Journal of Animal and Feed Sciences, 33, 1, 2024, 3–12, https://doi.org/10.22358/jafs/166576/2023
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Analysis of crude protein utilisation in ruminant rations: supplementation of limiting amino acids and their effect on the environment – an updated review

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KEY WORDS: crude protein, environment, limiting amino acids, rumen microbiota, ruminants

Received: 18 April 2023
Revised: 26 May 2023
Accepted: 26 May 2023

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ABSTRACT. Protein quality plays a pivotal and dynamic role in the growth, productivity, and reproduction of ruminants. Increasing the proportion of dietary protein (CP) alone cannot balance the concentration of limiting amino acids (AA) in the duodenum of high-yielding dairy cows. However, supplying rumen-protected AA is believed to improve productivity and reproduction rates. Malabsorption of CP-rich meals in the rumen leads to high nitrogen (N) excretion through urine and faeces in the form of nitric oxide, nitrous oxide, ammonia and nitrate in the environment. Research data indicate that lysine and methionine are the two most limiting AA in the ruminant ration. Supplementing these limiting AA in ruminant diets is one of the most effective strategies to improve CP usage and reduce the negative impact of CP in the diet. Several *in vivo* and *in vitro* experimental studies have demonstrated that even low-quality dietary CP, when supplemented with rumen-protected AA (Met + Lys), exhibited a greater ability to reduce N_2 or NH_3 losses, while also supporting a decrease in enteric fermentation gas production and minimising soil or water pollution associated with animal production. However, further research is necessary to explore the molecular and genetic mechanisms underlying the effects of AA dietary supplementation on the rumen microbiota in ruminants.

Introduction

Global livestock production is confronted with various challenges, including shifting consumer preferences, environmental concerns and economic stability. The financial aspects of livestock production are greatly affected by animal nutrition, particularly feed prices and availability. It is essential to tailor nutrition to the specific needs of each animal species, taking into account factors such as the type of cattle, lactation stage, animal health and production efficiency in order to avoid nutritional imbalances or diet-related issues. Key factors affecting ruminant nutrition include protein quality, low quantity, fibre content and its particle size, variety of feed, rumen microbiome population, feed digestion rate, weather conditions and nutrient sources (NRC, 2001). Protein is an essential nutrient for ruminants and plays a critical role in their physiological functions. Ruminants primarily acquire amino acids (AA) from two main sources: microbial protein (MP) and rumen undegradable protein (RUP), which are directly delivered to the abomasum. Both sources contribute significantly to meeting the maintenance requirements and metabolizable protein demands of ruminants (Abbasi et al., 2019).

