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# Beneficial effects of rumen-protected methionine on nitrogen-use efficiency, histological parameters, productivity and reproductive performance of ruminants

Mohamed S. Ayyat[a], Adham Al-Sagheer[a], Ahmed E. Noreldin[b], Mohamed E. Abd El-Hack[c], Asmaa F. Khafaga[d], Mervat A. Abdel-Latif[e], Ayman A. Swelum[f,g], Muhammad Arif[h], and Abdelfattah Z. M. Salem[i]

[a]Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig, Egypt; [b]Department of Histology and Cytology, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt; [c]Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig, Egypt; [d]Department of Pathology, Faculty of Veterinary Medicine, Alexandria University, Edfina, Egypt; [e]Department of Nutrition and Veterinary Clinical Nutrition, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt; [f]Department of Animal Production, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia; [g]Department of Theriogenology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt; [h]Department of Animal Sciences, University College of Agriculture, University of Sargodha, Sargodha, Pakistan; [i]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, México

**ABSTRACT**
Providing essential amounts of balanced nutrients is one of the most vital aspects of livestock production. Among nutrients, protein has an essential role in many physiological functions of animals. Amino acids in needs for both high and medium yielding ruminant animals are not fully covered by microbial degraded feed sources in the rumen of animals, and they must be met by protecting the proteins from being broken down in the rumen; hence, the dietary supplementation of rumen-protected proteins (RPP), including mainly rumen-protected methionine (RPM), became imperative. Many researchers are interested in studying the role of (RPM) in ruminant animals concerning its effect on milk yield, growth performance, digestibility, dry matter intake and nitrogen utilization efficiency. Unfortunately, results obtained from several investigations regarding RPM indicated great fluctuation between its useful and useless effects in ruminant nutrition particularly during early and late lactation period; therefore, this review article may be helpful for ruminant farm owners when they decide to supplement RPM in animal's diet. Conclusively, supplementation of RPM often has a balanced positive influence, without any reported negative impact on milk yield, growth performance and blood parameters especially in early lactating ruminant animals and when used with the low crude protein diet.

**KEYWORDS**
Rumen protected methionine; milk; reproductive performance; histological change

## Introduction

Protein is a very essential limiting nutrient, especially in ruminants fed low-quality forages. The nutrient requirements, including protein, are varied according to the animal's physiological status (growth, lactation and pregnancy). Protein is essential for optimum growth and maximum milk production. Two types of digestible protein are required by ruminant. The first is a protein that is degradable in the rumen (rumen degradable protein, RDP), which is utilized by microbes to generate microbial protein. The second is a bypass protein (rumen protected protein, RPP), which is digested by animal enzymes in the small intestine. Protecting dietary protein from microbial degradation in the rumen allows extra amino acids passing to the small intestine, and therefore supplies additional absorbable amino acids per unit of absorbable energy (Figure 1).[1]

Ruminant performance can be improved by dietary supplementation with rumen-protected amino acids (RPAA), mainly methionine (Met) and lysine (Lys). The rumen microbes rapidly degrade most amino acids into ammonia, organic acids and carbon dioxide. The produced ammonia, the main nitrogenous nutrient, is essential for bacterial growth. Therefore, the balance between RDP and RPP is very important

**CONTACT** Abdelfattah Z. M. Salem asalem70@yahoo.com Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca 50010, México.

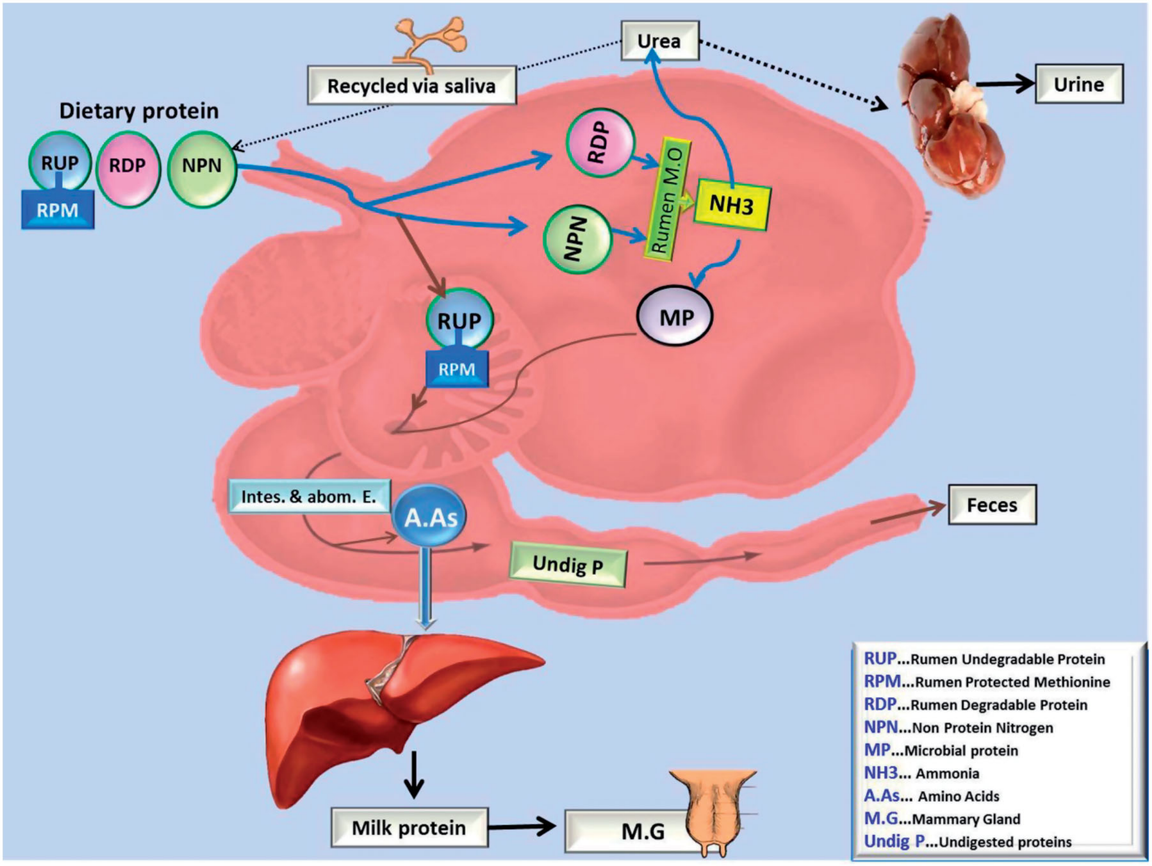


**Figure 1.** Protein digestion in ruminant; rumen undegradable protein (rumen protected methionine, RPM) bypassed directly without ruminal fermentation.

