frontiers | Frontiers in Animal Science

TYPE Review
PUBLISHED 17 December 2025
DOI 10.3389/fanim.2025.1713884

OPEN ACCESS

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RECEIVED 23 October 2025
REVISED 25 November 2025
ACCEPTED 28 November 2025
PUBLISHED 17 December 2025

CITATION
Costa TC, Nascimento KB, Danés MAC, Gionbelli MP and Duarte MS (2025) Rumen-protected methionine for dairy and beef cattle: current perspectives on methionine role, supplementation strategies, metabolism, health, and performance.
Front. Anim. Sci. 6:1713884.
doi: 10.3389/fanim.2025.1713884

Rumen-protected methionine for dairy and beef cattle: current perspectives on methionine role, supplementation strategies, metabolism, health, and performance

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Protein utilization by ruminants has unique characteristics due to the fractioning into ruminal degraded and undegraded portions. Because of these peculiarities, the amino acid (AA) profile that reaches the intestines for absorption differs from the known dietary protein sources. Among the essential AAs, methionine (Met) is the most limiting nutrient, especially in dairy cattle diets. Moreover, Met contributes to several biological processes, such as protein synthesis, epigenetic regulation, nuclear function, cellular redox potential, phospholipid homeostasis, among others. Hence, given these factors, there are considerable efforts to investigate the efficacy of this AA by employing technologies aimed at shielding it from rumen degradation in both dairy and beef cattle. In light of this, it is valuable to consolidate the findings available in the scientific literature regarding the performance, reproduction, fetal development, and health-related outcomes of rumen-protected methionine (RPM). This approach aims to offer a thorough and unbiased perspective concerning the potential outcomes achievable through the application of this technology. For instance, such insights can serve as a robust foundation for decision-making regarding the implementation of this technology in practical operations. Therefore, this literature review aims to: (1) explore Met metabolism and its importance as a precursor of methyl donors; (2) provide information on the existing methods for protecting Met in the rumen; and (3) compile research findings concerning the potential impacts of Met supplementation on milk production and composition, body weight gain, reproductive outcomes, immune function, and fetal development in both dairy and beef cattle.

KEYWORDS

bovine, bypass amino acid, epigenetic, fetal programming, immune system, N retention, performance, reproduction

1 Introduction

Methionine is one of the essential AA for humans and animals (François, 2023) and holds a distinctive position in driving various metabolic pathways. This sulfur-containing AA is intricately involved in protein synthesis, exerting potential effects on the Mammalian Target of Rapamycin (mTOR) pathway, a central molecular hub governing anabolic processes. Its metabolism encompasses transmethylation, remethylation, and transsulfuration (Brosnan et al., 2007). Thus, within the one-carbon cycle, Met contributes to the formation of S-adenosylmethionine, the primary methyl donor in reaction involving DNA, RNA, proteins, and phospholipids, which directly influence epigenetic regulation, and, thereby, the resulting phenotype (Clare et al., 2019). Methionine also supports cellular redox potential maintenance by generating antioxidants like taurine and glutathione (Alharthi et al., 2018), participates in phosphatidylcholine synthesis (Vance and Tasseva, 2013), and facilitates *de novo* synthesis of nucleotides (Girard and Matte, 2005). Therefore, given its involvement in these multifaceted processes, the utilization of Met plays a critical role in various aspects of dairy and beef cattle production, including milk yield and composition, performance, reproduction, developmental programming, and immunity.

Nevertheless, despite its benefits, most plant-based feeds (forage and cereals) are limited in Met (Brake et al., 2013). Additionally, in cattle, the rumen microbiota quickly breaks down ruminal protein components into peptides and AA, which are then utilized for microbial protein synthesis (Lopes et al., 2019). During these processes, partial protein degradation produces ammonia and carbon chain, through deamination and transamination. The released ammonia, a nitrogen source, is reutilized for the synthesis of microbial protein, having a distinct amino acid profile compared to the original diet. Consequently, an altered AA profile reaches the small intestine for absorption compared to what is initially provided in the diet (Wei et al., 2022). Therefore, it is essential to employ ruminal protection strategies to explore the impacts of this functional AA in livestock.

Based on this knowledge, Met has emerged as a significant commodity in the global economy (Neubauer and Landecker, 2021). To date, a majority of synthetic Met is produced by chemical processes from fossil resources (François, 2023) and commercialized either as chemical forms of DL-methionine or α-hydroxy analogs. Despite numerous studies exploring the effects of Met supplementation in dairy and beef cattle, findings often vary, likely attributed to differences in experimental methodologies, RPM sources, and the developmental stages of the animals (Wei et al., 2022). Additionally, variations in dietary composition, particularly in terms of crude protein (CP) content and the balance of other essential AA like lysine (Lys), can yield differing productivity responses. Consequently, it is essential to have a clear understanding of the anticipated outcomes when considering the adoption of such technologies on the farm, as they can significantly influence the ultimate cost of the diet.

Therefore, the current review aimed to (1) explore Met metabolism and its importance as a precursor of methyl donor; (2) present the main techniques to protect AA from ruminal degradation and commercial products available; (3) discuss the main results available in the literature regarding RPM supplementation on cattle performance, reproduction, fetal development, and immune system.

2 Methionine metabolism

Methionine and its metabolites, not only participate in protein synthesis but also have functional roles. Methionine contributes to synthesizing other essential amino acids, such as Cysteine (Cys), and to the supply of methyl (CH_3) donors in the one-carbon cycle (Bertolo and McBreairty, 2013). Moreover, Met is involved in epigenetic regulation by influencing DNA and histone methylation processes. Additionally, it contributes indirectly to nuclear function through polyamine synthesis. Methionine is also related to the maintenance of cellular redox potential by producing glutathione and taurine (Lauinger and Kaiser, 2021), as well as supporting to phospholipid homeostasis (Li and Vance, 2008). Additionally, Met has been shown to induce the mTOR complex 1 activation (Kitada et al., 2020), which regulates the synthesis of all proteins in the body, including enzymes, thereby reinforcing its importance for both milk yield and milk protein synthesis (Dong et al., 2018). Lastly, Met also has a distinctive function as the initiating amino acid in the synthesis of proteins (Praynat et al., 2009). Hence, considering its involvement in several biological processes that contribute to the observed effects on the performance, reproduction, fetal development, and health of both beef and dairy cattle, a more detailed examination of these aspects will be provided below.

2.1 One-carbon cycle and associated pathways

In the one-carbon cycle, Met metabolism is categorized into transmethylation, transsulfuration, and remethylation reactions. Transmethylation consists of the adenylation of Met to form S-adenosylmethionine (SAM). During this step, adenosine from ATP is transferred to the sulfur in Met through the action of methionine adenosyltransferase (Lauinger and Kaiser, 2021). S-adenosylmethionine acts as a universal methyl donor for several reactions, being the major biological methylating agent (Brosnan et al., 2007).

Before producing methylation potential, Met may also play an indirect role in polyamine (putrescine, spermidine, and spermine) synthesis (Figure 1). Polyamines are involved in cell division and proliferation, regulation of gene expression for cell survival, facilitation of DNA and protein synthesis, apoptosis and oxidative stress control, angiogenesis, and cell-to-cell communication (Lenis et al., 2017). Within polyamine metabolism, putrescine is produced