At present, protein quality is an important factor that has gained significant importance in ruminant nutrition. It should be emphasized that increasing crude protein (CP) levels in dairy cow ration does not directly enhance milk production (Wu and Satter, 2000; Gilbreath et al., 2021). Instead, it leads to elevated concentrations of NH_3 in the rumen, urea nitrogen in the blood and increased urinary nitrogen excretion rates (Castillo et al., 2001). Currently, research efforts are focused on improving CP quality and developing new nutritional requirements for ruminants, particularly through low-protein diets enriched with rumen-protected, encapsulated protein (limiting amino acids). Decreasing the CP concentration in ruminant diets has several positive effects, including reduced nitrogen (N_2) excretion, greenhouse gas (GHG) emissions, and ammonia pollution. It also improves the economics of animal feed, as well as reduces water and soil pollution (Wu and Satter, 2000). In dairy cows, the efficacy of dietary nitrogen conversion into milk protein is relatively low (approx. 26%), and the residual ingested nitrogen is excreted via the orofaecal route (Spears et al., 2003; Nadeau et al., 2007; Zhao et al., 2019). The losses of ruminal N_2 play a critical role in metabolic efficiency (Ayyat et al., 2021; Król et al., 2023; Zou et al., 2023). Indigestible N_2 derived from microorganisms can reduce the content of microbial protein, leading to a decrease in AA synthesis and its presence in milk protein. According to Roque et al. (2019), dairy calves excrete 72% of their nitrogen through the urinary tract. Various types and sources of protein, such as rumen degradable protein (RDP), RUP or MP, have been studied to minimise nitrogen losses in ruminants. However, it has been challenging to determine the specific benefits of RDP, RUP, and MP in reducing urinary N_2 losses in animals (Tufarelli et al., 2009). RDP is utilised for microbial protein generation (MPG) and provides energy in certain situations causing its limitation in ruminants. RDP is not used for MPG, it is converted to NH_3 and absorbed through the ruminal wall into the liver, where it is excreted in the urine following detoxification to urea (Hou et al., 2020). RDP is a vital component that not only improves the flow of duodenal AA, but also of peptides in ruminants (Choi et al., 2002). In addition, RDP has been shown to affect the production of ruminants, cost-effectiveness, and environmental toxic waste (Ali et al., 2009; Lobos et al., 2021). Consumption of RUP is an important source of AA, which can be assimilated through the small intestine and serve as the primary substrate for milk production and rumen development. Ruminal microbes are responsible for breaking down dietary protein into peptides, AA, and NH_3, which are then used by the rumen microbiome for MP production. However, the rate of MP synthesis is dependent on the velocity of feed transit in the rumen. While MP production contributes to the availability of amino acids, it cannot provide sufficient amounts of AA to meet the absolute AA balance requirements of high-producing dairy cows (Ali et al., 2009; Lobos et al., 2021). The primary objective of RDP administration is to supply adequate amounts of MCP to enhance the absorption of AA in the small intestine (SI) of animals. However, rumen undegradable AA must be enriched in the rations of high-producing ruminants to increase AA flux in the SI to maintain animal performance (Leonardi et al., 2003). Maintaining an appropriate amino acid (AA) balance is crucial for sustaining milk production, milk yield protein, and nitrogen utilisation in ruminants, surpassing the significance of RDP in ration formulation (Noftsger and St-Pierre, 2003; Laudadio and Tufarelli, 2010). Feeding practices play a significant role in the economics of animal production, accounting for 70–75% of overall production costs (Fathi et al., 2021; Agwaan, 2023). Studies have shown that supplementing sheep diets with 18% CP resulted in similar dry matter intake (DMI), feed conversion ratio (FCR), and body performance compared to diets supplemented with 16% CP. However the diet with 18% CP was economically more favourable (Archibeque et al., 2002; Abbasi et al., 2014). Similar findings were reported by Kaya et al. (2009). Therefore, dietary addition of the most limiting AA, even in low CP diets, can reduce excessive N_2 excretion into the environment, and decrease the contamination (Archibeque et al., 2002; Abbasi et al., 2014). Supplementing rumen-protected AA in the diet of high-yielding dairy cows, even those with low CP%, has the potential to bring about significant benefits, including reduced feeding costs, energy losses, and nitrogen excretion, as well as decreased environmental pollution (Abbasi et al., 2019).

This study aimed to assess the impact of incorporating rumen-protected AA in the diet of high-yielding dairy cows, specifically focusing on nitrogen utilisation efficiency, environmental sustainability, and the composition of the rumen microbiota. The findings of the study will contribute to the optimization of dietary strategies that enhance productivity, while simultaneously minimising N excretion and environmental pollution in ruminant farming practices.

Material and methods

A comprehensive literature search was conducted using various databases, including Pubmed (www.ncbi.nlm.nih.gov, last accessed April 2023); ISI Web of Science (www.webofscience.com, last accessed April 2023), Google Scholar (scholar.google.com, last accessed April 2023) and ScienceDirect (www.sciencedirect.com, last accessed April 2023), with a focus on original and primary research articles published in the last three decades. The search aimed to identify studies that investigated the supplementation of limiting amino acids in ruminant diets and examined their effects on the environment. Over 50% of the selected studies were published within the last 10 years, ensuring that the review reflects recent developments in the field. A total of 100 studies from 70 different sources were included in this review. The article is organized into four main sections, each addressing a specific aspect. The first section discusses the production of gases from livestock farms and their effect on the environment. The second section focuses on the supplementation of low dietary crude protein and its impact on nitrogen loss. The third section highlights the role of methionine as a limiting amino acid. Finally, the fourth section explores the use of rumen-protected amino acids in the diets of cows.