to meet the animal's AAs requirements. Methionine is an essential amino acid that could possibly be a limiting factor for the reproduction and lactation of dairy cows. A methionine codon is adopted for initiation of protein synthesis, and thus it plays an important role in cellular functions of mammals.[2,3] Supplementation with rumen-protected methionine (RPM) increases the percentage of dietary AAs that is absorbed from the intestine, especially Met, which addresses a serious limitation, enhances the overall utilization of nitrogen (N) in the diet,[4,5] and decreases the unfavorable environmental effects via modulation of urea kinetics. Several published studies indicated that, Met is the most limiting AA for milk production in dairy cattle fed diets containing legume forages, corn silage, soybean meal and corn grain.[6,7] Also, several studies[8–10] evaluated the effects of supplementation of rumen-protected methionine on milk production of dairy cows, and concluded an increment in milk protein percentage and milk protein yield. In addition, it has been demonstrated that RPM supplementation can enhance milk yield,[11] and milk protein levels.[12] Therefore, supplementing RPM with lower crude protein (CP) diets may allow farmers to feed their animals with this diet without losing milk and protein yields; the levels of urea in blood and milk, and its excretion through the urinary system are directly correlated with the dietary CP quantity;[13] as so the low dietary CP may increase the yield of milk and milk components.[14] As such, the current review article aimed to throw the light on the effect of RPM supplementation in ruminant diet regarding to its nutritional impacts, as well as its effect on nitrogen utilization efficiency, reproductive performance and hemato-histological parameters.

## General overview on rumen-protected methionine (RPM)

Extensive degradation of high-quality protein by microorganisms in the rumen results in some losses of nitrogen as urea in the urine. The objective behind the efforts for dietary protein protection is to maintain the valuable proteins from degradation and decrease wasteful ammonia generation in the rumen. The amino acid requirements for medium and high yielding cows and buffaloes are not fully covered by microbial sources, and therefore, they have to be covered by protecting the proteins from a breakdown in the rumen.[1] Dietary proteins can be protected from being broken down in the rumen via numerous procedures, including chemical modification, heat treatment and proteolytic activity inhibition, also natural protected proteins may be identified.[15]

All AA exist as the isomers D and L, which are chemically identical, the one being a mirror image of the other. Rumen protected methionine (RPM) fed in the D-form is absorbed into the plasma but needs to be converted into the L-isomer within tissues prior to its incorporation into animal proteins. The efficiency of conversion of commercial Met products from the D to L form has been of some concern, but studies have reported that the efficiency of use of D-Met, relative to L-Met, was 960 g/kg in growing steers.[16]

Methionine and cysteine are the two sulfur-containing essential proteinogenic AAs. In animals, Met can act as a precursor of cysteine, which is present mainly in structural proteins, including keratin or collagen in hair, skin, feathers and nails. The highest Met content, of approximately 5%, can be present in albumins, particularly egg albumin, which is a water-soluble protein. This is the main reason for the great Met need of poultry.[17]

L- and D-Met are the main isomers of Met. L-Met occurs most commonly in nature. In animals, L- and D-Met are metabolized by DL-racemase, which is essential for the use of chemically synthesized DL-Met racemase in industrial livestock farming as a feed additive (see below). In poultry diets, no notable difference exists in consuming L- or D-Met.[17] Many fungi, bacteria and plants can produce Met from organic or inorganic N, carbohydrates and sulfur sources. Nevertheless, animals rely on external Met sources. In organic farming, particularly pig and poultry breeding, Met supplementation becomes a problem, as Met is regarded as the first and third most limiting AA in poultry and piglet rations; respectively.[18]

Toledo et al.[19] concluded that the daily top-dressing of rumen-protected methionine might produce an acute increment in circulating methionine, and a minor improvement in percent milk protein. Furthermore, rumen-protected methionine feeding was reported to increase embryo development, and decrease pregnancy loss. But, these reproductive impacts were merely clear in multiparous cows. Thus, increasing plasma methionine levels after feeding cow the rumen-protected methionine was adequate to induce a small increment in milk protein percentage and to improve embryonic size and maintenance of pregnancy in multiparous cows.

Methionine deficiency in the diet has been associated with many health problems, including toxemia, muscle paralysis, childhood rheumatic fever, depression, hair loss, schizophrenia, liver deterioration, Parkinson's disease and altered growth.[20] This emphasizes the importance of Met in mammals. Additionally, a hereditary defect in Met metabolism in humans causes cystathioninuria, homocystinuria and hypermethioninemia, which have different signs including mental retardation, failure to thrive, clubfoot, thrombocytopenia, skeletal abnormalities, hearing defects and lens dislocation. When the metabolism of Met is deregulated, Met levels are highly increased.[21]

## Historical development of RPM

The concept of protecting free Met from ruminal degradation has been in place since 1960, when it became clear that the profile of absorbed Met from abomasal, intestinal and intravenous infusion experiments was not constantly ideal in ruminants;[22,23] these studies established that, sulfur AA were the main limiting AA for wool development and body weight increase of sheep and that Met was a limiting AA for both growing and lactating dairy cattle. Therefore, many laboratories tried to develop steps to guard Met from ruminal degradation.[22] Consequent interest in keeping Lys developed once its role was discovered as the second most essential limiting AA for growing lambs, and either the first or the second most important limiting AA for both growing and lactating dairy cattle. Numerous studies have been conducted to try to protect Met and Lys from ruminal degradation. Preliminary efforts focused on guarding Met with lipids, usually in mixture with inorganic materials and carbohydrates as softening agents, stabilizers and fillers. For instance, in the 1960s Delmar Chemicals of Canada produced a 20% Met product in which a core of DL-Met, tristearin and colloidal kaolin, was wrapped in a tristearin film. After a short time, Rumen Kjemi a/s of Oslo, Norway presented a more efficient product called Ketionin®, which offer a better intestinal liberation of the encapsulated Met. Ketionin® contains 30% DL-Met, 2% glucose, 4% stabilizers, antioxidants, flavoring agents, 6% $CaCO_3$, 58% tristearin and oleic acid.

