9	3.0	13.0	–
rumen microbes											
bacteria	15.8	5.2	10.2	4.0	11.5	16.3	10.2	11.7	2.7	12.5	–
protozoa	20.6	4.2	9.3	3.6	12.7	15.8	10.7	10.5	2.8	9.7	–

*Distillers dried grains.

1 – Adapted from Jurgens (2002), Wallace and Chesson (2008).

As depicted in Table 1, because of the closeness to the optimal ratio of lysine to methionine in fish meal, it is a high-quality RUP source among animal protein sources and has positive effects on cow performance (Schwab and Broderick, 2017). Fish meal was more effective in improving body weight gain in young than in finishing ruminants and in males than in females or castrated males (Hussein and Jordan, 1991). Edmunds et al. (2013) studied the amino acid profile of six forages: perennial ryegrass, white clover, ryegrass/white clover sown sward, alfalfa, and meadow grass. Based on this study, lysine had the greatest variations compared to other AAs. Variables that affected the AA profile in forages were the ensiling process and forage species. Except for lysine content in hay, differences between harvest, maturity, and conservation as hay or by artificial drying did not affect AA profile in forages. Givens and Rulquin (2004) stated that hay and dried forages had similar amino acid profiles to fresh forages, while they were around 50% different from ensiled forages. According to a 12-year literature review of Santos et al. (1998), treating most of the dietary protein sources for increasing RUP did not affect milk yield or milk protein content in milking cows which was speculated that these results may be associated with reducing intestinal digestibility and also a poor balance of AA in RUP sources. Feedstuffs cannot be the only source for preparing animal AA requirements for high producing animals or animals in the stages of pregnancy and growth. Therefore, there was a need to supplement diets with AAs. The amino acid profile of microbial protein was similar to milk and body tissue and also had more apparent digestibility. Ruminal bacteria had greater Met content and protozoa had greater Lys content in their EAA profiles; although it was assumed that this variation in the EAA content in microbial protein mixture upon leaving the rumen was fairly small and was not affected by changing population of microbiota.

Metabolism of amino acids in ruminants

The fate of amino acids is distributed between anabolic and oxidative reactions. Converting feed proteins to body tissues in ruminants had low efficiency (less than 35%). Some of this inefficiency can be related to the variation of the ruminal intestinal tract and metabolic events in the tissues. Underfed or fasting conditions enhance amino acid catabolism as compared to the metabolism of well-fed animals. Gluconeogenesis, one of the most studied catabolic reactions, is a metabolic pathway that provides carbon sources for synthesizing glucose from non-carbohydrate substrates such as lactate, glycerol, and amino acids. Using tracers, researchers showed that protein metabolism in ruminants, like non-ruminants, is dynamic, and daily protein synthesis elevates at supra-maintenance feed intakes compared to fasting ruminants. At similar intakes, these rates were greater for bovines compared to ovines, and this relates to the fact that bovines have a greater metabolic rate ($BW^{0.75}$) than the ovines at the same nutritional and physiological conditions (Lobley, 1992).

Amino acids have various metabolic duties in addition to providing the precursors for body protein (hormones, enzymes, etc.). The fasting condition allows the addition of AA to be more impactful. As the most limiting amino acid, methionine is an intermediate in trans methylation reaction, besides acting as cysteine precursor. These reactions provide one-carbon moieties for phospholipid biosynthesis (part of

membrane structure), methylation of DNA bases (transcription) and creatine production (storage and transfer energy) (Kumar et al., 2017).

The flux of the methyl-group of methionine exceeds that of the residual methionine carbons by three to five folds, with homocysteine as the immediate product of S-adenosylmethionine (SAM) (Soda, 2019). Homocysteine is one of the sulfur-containing amino acids produced during the metabolism of methionine by the trans-sulfuration pathway to cysteine. Homocysteine can be remethylated to methionine. Because ruminants have low availability of the methyl group in their diet, insufficiency in one-carbon substrates for remethylation may extend methionine oxidation. Increasing the supply of methyl groups or equivalent one-carbon structures in diet may reduce methionine catabolism. This approach may keep amino acids for protein anabolism (Kumar et al., 2017).

When intake is less than maintenance needs, insulin is one of the main factors that affects the rate of protein catabolism, especially through protein degradation (mostly in muscle tissue) along with decreasing branched-chain amino acid (BCAA) oxidation. At *ad libitum* feeding, protein and amino acid anabolisms and catabolism are more likely regulated by growth hormone/insulin-like growth factor 1 (GH/IGF-1), with a shift toward protein synthesis. Insulin and GH/IGF-1 function may supply overlapping regulatory mechanisms that can explain the cause(s) of biphasic alterations in amino acid dynamics and catabolism in ruminants with *ad libitum* intake compared to those with fasting conditions. The BCAAs may be considered as a regulator of integrating metabolisms in most peripheral tissues and rapidly oxidized into tricarboxylic acid (TCA) in the liver. This association seems well-set in the ruminants, as observed through the relation between the extent of BCAAs catabolism in peripheral and hepatic metabolism which remained fairly stable under different physio-nutritional conditions (Neinast et al., 2019).

Amino acid absorption in the gastrointestinal tract

The ruminant stomach (reticulorumen, omasum, abomasum) has the ability to absorb free AAs. According to Webb's study (Webb et al., 1993), it was shown that 22% of total free amino acids of portal blood in calves and 11% in sheep, were from stomach viscera. Rémond et al. (2000) demonstrated that free amino acids (glycine, serine, valine, methionine, and lysine) can be absorbed across the ruminal epithelium, although this absorption was not significant. The major absorption site was the small intestine (SI). According to Reynolds et al. (1994), amino acid disappearance values in SI were 57–76%. Also, Berthiaume et al. (2001) observed the disappearance of total amino acids in the small intestine in dairy cattle was 65.2%. Besides, the essential amino acids disappeared at a greater rate than the non-essential (66.5 and 63.9%, respectively). Therefore, the main site of AA absorption is the SI, and there is a need to keep AA from ruminal disposal and direct them to absorption sites.