Livestock farm gas production and its impact on the environment

The livestock industry plays a crucial role in the production of greenhouse gases (GHGs) (including CH_4, N_2O), contributing to approximately 18% of total GHG emissions, and has a significant impact on air quality and ruminant health (Steinfeld et al., 2006). In China, CH_4 discharge accounted for 11% of total GHG emissions, with 21% of livestock CH_4 production derived from ruminant enteric fermentation (Zhang and Chen, 2010). The livestock industry emits various types of gasses into the environment that add up to global warming. Manure from livestock contributes to approximately 37% of CH_4 emissions, 64% of ammonia (NH_3) emissions, and 65% of N_2O emissions (Kupper et al., 2020; Møller et al., 2022). This gas emissions can contaminate the environment and become a cause of acid rains (Epa, 2001). The production of CH_4 is influenced by factors such as feed composition, microbial diversity in the rumen, as well as the rumen fermentation rate (Steinfeld et al., 2006; Erisman et al., 2008). In addition to the environmental contamination caused by gas emissions, cows also participate in the release of nitrogen in manure and CH_4 production, which is a more potent greenhouse gas than CO_2 (Gerber et al., 2013). CH_4 production not only contributes to environmental contamination but also results in energy losses of approximately 2–12% of the gross energy intake (Vijn et al., 2020). Livestock globally accounts for approximately 14.5% of anthropogenic GHG emissions, with enteric CH_4 gas contributing to 25% of these emissions (Steinfeld et al., 2006). Insufficient levels of RDP in ruminant diets can lead to decreased animal performance and increased CH_4 emissions as a result of excess carbohydrate content in the ration (Vijn et al., 2020). Rumen microbial populations, including methanogens and protozoa, play a key role in the production of CH_4 (Clark et al., 2010). Modulating the rumen microbial population can potentially alter the synthesis of CH_4 in ruminants (Vijn et al., 2020). In cows, the production of enteric CH_4 is influenced by factors such as rumen microbial population, presence of methanogens in the rumen, and dietary feed intake. CH_4 is produced in the rumen during the fermentation carried out by anaerobic microorganisms. Methanogenic archaea, present in the rumen, produce CH_4 through anaerobic fermentation. These methanogens utilize metabolic H_2 and CO_2 as substrates for their metabolic activity (Bhatta et al., 2015; Makkar, 2016). Figure 1 and 2 show the process of CH_4 generation.

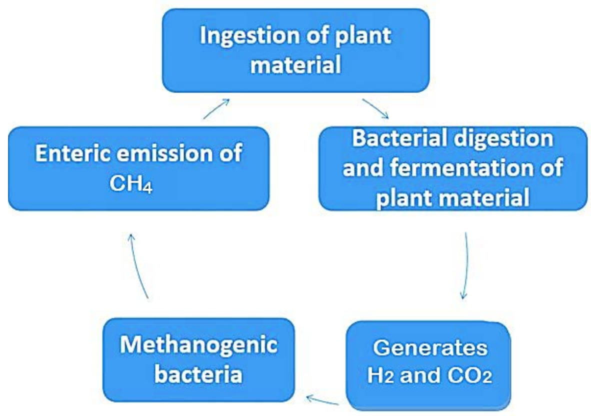

Figure 1. Illustration of feed fermentation in the ruminant body and H_2, and CO_2 gas emissions into the environment (own elaboration)

Figure 1 depicts the process of hydrogen production through enzymatic and bacterial digestion of plant material in the rumen. The produced H_2 is utilised by methanogens to generate enteric CH_4 and N_2, which are excreted through urine and manure (Janssen, 2010). This enteric CH_4 generated by rumen methanogens contributes significantly to environmental pollution as a GHG (Hristov et al., 2013).