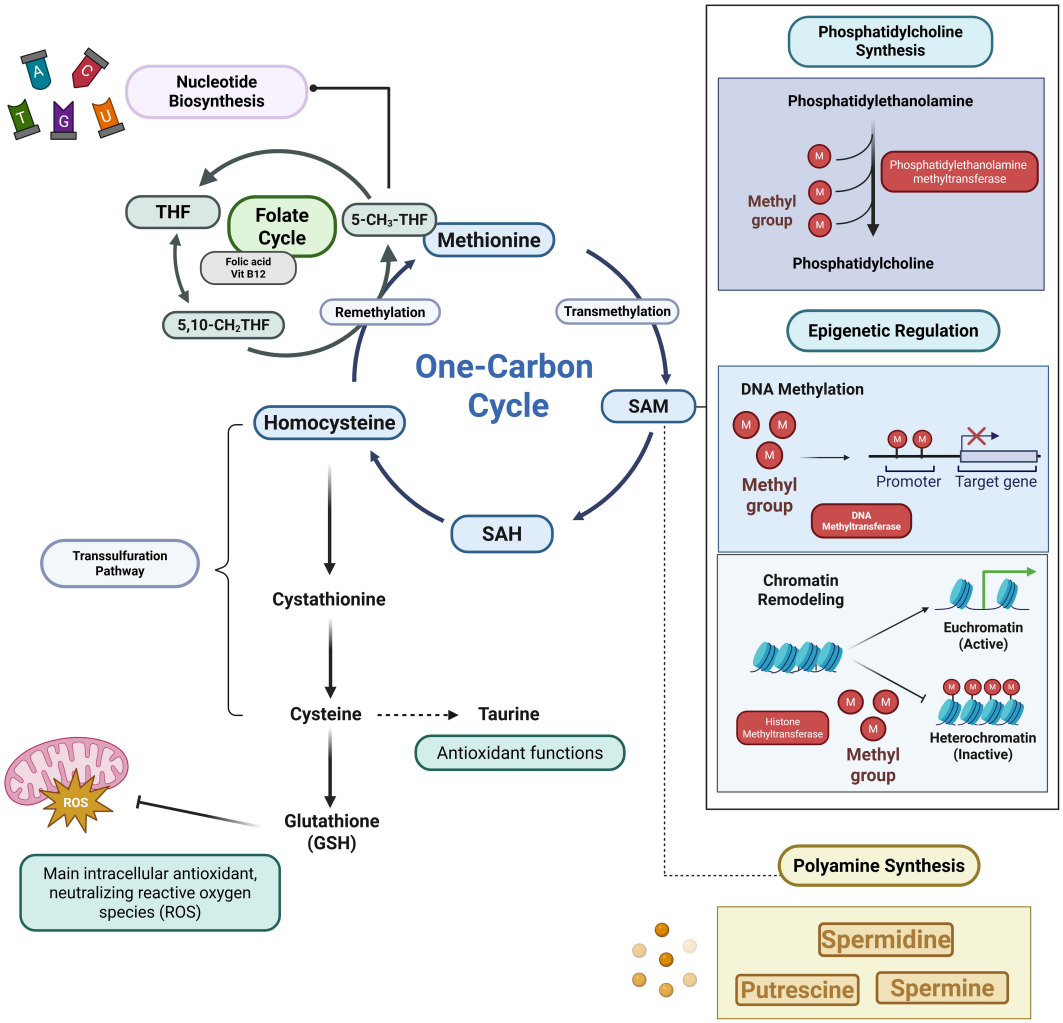

FIGURE 1
Schematic representation of methionine cycle. Adapted from: Cole et al. (2012); Clare et al. (2019); Girard and Matte (2005); Lauinger and Kaiser (2021); Preynat et al. (2009); Wallace et al. (2003). Abbreviations: SAH = S-adenosylhomocysteine; SAM = S-Adenosylmethionine; THF: Tetrahydrofolate; 5-CH3-THF: 5-methyltetrahydrofolate; 5,10-CH2THF: 5,10-methylene-tetrahydrofolate. Created in BioRender. Nascimento, K. (2025) https://BioRender.com/v97oxof)

through ornithine decarboxylation, a process originating from arginine (Wallace et al., 2003). Simultaneously, decarboxylated S-adenosylmethionine (dcSAM), derived from Met metabolism, serves as the source of aminopropyl groups for the synthesis of spermidine and spermine (Wallace et al., 2003). Following the transfer of aminopropyl groups, dcSAM transforms into 5'-deoxy-5'-methylthioadenosine, which then can undergo a series of steps in the Met salvage pathway to regenerate adenine and Met (Lauinger and Kaiser, 2021).

Furthermore, SAM serves as a methyl donor, methylating DNA and proteins, including histones (Zade et al., 2023), which in turn influences epigenetic regulation, and gene expression (Figure 1). The epigenetic regulation through the methylation of DNA and histones may act blocking or activating transcription. DNA methylation is associated with gene silencing, while hypomethylation is involved with the stimulation of the transcription process (Futscher et al., 2002). The enzymes DNA methyltransferases catalyze the transfer of methyl groups, derived from SAM, to the CpG islands in the promoter region of a gene, preventing the interaction of this region with the transcriptional machinery and consequently blocking gene expression (Triantaphyllopoulos et al., 2016) (Figure 1). DNA methylation also contributes to chromatin remodeling. Methylated CpG islands are typically associated with heterochromatin, partly due to the recruitment of methyl-CpG binding proteins, which in turn recruit histone deacetylases and other chromatin-remodeling complexes to promote transcriptional repression (Hashimoto et al., 2010).

In addition, histone methylation represents another major epigenetic mechanism that regulates chromatin structure and gene expression. The histones are a family of proteins associated with DNA in the nucleus, forming the chromatin structure. The histone tails are susceptible to the inclusion of a set of chemical

groups, including methyl groups from SAM donation (Triantaphyllopoulos et al., 2016) (Figure 1). Depending on the combination of the different chemical groups and which AA residue they are attached to, chromatin will assume distinct physical states (Jenuwein and Allis, 2001). The euchromatin is characterized by an open state and is amenable to transcription, while the heterochromatin is characterized by a compact DNA-protein structure that cannot be transcribed (Jenuwein and Allis, 2001). Therefore, Met utilization may alter the DNA and histones methylation level, influencing the overall abundance of mRNA and proteins, and, consequently, the upcoming phenotype.

The SAM also participates in the synthesis of phosphatidylcholine, the major phospholipid found in mammalian cells (Vance and Tasseva, 2013). Phospholipids act as crucial components of biological membranes and are secreted in protein-lipid complexes (Ridgway, 2021). Phosphatidylcholine is particularly important for facilitating the appropriate secretion of very low-density lipoproteins from hepatocytes (Gibellini and Smith, 2010), essential for preventing the accumulation of lipid droplets in hepatic tissue, which can ultimately lead to steatosis (Dahlhoff et al., 2013). In this sense, its *de novo* synthesis is possible through the choline pathway (considered the main pathway), but it can also occur via SAM (Figure 1). In this alternate pathway, phosphatidylethanolamine methyltransferase catalyzes a series of reactions by which three methyl groups derived from SAM are donated to phosphatidylethanolamine (Cole et al., 2012; Clare et al., 2019) (Figure 1).

In the next step of the one-carbon cycle, SAM donates its methyl group to a variety of acceptor molecules, leading to S-adenosylhomocysteine (SAH) formation (Figure 1) (Clare et al., 2019). The SAH is then hydrolyzed to produce homocysteine (Hcy) and adenosine through a reversible reaction catalyzed by the SAH hydrolase (Lu, 2013). Homocysteine has the potential to undergo transsulfuration, leading to the formation of Cys (Clare et al., 2019). Initially, Hcy combines with serine, giving rise to cystathionine (Finkelstein, 1990). Subsequently, cystathionine is cleaved, leading to Cys production (Finkelstein, 1990). The Cys is essential for taurine and glutathione (GSH) synthesis, both of which possess antioxidant properties (Métayer et al., 2008) (Figure 1). Therefore, an increased supply of Met may increase antioxidant production, potentially aiding in the relief of oxidative stress and inflammation triggered by the production of reactive oxygen metabolites (Alharthi et al., 2018).

Conversely, Hcy has the capacity for remethylation to regenerate methionine, which relies on the folate cycle and vitamin B_{12} (Figure 1) (Clare et al., 2019). Folic acid undergoes a reduction process to dihydrofolate (DHF) and then to tetrahydrofolate (THF), its biologically active form. Tetrahydrofolate is further converted into 5,10-methylene-tetrahydrofolate (5,10-CH_2-THF), which is subsequently reduced to 5-methyltetrahydrofolate (5-CH_3-THF) (Preynat et al., 2009). Following this, vitamin B_{12} serves as a co-enzyme for methionine synthase, the essential enzyme facilitating the transfer of a methyl group from 5-methyl-tetrahydrofolate to Hcy, thereby promoting the regeneration of Met (Preynat et al., 2009).

Methionine is also indirectly involved in the *de novo* synthesis of nucleotides (Figure 1). The purine ring synthesis involves the incorporation of one-carbon groups from folate, contributing specifically to the C2 and C8 atoms (James et al., 1994). Additionally, in pyrimidine synthesis, the conversion of uridine monophosphate (UMP, the primary precursor) takes place, leading to the formation of deoxyuridine monophosphate (dUMP). Following this, the conversion of this compound into thymidine monophosphate (dTMP) takes place, utilizing the one-carbon unit supplied by 5,10-CH_2-THF as a substrate. This step, in turn, constitutes a rate-limiting factor for DNA synthesis (Girard and Matte, 2005).