Methods of decreasing AA breakdown in the rumen

Some of the amino acids are considered as limiting AAs for ruminants; these amino acids are not synthesized in the animal's body, and their requirements are met from exogenous sources (dietary). Previously, it was mentioned that most protein sources that

are used for formulating a diet for high yielding dairy cows had an AA deficiency and did not support the requirements for milk production. Microbial proteins are the major sources of AA for ruminants but are not enough to fulfill the AA requirements of dairy cows (NRC, 2001). Adding Met in diet can be a suitable method for supplying this amino acid, but rumen microorganisms break down free AAs and utilize them for their own needs. Until recently, several ways were suggested by feed scientists to decrease this problem, like defaunation and debacterization of rumen against AA-fermenting fungi and bacteria, inhibitors or antagonists of vitamin B₆ enzymes, diet composition and also protecting AA from rumen degradation, as discussed below.

Defaunation and debacterization of rumen

Amino acids are split into amino groups and used by bacteria. Most common amino acid-fermenting bacteria are *Clostridium aminophilum*, *Clostridium sticklandii*, *Megasphaera elsdenii*, *Prevotella ruminicola*, *Butyrivibrio fibrisolvens*, *Selenomonas ruminantium*, and *Streptococcus bovis*. The inhibition of the growth of the AA-degrading bacteria and protozoa may decrease AA degradation and metabolism in the rumen and ruminal breakdown of amino acids (Puniya et al., 2015).

Amino acid sparing effects

The protein (amino acid) sparing effect is accrued when the body derives energy from other sources instead of proteins such as carbohydrates and fats, and this process decreases amino acid metabolism as an energy source. Infusing propionate into sheep rumen showed evidences of AA sparing effects and increasing N retention. Due to this metabolic effect, AA gets refrained from deamination and more AA will continue on to the post ruminal and intestine (Schwab and Broderick, 2017).

Using antibiotics

Some studies reported that using antibiotics inhibited ruminal ammonia production and ruminal escape of AAs. Schelling et al. (1973) fed oxytetracycline to sheep that received Met and Lys orally and observed the enhancement of ruminal escape of the AAs. Researchers in another study fed diphenyliodonium salts and observed notable inhibition of AA deamination and enhanced N-retention in the rumen of growing sheep and cattle (Chalupa et al., 1975; Mazinani and Rude, 2020). Broderick and Balthrol (1979) tested the effects of several chemical inhibitors of AA deamination (toluene, thymol, penicillin G, oxytetracycline, chlortetracycline, and hydrazine) in non-lactating cows and observed reduction in amino acid deamination. Transaminase enzymes require pyridoxal phosphate (B₆) as a coenzyme to transfer amino groups of one AA to a keto acid to form another AA. Hence, inhibitors of vitamin B₆ enzymes or antagonists of vitamin B₆ like hydrazine showed some effects on AA deamination; it is postulated that this is by attacking the carbonyl group of B₆ (Broderick et al., 2004). Also supplementing monensin can enhance the metabolizable energy of diet by increasing propionate production as the result of increasing dry matter digestibility and utilizing spare AA from deamination for gluconeogenesis. Monensin can reduce the deamination of proteins in the diet by reducing hyper-ammonia producing (HAP) bacteria populations that can deaminate more than 25% of feed proteins (Tedeschi et al., 2011).

Presence of other AAs

Some experiments indicated that the degradation rate of a specific AA is reduced in the presence of the other AAs. Velle et al. (1998) studied the escape and apparent ruminal degradation of AAs. Two different treatments included mixtures of 9 EAA and 8 NEAA, respectively. According to the results, the mean of degradation rate in EAAs was 26% when the AAs were used in mixtures and it was 45% when the AA were administered individually, and the corresponding values were 34 and 54% for NEAA. Supplementing AA in low-quality forage-based diets promotes protein accretion and N retention (Volden et al., 2001). Sulu et al (1989) fed cattle two different diets supplemented with AA and observed that the degradation rates of these AAs in the forage-based diet were less and escaped more from the rumen as compared with the high concentrate diets.

Innovative approaches to protect amino acids for precision ruminant nutrition

The first attempts to produce rumen-protected amino acids date back to the late 1960s. As Met was considered the first limiting AA, most of the research was done with this AA. Several researchers and institutes started studies to develop a procedure to protect AAs from rumen degradation (Chalupa, 1975). Most of these procedures were conducted on physical protection of AA, and protection materials were often a combination of a lipid with inorganic materials or carbohydrates. The problems associated with mechanical and physical protection are their sensitivity to scratches which destroys the protecting layer that protects them from abrading in the rumen. Common physical protection techniques are coating, encapsulation, nano-capsulation, micro-capsulation, and macro-capsulation (Mazinani et al., 2020). Chemical methods and use of amino acid analogs and their derivatives were employed for this issue, and all of these methods had benefits and disadvantages as summarized in the following sections.

Physical protection

One example of these products was a core of 20% DL-Met wrapped in 80% tristearin film. The other one was an encapsulated Met containing 30% DL-Met, 58% tristearin and oleic acid, 6% CaCO₃, 4% stabilizing, antioxidant, and flavoring agents and 2% glucose. The latter product had a greater intestinal release. Following that, several protected AAs were developed by lipid encapsulation (Schwab, 1995). The major problem associated with using lipid encapsulating material was the greater release of Met into the rumen. The next approaches for protecting amino acids were involved using enzyme-resistant coated materials and pH-sensitive polymers; some of them were patentable. These products were stable in the rumen's pH and had a post-ruminal delivery system that was released into the intestine, and they were more efficient as compared to the previous protection procedures. Most of these capsulation methods were associated by some problems such as abrading protection layers during processing and handling diet or chewing by the animals. Disturbed coats makes amino acids available for rumen microbes, and therefore, it leads to wasted AAs (Schwab and Broderick, 2017).

Chemical methods

Chemical treatments can decrease rumen degradation of protected AAs. These agents may include aldehydes, alcohol, tannins, alkalis, cations, bentonite, etc. Usu-

ally, these treatments create pH-sensitive modification that decreases rumen digestibility and the treatment reverses in the acidic pH of the abomasum and duodenum. Mazinani et al. (2019) revealed that rumen chemical protected AAs with aldehyde showed some antibacterial effects and increased performance gain in feedlot calves.