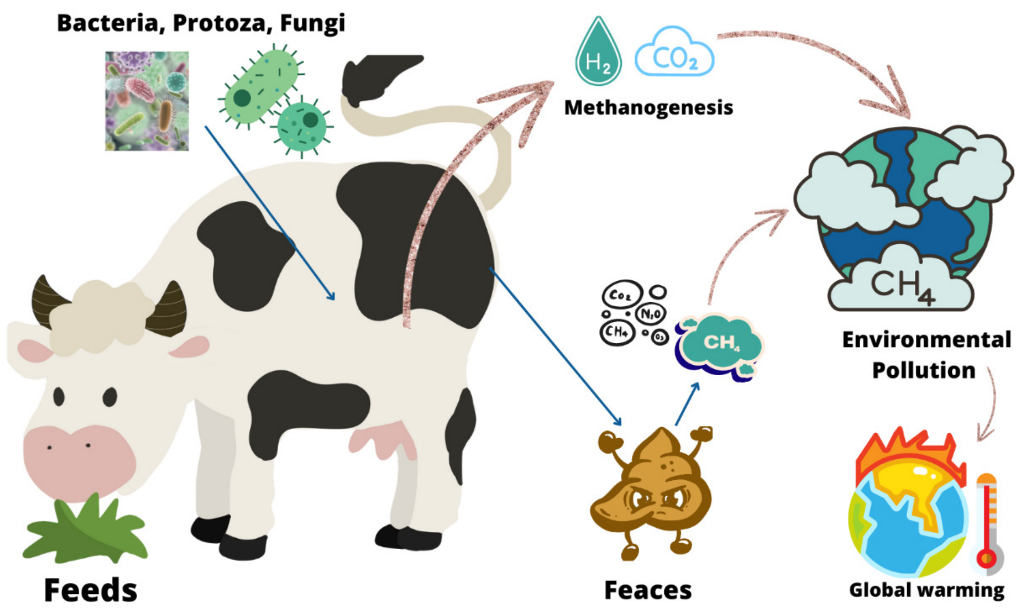

Figure 2. Illustration of feed fermentation in the ruminant body and N_2O, H_2, and CO_2 gas emissions into the environment (own elaboration)

Efficient nitrogen utilisation by dairy cows

Nitrogen emitted by ruminants is released into the atmosphere and undergoes transformations into varieties, such as nitrate, nitrous oxide, nitric oxide, di-nitrogen and ammonia. These nitrogen compounds can have detrimental effects on human health as well as on soil, water, and air quality (Chojnacka et al., 2021). Farmers face several environmental challenges due to excessive nitrogen excretion. However, when animal ration is supplemented with a lower CP content, the amount of nitrogen released is also lower (by about 12–21%) (Kirwan et al., 2021).

Nitrogen excretion was significantly lower when the ration of dairy cows had a low CP content during their production. A reduction of 1% in CP content in the ration resulted in an 8–10% decrease in nitrogen release. Furthermore, when the CP level was reduced by 3–4% with the addition of limiting amino acids, the decrease in nitrogen excretion reached up to 30% (Cole et al., 2005; Vasconcelos et al., 2007). Feeding ruminants with diets low in CP% allows for a reduced excretion of nitrogen in manure, which in turn affects the mineralisation rate of nitrogen, leading to a lower release of plant-available nitrogen. The efficiency of dietary nitrogen utilisation by the animal during the production period was approx. 30%, while the remaining N_2 was released from the body to the atmosphere, with approx. 30% excreted in faeces and 40% in urine (Powell and Broderick, 2011).