Many other lipid-protected Met products have also been assessed. The main difficulty with lipids using as the principal encapsulating material is to find a mixture of materials and processes that have a great ruminal escape and intestinal release of Met. Nowadays, the most efficient method is surface-coating of Met with enzyme-resistant, pH-sensitive synthetic polymers that are insoluble in the neutral pH environment of the rumen, but are highly soluble in the acidic abomasum. This method depends on the pH differences between the rumen and the abomasum for ruminal guard and intestinal release. Polymer-protected Met has greater ruminal protection and intestinal release coefficients, compared with other products. Currently, the patent rights for the utilization of pH-sensitive polymers for keeping nutrients from ruminal degradation are held by Adisseo, Inc., Antony, France.

The adoption of analogs and derivatives of Met is another method that has been utilized for incrementing the supply of Met to ruminants. Amino acid derivatives are free AA to which a chemical hindering group has been added to the α-amino group, or in which the acyl group has been manipulated. Isopropyl-DL-Met, t-butyl-DL-Met, N-stearoyl-DL-Met, N-oleoyl-DL-Met and capryl-caproylic-DL-Met are examples of Met derivatives that have some resistance to ruminal degradation.[24] The shorter chain has greater ruminal escape than longer chain alkyl esters. However, most of the Met derivatives have not been sufficiently evaluated regarding their capacity to increase the post-ruminal sources of absorbable Met. Amino acid analogs result from the replacement of the α-amino group of the AA with a non-nitrogenous group, such as a hydroxyl group. Met hydroxy analog (MHA; DL-α-hydroxy-γ-mercaptobutyrate), more commonly known as 2-hydroxy-4-methylthio butanoic acid (HMB), is the most studied AA analog. Free HMB is more unaffected with ruminal degradation than free Met. Ruminants possess the enzymes that convert HMB into Met. Additionally, it can be absorbed via passive diffusion from the rumen and omasum. Nevertheless, owing to its slight influences on blood Met levels and milk protein amounts when given to lactating dairy cows fed Met-deficient feeds, it seems that HMB has the low capability to replace absorbed Met in dairy cows. Also, it was reported that numerous esters of HMB improve its ruminal escape, probably in part due to their obvious capacity to be absorbed via the rumen wall. The isopropyl ester of HMB has an excellent ability to substitute absorbed Met.[25,26]

## A comparison of some available products

Smartamine™ M has the highest efficiency as a source of absorbable Met compared to the other products described in the current review. Nylon bag studies show that ruminal stability of Smartamine™ M exceeds 90% at 24 h. Furthermore, its intestinal release value is around 90%, which is estimated either by the quantity released after 1 h in an HCL-pepsin (pH 2.0) solution or by the mobile bag technique following exposure to the HCL-pepsin solution. In in-vivo experiments using early lactation cows, a cannulated cow had 90% ruminal escape values across four different diets, and an intestinal disappearance value of 98%, resulting in an average Met bioavailability value of 88%.[27]

The efficiency of Mepron® M85 as a source of absorbable Met is lower than that of Smartamine™ M and higher than that of lipid protected products. This result has been confirmed by *in vitro* studies and other studies using the above-mentioned approaches. Mepron® M85 degrades in the rumen faster than Smartamine™ M, and results in a significantly greater increment in plasma sulfur AA levels than Smartamine™ M. A conclusion of in situ nylon trials demonstrates ruminal protection values that approximate 90% at 2 h, 85% at 6 h, 70% at 12 h, 60% at 24 h and 15% at 96 h. Assessments of passage rates from the rumen, which have not been reported, are required to estimate ruminal escape. Additionally, Mepron® M85 is less digestible in the small intestine than Smartamine™ M. Usage of the mobile bag technique has shown that 25–70% of the Mepron® M85 arriving in the small intestine is lost in the feces, and the amount of loss increases with increased feed intake. It seems doubtful that the mobile bag technique is a suitable process for evaluating the intestinal Met release from an RPM product such as Mepron® M85, as it depends on abrasion and physical forces for its degradation. The effects of supplementary RPM on productive performance of dairy animals are listed in Table 1.

## Impacts of using RPM in nutrition

### *Digestibility and dry matter intake (DMI)*

#### *Cattle*

Normally, feeding cattle with silage and hay, which contain a low amount of dietary protein, is enough for limited milk production or growth. Researchers may supplement cattle feed with RPP to elevate the production levels.[28] The bioavailability of protected Met in the intestine varies from 22 to 32%.[29] Researchers have detected variable influences on dry matter intake (DMI) after supplementing basal diets of cattle with various levels of RPM. Supplementing cattle with RPM alone did not affect the performance of finishing calves, although the combination of Met and Lys more effectively improved the performance of ruminants.[30] Animals fed with high forage and maize-based ration balanced with RPMet either alone or in