Amino acid analogs

Another way for solving this issue is the use of analogs and derivatives of AAs that were mostly based on Met. They are usually free AAs with a chemical blocking group that are added to the acyl group or amino group. Some of the Met derivative examples that have been shown to have ruminal degradation resistance are isopropyl-DL-Met, t-butyl-DL-Met, N stearoyl-DL-Met, N-oleoyl-DL-Met, and caproyl-caprylic-DL-Met (Oke and Loerch, 1989). Most studies on AA analogs are methionine hydroxy analogs (MHA), also called 2-hydroxy-4-methylthio butanoic acid (HMB). The experiments showed that compared to free Met, HMB was more resistant to ruminal degradation. Although supplementing HMB resulted in negligible effects on blood Met and milk protein of dairy cows fed diets with Met deficiency, the results were inconsistent. In a recent study, it was observed that supplementing HMB increased the production performance of ruminants (Chen et al., 2020). Two-hydroxy-4-(methylthio) butanoate reduced milk fat depression in dairy cows and the researchers hypothesized that it was linked to the changes in the rumen bacterial community (Pitta et al., 2020).

Economic importance of supplementing rumen-protected amino acids to enhance ruminant performance

Amino acid supplementation benefits animal agriculture and provides financial benefits to animal farmers for sustainable livestock farming. This nutritional strategy is especially important to feed the ever-increasing world population in the face of shrinking farmlands. As Met is considered the most essential or the first-limiting AA for dairy cows, it has received more attention. The roles of Met in ruminants concerning its effects on milk yield, growth, digestibility, feed intake and efficiency of nitrogen utilization have been evaluated in many studies (Apelo et al., 2014).

Generally, ruminants can only use 10–40% of the protein-nitrogen in the feed. The remaining nitrogen is excreted in urine and feces and contributes to the emissions of ammonia and nitrous oxide into the atmosphere, and nitrate leaching to surface and ground waters. One of the feeding strategies that can help minimizing these environmental effects is low-N feeding; this strategy includes shifting from high-protein feeds to low-N feeds. These strategies are required to maintain animal performance. Therefore, a possible deficiency in essential amino acids is compensated by using synthetic amino acids. Improving gross N efficiency would improve dairy economics, reduce environmental impacts, and reduce the demand for sources of animal feed proteins (van Vuuren et al., 2015).

Amrutkar et al. (2014) conducted an experiment on economic benefits of supplementing rumen-protected Met (RPM) and RPL to the high yielding lactating cows, and they estimated net return of feed cost per milk yield of animal. Their experiment results showed that supplementing RPM and RPL in the ration of high yielding lactating cows was cost effective. During the transition period, supplement-

ing protected AA minimizes negative energy balance and increases recovery of body weight loss and body condition score (Shelke et al., 2012), protected the liver and can be used for prevention of subclinical ketosis (Vudmaska et al., 2021). Researchers in another study reported that adding RPM in the ration of high producing cows had significant economic benefits by enhancing milk performance and milk components (Sun et al., 2010). Maintaining the health of the animals has its economic benefits (Ordway et al., 2009) and when evaluating the effects and benefits of using supplemental AA sources, health responses should be considered too.

Effect of supplementing Met on milk quality and quantity

Initial studies on feeding RPM were inconsistent about their effects on ruminants' performance. Broderick et al. (1970) published the first paper about feeding rumen-protected AA; early- to mid-lactation Holstein cows were fed an encapsulated Met supplement, and the results showed no effect on feed intake, milk yield, and milk component percentages; although, plasma Met concentrations increased. The second study with the same rumen-protected Met source was conducted by Williams et al. (1970) which had reported no benefits of feeding Met and no change on Met concentrations in plasma. Observing these inconsistent responses associated with Met supplementation in these papers, Schwab et al. (1976) conducted a series of experiments to determine the limiting AAs in early and mid-lactation cows and concluded that the milk protein content was sensitive to AA supplementation; adding Met alone had no effect on production performance; whereas, Met and Lys together accounted for 43% of milk protein response. Since then, numerous studies have identified benefits of using rumen protected AA. Several recent studies evaluated the effects of using protected Met on the productive performance of dairy cows. According to a meta-analysis with 35 experiments, it was observed that milk proteins increased in cows fed with supplemented Met (Vyas and Erdman, 2009). Osorio et al. (2013) investigated supplementing multiparous dairy cows with Met; this increased milk production, milk protein and milk fat percentage. A recent study conducted on transition cows supplementing their diet with Met showed that the supplemented group had greater milk production and milk protein percentage (Zhou et al., 2016). Using ethyl cellulose protected methionine by the same research group showed greater dry matter intake, increased milk yield, milk fat and protein yield in a supplemented group of transition cows (Batistel et al., 2018). Therefore, most of the experiments about supplementing protected Met concerning productivity showed an increase in milk protein. Also, some results regarded the potential of increasing milk production and milk fat percentage. However, a few results showed the correlation between the supplementation of Met in Met-deficient diets and casein synthesized in the mammary gland.

Beef performance under supplementing Met

Evidence about benefits of Met in beef cattle stems from the finding the Met-supplemented group obtained greater weight, compared to the control group (Lopes et al., 2019). In another study, no difference was found between the control group and Met supplemented group (Clements et al., 2017). When Waterman et al. (2012) in-

investigated the effects of protected Met on plasma amino acid concentrations in pregnant beef heifers at the end of pregnancy, similar results were observed, and there was no difference between treatments in body condition scores (BCS) or weight gains and in calves' birth weights.

Benefits of supplementing Met on reproduction

Another identified effect of supplementing protected methionine is on reproduction. Acosta et al. (2016) determined that in multiparous postpartum cows that were supplemented with protected Met, embryo preimplantation and survival capacity of the embryo were improved compared to the control group. In this study, the total methylation and lipid contents of embryos were evaluated after superovulation and embryo washing following artificial insemination. According to a study (Sturmey et al., 2009), lipid reserves served as a source of energy for embryos in the pre-implantation stage and were associated with the synthesis of membranes. Thus, greater lipid accumulation in pre-implanted embryos increases their survivability. Also, Acosta et al. (2017) observed the effects of methionine supplementation on the steroidogenic potential of the first postpartum dominant follicle. Expression of mRNA in follicular cells of the cows that received Met supplement was changed, and they had greater methionine concentrations in the follicular fluid of the first postpartum follicle. Most of the experiments evaluating the effects of Met on the reproductive performance concluded that it had some benefits, especially in embryos during the implantation stage when vascular communication with the mother is finished, and the embryo depends on intrauterine fluids for development (Alonso et al., 2008). Also, the results led to the conclusion that ovarian activity, follicular growth, and size of dominant follicle was increased (Dominguez et al., 2017; Jaenisch and Bird, 2003; Wrenzycki et al., 2005).