Excretion of nitrogen by ruminants acts as soil and water contamination

Feeding diets supplemented with maize silage and alfalfa increases urinary nitrogen excretion and NH_3 generation from manure. This is due to their higher nitrogen and protein content, as well as an imbalanced carbon-to-nitrogen ratio, which promotes rapid decomposition and the development of anaerobic conditions that stimulate microbial activity and NH_3 production. However, a diet with a lower CP content can be an effective approach to reduce NH_3 and N_2O emissions from manure (Weiss et al., 2009; Dijkstra et al., 2011). Excessive nitrogen loss can lead to water pollution and have a significant impact on marine ecosystems (Howarth and Marino, 2006). The release of excess N_2 into the atmosphere contributes to nutrient enrichment, resulting in increased algae growth, acidification, organic matter decomposition, and reduced oxygen concentration in the environment (Wright et al., 2001; Steinfeld et al., 2006). Elevated levels of released N_2 pose risks to aquatic systems as animal manure can be converted into nitrate by soil bacteria, leading to groundwater contamination. Nitrate derived from groundwater can penetrate to drinking water and cause health problems, including methanoglobinaemia (Majumdar and Gupta, 2000; Nadeau et al., 2007). Excessive nitrogen excretion not only pollutes the environment but also has consequences for animal farm productivity (Bouwman et al., 2013). To reduce the amount of nitrogen in groundwater, it is necessary to address the excessive nitrogen load in the soil. Water excreted from feed production and processing units is a major source of nitrogen in water systems (De-Bashan and Bashan, 2004; Stošić et al., 2021). In some cases, the use of low-cost rations for ruminants can help maintain nutritional balance, reduce water and air pollution, and alleviate deforestation (Makkar, 2016).

Excessive nitrogen loss and ammonia emissions from the livestock industry

All ruminants excrete excess nitrogen, which contributes to the increased levels of NH_3 in the surrounding atmosphere. The amount of nitrogen excreted in N_2 in faeces and urine is influenced by factors such as dietary nitrogen supplementation, digestibility rate, and animal species (Mohd Azmi et al., 2021). The ingestion of excess dietary N_2 and its subsequent decomposition in the rumen is directly related to the release of NH_3 from cow manure. NH_3 is highly reactive and undergoes various atmospheric reactions, resulting in the formation of compounds such as fine particles, ammonium sulphate, ammonium nitrate, ammonium bisulphate, sulphuric acids, nitric acid, and ammonium hydroxide in the form of a white fog (Higueras et al., 2004). These compounds contribute to severe air contamination on a global scale and are associated with approximately 2 mln premature mortalities annually (Ahmadi et al., 2015). Protein and non-protein dietary supplements are degraded into AA, peptides, NH_3, and excess urine nitrogen, 60–80% of which is lost to the environment (Kröber et al., 2000; Reynal and Broderick, 2005; Hristov et al., 2013). Reducing the amount of protein supplementation in animal feed may also minimise NH_3 evaporation from animal waste. The evaporation of NH_3 appears to be the primary cause of surface water contamination, soil contamination, and acidification. Approximately 50% of ammonia emissions originate from animal agriculture, with animal waste being a significant contributor. This release of ammonia from animal waste contributes to anthropogenic GHG emissions (Nocek et al., 2006). Air pollution with N_2O, NH_3, and sulphur oxide increases the likelihood of acid rain, which has significant implications for biodiversity and human well-being (McGinn et al., 2003; Erisman et al., 2008). Moreover, NH_3 contamination in the environment has detrimental effects on the health and performance of both animals and humans. The unpleasant odour associated with NH_3 emissions also disrupts the daily lives of people living near ruminant farms or working on them (Arogo et al., 2003; Wathes et al., 2004). Additionally, the presence of excess NH_3 affects the performance of monogastric species. Several factors contribute to NH_3 production, including soil conditions, fertilisation, feed nitrogen levels, management of ruminant manure, and environmental factors (Sommer et al., 2004; Ho et al., 2021). NH_3 emissions from animal manure is also affected by the level of dietary CP in animal rations (Hristov et al., 2019). It is estimated that approximately 79% of urinary nitrogen is lost to the environment, and vaporisation losses ranging from 64 to 124% of nitrogen have been documented. While urea itself is not a volatile substance, when it comes into contact with animal manure, it undergoes rapid hydrolysis into NH_3 and CO_2 due to the presence of urease in the manure. Urine nitrogen has been identified as the primary source of ammonia nitrogen evaporation (Schumacher, 2020; Smith et al., 2021).