2.2 Methionine and mTOR pathway

The mTOR serves as a central molecular hub that positively governs anabolic processes while simultaneously exerting negative regulation on certain catabolic pathways (Xie and Proud, 2013). Within mammalian cells, mTOR assembles into at least two functionally and structurally distinct complexes, namely the mTOR complex 1 (mTORC1) and the mTOR complex 2 (mTORC2) (Takahara et al., 2020). The mTORC1 is associated with processes such as protein synthesis, including ribosome biogenesis, translation initiation, and elongation (Xie and Proud, 2013; Takahara et al., 2020). On the other hand, mTORC2 is linked to cell survival and actin organization (Takahara et al., 2020). The mTORC1 and mTORC2 respond to growth factors, but only mTORC1 is influenced by glucose and amino acids (Jewell and Guan, 2013).

However, mTORC1 is not equally sensitive to all AAs. Unlike leucine and arginine, which directly bind to their respective sensors upstream of mTORC1 (Sestrin2 and CASTOR1), methionine is sensed indirectly through SAM (Gu et al., 2017). The mechanism involves the interaction of SAM with SAMTOR, an S-adenosylmethionine sensor that regulates the mTORC1 pathway (Gu et al., 2017). When methionine availability increases, SAM levels rise and promotes the dissociation of the SAMTOR-GATOR1 complex. This dissociation releases the inhibitory effect of GATOR1, facilitanting mTORC1 activation (Gu et al., 2017).

The mTORC1 pathway has been extensively elucidated in various investigations (Wang and Proud, 2006; Hall, 2008; Kitada et al., 2020; Xie and Proud, 2013). Essentially, upon activation, mTORC1 initiates the phosphorylation of eukaryotic initiation factor 4E binding protein 1 (4EBP1) and ribosomal protein S6 kinase 1 (Dong et al., 2018). Subsequently, ribosomal protein S6 (RPS6), the α subunit of eukaryotic translation initiation factor 2 (eIF2α), and eukaryotic translation elongation factor 2 (eEF2) undergo phosphorylation (Dong et al., 2018). The consequential outcome of these processes is the facilitation of mRNA translation initiation and elongation.

3 Methods for protecting free amino acids from ruminal degradation

In ruminants, protein degradation in the rumen complicates the targeted delivery of specific amino acids to the small intestine for absorption (Bach et al., 2005). Despite ingredient choices with a significant proportion of RUP, most of them lack sufficient Met (Schwab, 1995). Consequently, there is considerable interest in developing strategies to protect this AA from ruminal degradation and deliver it directly to the intestine. In this sense, before formulating a diet and including a source of protected AA, it is necessary to understand the main methods inherent to the protection of AA nowadays. Thus, these methods are better detailed in the following sections.

3.1 Physical protection

The first study aimed at producing protected AA occurred in the 1960s when most research focused on developing methods to protect Met from ruminal degradation (Mazinani et al., 2022). Initially, efforts were made to physically protect Met from ruminal degradation using lipids, usually combined with inorganic materials, carbohydrates, stabilizers, and fillers. The first product developed produced by Delmar chemicals of Canada contained a core of 20% DL-Met, tristearin and colloidal kaolin wrapped in a tristearin film (Ayyat et al., 2021). Approximately 60-65% of the Met bypassed the rumen and was available for intestinal absorption, resulting in only 12-13% of the original as fed product being bioavailable Met (Ordway, 2005).

Subsequently, a more efficient product, called Ketionin®, containing DL-Met, stabilizers, antioxidants, flavoring agents, $CaCO_3$, tristearin, and oleic acid was produced and presented a better intestinal release of Met (Ayyat et al., 2021). Ketionin® allowed approximately 80% of the Met to escape ruminal degradation, with approximately 19% of the original as-fed product being bioavailable Met (Ordway, 2005). Nevertheless, due to the challenges of obtaining an ideal mixture of material and processes and achieving optimal ruminal escape and a high intestinal release of Met, a more efficient method was created to substitute the initial encapsulation method (Schwab and Ordway, 2003).

Currently, the most effective method to protect Met is the surface-coating of the AA with a modified carbohydrate and coated by pH-sensitive synthetic polymers. In this sense, two commercial rumen-protect Met products are particularly popular in animal production, namely Mepron® M85 (Evonik Nutrition and Care GmbH, Hanau-Wolfgang, Germany) and Smartamine™ - M (Adisseo Inc., Antony, France). In Mepron pellets, Met and starch are coated by ethyl-cellulose and stearic acid, which releases Met slowly over time by abrasion and physical force in the intestine (Schwab and Ordway, 2003). The end product comprises at least 85% DL-methionine (Schwab and Ordway, 2003).

The other product available is Smartamine, which contains 75% DL-Met. The coating-protected method of Smartamine is based on the pH differences between the rumen and abomasum, and the release of the AA will depend on the environmental pH. The rumen pH varies depending on the diet composition. However, it fluctuates between 5.5 and 7.0 (Mazinani et al., 2020). In contrast, the abomasum pH is around 2 and 3 (Mazinani et al., 2020). Hence, Smartamine is resistant to the rumen pH, but is soluble in lower pHs, such as the post-ruminal environment, being undegradable in the rumen and immediately released in the abomasum.

Various experimental techniques are currently available to assess the bioavailability of rumen-protected products. One approach is the continuous infusion of isotope-labeled amino acids as tracers (Estes et al., 2018; Rebelo and Lee, 2024). Another method involves the use of selenium-enriched yeast containing selenomethionine, a methionine analog where selenium replaces sulfur, which serves as a marker for digestible methionine. This allows for the evaluation of methionine utilization by monitoring changes in selenium concentration in milk (Weiss and St-Pierre, 2009). Other notable techniques include plasma-free amino acid dose-response (Whitehouse et al., 2017), *in situ* and *in vitro* methods (Estes et al., 2022), and fecal amino acid excretion (Räisänen et al., 2020), all of which are employed to evaluate rumen-protected amino acids.

However, employing different techniques can lead to varying results when comparing the bioavailability of products. For instance, Whitehouse et al. (2016) utilized the plasma-free amino acid dose-response method to compare the bioavailability of methionine in Smartamine M versus Mepron, finding that Mepron had 28% of the bioavailability of Smartamine. In contrast, Clark et al. (2023) evaluated the bioavailability of Mepron and Smartamine by supplementing with selenium-enriched yeast, using selenomethionine as a tracer for metabolizable methionine. This study determined a bioavailability of 74% for Mepron and 80% for Smartamine. These findings highlight that bioavailability values can vary depending on the technique used. While these metrics are crucial for developing nutritional matrices and formulation models, the most reliable indicator of a product's effectiveness in terms of bioavailability is the phenotypic outcomes observed in the animal, which will be discussed in more detail below.

In addition, the physical protection methods are susceptible to some problems. First, the products may be abraded by human handling, diet mixing, or chewing by the animal, which will expose the protected AA to the ruminal microorganism and influence the availability for intestinal absorption. Additionally, the resistance of the protection layer to ruminal enzymes will influence the ability of digestion by the post-ruminal enzymes.

3.2 Chemical protection

In addressing the challenges linked to the vulnerability of physical protection techniques to abrasion degradation, chemical protection approaches, comprising the use of amino acid analogs and their derivatives were developed (Mazinani et al., 2022). While AA derivatives consist of a chemical blocking group added to an

alpha-amino group or a modified acyl group in a specific free AA, AA analogs are developed by replacing the alpha-amino group with a non-nitrogenous group (Schwab, 1995).