**Table 1.** The effect of rumen-protected methionine (RPM) supplementation on ruminant productive performance

| Animal species | RPM source | RPM level | Duration | Effects | Reference |
|---|---|---|---|---|---|
| Holstein cows | Mepron, Evonik Corp., Kennesaw, GA | 4.2–12.4 g/d | 4 weeks | 15% CP diet + 10.3 g RPM/d increased milk protein, reduced N excretion, and improved N efficiency without altering the digestibility of DM, OM, and fiber. | Nursoy et al.[106] |
| Holstein cows | Smartamine M; Adisseo, Antony, France | 0.07% | 21 days | DMI, nutrient digestibility, and milk protein synthesis were not influenced by supplementing ground field peas with RPM | Pereira et al.[96] |
| Holstein cows | Mepron, Degussa Corp. | 5–15 g/d | 4 weeks | • Feeding 14.8% CP diet + 15 g RPM/day improved N efficiency and reduced urinary N excretion.<br>• 16.1% CP diet and 10 g/d of RPM (17 g/d of Mepron) achieved the best compromise between milk production and lowered N excretion. | Broderick et al.[93] |
| Holstein cows | Smartamine M; Adisseo Inc., Alpharetta, GA | 13.7 g/ day (estimated to deliver 8.0 g of intestinally absorbable) | 28 days | 17% canola meal rations + RPM increased milk yield and protein and fat content, but reduced milk lactose yield and concentration. | Swanepoel et al.[107] |
| Holstein cows | Commercial product | 30 g/d (supplied 7.65 g of metabolisable Met) | 8 weeks | Enhanced milk yield when metabolisable protein supply was adequate | Awawdeh[84] |
| Dairy cows | Mepron (Evonik Industries, Hanau, Germany) and Smartamine (Adisseo, Antony, France) | Meta-analysis study | | True milk protein (TMP) % and yield was increased<br>DMI and milk fat % were slightly reduced<br>Milk yield and milk fat yield were slightly increased.<br>Mepron-fed cows producing less TMP % but, more milk production, and twice TMP yield. | Patton[9] |
| Merino ewes | Met-Plus, Niso Shoji Co., Japan | 6.3 g/d | Day 111 of pregnancy to day 7 after lambing | • 10% higher lamb birth weights<br>• Elevated total IgG in lambs aged 4 and 6 weeks.<br>• RPM did not alleviate the oxidative stress of ewes during the transition period | Liu et al.[108] |
| Massese ewes | Methioby® | 5 g/d | 17 days | • Increased fat corrected milk (6.5%) and blood urea.<br>• DMI and nutrient digestibility were not affected. | Antongiovanni et al.[109] |
| Comisana ewes | (Silo, I-50100 Florence, Italy) | 3.5 or 7.0 g/kg | 16 weeks | Increased 16:0 and 18:3 but, decreased 4:0 and 12:0 milk fatty acids. | Sevi et al.[59] |
| Lactating Saanen goats | Mepron M85, Degussa Co, Allendale, NJ | 2.5 or 5 g/d | 30 days | 2.5g RPM improved milk production and milk protein. | Flores et al.[110] |
| Toggenburg × French Alpine × Saanen crossbred goat | Mepron 85 Degussa México Co. | 1, 2 or 3 g/d | 110 days | • DMI, milk yield, and milk composition were not changed.<br>• Positive energy balance. | Alonso-Melendez et al.[111] |
| Shami goats | Calcium salt of methionine hydroxyl analogue, min. 84 %; Novus, Saint Charles, MI | 2.5 or 5 g/d | 60 days | • Feed intake, milk production, and fatty acid composition were not altered.<br>• Weaning weight and average daily gain of kids were not changed | Al-Qaisi and Titi[112] |
| Danish Landrace × Saanen crossbred goats | Smartamine™, Aventis Animal Nutrition, Rhone-Poulenc, Antony Cedex, France | 6 g combined with 15 g ruminally protected lysine | 2 weeks | Positive response on mammary uptake of lysine and methionine in late compared to early lactation. | Madsen et al.[113] |

combination showed an increased digestibility of CP.[31] Noftsger et al.[32] evaluated the influences of RPM on rumination in lactating cows. They noticed that the apparent digestibility level of organic matter and *in vitro* neutral detergent fiber (NDF) of the diets fortified with RPM were higher than in control diets. However, the bacterial N entering the omasum, the concentration of ammonia and rumen volatile fatty acids profile were not altered.

Pre-partum and post-partum dairy cows fed basal rations supplemented with Lys and RPM that contained 16–18.5% CP showed no elevation in DMI level.[33] Instead, Piepenbrink et al.[34] observed higher DMI of the ration with 18% CP without any supplement relative to the ration containing 14% CP supplemented with Lys and RPM. Moreover, basal rations of multiparous cows supplemented with 2% of RPM did not influence DMI.[35] Nevertheless, Armentano et al.[35] reported that the threshold response to RPP fortification is when rations have at most 7% CP. Socha et al.[33] observed an improvement in the absorption of intestinal AAs in pre-partum and post-partum cows. However, there was no influence on body weights for cows receiving diets supplemented with a mixture of RPM and Lys, and containing 16 and 18.5% CP.

### Sheep

Haddad et al.[36] recorded an enhancement in the digestibility of dry matter (DM) and CP with an increase in RPM supplementation (positive correlation). On the contrary, Soto-Navarro et al.[37] observed an increase in N digestibility and ruminal organic matter after using 12% and 15% fish meal in diets of goats. The increased duodenal N flow when dietary levels of a fish meal increased is due to increases in ruminal N recycling.[38] Fahmy et al.[39] observed a decrease in the digestibility of NDF in lambs fed with corn gluten meal and fish meal versus soya-bean meal in roughage based diets. They suggested that soya-bean meals result in high-quality AAs in the intestine.

Collectively, previous investigations still fluctuated between the useful and useless impact of RPM supplementation in ruminant diets; therefore more studies concerning the effect of RPM on digestibility, rumination and DMI are still needed.

## Growth performance and microbial protein absorption

### Cattle

Feeding cattle with low amounts of RPM is useful for animals with AA limitations. Greenwood and Titgemeyer[40] observed reduction in N emissions after supplementation of a low CP diet with RPM. In addition, digestibility, rumen fermentation, microbial crude protein, and *R. albus* population were similar to low protein with high Met to that of high protein diet. However, production of $NH_3$–N, total gas and methane volume were significantly higher in the high protein group than low protein low Met, low protein high Met and low protein diets.[41] Moreover, Klemesrud et al.[30] reported that addition of metabolizable Lys to rations improved the performance of finishing calves.

Diets containing insufficient amounts of metabolizable AAs may reduce protein accumulation during pregnancy. Waterman et al.[42] observed that supplementation with a mixture of 5 g/d of RPM and urea improved protein accretion and N efficiency during late pregnancy. Supplementing RPM to tall fescue hay increased both N retention and N digestion, and decreased daily urine N excretion in beef steers. Archibeque et al.[4] observed that supplemented RPM can meet the protein daily requirements by increasing the amount of N utilization by steers.

### Sheep

The protein requirements of fast-growing sheep exceed the protein generated by bacteria.[43] The new theory of protein supply for small ruminants is focused on increasing microbial protein absorption by the intestine through the provision of dietary protected protein that escapes without degradation in the rumen.[44] Usually, ruminants do not require EAAs in the diet. But, after microbial protein synthesis in the rumen is decreased, or AA needs are not sufficiently met animal production decreases.[45] The quality and quantity of AAs absorbed from the small intestine is greatly affected by supplemental RPP sources and microbial protein synthesis.[46] Dove and Robards[47] observed a positive effect on wool-yielding sheep production after abomasum infusion with Met and casein. Can et al.[48] reported an improvement in feed efficiency and DMI in male lambs fed rations composed of 5% RPM and 16% CP, although Hussein and Jordan[49] observed the opposite. Ponnampalam et al.[50] observed that supplementation of RPM to low-quality diets increased the CP and DM in small ruminants maintained at high ambient and neutral temperatures. Haddad et al.[51] investigated the influence of optimal dietary CP on the finishing rations of lambs. They used 10-18% CP in the diets and detected an increment of CP and DMI with increasing protein levels in the diets. Moreover, Habib et al. [44] detected an improvement in growth performance of lambs supplemented with RPM, due to the high Met requirement of growing Awassi lambs throughout the last stage of finishing.[52] Later, the results reported by Abdelrahman et al.[52] were confirmed by Ponnampalam et al.,[50] who observed that fish meal increased feed efficiency and growth rates, as compared with soybean meal and canola meal. In contrast to the previous report, Fahmy et al.[39] reported that roughage based diets supplemented with soybean meal resulted in more average daily gains than those obtained from supplemented corn gluten meal or fish meal. In general, supplementation of rumen protected protein in ruminant diet can play a part in improvement of animal growth performance and absorption of microbial protein.