Low dietary CP supplementation and effect on nitrogen loss

Protein is probably the most important nutrient in the diet of ruminants. In the rumen, protein undergoes degradation with the help of microorganisms, resulting in the formation of peptide-bound amino acids, free amino acids, and ammonia. On the other hand, peptide-bound amino acids and free amino acids serve as the building blocks for protein synthesis, while ammonia can be utilised by ruminal microorganisms for microbial protein (MP) production. However, certain dietary proteins may resist degradation in the rumen and pass through to the intestine, where they contribute to the absorption of amino acids and support milk production in high-yielding dairy cattle (Choi et al., 2002). Providing higher amounts of nitrogen to dairy animals not only leads to increased MP production, but also results in elevated levels of NH_3 and higher nitrogen loss via urinary excretion (Khan et al., 2020). Increasing the CP content in dairy cow feeds has been found to enhance milk production, but also increase ruminal NH_3 generation and blood urea nitrogen levels, leading to higher nitrogen losses through urine (Wu and Satter, 2000; Castillo et al., 2001; Broderick et al., 2008). Bahrami-Yekdangi et al. (2016) reported that lowering CP concentrations in a ruminant diet from 18.4 to 15.1% significantly reduced N_2 urine loss. Microbes play an important role in the rumen, as they ferment fodder and produce volatile fatty acids, such as H_2, CO_2, and CH_4. They also play a dynamic role in the breakdown of dietary N_2 for the synthesis of MP, which serves as a crucial source of AA for ruminants (Abbasi et al., 2019). MP participates in the distribution of AA in the SI, accounting for 40–90% of total absorbable protein (Koenig et al., 2000). Approximately 25–35% of the dietary protein is utilised and subsequently passed on through milk, while the remaining nitrogen is excreted from the body in faeces and urine (Katongole and Yan, 2020). In addition, a significant relationship has been discovered between manure nitrogen production and dietary protein intake. Reducing the percentage of CP in ruminant rations can help minimise N_2 excretion into the environment (Yan et al., 2010). However, supplementing the feed with a moderate concentration of CP has been proposed as a viable option for reducing N excretion without compromising the ruminant's energy balance (Agle et al., 2010). A basic ration, even with low CP%, when supplemented with limiting AA, can reduce feeding costs, energy losses, N_2 excretion, and environmental pollution compared to a ration with high CP%, which is responsible for increased heat increment, high energy losses and environmental pollution (Abbasi et al., 2019).

The role of methionine as a limiting amino acid

Methionine (Met) plays a crucial role as a limiting AA in the diet of ruminants. It is involved in various metabolic processes, including its incorporation into polypeptide chains and its contribution to the production of α-ketobutyrate and cysteine. Additionally, it is an essential component in the synthesis pathway of S-adenosylmethionine (SAM). In ruminants, Met is recognised as a critical limiting AA (Park et al., 2020), and humans and animals are unable to naturally synthesise Met, making it an essential dietary requirement. The demand for this AA can be met by ingestion of protein-rich foods containing Met or the use of Met supplements. In microbial and plant metabolism, the aspartate family is involved in the biosynthesis of Met (Ferla and Patrick, 2014). The majority of proteins, including those commonly found in livestock feed, lack essential amino acids, such as Met, necessary for sustaining optimal milk production and supporting tissue development in high or medium-producing dairy cattle. While protein produced by bacteria are an important source of AA, their quantity is insufficient to meet the demands of tissue growth and milk yield in dairy cows (Robinson et al., 2004; Mavrommatis et al., 2021). Supplementing limited AA has emerged as a valuable strategy to meet the dietary requirements of high or medium-yielding dairy cows. However, direct supplementation of Met in dairy cow rations faces challenges due to microbial activity in the rumen. Microorganisms tend to break down Met and utilise it for their own purposes, thereby limiting its availability. To overcome this problem, animal nutritionists can provide rumen-undegraded or rumen-encapsulated versions of this AA to improve its passage directly into the SI for optimal bioavailability and availability. Furthermore, optimising the ratio between Lys and Met in the diet is crucial for maximising microbial protein synthesis. The recommended ratio of Lys to Met is 3:1, and achieving this balance allows to reduce the crude protein (CP%) content in the diet from 17–19% to 15–16%. This reduction not only improves dairy cow reproduction, but also reduces nitrogen excretion in urine and faeces, contributing to environmental sustainability (Park et al., 2020).