Effects of lysine and other EAA on animal performance

Not much research has been done on the effects of other amino acids. They are not commercially produced; perhaps it is because of low demand or high costs of protecting them. Although Lys has received more attention as the second limiting AA for dairy cattle or the most limiting AA in corn-based diets since Lys was one of the most limiting AAs in supplemented diets, it was expected that adding rumen protected lysine (RPL) in animal diets would have beneficial effects on milk yield; these expectations were observed in some studies conducted on supplementing RPL (Blauwiekel et al., 1997; Robinson et al., 2011). Robinson et al. (2011) reported an increase in milk yield of dairy cows fed a diet with 15 to 21 g RPL, and there was no effect on milk fat percentage. In contrast, Swanepoel et al. (2010) did not observe any effects on milk production when dairy cows were fed a diet with 10 g RPL. In another study, adding RPL increased DM intake and decreased free fatty acid and β -hydroxybutyrate concentrations of serum in postpartum cows, but no effects were observed for milk yield, milk fat, or milk lactose (Girma et al., 2019).

Leucine, isoleucine, and valine

Leucine, isoleucine, and valine are a group of EAA collectively referred to as branched-chain amino acids (BCAAs). It has been shown that leucine increased pan-

creatic α -amylase secretion in the SI of heifers cannulated with abomasal, duodenal, and ileal catheters (Ren et al., 2020). In that study, Leu had no effects on rumen fermentation profile or starch digestibility, but blood glucose concentration and fecal pH increased. Additionally, Leu decreased the amylolytic genus of *Faecalibacterium* and increased *Pseudobutyrvibrio* and *fibrolytic* genera *Ruminiclostridium* (Cao et al., 2018; Li et al., 2011). The results lead to the conclusion that among the EAA, Leu had the most potential for activating protein translation. This occurred through Leu binding to Sestrin2 in the cytoplasm, which disrupted the Sestrin2-GATOR2 interaction in cells (Saxton et al., 2016). Based on several experiments, evidence showed that BCAA increased milk protein yield (Doelman et al., 2015; Nichols et al., 2017). Recently, several studies have been conducted for investigating the endogenous metabolism of His in dairy cows (Yoder et al., 2020; Zang et al., 2019). In a study on milk-fed calves which received Leu and Phe in milk, phenylalanine tended to increase pancreatic cell number; whereas, leucine tended to increase pancreatic cell size. Besides that, both of them did not affect the activities of the pancreatic enzymes. The results indicated that supplementing Leu and phenylalanine in calves affected the growth and development of the pancreas in different ways (Cao et al., 2018; Yu et al., 2014).

Phenylalanine

Phenylalanine is one of the EAA and an aromatic AA that can increase the secretion of cholecystokinin through calcium-sensing receptors (Cao et al., 2018). Cholecystokinin can promote the synthesis of pancreatic amylase, trypsin, and trypsinogen, stimulate the release of these pancreatic enzymes and enhance their activities. Yu et al. (2014) conducted a study about the regulation of pancreatic exocrine secretion in goats and reported that phenylalanine had a role in regulating the secretion of pancreatic α -amylase and also Liu et al. (2015) observed the same results for leucine in Holstein heifers.

Histidine

Lapierre et al. (2008) and Lee et al. (2012) suggested that the digestible His requirement should be similar to that of digestible Met (2.2% of metabolizable protein) based on their concentrations in milk protein and the same metabolism of both of them in the mammary gland and the splanchnic tissues. Lapierre et al. (2014) claimed that muscle carnosine and anserine had quadratic responses when His was infused to the abomasum. Although it was in contrast with the results of Giallongo et al. (2015) study which reported that muscle carnosine and anserine had no differences in His-deficient and His-adequate diets. In both studies, RP-His did not affect blood hemoglobin in lactating dairy cows. These results were in agreement with Zang et al. (2019) who postulated that low dietary His supply in dairy cows can decrease dry matter intake (DMI), milk yield, milk protein, and blood hemoglobin. In this study, concentrations of EAAs (His, Leu, Lys, Val) in plasma decreased in the His-deficient group as compared with the His-adequate group. The concentrations of His, Leu, and Val in muscle decreased in His-deficient diet, and this group had less blood hemoglobin concentrations.

Table 2. Summarizing the finding of the studies of beneficial effects of consuming RPM

Animal species	RPM amount	Study duration	Effects	References
1	2	3	4	5
Dairy Holstein cows	4.2–12.4 g/d	4 weeks	Increased milk protein, reduced N excretion, and improved N efficiency without altering the digestibility of DM, OM, and fiber.	Nursey et al., 2018
Dairy Holstein cows	0.07% of diet	21 days	DMI, nutrient digestibility, and milk protein synthesis were not influenced by supplementing ground field peas with RPM.	Pereira et al., 2020
Dairy Holstein cows	5–15 g/d	4 weeks	Improved N efficiency and reduced urinary N excretion. Treatment with 16.1% CP and 10 g/d of RPM achieved the best compromise between milk production and lowered N excretion.	Broderick et al., 2009
Dairy Holstein cows	13.7 g/d	28 days	Diet with 17% canola meal and RPM increased milk yield and protein and fat content, but reduced lactose yield and concentration in milk.	Swanepoel et al., 2015
Dairy Holstein cows	30 g/d	8 weeks	Enhanced milk yield when metabolizable protein supply was adequate.	Awawdeh, 2016
Merino ewes	6.3 g/d	Meta-analysis study		Patton, 2010
		Day 111 of pregnancy to day 7 after lambing	True milk protein % (TMP) was increased. DMI and milk fat % were slightly reduced. Milk yield and milk fat yield were slightly increased.	Liu et al., 2016
			Lamb birth weight was increased 10%. Elevated total IgG in lambs aged 4 to 6 weeks. RPM did not alleviate the oxidative stress of ewes during the transition period.	
Massese ewes	5 g/d	17 days	Increased fat corrected milk (6.5%) and blood urea. DMI and nutrient digestibility were not affected.	Antongiovanni et al., 2002
Comisana ewes	3.5 or 7.0 g/kg	16 weeks	Increased 16:0 and 18:3 milk fatty acids. decreased 4:0 and 12:0 milk fatty acids.	Sevi et al., 2000
Lactating Saanen goats	2.5 or 5 g/d	30 days	2.5 g RPM improved milk production and milk protein.	Flores et al., 2009
Toggenburg×French Alpine×Saanen crossbred goats	1. 2 or 3 g/d	110 days	DMI, milk yield, and milk composition were not changed. Positive effects on energy balance.	Alonso-Mélendez et al., 2016

Table 2 – contd.