## Reproductive performance

Methionine could potentially be limiting for reproduction in lactating dairy cows.[3] Several studies have linked concentrations of Met with optimal early embryonic development.[53] A recent *in vivo* study demonstrated that feeding RPM in lactating dairy cattle produced dramatic alterations in gene expression in embryos, generally decreasing concentrations of mRNA in early embryos.[54] Also, Met is concentrated in uterine and embryonic fluids in both sheep[55] and cattle,[56] suggesting a role for elevated uterine Met in normal embryonic development and survival. Recently, Toledo et al.[19] evaluated the effects of daily top-dressing (individually feeding on the top of the total mixed ration) with RPM from 30 ± 3 until 126 ± 3 days in milk on productive and reproductive performance in lactating dairy cows and they concluded that the increase in plasma Met concentrations after feeding RPM was sufficient to produce a small increase in milk protein percentage and to improve embryonic size and pregnancy maintenance in multiparous cows.

**Cattle:** In early lactating cows, supplementation with rumen-protected methionine (RPM) and Lys (RPL) or RPM alone had no influence on milk fat and true protein contents.[33,57,58] On the contrary, a significant increase in milk protein was reported after addition of RPL and RPM with two levels of protein in basal rations during mid or early lactation, although there was little effect on milk production.[28,59] The supplementation of ruminally protected methionine (Mepron®) at 18.2 g per head per day had beneficial, but small and mostly statistically insignificant effects on milk performance and milk composition.[60] Piepenbrink et al.[34] reported that supplementing RPM and Lys to diets containing 14 and 18% CP yielded high protein-content of milk and milk production. Also, Holstein cows with a high milk production need supplementation of RPM (16 g/d) to enhance milk production.[61]

Alfalfa hay-concentrate based diets supplemented with a mixture of 1.03% Lys and 0.52% Met provided optimal levels of Lys and Met to the ruminal microorganisms and enhanced ruminal fermentation and intestinal absorption of protected AAs. The results obtained from the *in vitro* study were confirmed by the findings of the *in vivo* study, which demonstrates that ruminants respond positively to diets supplemented with RPM and Lys. Data obtained from an *in vivo* study indicated alteration of post-ruminal supplies of EAAs and ruminal fermentation during late lactation. However, these effects may be related to energy partitioning in body tissues more than significant effects on milk contents and milk production. The author thought these results could be explained by a decrease in the utilization of RPM and Lys mixture to a less than optimal dose, a short experimental period, and the late lactation stage of cows.[62] In another study, supplementing RPM led to elevated concentration of glucose and urea in arterial plasma, but milk composition and production were not affected.[12,34] However, Misciattelli et al.[63] and Rulquin et al.[64] found that supplementing dairy cows with RPM resulted in an elevation in milk fat and protein contents; respectively. The authors also found that supplementing dairy cows with RPL and RPM elevated protein yield in comparison with previous reports, although these effects were not significant. Feeding dairy cows high levels of RPM increased the use of N in milk production. Hence, using RPM led to maximum N efficiency which consequently resulted in higher milk production with high protein content. This demonstrates that post-ruminal digestibility of RPM has more impact than total RPM supplementation. No interaction effects were noted between RPM and CP levels on milk composition and production in dairy cows. Supplementing cows with RPM did not influence N levels in milk, feces, or urine.[64] Fahey et al.[65] detected significant positive effects of supplementation with Met hydroxyl analogs and fatty acid calcium salts on blood cholesterol concentration, milk lactose production and milk yield. However, a positive effect on reproductive performance was limited to cows at first lactation.

Bach and Stern[66] detected high plasma Met concentrations after supplementing the diet of Holstein cows with 0, 30 and 60 g of RPM/day. Supplementing the diet of cows with RPM having a low CP content had little effect on milk protein content and milk yield.[67] Girard and Matte[68] observed positive effects of vitamin $B_{12}$ injections on lactating cows fed diets fortified with RPM and folic acid. They reported an increase in lactose, milk solids, fat and energy corrected milk yield. On the contrary, few studies[69,70] concluded that milk composition and its yield were not influenced by supplementing cows with RPM and Lys. Also, supplementation of RPM did not change the net mammary intake of Met.[12] The intestinal absorption of Met and Lys is very low in cattle fed silage diets because most of the microbial protein synthesis in the rumen is used up during milk production.[71]

Dinn et al.[58] investigated the outcome of the addition of rumen-protected (RP) Lys and Met to rations with low CP on milk protein and total milk yields. The authors observed no changes in milk protein content between cows fed rations fortified with RPM and cows fed diets with normal CP.[58] However, the CP digestibility, milk production, blood urea N and urinary N excretion declined as the percentages of CP decreased in the rations. Moreover, milk fat was affected.[59] The supplementation of cows with RP Lys and Met resulted in increased ratios of palmitic, palmitoleic, stearic and oleic fatty acids in milk. Additionally, the blood Met, cysteine and taurine concentrations were increased after adding RPM to rations, also a positive effect of RPM in minimizing milk urea was observed.[72] Interestingly, a substantial effect of grass silage and grain compound mixture supplemented with RPM was evident on milk protein (casein), milk fat and daily milk yields.[73] Additionally, fortification of RPM with meat-bone meal and hydrolyzed feather meal improved milk fat and protein contents.[74]

Supplementation of RPL and RPM improved the lactation performance of dairy cows. The Met hydroxyl analog and D, L-Met have similar actions in improving milk fat, milk yield, days to first service, body condition score and somatic cell count in dairy cows.[75] The best results were obtained in animals at the early lactation stage and fed low protein diets supplemented with RPM.[76,61] No change in total digestive tract N digestion was detected when supplementing diets of non-lactating dairy cows with Met and Lys. Moreover, no alterations in ruminal fermentation or microbial protein production were reported. Supplementing diets of late lactating cows with Met and Lys did not improve the efficacy of milk production.[77]