Effect of supplementation of low CP and rumen-protected AA in the diet of cows

Lowering CP concentrations through rumen-protected amino acid (AA) supplementation is a favourable strategy for maintaining milk production and composition in dairy cows, while reducing environmental pollution associated with the ruminant industry (Leonardi et al., 2003). Cows frequently struggle to maintain their protein and energy balance in the body during lactation. As a result, a significant proportion of AA intake by cows is utilised for protein synthesis, with a substantial portion being metabolised in the liver to provide energy for the animals (Hill et al., 2013). In pregnant cows, more than 50% of AA intake is utilised by the developing foetus to support proper growth and maintain glucose concentration, which is essential for overall metabolic balance (McCabe and Boerman, 2020). Cattle can acquire these AA in two ways: from protein produced by microbes in the rumen, and through protein derived from sources outside of the rumen. The absorption of free AA in the small intestine is crucial for animal production. Under critical conditions, when the blood concentration decreases in the mammary glands, animals have the ability to modulate its flow and extract ratios to obtain more AA to sustain milk production and its properties (Vanhatalo et al., 2003). Administration a low amount of CP along with rumen-protected Met to ruminants have shown promising results, particularly during the early lactation period (Kröber et al., 2000). Lys or Met supplementation alone or in combination has provided remarkable effects during initial phase of lactation in dairy cows (Patton, 2010).

These days, nutritionists are more enthusiastic about introducing limiting AA into ruminant diets in the form of rumen-encapsulated Lys and Met (Lapierre et al., 2006). This attractive technique is aimed to elevate the proportion of rumen-protected Met and Lys during the early lactation phase to increase milk production, as well as milk protein and fat content (Davidson et al., 2008; Fleming et al., 2019; Lee et al., 2019; Gilbreath et al., 2021; Kim and Lee, 2021). Numerous studies have reported that supplementing ruminant diets with rumen-protected AA leads to an increase in milk production. The availability and composition of Lys and Met in the SI are crucial factors for milk production. By supplementing these limiting AA in ruminant diets, the amount of essential AA reaching the SI is improved, resulting in increased milk production (Schwab et al., 2003; Park et al., 2020; Kim and Lee, 2021).

Nutritionists worldwide are actively investigating the relationship between supplemented protein and limiting amino acids (AA) in order to enhance ruminant performance. A comparison between a ration containing 16.2% CP and rumen Met supplements resulted in the same milk production as a ration with 17.4% CP without any AA treatment (Broderick et al., 2008). In a study conducted by Kim and Lee (2021), supplementation of rumen-encapsulated Met to the ration during mid-lactation resulted in an increase in milk yield. Similarly, during the pre and post-partum phases, supplementation of the basic ration with rumen-encapsulated Lys and Met was found to increase dairy cow milk output (Socha et al., 2005). Incorporating supplementation of limiting AA into a basic diet with a reduced CP content has been shown to potentially increase nitrogen utilisation. Research conducted by Haque et al. (2012) demonstrated that a basic diet containing 15% CP, along with the addition of limiting AA, could lead to a notable improvement in nitrogen utilisation, with an increase of 30% or more reported. Moreover, the formulation of basic ration for dairy cows with 14% CP and supplementation of rumen-protected Met (26 g per cow per day) significantly increased milk production, solid fat content (SFC), casein yield, and total solids compared to a control diet containing 16% CP (Titi et al., 2013). This indicates that supplying a sufficient quantity of limiting AA directly into the SI of lactating cows can lead to increased milk production, milk fat content, and casein protein concentration (Xie et al., 2013; Abbasi et al., 2019).

Conclusions

High concentrations of crude protein in ruminant diets have been associated with increased nitrogen excretion, leading to environmental pollution and increased feeding costs. To address this issue and improve the performance and reproductive status of ruminants, while reducing nitrogen excretion (N_2 and NH_3), it is recommended to supplement ruminant diets with limiting amino acids (AA). The present meta-analysis have demonstrated that reducing the excess protein content in ruminant diets through the addition of limiting AA sustained the balance of microbial protein and enhanced milk production. Furthermore, there are opportunities to explore the use of other rumen-protected AA and manipulate rumen microbes, bedding, and diverse feed additives to mitigate nitrification and the release of greenhouse gases.

Conflict of interest

The Authors declare that there is no conflict of interest.

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