Reviewing the potential of AA derivatives to escape from ruminal degradation, Loerch and Oke (1989) reported three main products that promote an increase in Met absorption post-ruminally, known as N-stearoyl-DL-Met, N-oleoyl-DL-Met, and Capryl- caprylic-Met. The Met hydroxy analogs 2-hydroxy-4-(methylthio) butanoic acid (called HMB or HMTBa) and isopropyl ester of 2-hydroxy-4-(methylthio) butanoic acid (HMBi) have been widely used as a precursor of methionine in ruminant nutrition (Noftsger et al., 2003). For instance, HMB may be provided as either liquid or in Ca salt form, being the Alimet®, MFP®, or MHA® (Novus International, St. Charles, MO) examples of available commercial products. Based on the technical details supplied by the manufacturer, the Alimet feed additive serves as a liquid methionine source, MFP delivers methionine in a dehydrated state, and MHA is a dry, granular powder. The HMB distinguishes itself from methionine due to the presence of a hydroxyl group on the alpha carbon, in contrast to methionine's amino group (Dibner, 2003). Although HMB can suffer ruminal degradation, when compared to free methionine, HMB was less degraded by the ruminal microorganism (Mazinani et al., 2022). The HMB can be absorbed across ruminal, omasal, and abomasal epithelium (McCollum et al., 2000), being converted to Met in peripheral tissues (mainly nonhepatic tissues) after absorption (Lobley et al., 2006a). In addition, forestomach tissues have the potential to convert HMB into Met, given the high enzymatic activities found in the rumen and omasum, which allows the conversion of D- and L-isomers of HMB into the L-Met precursor (Lobley et al., 2006b). Lastly, ruminal microorganisms can degrade HMB, and these degradation products persist as the fluid phase passes between the rumen and the abomasum (Lobley et al., 2006b). In the past years, several studies evaluating the effects of HMB, mainly in cow's milk production and composition, found a variety of responses including benefits or no effects. Such inconsistency may be explained by the variance of HMB's rumen degradability, which may fluctuate between 21 (Koenig et al., 1999; Vázquez-Añón et al., 2001) to 99% (Jones et al., 1988).

An HMBi product available in the market is the MetaSmart (MS; Adisseo Inc., Antony, France). The process of esterifying HMB to isopropanol reduces its magnitude of degradation in the rumen (St-Pierre and Sylvester, 2005). Thus, HMBi exhibits partial resistance to microbial degradation due to its enhanced ability to permeate biological membranes, facilitating rapid absorption through the rumen wall (Graulet et al., 2004). Approximately 50% of HMBi is shielded from ruminal degradation, with the remaining 50% undergoing hydrolysis by rumen microorganisms to HMB and isopropanol (Graulet et al., 2005). The HMB remaining available in the rumen, in turn, exhibits comparable behavior to unaltered HMB (Noftsger et al., 2005).

4 Rumen-protected methionine and its effects on dairy and beef cattle

All data collection was performed using the available published scientific literature. Relevant articles were searched through the PubMed®, ScienceDirect, Google Scholar, and Web of Science platforms without restrictions on publication date. The search strategy was developed using keywords and Boolean operators such as "rumen-protected methionine," "methionine supplementation AND dairy cows," and "methionine supplementation AND beef cattle." After retrieval, duplicate records, *in vitro* studies, literature reviews, and meta-analyses were removed. The remaining studies were screened based on title and abstract. Eligible studies were required to report at least one relevant outcome, depending on the focus of each section: milk yield or composition, animal performance (e.g., weight gain), reproductive responses, offspring response to maternal supplementation, or immune and inflammatory responses. The selected studies are summarized in Tables 1-4.

4.1 Milk yield and composition in dairy cows

Great efforts have been made to improve milk production and milk composition in dairy cows, including genetic selection and nutritional advances (Weiss, 2017). Hence, RPM supplementation allows the investigation of the role of Met in the metabolism of dairy cows, specifically in lactation performance and milk composition. The impact of supplementary RPM on dairy cows is evident; however, the response intensity is difficult to evaluate due to the differences in experimental designs, RPM sources, and purity among studies (Wei et al., 2022).

A meta-analysis to elucidate the main impacts and extent of RPM on dairy cows' performance (Patton, 2010) gathered a total of 129 studies and allowed 75 dietary comparisons between the control and RPM-enriched diets. The main results showed that RPM-enriched diets decreased cows' dry matter intake (DMI) and milk fat percentage, while the milk production and the production of true milk protein increased both as a percentage (0.07%) and yield (27 g/d). Among the physically protected Met products, Mepron-fed cows showed lower DMI, greater milk production, and twice as much milk protein yield than Smartamine-fed cows (37 g/d vs. 16 g/d) (Patton, 2010).

Furthermore, in another meta-analysis (Zanton et al., 2014) utilizing a compilation of 64 articles investigating the utilization of postruminally administered DL-methionine, HMTBa (offered in either a liquid form or as a calcium salt), Mepron, and Smartamine, it was observed that cows receiving Smartamine exhibited higher feed intake, whereas those supplemented with Mepron showed reduced DMI compared to the control group. Although milk yield did not exhibit

a significant response to Met supplementation, there was a tendency for an increase of 0.28 kg/d and 0.31 kg/d in cows supplemented with HMTBa and Mepron, respectively (Zanton et al., 2014). Milk protein yield experienced an elevation in average of 20.8 g/d due to supplementation from all sources or administration methods. The magnitude of this increase was greater for Mepron (35 g/d) compared to HMTBa (13 g/d), whereas no statistically significant differences were observed among Mepron, Smartamine, and infused DL-Met (Zanton et al., 2014). Except for cows infused with HMTBa, milk protein concentration increased by 0.07% in cows treated both Mepron and Smartamine, while infusions of DL-Met contributed for an 0.08% increase in this parameter (Zanton et al., 2014). Milk fat yield showed an increase for Mepron and HMTBa, while milk fat concentration rose for DL-Methionine infusion and cows supplemented with HMTBa (Zanton et al., 2014).

Based on scientific evidence (Vyas and Erdman, 2009; Patton, 2010; Zanton et al., 2014), it is possible to say that the most consistent outcome resulting from dietary RPM is the enhancement of milk protein production. This response can be explained by the significant role of Met in the mTOR pathway, as Met consistently appears as the most limiting amino acid affecting milk protein synthesis in dairy cows. Similarly, an *in vitro* study (Nan et al., 2014) using bovine mammary cells demonstrated that Lys, Met, and a combination of these amino acids (especially in a 3:1 Lys: Met ratio) positively regulated the expression of genes associated with the JAK2-STAT5 and mTOR pathway, and increased casein concentration, suggesting a potential effect on enhancing milk protein synthesis.

Additionally, although the use of RPM can promote benefits on milk production (Osorio et al., 2013; Zhou et al., 2016; Batistel et al., 2017a), it is important to highlight that in certain instances, the Met supplementation failed to increase the milk yield. For instance, in a study conducted by Cardoso et al. (2021), where they assessed three dietary interventions from approximately 18 days before to 45 days after parturition, comprising low protein, high protein, or high protein along with rumen-protected Met (Mepron), it was verified that the inclusion of rumen-protected methionine to the HP regimen did not result in any change in milk yield. Additionally, in a more recent meta-analysis (Wei et al., 2022), conducted from 14 studies covering data from 623 dairy cows, and considering the content of metabolizable amino acids that eventually reach the small intestine from different sources of RPM, it was found that adding RPM to the diet did not significantly improve the milk yield of the dairy cows. Hence, it becomes crucial to assess the specific conditions under which the utilization of RPM will be implemented, ensuring that the financial investment in this technology yields the anticipated outcomes. The duration of the treatment and RPM doses are summarized in Table 1.

TABLE 1 Information about the breed, physiological status, RPM doses and sources, and duration of treatments used in *in vivo* studies addressed in the sections milk yield and composition, and performance in the current review.