1	2	3	4	5
Shami goats	2.5 or 5 g/d	60 days	Feed intake, milk production, and fatty acid composition were not altered. Weaning weight and average daily gain of kids were not changed.	Al-Qaisi and Titi, 2014
Danish Landrace×Saanen crossbred goats	6 g combined with 15 g rumen-protected lysine	2 weeks	Positive effect on mammary uptake of lysine and methionine in late lactation compared to early lactation.	Madsen et al., 2005

Conclusions

Considerable development has been made in AA supplementation for ruminant nutrition. A number of nutritionists around the world use crude proteins to balance diet proteins, and balancing diets according to limiting amino acids is not common. However, this is changing, and the positive results of using AA balanced diets should encourage researchers, industry and dairy farmers to feed lower protein diets and use diets containing rumen-protected amino acids. However, some amino acids still need more research to properly understand their effects on livestock performance. In the technical aspects, most of the challenges related to protecting AA have been overcome, and commercial RPAA are increasing in animal nutrition markets. However, there is a need for developing new technologies and/or modifying the existing to increase ruminal escape and delivery to the intestine, the absorption site. Predicting precise AA needs of animal is another issue faced in this field that requires development of new and innovative models for predicting their needs. As such, continuous improvements are necessary in predicting and reaching the AA needs of ruminants, as well as the efficiency, sustainability and profitability of commercial farms. Research in this area can lead to a better understanding of amino acid needs of animals, define better diets to meet these needs and a more reliable characterization of AA supplements.

References

- Acosta D.A.V., Denicol A.C., Tribulo P., Rivelli M.I., Skenandore C., Zhou Z., Luchini D., Correa M.N., Hansen P.J., Cardoso F.C. (2016). Effects of rumen-protected methionine and choline supplementation on the preimplantation embryo in Holstein cows. *Theriogenology*, 85: 1669–1679.
- Acosta D.A.V., Rivelli M.I., Skenandore C., Zhou Z., Keisler D.H., Luchini D., Corrêa M.N., Cardoso F.C. (2017). Effects of rumen-protected methionine and choline supplementation on steroidogenic potential of the first postpartum dominant follicle and expression of immune mediators in Holstein cows. *Theriogenology*, 96: 1–9.
- Al-Qaisi M., Titi H.H. (2014). Effect of rumen-protected methionine on production and composition of early lactating Shami goats milk and growth performance of their kids. *Archiv. Anim. Breed.*, 57: 1–11.
- Alonso L., Maquivar M., Galina C.S., Mendoza G.D., Guzmán A., Estrada S., Vilalareal M., Molina R. (2008). Effect of ruminally protected methionine on the productive and reproductive performance of grazing *Bos indicus* heifers raised in the humid tropics of Costa Rica. *Trop. Anim. Health Prod.*, 40: 667–672.
- Alonso-Mélendez E., Mendoza G.D., Castrejón-Pineda F.A., Ducoing-Watty A.E. (2016). Milk production in dairy goats supplemented with different levels of ruminally protected methionine. *J. Dairy Res.*, 83: 148.
- Amrutkar S.A., Thakur S.S., Pawar S.P. (2014). Economics of supplementing rumen protected methionine and lysine in the ration of lactating crossbred cows. *Indian J. Anim. Nutr.*, 31: 14–19.
- Antongiovanni M., Secchiari P., Mele M., Buccioni A., Serra A., Ferruzzi G., Rapaccini S., Pistoia A. (2002). Olive oil calcium soaps and rumen protected methionine in the diet of lactating ewes: effect on milk quality. *It. J. Anim. Sci.*, 1: 55–63.
- Arriola Apelo S.I., Knapp J.R., Hanigan M.D. (2014). Invited review: Current representation and future trends of predicting amino acid utilization in the lactating dairy cow. *J. Dairy Sci.*, 97: 4000–4017.
- Awawdeh M.S. (2016). Rumen-protected methionine and lysine: effects on milk production and plasma amino acids of dairy cows with reference to metabolisable protein status. *J. Dairy Res.*, 83: 151–155.