Pruekvimolphan and Grummer[74] reported that adding RPM, meat meal and bone meal to a mixture of 50% alfalfa silage and 50% corn-based concentrate rations increased milk CP percentage, milk yields and DMI in cattle. Socha et al.[33] observed increased milk true protein yield and milk fat but decreased plasma glucose concentrations in early-lactation cows after they were supplemented with RPM. This positive response of early-lactation cows depends on the intestinal digestibility of the RPL and RPM, dietary CP concentration and metabolizable protein supply. Girard et al.[78] studied the synergistic effects of mixing folic acid and RP Met on indicators of folate metabolism and lactational performance. RPM increased the concentration of total milk solids but did not affect milk yield. Supplementing the diet of cows fed on RPM with folic acid had adverse effects on milk lactose, casein and CP concentrations. Cows supplemented with RPM had lower serum cysteine, but there was no effect in cows fed control diets. Ayala et al.[79] studied the effects of supplementing cow diets with fat and RPM (fat alone, RPM alone and fat plus RPM elevated milk production) on energy balance, milk quality and milk production; cows supplemented with RPM showed the lowered percentage of milk fat and elevated the percentage of milk protein. Moreover, fat alone, RPM alone and fat plus RPM elevated milk lactose, milk protein and milk fat concentrations. In addition, supplementing cows with RPM resulted in decreased urea and increased plasma glucose levels. Furthermore, Yang et al.[80] studied the impact of different levels of RBM on serum AA metabolism and dairy performance. No change was detected in the percentage of lactose and milk protein. However, cows fed 42 g RPM had significantly higher milk yield than cows in the control group. Additionally, supplementing cows' diets with 56 g RPM resulted in a remarkable increase in the percentage of milk fat. The supplementation of cows' diets with RPM decreased the level of serum AAs, with the exception of Arg and Met.

Lee et al.[81] evaluated the effect of adding RP histidine (His) (RPH), Met and Lys to a metabolizable protein-deficient diet on the performance of dairy cows. The incompletely metabolized protein diets decreased urinary N excretion, plasma urea N and the total tract digestibility of all measured nutrients. Milk protein yield was significantly increased by supplementation of deficient metabolized protein diet with Lys, Met and His, and was not different from adequate metabolized protein. Therefore, the supplementation of the deficient metabolized protein diet with RPM and RPL lowered the variance in milk yield and DMI compared with a diet containing adequate amounts of metabolized protein. The metabolized protein-deficient diets lowered urinary N losses and elevated milk N efficiency. Furthermore, Li et al.[82] measured the level to which milk protein was affected by feeding cows with diets supplemented with RPL and RPM and with distillers dried grains with solubles (DDGS). Compared with the control group, Met and Lys elevated 4% fat-corrected milk, milk yield, protein in milk and energy-corrected milk yield. Lys and Met elevated the EAAs and total AAs in milk, which could be attributed to the high available amount of Lys and Met in the intestine by DDGS.

Amrutkar et al.[83] studied the effect of adding RPM, RPL and rumen-protected choline (RPC) on high-yielding crossbred cows. Results revealed that cows in the Met-Lys group had better body conditions and higher milk components (lactose, protein and fat) yield. However, cows in the choline group had the highest concentrations of choline, vitamin E, unsaturated fatty acids and monounsaturated fatty acids in milk. All groups had similar amounts of non-esterified fatty acids, glucose, plasma urea N and cholesterol. Furthermore, cows in the Met-Lys and choline groups had the lowest concentration of very low-density lipoproteins and triglycerides. Therefore, supplementing the diets of high-yielding crossbred cows with RPM, RPL, or RPC was beneficial during the early lactation period.

Recently, Awawdeh[84] studied the impacts of RPM alone or with RPL on plasma AAs and milk yield of dairy cows. The average of daily milk yield was 28 and 30 kg/cow for the control and Met-Lys group, respectively. There was no effect of several treatments on milk contents of lactose, fat, total solids, or solid non-fat. Cows in the Met-Lys group had the greatest milk protein content. Supplementation with RPM had no effect on plasma levels of all AAs. Therefore, supplementing the diets of cows with RPM plus RPL resulted in a greater milk protein content and milk yield than supplying RPM alone.

The beginning of lactation in dairy cows is characterized by a severe negative protein and energy balance. Increasing Met availability during this period may improve immune function, lipid metabolism in the liver and milk production. Therefore, Batistel et al.[85] tried to assess the performance of dairy cows fed with ethyl-cellulose RPM (Mepron®, Evonik Nutrition and Care GmbH, Hanau-Wolfgang, Germany) throughout the pre-partum and early lactation periods. Cows fed ethyl-cellulose RPM had overall greater cumulative DMI than cows in the control group. Compared with controls, throughout the fresh period (1–30 days in milk; DIM) feeding ethyl-cellulose RPM incremented milk yield by 4.1 kg/d, fat yield by 0.17 kg/d, milk protein yield by 0.20 kg/d, 3.5% fat-corrected milk by 4.3 kg/d, and energy corrected milk by 4.4 kg/d. The addition of ethyl-cellulose RPM increased milk protein content by 0.16 percentage units relative to the control throughout the fresh period. No differences were noted for milk fat, lactose and milk urea N concentration. Throughout the high-producing period, cows fed ethyl-cellulose RPM increased DMI and milk yield by 1.45 and 4.4 kg/d, respectively. Ethyl-cellulose RPM also increased fat yield by 0.19 kg/d, milk protein yield by 0.17 kg/d, 3.5% fat-corrected milk by 4.7 kg/d, and energy-corrected milk by 4.8 kg/d compared with those in control group cows. Ethyl-cellulose RPM addition decreased plasma fatty acids in the fresh period, and decreased $\gamma$-glutamyl transferase, demonstrating improved liver function. Therefore, the greater milk production was, at least in part, driven by the greater voluntary DMI and better liver function. Recently, Titi[86] studied the influence of adding RPM to the diets of Shami goats during early lactation and the last 60 days of pregnancy on the growth performance of kids, and on the composition, production, and fatty acid profile of goat milk. Results revealed that supplementing diets with RPM had no effect on birth and weaning weights, or on the average daily weight gain in kids. However, there was a substantial improvement in the milk to gain ratio of kids. Goats in the RPM group had a significant increase in milk production. Milk protein content, energy-corrected milk and casein were also highest in the milk from goats in the RPM group. However, there was no difference in milk fat content among the different groups. Moreover, goats in the RPM group had the best feed to milk ratio. There was no change in milk fatty acid composition after RPM treatment. Therefore, supplementing goat diets with RPM during late pregnancy increased milk protein content and milk production in Shami goats, but did not influence the birth weight or growth rates of suckling kids. Conclusively, it may be suggested that dietary supplementation of rumen-protected proteins has a positive response on increasing milk yield and milk constituents from fats and protein particularly in early lactating cow, however, it was less effective in late lactating cows.