References	Breed/physiological status	RPM level and source	Duration
Milk yield and composition			
Osorio et al. (2013)	Multiparous Holstein cows	0.19% RPM (DM basis; MetaSmart) 0.07% of RPM (DM basis; Smartamine® M)	-21 d before expected calving to 30 d in milk
Batistel et al. (2017a)	Multiparous Holstein cows	0.09% and 0.10% of RPM (DM basis; Mepron®M85)	-28 to 60 d relative to parturition
Cardoso et al. (2021)	Primiparous and multiparous Holstein cows	0.09% and 0.13% of RPM (DM basis; Mepron®M85)	-18 to 45 d relative to parturition
Performance			
Liker et al. (2006)	Charolais growing cattle	10 g/RPM/d (Mepron®M85)	94 d
Waterman et al. (2012)	Angus pregnant heifers	15 g/RPM/d methionine hydroxy analog (MHA) (Mepron®M85)	Last 49 to 66 d of pregnancy
Clements et al. (2017)	Simmental-Angus cows	10 g of MHA (MFP® Feed Supplement)	23d before calving to 73d postpartum
Cantalapiedra- Hijar et al. (2020)	Charolais bulls	7 g/RPM/d (Smartamine® M)	210d
Dominguez et al. (2020)	Brangus heifers	4g/RPM per 100g of mineral salt (Smartamine® M)	45d prior to the FTAI
Inhuber et al. (2021)	Fleckvieh bulls	1.6 g of RPM/Kg of DM (Smartamine® M)	105d
Baggerman et al. (2021)	Crossbred steers	4g, 8g, or 12g/RPM/head/d (MetiPEARL)	111d or 139d (experiment 1 56d (experiment 2)
Cabezas et al.(2023)	Montbeliard steers	1.5 g of RPM/Kg of FM[1] (KESSENT® MF) 1.8g of RP-Lys2/Kg of FM (LysiGEM™)	202d

[1]FM: Fresh matter; [2]RP-Lys: rumen-protected lysine

4.2 Beef cattle performance

Although many studies have been performed in dairy, limited research has been conducted investigating the effect of RPM in beef cattle. Liker et al. (2006) observed the lack of difference in beef cattle average daily gain (ADG) during the growing phase after a period of RPM supplementation (Mepron) and assumed that possibly Met is not a limiting AA at this stage of development. Indeed, a study performed to evaluate whether Met is the main AA that limits the growth performance of fattening bulls, showed that the supplementation of RPM in low CP diets did not change the final body weight (BW), ADG, and carcass characteristics (Inhuber et al., 2021). Moreover, blood Lys concentration was lower in the groups that received RPM in low CP diets, compared to the negative control groups (low CP without RPM supplementation) supporting the idea that Lys could have been the first-limiting AA for growth instead of Met (Inhuber et al., 2021). In an early study, which administered postruminally adequate amounts of Lys and varying amounts of Met, it was already demonstrated the fact that Met was not limiting for the growth of steers (Hill et al., 1980). Similarly, during the finishing period on feedlot, increasing levels of RPM, along with 4 g/head/day of supplemental lysine, did not affect animal performance (BW, ADG, DMI) (Baggerman et al., 2021). However, there was a 55% increase in myonuclei density in the muscle of cattle supplemented with 8 g/head/day of RPM, which may explain the significant linear relationship observed between increasing dietary RPM levels and Longissimus muscle area (Baggerman et al., 2021).

It is important to emphasize that the inefficient utilization of high-protein diets in the rumen can result in excessive nitrogen losses to the environment, thereby contributing to the production of environmentally harmful gases (Abbasi et al., 2018). In finishing cattle diets, approximately 10% to 20% of the nitrogen consumed is retained in body tissues, whereas 30% to 50% is excreted in the feces. The majority of the remaining nitrogen, approximately 40% to 70% of what is ingested, is eliminated via urine (Cole and Todd, 2009). Reducing crude protein (CP) concentrations in the diet, while maintaining animal performance, has therefore been proposed as a strategy to decrease nitrogen excretion and lower feeding costs. Based on this, Cabezas et al. (2023) evaluated whether incorporating RPM and rumen-protected lysine into a diet with a 3% reduction in CP would affect the growth performance of beef cattle. Their findings showed that the inclusion of protected amino acids enables the formulation of lower-CP diets while enhancing nitrogen-use efficiency for protein synthesis, particularly during the finishing phase, without compromising meat quality (Cabezas et al., 2023). Under a forage-based dietary scenario, RPM supplementation improved the growth performance of Charolais bulls, with a more pronounced effect when diets were formulated with high protein content (16.2% CP) (Cantalapiedra-Hijar et al., 2020). These improvements were accompanied by metabolic shifts, including a more favorable plasma amino acid profile and several indicators of enhanced nitrogen-use efficiency and nitrogen retention. Altogether, these results highlight the importance of considering amino acid balance when formulating diets for growing-fattening ruminants (Cantalapiedra-Hijar et al., 2020).

In terms of maternal performance, gestating beef cows appear not to respond to RPM supplementation, as no effects have been observed on BW or body condition score (BCS). For instance, Waterman et al. (2012) investigated the effects of RPM supplementation (Mepron) in grazing beef heifers during late gestation and reported similar results in maternal BW, BCS, and plasma amino acid concentrations, suggesting a limited response to supplemental AA due to the limitation of energy and the weight loss occasioned by the pasture quality. Beef cows supplemented with RPM (HMA) during pre and postpartum did not show improvement in BW and BCS at any time point when compared to the non-supplemented cows (Clements et al., 2017). Contrary to what was observed during gestation, the RPM supplementation (Smartamine) 45 d before the fixed-time artificial insemination (FTAI), contributed to the increase in beef heifers' BW on the day of the artificial insemination and 30 d after (Dominguez et al., 2020), which could favor the maintenance of gestation.

Finally, it is important to mention that despite the lack of differences between RPM and control-treated cows, clear changes were reported regarding maternal blood parameters, nutrient delivery through the placenta, in addition to positive effects on follicular and fetal development, and offspring's postnatal performance. Hence, these topics are better detailed in the following sections. The duration of the treatment and RPM doses are summarized in Table 1.

4.3 Reproduction

To ensure a required gene expression pattern to support embryonic development, the maternal (oocyte) and paternal (sperm) epigenome are remodeled to reach totipotency, driving cellular differentiation toward all cell types (Ross and Sampaio, 2018), including embryonic and extraembryonic (*i.e.*, placenta) cell types. The transition from maternal to embryonic control of development includes the degradation of a subset of maternal products (mRNAs, proteins), followed by the embryonic genome activation (EGA), which is an essential process for embryonic development (Tadros and Lipshitz, 2009). Although the levels of demethylation during the pre-implantation period are significant, a portion of DNA methylation will be kept during the reprogramming after fertilization, including the imprinted genes, which represent an inheritance from the paternal environment to the offspring phenotype (Jiang et al., 2018). Evaluating the effects of the restriction of total metabolizable energy during the second half of cows' gestation, Paradis et al. (2017) reported hypomethylation of the imprinted IGF2 gene of the differentially methylated region 2 (DMR2) in the *Longissimus dorsi* muscle of the restricted offspring.

Nutrition plays a crucial role in shaping the reproductive outcomes of ruminants (Robinson et al., 2006). Maintaining an optimal maternal condition is essential for achieving favorable pregnancy rates, high-quality embryos, and ultimately, successful

gestation and lactation to ensure the production of healthy calves. However, the period before the breeding season is characterized by the scarcity of forage in tropical regions and may impair cow's reproductive performance and the establishment of pregnancy. Thus, strategic supplementation with RPM before a breeding program allowed the increase in follicle size (> 9 mm) and reflected in the greater percentage of ovulation (Alonso et al., 2008). Similarly, the RPM supplementation (Smartamine) 45 d before the FTAI protocol in a pasture-based diet tended to increase the dominant follicle size in beef heifers (Dominguez et al., 2020). Evaluating the main effects of RPM (Smartamine) and its interaction with parturition order, Toledo et al. (2017), reported that RPM supplementation was effective for multiparous, while no significant impact was observed in primiparous cows. In this study, RPM supplementation after the transition period (30-126 d postpartum) increased the embryonic size and lowered the pregnancy loss rates in multiparous cows (Toledo et al., 2017).

Evaluating the AA composition in the uterine lumen during early pregnancy in cattle, Groebner et al. (2011) showed that the concentration of most essential amino acids is enhanced as gestation advances. Hence, maternal supplementation of rumen-protected AA may be a valuable strategy to ensure an adequate nutrient supply for oocyte maturation, early embryo development, and the maintenance of gestation. The supplementation of RPM (Smartamine) for cows during the transition period contributed to the increase in Met concentration in the follicular fluid of the first postpartum dominant follicle (DF) and higher expression of *3β-HSD*, which may be related to the selection of DF in cattle and affect oocyte quality (Acosta et al., 2017). Moreover, the findings of Peñagaricano et al. (2013) support the hypothesis that maternal nutrition, specifically by supplying cows with RPM (Smartamine), largely influences oocyte maturation, fertilization, and preimplantation embryonic development.