- Ayyat M.S., Al-Sagheer A., Noreldin A.E., Abd El-Hack M.E., Khafaga A.F., Abdel-Latif M.A., Swelum A.A., Arif M., Salem A.Z.M. (2019). Beneficial effects of rumen-protected methionine on nitrogen-use efficiency, histological parameters, productivity and reproductive performance of ruminants. *Anim. Biotechnol.*, 32: 51–56.
- Batistel F., Arroyo J.M., Garces C.I.M., Trevisi E., Parys C., Ballou M.A., Cardoso F.C., Lóor J.J. (2018). Ethyl-cellulose rumen-protected methionine alleviates inflammation and oxidative stress and improves neutrophil function during the periparturient period and early lactation in Holstein dairy cows. *J. Dairy Sci.*, 101: 480–490.
- Berthiaume R., Dubreuil P., Stevenson M., McBride B.W., Lapierre H. (2001). Intestinal disappearance and mesenteric and portal appearance of amino acids in dairy cows fed ruminally protected methionine. *J. Dairy Sci.*, 84: 194–203.
- Blauwiel R., Xu R., Harrison J.H., Loney K.A., Riley R.E., Calhoun M.C. (1997). Effect of whole cottonseed, gossypol, and ruminally protected lysine supplementation on milk yield and composition. *J. Dairy Sci.*, 80: 1358–1365.
- Broderick G.A., Balthrol J.E. (1979). Chemical inhibition of amino acid deamination by ruminal microbes *in vitro*. *J. Anim. Sci.*, 49: 1101–1111.
- Broderick G.A., Kowalczyk T., Satter L.D. (1970). Milk production response to supplementation with encapsulated methionine per os or casein per abomasum. *J. Dairy Sci.*, 53: 1714–1721.
- Broderick G.A., Murphy M.L., Udén P. (2004). Effect of inhibitor concentration and end-product accumulation on estimates of ruminal *in vitro* protein degradation. *J. Dairy Sci.*, 87: 1360–1371.
- Broderick G.A., Stevenson M.J., Patton R.A. (2009). Effect of dietary protein concentration and degradability on response to rumen-protected methionine in lactating dairy cows. *J. Dairy Sci.*, 92: 2719–2728.
- Cao Y.C., Yang X.J., Guo L., Zheng C., Wang D.D., Cai C.J., Liu S.M., Yao J.H. (2018). Effects of dietary leucine and phenylalanine on pancreas development, enzyme activity, and relative gene expression in milk-fed Holstein dairy calves. *J. Dairy Sci.*, 101: 4235–4244.
- Chalupa W. (1975). Rumen bypass and protection of proteins and amino acids. *J. Dairy Sci.*, 58: 1198–1218.
- Chalupa W., Chow A.W., Parish R.C. (1975). Methods and compositions for inhibiting rumen microbial deamination. United States Patent 1191. Smithkline Corporation, Philadelphia, Pa.
- Chen D., Yan J., Shen W., Song Y., Lan X., Yi K., Muhammad A.R. (2020). Effect of inclusion of HMBi in the ration of goats on feed intake, nutrient digestibility, rumen bacteria community and blood serum parameters. *J. Anim. Physiol. Anim. Nutr.*, 104: 987–997.
- Clements A.R., Ireland F.A., Freitas T., Tucker H., Shike D.W. (2017). Effects of supplementing methionine hydroxy analog on beef cow performance, milk production, reproduction, and preweaning calf performance. *J. Anim. Sci.*, 95: 5597–5605.
- Doelman J., Kim J.J.M., Carson M., Metcalf J.A., Cant J.P. (2015). Branched-chain amino acid and lysine deficiencies exert different effects on mammary translational regulation. *J. Dairy Sci.*, 98: 7846–7855.
- Dominguez J.H., Lopes M.G., Machado F.A., Santos E., Lopes F., Franck J.S., Del Pino F.A.B., Fischer G., Corrêa M.N., Schmitt E. (2017). Efeito da suplementação de metionina sobre o ganho de peso e diâmetro folicular em novilhas de corte submetidas a protocolo de inseminação artificial em tempo fixo. In: *Simposio Internacional de Reproduccion Animal*, 12. IRAAC, Cordoba.
- Edmunds B., Südekum K.H., Bennett R., Schröder A., Spiekers H., Schwarz F.J. (2013). The amino acid composition of rumen-undegradable protein: a comparison between forages. *J. Dairy Sci.*, 96: 4568–4577.
- Flores A., Mendoza G., Pinos-Rodriguez J.M., Plata F., Vega S., Bárcena R. (2009). Effects of rumen-protected methionine on milk production of dairy goats. *It. J. Anim. Sci.*, 8: 271–275.
- Giallongo F., Hristov A.N., Oh J., Frederick T., Weeks H., Werner J., Lapierre H., Patton R.A., Gehman A., Parys C. (2015). Effects of slow-release urea and rumen-protected methionine and histidine on performance of dairy cows. *J. Dairy Sci.*, 98: 3292–3308.

- Girma D.D., Ma L., Wang F., Jiang Q.R., Callaway T.R., Drackley J.K., Bu D.P. (2019). Effects of close-up dietary energy level and supplementing rumen-protected lysine on energy metabolites and milk production in transition cows. *J. Dairy Sci.*, 102: 7059–7072.
- Givens D., Rulquin H. (2004). Utilisation by ruminants of nitrogen compounds in silage-based diets. *Anim. Feed Sci. Technol.*, 114: 1–18.
- Hussein H.S., Jordan R.M. (1991). Fish meal as a protein supplement in ruminant diets: A review. *J. Anim. Sci.*, 69: 2147–2156.
- Jaenisch R., Bird A. (2003). Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nature Genet.*, 33: 245–254.
- Jurgens M.H. (2002). *Animal Feeding and Nutrition*. Kendall Hunt.
- Kumar A., Palfrey H.A., Pathak R., Kadowitz P.J., Gettys T.W., Murthy S.N. (2017). The metabolism and significance of homocysteine in nutrition and health. *Nutr. Metab.*, 14: 78.
- Lapierre H., Ouellet D.R., Doepel L., Holtrop G., Lobley G.E. (2008). Histidine, lysine and methionine: from metabolism to balanced dairy rations. *Proc. 44th Eastern Nutrition Conference of the Animal Nutrition Association of Canada (ANAC)*, University of Guelph, Guelph, ON, Canada, pp. 19–36.
- Lapierre H., Ouellet D.R., Lobley G.E. (2014). Estimation of histidine requirement in lactating dairy cows. *J. Dairy Sci.*, 97(E-Suppl. 1): 757.
- Lee C., Hristov A.N., Cassidy T.W., Heyler K.S., Lapierre H., Varga G.A., De Veth M.J., Patton R.A., Parys C. (2012). Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. *J. Dairy Sci.*, 95: 6042–6056.
- Li X., Rezaei R., Li P., Wu G. (2011). Composition of amino acids in feed ingredients for animal diets. *Amino Acids*, 40: 1159–1168.
- Liu K., Liu Y., Liu S.M., Xu M., Yu Z.P., Wang X., Cao Y.C., Yao J.H. (2015). Relationships between leucine and the pancreatic exocrine function for improving starch digestibility in ruminants. *J. Dairy Sci.*, 98: 2576–2582.
- Liu S., Lei J., Hancock S., Scanlan V., Broomfield S., Currie A., Thompson A. (2016). Lamb survival, glutathione redox state and immune function of neonates and lambs from periparturient Merino ewes supplemented with rumen-protected methionine. *Archiv. Anim. Nutr.*, 70: 389–401.
- Lobley G.E. (1992). Control of the metabolic fate of amino acids in ruminants: a review. *J. Anim. Sci.*, 70: 3264–3275.
- Lopes M.G., Echenique Dominguez J.H., Corrêa M.N., Schmitt E., Fischer G. (2019). Rumen-protected methionine in cattle: influences on reproduction, immune response, and productive performance. *Arquiv. Instituto Biológico*, 86.
- Madsen T.G., Nielsen L., Nielsen M.O. (2005). Mammary nutrient uptake in response to dietary supplementation of rumen protected lysine and methionine in late and early lactating dairy goats. *Small Rumin. Res.*, 56: 151–164.
- Mazinani M., Rude B. (2020). Population, world production and quality of sheep and goat products. *Am. J. Anim. Vet. Sci.*, 15: 291–299.
- Mazinani M., Naserian A.A., Rude B., Valizadeh R., Tahmasbi A. (2019). Production of rumen-protected essential amino acids with chemical technique. *Biosci. Biotechnol. Res. Asia*, 16: 789–795.
- Mazinani M., Naserian A.A., Rude B.J., Tahmasbi A.M., Valizadeh R. (2020). Effects of feeding rumen-protected amino acids on the performance of feedlot calves. *J. Adv. Vet. Anim. Res.*, 7: 229–233.
- Neinast M.D., Jang C., Hui S., Murashige D.S., Chu Q., Morscher R.J., Li X., Zhan L., White E., Anthony T.G. (2019). Quantitative analysis of the whole-body metabolic fate of branched-chain amino acids. *Cell Metab.*, 29: 417–429.
- Nichols K., Doelman J., Kim J.J.M., Carson M., Metcalf J.A., Cant J.P. (2017). Exogenous essential amino acids stimulate an adaptive unfolded protein response in the mammary glands of lactating cows. *J. Dairy Sci.*, 100: 5909–5921.
- NRC (2001). *Nutrient Requirements of Dairy Cattle*, 7: 381.
- Nursoy H., Gonzalez Ronquillo M., Faciola A.P., Broderick G.A. (2018). Lactation