## Blood parameters

Blood parameters are the real indicators of the health status of an animal. Liker et al.[87] studied the influence of RPM on some hematological values and blood biochemistry of cows. A significant decline in plasma glucose concentrations in cows was observed in the RPM group. However, no clear variations were detected in plasma concentrations of alkaline phosphatase, gamma-glutamyl-transferase (GGT), and asparate amino-transferase (AST) between groups. RPM decreased the red blood cell count of cows. The authors concluded that RPM had anti-stress effects on cows in winter stable feeding during late gestation. Furthermore, Liker et al.[88] studied the effect of RPM on hematological and biochemical parameters in the blood of growing beef cattle (including albumin, total protein, total cholesterol, triacylglycerol, urea, glucose, alanine aminotransferase (ALT), creatinine, GGT, AST, red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), hematocrit value (Hct), hemoglobin concentration (Hb), differential blood count and white blood cell count (WBC)). RPM increased the activity of ALT, and the total cholesterol and plasma glucose concentrations. However, RPM decreased the plasma urea concentration. No significant effects of RPM on albumin, total protein, creatinine plasma concentrations, triacylglycerol, GGT and AST activities were reported.

Ardalan et al.[89] investigated the effects of supplementing cows with RPC and RPM on plasma metabolites. No effects of feeding cows with RPM and RPC were detected on the levels of glucose, plasma triglycerides, non-esterified fatty acids (NEFA), total protein, $\beta$-hydroxybutyrate (BHBA), AST, or plasma urea nitrogen (PUN) during the pre-partum period. On the contrary, RPM decreased plasma protein concentrations and increased plasma AST concentrations in post-partum cows. Following calving, feeding RPC increased 40 g/kg fat-corrected milk yield, milk yield and DMI.

Jacometo et al.[90] studied the impacts of RPM on blood indicators and liver functions of neonatal calves. They detected a rise in plasma insulin concentrations and greater expression of insulin receptors, which led to a lower blood glucose level. Collectively, the results indicated that RPM improved the maturation of fatty acid oxidation and gluconeogenesis in the liver, which would be beneficial for extrauterine life. Moreover, Jacometo et al.[91] observed the effects of adding RPM throughout the last four weeks of pregnancy on hepatic amply of genes linked with trans-sulfuration, DNA methylation and Met metabolism, which confirms the processes of maturation of fatty acid oxidation and gluconeogenesis in the liver.

Moreover, oxidative stress parameters were also measured in milk and plasma of ewes, including free radical scavenging action and total antioxidants. The results verified that diets supplemented with RPM significantly elevated the ewe's total milk solids and fat content. RPM supplemented diets had no significant effect on the fatty acid profile of ewe milk. Ewes fed with RPM supplemented diets had significantly higher free radical scavenging potential in their milk than that of the control group ewes. Moreover, the glutathione transferase level was significantly higher in the plasma of ewes fed the RPM ration, than in the control. Moreover, a significant increase and a trend for an increase (20 and 16.72%, respectively) were recorded in the growth rates of lambs suckling from ewes fed with RPM diets, compared with that in the control group lambs. In general insight, dietary supplementation of RPP has limited effect on blood parameters and liver enzymes.

## Nitrogen utilizing efficiency and nitrogen pollution

A high CP content results in high N volatilization. Therefore, to decrease N emissions, the CP content in rations should be reduced,[92] but this will also reduce dairy milk production. To solve the problem of N pollution without a decrease in milk production, many studies have aimed to produce a standard ration that results in ideal N emission and dairy production. The most suitable way to lower dietary CP content without losing milk production is to supply the exact amount of the indispensable AAs at par with their limiting amounts.[58] The influences of RPM supplementation on N volatilization and N use were studied by Kröber et al.;[92] the authors found no effects of RPM supplementation on fecal and milk N excretion. However, the feed N utilization increased with a decreasing CP proportion in rations supplemented with RPM. Therefore, the amount of ammonia released from fresh slurry significantly decreased. Moreover, the authors reported that the usage of additional Met supplemented to low CP rations reduced the urinary excretion of total protein. Broderick et al.[93] studied the effects of RPM with low CP rations, and found Apparent N efficiency was the highest for the lowest CP diet with the greatest RPM. Moreover, low CP diets reduced milk urea N and urinary N excretion.

Chen et al.[94] compared the effects of diets supplemented with isopropyl-2-hydroxy-4-(methylthio)-butanoic acid (HMBi) and diets supplemented with RPM on N-utilization in lactating dairy animals. Diets supplemented with RPM resulted in 80% of the total absorption of Met from the intestine, while for diets supplemented with HMBi, this was 50% only. However, the authors detected similar improvements for milk production and N use in HMBi and RPM groups. The authors reported that when HMBi or RPM was added to a 15.6% CP diet, the response was superior to that in cows fed a 16.8% CP diet, but with improved N utilization and reduced N excretion, and this was particularly true for supplementation with RPM rather than supplementation with HMBi.

Arriola Apelo et al.[95] tried to reverse the trend of milk yield reduction resulting from feeding cows a decreased protein diet to transition lactating dairy cows by adding one or more ruminal protected AAs. They studied the effects of low CP diets fortified with rumen-protected Met, Lys, Leu, or a mixture of these. The authors did not observe any adverse effects of the low CP diet combined with supplemented AAs on fat and milk yield. Yet, milk protein and lactose yields declined for cows fed a low CP diet mixed with supplemented AAs. Moreover, milk urea N concentrations were lesser for all diets, proposing that higher N efficiency was achieved by a mixture of rumen-protected AAs.