The effects of RPM on the transcriptome profile during follicular and early embryo were investigated, and the results showed that it is likely that DNA methylation may have been a mechanism utilized to modulate gene expression patterns (Peñagaricano et al., 2013). Indeed, the global alterations in DNA methylation may result in embryo death and developmental defects (Reik et al., 2001). Notably, by using a Met antagonist (ethionine) and a product of Met metabolism (SAM) *in vitro*, Ikeda et al. (2012) reported that the disturbance in methionine metabolism, through SAM deficiency and consequently DNA hypomethylation impairs the morula-to-blastocyst transition during bovine preimplantation. Conversely, although the medium containing Met was able to enhance the percentage of culture bovine oocytes developing into viable blastocysts, the global methylation was not altered by Met concentration (Bonilla et al., 2010). Similarly, supplementation of RPM (Smartamine) for Holstein multiparous cows during the transition period followed by the removal of RPM supplementation until day 72 postpartum, showed a decrease in global DNA methylation compared to cows that did not receive RPM supplementation during the entire period of evaluation (Acosta et al., 2016). However, the greater lipid concentration in embryos from RPM-supplemented cows after the transition period (31–72 d postpartum) suggests an enhancement in embryonic survival (Acosta et al., 2016), validating the indispensable role of Met during this period.

While the maternal effects of RPM on embryonic development are well understood, the dietary paternal effects on sperm functionality and reproductive performance need further evaluation. Moreover, there is a scarcity of studies addressing these effects in bulls. However, research conducted with rams has shown promising results regarding the use of RPM supplementation, which may serve as a valuable reference for future studies in other species, including cattle. The evaluation of RPM supplementation over a 3-month period in rams showed positive effects in increasing serum testosterone and scrotal circumference (Alkhashab et al., 2021). Moreover, the experimental diet improved sperm quality, as evidenced by a higher proportion of live sperm and a reduction in dead and abnormal sperm (Alkhashab et al., 2021).

Considering the importance of methionine (MET) as a methyl donor in DNA methylation processes, studies have investigated the effects of RPM supplementation in males on epigenetic regulation, as well as on gene and protein expression profiles related to sperm quality and seminal plasma composition. Gross et al. (2020) reported that supplementing prepubertal rams with RPM led to an earlier age at puberty and altered the differentially methylated regions (DMRs) associated with genes involved in sexual development in F0 generation, which was inherited by the F1 and F2 generations (Braz et al., 2022). Furthermore, the embryos produced by RPM-treated rams also displayed differential gene expression, demonstrating that inheritance of methylation patterns may alter gene expression in future generations (Townsend et al., 2022).

In addition to serving as a transport medium for sperm, the seminal plasma also plays a role in direct communication with the female reproductive tract to alter uterine function and drive physiological changes that increase pregnancy success. The treatment of prepubertal rams with RPM enriched diets altered the proteome of their seminal plasma by upregulating proteins associated with sperm function, including maturation, capacitation, and interaction with the zona pellucida, as well as proteins involved in supporting motility and acrosomal function (Townsend et al., 2025). Moreover, the seminal plasma of RPM-treated rams exhibited a greater abundance of proteins known to activate pathways that promote an immunologically receptive environment for embryo implantation (Townsend et al., 2025). It is evident that RPM supplementation not only altered the seminal plasma composition toward an improvement in semen functionality but also influenced epigenetic regulation. In this regard, seminal plasma from RPM-treated rams showed higher expression of the microRNA miR-543-3p, which targets the ten-eleven translocation (TET) family of enzymes responsible for DNA demethylation (Townsend et al., 2025). These findings suggest that RPM supplementation may influence epigenetic remodeling through seminal plasma miRNAs that regulate DNA methylation

machinery, with potential downstream effects on both sperm function and early embryo development. The duration of the treatment and RPM doses are summarized in Table 2.

4.4 Maternal RPM supplementation and offspring performance

Considering that embryonic development is the period of extensive epigenetic reprogramming, maternal nutrition during the periconceptional and early pregnancy stages plays a crucial role in establishing the molecular and physiological foundations for fetal growth and long-term offspring performance. Indeed, the primary transcripts affected in fetal liver under a maternal nutrient restriction scenario are those associated with AA metabolism and epigenetic modification, such as methyltransferases (Crouse et al., 2019).

Evaluating the impacts of maternal RPM supplementation during the periconceptional period (115 d) on postnatal beef calves, Silva et al. (2021) verified greater post-weaning BW, ADG, and improvement in feed efficiency in the offspring from the RPM group. In contrast, a shorter period of RPM supplementation from day -7 to +7 relative to artificial insemination was not sufficient to affect calves' birth and weaning weights or induce prenatal morphological alterations in the embryo and fetuses (Heredia et al, 2025a). However, the upregulation of genes involved in muscle development in the skeletal muscle of pre-weaned calves (Heredia et al, 2025a) may have contributed to increasing the calves ADG and final BW at post-weaning (Heredia et al, 2025b). Despite the positive effects on weight gain, preconception RPM supplementation may have reduced subcutaneous fat deposition in the offspring, indicating a possible shift in energy partitioning toward lean tissue accretion (Heredia et al, 2025b).

A series of studies have investigated the effect of RPM in combination with other components of the one carbon metabolism, which includes folate, vitamin B_{12} and choline, during early gestation in beef heifers. Supplementing beef heifer during the first 63d of gestation with one-carbon cycle components (dietary RPM, rumen-protected choline, and injection of vitamin B_{12} and folic acid) increased the availability of vitamin B_{12}, folate and folate intermediates in maternal serum and promoted greater levels of 5-CH_3-THF in the allantoic and amniotic fluid (Syring et al., 2024). These findings suggest enhanced maternal-fetal transfer and activation of one-carbon metabolism during early gestation, potentially supporting epigenetic programming and impacting fetal development outcomes.

In fact, using the same experimental design, Safain et al. (2025) showed that fetal tissues responded differentially to one-carbon components supplementation. The fetal liver exhibited higher mitochondrial activity and increased mitochondrial DNA copy numbers—both indicative of enhanced energy metabolism—without major shifts in gene expression, suggesting that regulation may occur at the protein level (Safain et al., 2025). In contrast, fetal muscle showed no changes in mitochondrial activity but displayed reduced expression of genes involved in fat metabolism and energy production, suggesting early transcriptional changes that could affect postnatal muscle energy utilization (Safain et al., 2025).

The effect of one-carbon components supplementation during early gestation on fetal jejunum morphology was recently evaluated (Daneshi et al., 2025). The study reported that when heifers were fed restricted during early gestation, the supplementation of one-carbon metabolism components increased the thickness of the muscularis externa of fetal small intestine and promoted deeper crypts in the jejunum of the offspring (Daneshi et al., 2025). These findings may suggest an enhance in intestinal motility, which is

TABLE 2 Information about the breed, physiological status, RPM doses and sources, and duration of treatments used in *in vivo* studies addressed in the reproduction section of the current review.

References	Breed/physiological status	RPM level and source	Duration
Reproduction			
Alonso et al. (2008)	Heifers (*Bos indicus x Bos taurus*)	10 g/RPM//d (Mepron®M85)	45 d
Peñagaricano et al. (2013)	Holstein cows	2.43% RPM/d of the metabolizable protein (Smartamine® M)	~70 d postpartum
Acosta et al (2016; 2017)	Multiparous Holstein cows	0.08% of RPM/d (DM basis; Smartamine® M)	21 d before calving to 30 d in milk
Toledo et al. (2017)	Holstein cows	21.2 g of RPM/d (Smartamine® M)	from 30 to 126 d in milk
Dominguez et al. (2020)	Brangus heifers	4g/RPM per 100g of mineral salt (Smartamine® M)	45 d prior to the FTAI
Gross et al. (2020)	Polypay rams	3 g of RPM/d (Smartamine®)	10.7 to 13.6 weeks of age until puberty
Alkhashab et al. (2021)	Awassi male lambs	5% RPM of daily ration intake/d (Mepron®M85)	3 months
Braz et al. (2022)	Polypay rams	3 g of RPM/d (Smartamine®)	10–12 weeks
Townsend et al. (2022)	Polypay rams	3 g of RPM/d (Smartamine®)	77 weeks
Townsend et al. (2025)	Polypay rams	3 g of RPM/d (Smartamine®)	105d

essential for the effective digestion and nutrient absorption, accompanied by protective effect against villus atrophy caused by undernutrition (Daneshi et al., 2025).