- response to soybean meal and rumen-protected methionine supplementation of corn silage-based diets. *J. Dairy Sci.*, 101: 2084–2095.
- Oke B.O., Loerch S.C. (1989). The effects of dietary level and formaldehyde treatment of corn on site and extent of starch digestion in sheep. *J. Anim. Sci.*, 67(Suppl. 1): 538.
- Ordway R.S., Boucher S.E., Whitehouse N.L., Schwab C.G., Sloan B.K. (2009). Effects of providing two forms of supplemental methionine to periparturient Holstein dairy cows on feed intake and lactational performance. *J. Dairy Sci.*, 92: 5154–5166.
- Osorio J.S., Ji P., Drackley J.K., Luchini D., Looor J.J. (2013). Supplemental Smartamine M or MetaSmart during the transition period benefits postpartal cow performance and blood neutrophil function. *J. Dairy Sci.*, 96: 6248–6263.
- Patton R.A. (2010). Effect of rumen-protected methionine on feed intake, milk production, true milk protein concentration, true milk protein yield, and the factors that influence these effects: a meta-analysis. *J. Dairy Sci.*, 93: 2105–2118.
- Pereira A.B.D., Moura D.C., Whitehouse N.L., Brito A.F. (2020). Production and nitrogen metabolism in lactating dairy cows fed finely ground field pea plus soybean meal or canola meal with or without rumen-protected methionine supplementation. *J. Dairy Sci.*, 103: 3161–3176.
- Pitta D.W., Indugu N., Vecchiarelli B., Hennessy M., Baldin M., Harvatine K.J. (2020). Effect of 2-hydroxy-4-(methylthio) butanoate (HMTBa) supplementation on rumen bacterial populations in dairy cows when exposed to diets with risk for milk fat depression. *J. Dairy Sci.*, 103: 2718–2730.
- Puniya A.K., Singh R., Kamra D.N. (2015). *Rumen microbiology: from evolution to revolution*. Springer, 379 pp.
- Rémond D., Bernard L., Poncet C. (2000). Amino acid flux in ruminal and gastric veins of sheep: effects of ruminal and omasal injections of free amino acids and carnosine. *J. Anim. Sci.*, 78: 158–166.
- Ren H., Bai H., Su X., Pang J., Li X., Wu S., Cao Y., Cai C., Yao J. (2020). Decreased amyolytic microbes of the hindgut and increased blood glucose implied improved starch utilization in the small intestine by using rumen-protected leucine in dairy calves. *J. Dairy Sci.*, 103: 4218–4235.
- Reynolds C.K., Harmon D.L., Cecava M.J. (1994). Absorption and delivery of nutrients for milk protein synthesis by portal-drained viscera. *J. Dairy Sci.*, 77: 2787–2808.
- Robinson P.H., Veira D.M., Ivan M. (1998). Influence of supplemental protein quality on rumen fermentation, rumen microbial yield, forestomach digestion, and intestinal amino acid flow in late lactation Holstein cows. *Can. J. Anim. Sci.*, 78: 95–105.
- Robinson P.H., Swanepoel N., Shinzato I., Juchem S.O. (2011). Productive responses of lactating dairy cattle to supplementing high levels of ruminally protected lysine using a rumen protection technology. *Anim. Feed Sci. Technol.*, 168: 30–41.
- Santos F.A.P., Santos J.E.P., Theurer C.B., Huber J.T. (1998). Effects of rumen-undegradable protein on dairy cow performance: a 12-year literature review. *J. Dairy Sci.* 81: 3182–3213.
- Saxton R.A., Knockenhauer K.E., Wolfson R.L., Chantranupong L., Pacold M.E., Wang T., Schwartz T.U., Sabatini D.M. (2016). Structural basis for leucine sensing by the Sestrin2-MTORC1 pathway. *Science*, 351: 53–58.
- Schelling G.T., Richardson C.R., Tucker R.E., Mitchell G.E. (1973). Lamb responses to dietary methionine and oxytetracycline. *J. Anim. Sci.*, 37: 356.
- Schwab C.G. (1994). Optimizing amino acid nutrition for optimum yields of milk and milk protein. *Proc. Southwest Nutrition and Management Conference*, pp. 114–132.
- Schwab C.G. (1995). Protected proteins and amino acids for ruminants. *Biotechnol. Anim. Feeds Anim. Feed.*, 141.
- Schwab C.G., Broderick G.A. (2017). A 100-year review: protein and amino acid nutrition in dairy cows. *J. Dairy Sci.*, 100: 10094–10112.
- Schwab C.G., Satter L.D., Clay A.B. (1976). Response of lactating dairy cows to abomasal infusion of amino acids. *J. Dairy Sci.*, 59: 1254–1270.
- Sevi A., Taibi L., Albenzio M., Muscio A., Annicchiarico G. (2000). Effect of parity on milk yield, composition, somatic cell count, renneting parameters and bacteria counts of Comisana ewes. *Small Rumin. Res.*, 37: 99–107.