Previous research has found that feeding cows ≥24% of the diet dry matter (DM) in the form of field peas decreased milk yield and concentration and yield of milk protein; probably due to decreased DMI and limited amounts of Lys and Met. Pereira et al.[96] divided cows into four groups fed different supplemented rations: (1) 36% ground corn, 2.4% soybean meal and 1.3% urea (UR), (2) 29.7% ground corn, 9.8% soybean meal, 0.13% RPL and 0.07% RPM (CSBAA), (3) 25% ground field peas, 12.3% ground corn and 2.4% soybean meal (FP), or (4) FP containing 0.15% RPL and 0.05% RPM (FPAA). The authors' aim was to assess the impacts of FP versus UR, FPAA versus CSBAA and FPAA versus FP on milk yield and composition, N usage, nutrient digestibility, ruminal fermentation profile and plasma content of AAs. Milk yield did not vary between diets. Relative to cows fed UR, those fed FP had higher DM intake, the concentration of milk true protein, apparent total tract digestibility of fiber, urinary release of purine derivatives and total volatile fatty acid concentrations in the rumen and Lys in plasma, as well as lower concentrations of milk urea N and ruminal $NH_3$–N. The milk urea N concentration, together with the level and yield of milk fat, was higher in cows fed FPAA diets than in cows fed CSBAA diets. Additionally, cows fed FPAA diets had a higher concentration of ruminal total volatile fatty acids, higher amounts of isobutyrate and acetate and lower levels of valerate and propionate than cows fed CSBAA diets. The plasma proportions of His, leucine and Phe were lower, while plasma Met was higher and plasma Lys tended to be higher in cows fed FPAA vs. CSBAA diets. Milk true protein concentration, but not its amount, was increased in cows fed FPAA vs. FP diets. Nonetheless, cows fed FPAA diets exhibited lower levels of His and leucine in plasma relative to that fed FP. Overall, feeding cows RPP- supplemented diets did not adversely affect milk yield and synthesis of milk protein. However, it did not improve milk yield or milk protein synthesis, but decreased the excretion of N as urea in urinary and improve N efficiency.

## Histological parameters

The effect of supplementing cows' diets with RPM on lymphocyte proliferation and mononuclear cell composition was studied by Soder and Holden;[97] the authors detected an increment in the proliferative ability of peripheral blood T lymphocytes after the cows had consumed 22 g of RPM per day, while the phenotypic mononuclear cell composition of blood and milk were not affected. Furthermore, Acosta et al.[53] detected that the addition of RPM to the basal diet ration until 30 days after calving increased the lipid content and decreased DNA methylation of the preimplantation embryos, and subsequently increased the survival rate of the embryos due to the fact that the endogenous lipid stores serve as an energy substrate.[98] Recently, Stella[99] studied the effect of dietary fortification of RPM on immunohistochemistry of superoxide dismutase 1 and glutathione peroxidase 1, uterine cytology, plasma amino acid concentrations and lipid profiles of the endometrial tissue and the preimplantation embryo; RPM was found to enhance neutrophil infiltration of the endometrium at 30 DIM, suggesting improved uterine and systematic immunity.[100] Moreover, cows in the RPM supplemented group tended to have higher polymorphonuclear neutrophil proportions than cows in the control group at 15 DIM, which decreased in subsequent days.[99] Furthermore, RPM increased polyunsaturated lipid concentrations and decreased monounsaturated lipid concentrations in embryos, compared to control cows, which may be an indication of enhanced embryonic vitality. In addition, RPM can improve the reproductive ability of cows during and after the transition period, via increasing the levels of un/monounsaturated lipids, saturated lipids and unsaturated lipids in uterine tissue.

Acosta et al.[98] studied the effect of RPM on the expression of immune indicators in follicular cells of the first postpartum dominant follicle in Holstein cows. RPM was found to downregulate the pro-inflammatory genes, which is an indication of lower inflammatory processes in follicular cells of the first postpartum dominant follicle. The inhibitory action on the systemic hyper-response probably results from acting on the oxidative status of the animal, or indirectly through its immunologically active metabolites (e.g., taurine).[100] Moreover, supplementing cows with Met increased the 3b-HSD expression in the follicular cells during the transition period, which is necessary for the biosynthesis of all categories of steroid hormones. In addition, RPM induced a great increase of Met concentrations in the follicular fluid, which can improve oocyte quality of multiparous Holstein cows.[101]

Li et al.[102] studied the effect of RPM on the energy-overfeeding rations. It was found that RPM decreased the negative effects of energy-overfeeding rations on transition cows. Therefore, RPM is effective in decreasing the upregulation of some pro-inflammatory genes, such as TLR4. Moreover, the similar milk yield between cows fed the high-energy diet with RPM and cows fed the lower-energy diet proves the idea that Met can overcome the limitations of high-energy diet during the pre-partum period. Additionally, Zhou et al.[103] studied the impacts of RPM supplementation on the immune-metabolic status in transition cows by measuring concentrations of biomarkers in milk, liver tissue and plasma, in addition to the assessment of polymorphonuclear leukocyte function; the authors detected great rise in plasma paraoxonase levels, which indicates a perfect liver function, where it associates with high-density lipoprotein and protects it from oxidative damage. Moreover, cows fed diets supplemented with RPM had higher levels of reduced and total glutathione (a potent intracellular antioxidant) in the liver. In addition, an increase in blood polymorphonuclear leukocyte phagocytosis capacity was noted in RPM supplemented cows. These results were also confirmed by Zhou et al.[104] Furthermore, supplementing cows' diets with RPM has a great impact on transition cow performance; it decreases the prevalence of retained placenta and clinical ketosis, in addition to increasing the blood glucose level, milk yield and milk protein in dairy cows.[105]

## Conclusions

In ruminant animals, protein requirements are double those of non-ruminant animals, where protein plays a key role in supporting the rumen anaerobic ecosystem, and to cover the animal's requirements. However, because of ruminal fermentation, some of the dietary protein is degraded (referred to as rumen-degradable protein, RDP), Therefore, needs to dietary supplementation of RPP (including RPM) are growing to support the productive and physiological requirements of livestock. Evidences from the previous investigations may draw the attention to the positive animal response against RPM supplementation particularly during lactation period where the needs to proteins are greaten. However, several research programs are still needed to paint a clearer picture of RPM and its vital role in different stages of animal life.

## ORCID

Mohamed S. Ayyat https://orcid.org/0000-0003-0668-1823
Adham Al-Sagheer https://orcid.org/0000-0002-9895-4106
Mohamed E. Abd El-Hack https://orcid.org/0000-0002-2831-8534
Abdelfattah Z. M. Salem http://orcid.org/0000-0001-7418-4170

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