It is evident that the manipulation of the one-carbon metabolism and the methylation processes through the diet can influence long-term metabolic and performance outcomes. Therefore, there is an attempt to induce methyl deficiency through feed additives, such as guanidinoacetic acid (GAA), to better evaluate the role of RPM in supporting methyl donor supply. Preliminary results from a study conducted with pregnant beef cows in late gestation reported increased maternal plasma MET concentrations in cows fed RPM and lower concentrations in those supplemented with GAA, with intermediate values in cows receiving both RPM + GAA (Motta et al., 2025), highlighting the role of GAA as a methyl consumer. Although the GAA altered MET availability in the dams (Motta et al., 2025), supplementation of GAA to heifers 63 days before breeding until d 63 of gestation, did not alter the methyl supply in fetal liver (Crouse et al., 2023). Moreover, fetuses from dams supplemented with RPM were able to maintain methionine homeostasis by shunting methionine through adjacent pathways (transsulfuration), contradicting the hypothesis that GAA supplementation would induce a methionine deficiency and compromise fetal methyl balance (Crouse et al., 2023). Taken together, these findings suggest that GAA has a more pronounced effect on inducing methionine deficiency at the maternal level, while its impact on fetal methyl metabolism and postnatal phenotype appears to be limited.

Maternal nutrition modulates fetal development and influences the offspring's performance postnatally (Barcelos et al., 2022; Santos et al., 2022; Nascimento et al., 2024) mainly by affecting skeletal muscle development (Carvalho et al., 2022). Due to the placental structure that impedes the direct contact between maternal and fetal blood, the transport of nutrients to fetal circulation depends on protein transporters, concentration gradients, and diffusion channels (Brett et al., 2014).

The RPM supplementation during the last 28 days of gestation promoted an up-regulation in amino acid and glucose transporters in the placentome of cows, mediated by changes in gene transcription and mTOR signaling (Batistel et al., 2017b). Moreover, RPM supplementation contributed to the birth of heavier offspring (Batistel et al., 2017b), which may be beneficial for beef calves to reach the slaughter weight sooner. In the complementary study from the same research group (Batistel et al., 2019), Met supply during late gestation also led to sex-dependent responses in placental metabolism and DNA methylation, improving the calf birth body weight (Batistel et al., 2019). Placenta from pregnant cows carrying male fetuses and fed an enriched Met diet presented greater energy production in the placenta TCA cycle. In contrast, lower global DNA methylation, and a greater abundance of DNMTs were detected in Met-supplemented pregnant cows carrying females (Batistel et al., 2019). Therefore, these findings demonstrate the importance of Met for fetal growth at late gestation in cattle and their complex interaction with other factors.

Efforts have been made to evaluate the effect of RPM supplementation during late pregnancy and its long-term effects on offspring performance. Studies have reported that maternal RPM supplementation during late gestation (last 28 days of gestation) increased calves BW (Alharthi et al., 2018), ADG (Alharthi et al., 2018), and hip and wither height (Alharthi et al., 2018; Palombo et al., 2021) from birth to 9 weeks of age. Intriguingly, these improvements were not entirely associated with maternal DMI and colostrum quality, suggesting the mechanisms of nutrient transport through utero-placenta may play a significant role in RPM diets (Alharthi et al., 2018). In addition to evaluating RPM supplementation during the last trimester of gestation (last 90 days), Alfaro et al. (2024) extended supplementation through 80 days of lactation and also supplemented early-weaned beef calves with RPM for 100 days. This prolonged (pre- and postnatal) supplementation did not affect BW or BCS but increased the expression of adipogenic genes (*PPARG*, *LPL*, and *CEBPD*), suggesting a potential to enhance adipose tissue development and increase marbling during the finishing phase (Alfaro et al., 2024). However, caution is warranted in interpreting these findings since the study did not evaluate whether the upregulation of these genes was translated into better carcass quality.

Indeed, the three-year studies conducted by Tadich et al. (2024), involving both primiparous and multiparous cows managed under different late-gestation feeding regimes (non-supplemented, dried distillers grains [DDG]-based diets, and RPM supplementation), showed limited effects on the performance of both dams and progeny. This research also evaluated offspring carcass traits and found no differences among treatments, indicating that RPM supplementation may not be necessary when adequate levels of rumen-degradable and undegradable protein are supplied through DDG-based diets (Tadich et al., 2024).

Since the liver is essential in regulating one-carbon metabolism and may influence the immunological responses in newborn animals, this organ may be an exciting objective for studying the fetal programming effects influenced by dietary methyl donors, such as Met. Palombo et al. (2021) tested whether the Met supplementation postruminally during late gestation would program the Met cycle in the calf liver. The results showed that maternal RPM supplementation in late gestation promoted more significant global DNA methylation in the offspring's liver, accompanied by a general alteration in the hepatic transcriptome, proteome, and metabolome, favoring the enrichment in glucose and lipid metabolism, glutathione, and immune system pathways (Palombo et al., 2021). The authors also pointed out some markers (*i.e.*, FOXO1, PPARG, E2F1, and CREB1) and suggested that they may play a role in coordinating the effects induced by maternal treatments (Palombo et al., 2021). Similarly, the RPM supplementation for cows during late pregnancy induced a rapid maturation of gluconeogenesis and fatty acid oxidation pathways in the offspring's liver at birth, which could favor the metabolic demands in postnatal life (Jacometo et al., 2016). Moreover, the calves' blood metabolites assessment indicates that RPM contributed to increasing systemic insulin sensitivity and reducing

stress (Jacometo et al., 2016). In agreement with these results, calves from RPM-supplemented cows experienced alteration in Met metabolism to favor the synthesis of taurine and glutathione, suggesting an advantage for controlling metabolic-related stress (Jacometo et al., 2017).

Finally, as discussed previously, RPM diets influence the ruminal microbiota community and contribute to improving nutrient utilization, and production of volatile fatty acids, among others. Although studies evaluating the offspring's rumen microbiome as a result of maternal RPM supplementation during distinct stages of gestation are still lacking, the evidence showing that fiber digestibility was the most affected parameter in the offspring from maternal RPM treatment (Silva et al., 2021) shed light on the importance of further research in this area. The duration of the treatment and RPM doses are summarized in Table 3.

4.5 Immune system

Typically, during the last months of gestation and the first month of lactation, there is an increase in fat mobilization from maternal depots and an accumulation of hepatic non-esterified fatty acid (NEFA) (Pullen et al., 1990). Since the bovine liver is limited to oxidizing NEFA, triacylglycerol (TAG) accumulation may frequently occur (Dodson et al., 2010). The increase in inflammation and oxidative stress during this period impairs liver function (Bionaz et al., 2007; Trevisi et al., 2012). Although TAG accumulation is more pronounced in dairy (Pullen et al., 1990), beef cows may also be affected, depending on body condition status and the extent of fat mobilization.

Among a variety of mechanisms, inflammation is characterized by the increase in the synthesis of positive acute phase proteins cytokines (*i.e.*, haptoglobin, serum amyloid A) (Bertoni et al., 2008) and in the production of pro-inflammatory cytokines (*i.e.*, IL-6, IL-1, TNF-α) (Owen et al., 2013). In this sense, Met plays a fundamental role in the liver by stimulating the synthesis of very low-density lipoprotein (VLDL), and therefore, helps reduce TAG accumulation (Martinov et al., 2010). Maternal RPM supplementation, utilizing two different products available in the market (Smartamine M or MetaSmart) during the last 21 days of gestation, promoted a reduction in plasma positive acute-phase proteins, accompanied by a decrease in plasma IL-6 after calving, indicating a reduction in proinflammatory responses (Osorio et al., 2014b). Moreover, the antioxidant and β-oxidation capacity in the liver was improved in RPM-supplemented cows, through the enhancement in *de novo* production of glutathione and carnitine (Osorio et al., 2014b). More recently, Batistel et al. (2018), showed

TABLE 3 Information about the breed, physiological status, RPM doses and sources, and duration of treatments used in *in vivo* studies addressed in the maternal RPM supplementation and offspring performance section of the current review.