- Shelke S.K., Thakur S.S., Shete S.M. (2012). Protected nutrients technology and the impact of feeding protected nutrients to dairy animals: a review. *Int. J. Dairy Sci.*, 7: 51–62.
- Soda K. (2019). Spermine and gene methylation: a mechanism of lifespan extension induced by polyamine-rich diet. *Amino Acids*, 52: 213–224.
- Sturmey R.G., Reis A., Leese H.J., McEvoy T.G. (2009). Role of fatty acids in energy provision during oocyte maturation and early embryo development. *Reprod. Domest. Anim.*, 44: 50–58.
- Sulu N., Bjørnstad K., Grønseth D., Velle W. (1989). Ruminal degradation and outflow of amino acids in cows. *J. Vet. Med. Ser. A*, 36: 55–63.
- Sun H., Zhang X.-M., Wang X., Luo Y., Bi X.-H., Li H.-X., Zhang X.-S., Zhao S.-F. (2010). Effect of supplementing rumen protected methionine on milk performance of dairy cow and economic benefits analysis. *China Dairy Cattle*, 11.
- Swanepoel N., Robinson P.H., Erasmus L.J. (2010). Amino acid needs of lactating dairy cows: impact of feeding lysine in a ruminally protected form on productivity of lactating dairy cows. *Anim. Feed Sci. Technol.*, 157: 79–94.
- Swanepoel N., Robinson P.H., Erasmus L.J. (2015). Effects of ruminally protected methionine and/or phenylalanine on performance of high producing Holstein cows fed rations with very high levels of canola meal. *Anim. Feed Sci. Technol.*, 205: 10–22.
- Tedeschi L.O., Callaway T.R., Muir J.P., Anderson R.C. (2011). Potential environmental benefits of feed additives and other strategies for ruminant production. *Rev. Bras. Zootec.*, 40: 291–309.
- Toledo M.Z., Baez G.M., Garcia-Guerra A., Lobos N.E., Guenther J.N., Treviño E., Luchini D., Shaver R.D., Wiltbank M.C. (2017). Effect of feeding rumen-protected methionine on productive and reproductive performance of dairy cows. *PLoS One*, 12(12):e0189117.
- Velle W., Kanui T.I., Aulie A., Sjaastad Ø.V. (1998). Ruminal escape and apparent degradation of amino acids administered intraruminally in mixtures to cows. *J. Dairy Sci.*, 81: 3231–3238.
- Volden H., Velle W., Sjaastad Ø.V., Aulie A., Harstad O.M. (2001). Concentrations and flow of free amino acids in ruminal and duodenal liquid of dairy cows in relation to feed composition, time of feeding and level of feed intake. *Acta Agric. Scand. A - Anim. Sci.*, 51: 35–45.
- Vudmaska I., Petrukh I., Sachko S., Vlizio V., Kosenko Y., Kozak M., Petruk A. (2021). Using hop cones, vitamin E, methionine, choline and carnitine for treatment of subclinical ketosis in transition dairy cows. *Adv. Anim. Vet. Sci.*, 9: 55–62.
- van Vuuren A.M., Pineiro C., van der Hoek K.V., Oenema O. (2015). Economics of low nitrogen feeding strategies. Pp. 35–51 in *Costs of ammonia abatement and the climate co-benefits*. Springer.
- Vyas D., Erdman R.A. (2009). Meta-analysis of milk protein yield responses to lysine and methionine supplementation. *J. Dairy Sci.*, 92: 5011–5018.
- Wallace R.J., Chesson A. (2008). *Biotechnology in Animal Feeds and Animal Feeding*. John Wiley & Sons.
- Waterman R.C., Ujazdowski V.L., Petersen M.K. (2012). Effects of rumen-protected methionine on plasma amino acid concentrations during a period of weight loss for late gestating beef heifers. *Amino Acids*, 43: 2165–2177.
- Webb K.E. Jr, Dirienzo D.B., Matthews J.C. (1993). Recent developments in gastrointestinal absorption and tissue utilization of peptides: a review. *J. Dairy Sci.*, 76: 351–361.
- Williams L.R., Martz F.A., Hilderbrand E.S. (1970). Feeding encapsulated methionine supplement to lactating cows. *J. Dairy Sci.*, 53: 1709–1713.
- Wrenzycki C., Herrmann D., Lucas-Hahn A., Gebert C., Korsawe K., Lemme E., Carnwath J.W., Niemann H. (2005). Epigenetic reprogramming throughout preimplantation development and consequences for assisted reproductive technologies. *Birth Defects Research Part C: Embryo Today: Reviews*, 75: 1–9.
- Yoder P.S., Huang X., Teixeira I.A., Cant J.P., Hanigan M.D. (2020). Effects of jugular infused methionine, lysine, and histidine as a group or leucine and isoleucine as a group on production and metabolism in lactating dairy cows. *J. Dairy Sci.*, 103: 2387–2404.
- Yu Z.P., Xu M., Wang F., Liu K., Yao J.H., Wu Z., Qin D.K., Sun F.F. (2014). Effect of duodenal infusion of leucine and phenylalanine on intestinal enzyme activities and starch digestibility in goats. *Livest. Sci.*, 162: 134–140.

- Zang Y., Silva L.H.P., Ghelichkhan M., Miura M., Whitehouse N.L., Chizzotti M.L., Brito A.F. (2019). Incremental amounts of rumen-protected histidine increase plasma and muscle histidine concentrations and milk protein yield in dairy cows fed a metabolizable protein-deficient diet. *J. Dairy Sci.*, 102: 4138–4154.
- Zhao K., Liu W., Lin X.Y., Hu Z.Y., Yan Z.G., Wang Y., Shi K.R., Liu G.M., Wang Z.H. (2019). Effects of rumen-protected methionine and other essential amino acid supplementation on milk and milk component yields in lactating Holstein cows. *J. Dairy Sci.*, 102: 7936–7947.
- Zhou Z., Vailati-Riboni M., Trevisi E., Drackley J.K., Luchini D.N., Looor J.J. (2016). Better postpartal performance in dairy cows supplemented with rumen-protected methionine compared with choline during the peripartal period. *J. Dairy Sci.*, 99: 8716–8732.

Received: 17 XII 2020

Accepted: 5 III 2021