References	Breed/physiological status	RPM level and source	Duration
Maternal RPM supplementation and offspring performance			
Jacometo et al. (2016) Jacometo et al. (2017)	Holstein cows	0.08% of RPM/d (DM basis; Smartamine® M)	51d (21 d before calving to 30 d in milk)
Batistel et al. (2017b) Batistel et al. (2019)	Multiparous Holstein cows	0.9 g/kg of RPM (DM basis; Mepron®)	Last 28 d of pregnancy
Alharthi et al. (2018)	Holstein cows	0.09% of RPM/d (DM basis; Mepron®)	Last 28 d of pregnancy
Silva et al. (2021)	Brangus- Angus crossbred cows	9.5 g/d of RPM (Metasmart©Liquid)	115 d (periconception period)
Palombo et al. (2021)	Holstein cows	0.09% of RPM/d (DM basis; Mepron®)	Last 28 d of pregnancy
Crouse et al. (2023)-preliminary results	MARC II heifers	10 g/d of RPM 40 g/d of GAA	126d (63d before breeding and 63d of gestation)
Alfaro et al. (2024)	Primiparous Angus, Angus-Simmental crossbred, Simmental cows	8 g/d of RPM (Smartamine®)	Last trimester of gestation (~90d) and first ~80d of lactation
Tadich et al. (2024)	Angus crossbred cows	1oz/d of RPM (MFP® Feed Supplement)	Late gestation (90 d)
Syring et al. (2024) Safain et al. (2025) Daneshi et al. (2025)	Angus crossbred heifers	7.4 g/d RPM (Smartamine®) 44.4 g/d RP-Cho[1] (ReaShure) 20 mg Vit B12 (IM[2] injection) 320 mg folic acid (IM injection)	First 63d of pregnancy
Heredia et al. (2025a) Heredia et al. (2025b)	Angus crossbred cows	15g/d of RPM (Smartamine® M)	14d (-7 and +7d relative to artificial insemination)
Motta et al. (2025)-preliminary results	Nelore cows	0.02 g/kg BW of RPM (Mepron®) 0.12 g/kg BW of GAA	Last ~88d of pregnancy

[1]RP-Cho: rumen-protected choline; [2]IM: intramuscular

that the supply of RPM during the transition period for dairy cows ensuring a ratio of 2.8: 1 Lys to Met (% metabolizable protein) was effective by reducing the inflammation and oxidative stress, in addition to improve the liver and neutrophil function. Consistently, other studies using dairy cattle as a model (Osorio et al., 2016, 2013) also demonstrated the effectiveness of Met use during the periparturient period through reduced inflammation and oxidative biomarkers levels, indicating a greater health status in RPM cows.

Similarly, the liver transcriptome profile of the cows receiving RPM supplement during late gestation and early lactation revealed that the methyl donor SAM and antioxidants (*i.e.*, glutathione) are increased by the supply of Met (Osorio et al., 2014a). The improvement in lipid metabolism and immune function was further confirmed in companion publications by the same research group, which showed connections between global DNA methylation and its effect on proliferator-activated receptor alpha (PPARα) (Osorio et al., 2016). In summary, the upregulation of hepatic PPARα and its target genes in RPM cows was related to enhanced lipid metabolism, favoring their immune status and performance (Osorio et al., 2016). Lastly, Vailati-Riboni et al. (2017) also verified improvements in immune function related to enhanced neutrophil and monocyte phagocytic capacity, as well as their oxidative burst activity in RPM cows.

Not only the dams may benefit, in terms of immunological responses, from RPM supplementation, but also their offspring. One of the leading causes of newborn calves' death is their immature immune system (Gruse et al., 2016). The first two weeks of life are the period of susceptibility to enteritis, diarrhea, septicemia, and pneumonia in calves (Svensson et al., 2006; Windeyer et al., 2014). A study isolated polymorphonuclear leukocytes (PMNL), which are the primary mediators of the innate immune response, from 3 week-old calves and cultured in different media (Abdelmegeid et al., 2017). The results showed that Met supplementation downregulated pro-inflammatory genes in PMNL, and enhance the synthesis of compounds, which may be beneficial for neonatal calves with immature immune system (Abdelmegeid et al., 2017).

Furthermore, despite there being limited research investigating the effects of RPM during heat stress in cattle, existing findings suggest positive outcomes linked to the administration of Met in animals under such conditions. For instance, in a study involving lactating Holstein cows (Pate et al., 2020), supplementation with RPM (Smartamine) was observed to preserve milk protein and fat concentration amidst a heat stress challenge. Nevertheless, DMI, milk yield, and feed efficiencies remained unaffected by RPM supplementation. Moreover, another investigation conducted by Pate et al. (2021) examined the impact of RPM supplementation on dairy cows experiencing heat stress. Although RPM did not attenuate the systemic immune activation induced by heat-stress challenge, the study demonstrated that RPM had a protective effect on the mammary gland (Pate et al., 2021). Under the heat-stress, the ratio of apoptotic cells to proliferating cells in the mammary tissue was drastically decreased in the RPM group (from 13.2 in the Control group to 3.9 in the RPM group) (Pate et al., 2021). The authors inferred that the increased plasma Met concentration in the RPM group, likely supplied the elevated demand in the mammary tissue required to synthesize cytoprotective proteins, such as heat-shock proteins, which are necessary for cellular defense against thermal stress (Pate et al., 2021). The duration of the treatment and RPM doses are summarized in Table 4.

5 Conclusions and future perspectives

Due to the aspects of protein digestion sites in ruminants, considerable efforts have been made to protect specific amino acids from ruminal degradation. Although there are some differences in

TABLE 4 Information about the breed, physiological status, RPM doses and sources, and duration of treatments used in *in vivo* studies addressed in the immune system section of the current review.

References	Breed/ physiological status	RPM level and source	Duration
		Immune System	
Osorio et al. (2013) Osorio et al. (2014a) Osorio et al. (2014b) Osorio et al. (2016)	Multiparous Holstein cows	0.19% of RPM/d (DM basis; MetaSmart™) 0.07% of RPM/d (DM basis; Smartamine® M)	51 d (21 d before calving to 30 d in milk) (Osorio et al., 2013, 2014a, 2016) Last 21 d of pregnancy (Osorio et al., 2014b)
Vailati- Riboni et al. (2017)	Multiparous Holstein cows	0.07% of RPM/d (DM basis; Smartamine® M)	51 d (21 d before calving to 30 d in milk)
Batistel et al. (2018)	Multiparous Holstein cows	0.09 and 0.10% of the DMI during pre and post-parturition respectively (Mepron®)	88 d (28 d before calving to 60 d in milk)
Pate et al. (2020) Pate et al. (2021)	Multiparous Holstein cows	1.05 g of RPM/kg of DMI (Smartamine® M)	–

the protection methods, they all aim to enhance the AA delivery to the intestine and improve animal performance. As an essential AA, the supplementation of Met is necessary for the maintenance of protein synthesis and influences overall metabolism in various functions. By providing methyl donors for the methylation of DNA and histones, Met actively establishes the epigenetic state of the early embryo. In terms of animal performance, RPM supplementation appears to have divergent responses depending on the developmental stage. In dairy cows, RPM alters milk composition, mainly by the enhancement of milk fat and protein. However, the extension of the accretion of these components varies according to the RPM source and the level of CP in the diet. For beef cattle, rumen-protected lysine appears to be more limited for growth than RPM, and therefore, should be the focus of further studies. The immune system of bovines and their offspring benefits from sources of RPM. While in the reproduction and performance of the future generations, RPM may improve oocyte quality, contribute to the birth of heavier calves, and alter the offspring's metabolism by activating adaptive mechanisms to face the metabolic demands of the postnatal period. Although there is an increasing number of studies evaluating the effects of RPM supplementation during distinct stages of an animal's life, mainly in dairy cows, a limited number of studies have adressed these effects in beef cattle, particularly regarding muscle development and beef quality. Furthermore, greater efforts to develop new and innovative models for predicting precise AA requirements in ruminants are still needed, which would improve the commercial system's efficiency and sustainability. Hence, future studies in this area can enhance precision nutrition and lead to a better understanding of the impacts of RPM on the final product and environment.

Author contributions

TCC: Writing – review & editing, Writing – original draft, Visualization, Investigation, Conceptualization, Methodology. KBN: Methodology, Conceptualization, Visualization, Investigation, Writing – review & editing, Writing – original draft. MACD: Writing – review & editing, Investigation, Visualization. MPG: Visualization, Conceptualization, Writing – review & editing, Methodology, Investigation, Writing – original draft. MSD: Conceptualization, Writing – review & editing, Methodology, Visualization.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This study was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC; Grant#401862), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Grant 315247/2020-7), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG; Grants APQ-02904-24; APQ-04181-22 and BDP-00215-22), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; Grant 001).

Conflict of interest

The authors declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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