Review Article



Supplementation of Ruminally Protected Proteins and Amino Acids: Feed Consumption, Digestion and Performance of Cattle and Sheep

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ABSTRACT

Provision of nutrients in balanced form and required amounts is essential to meet the productive targets in livestock. Among nutrients, protein plays a pivotal role in growth, production and reproduction of farm animals. However, nature and level of dietary protein determine the supply of both physiologically and dietary essential amino acids in animals. In ruminants, protein requirements are twofold; to support the anaerobic ecosystem in the rumen and to meet the animal needs. However, because of ruminal anaerobic fermentation, a portion of dietary protein is degraded in the rumen (RDP) and the rest escape from ruminal degradation (RUP). The RDP is used to support the growth of anaerobic bacteria and thus profile of microbial protein along with the nature of RUP determines the availability of dietary and physiologically essential amino acids in ruminants. Generally, the requirements of high producing animals for dietary essential amino acids are increased from those supplied by the microbial and escaped protein pool. Thus the dietary supplementation of rumen protected protein and amino acids are recommended to support the physiological and productive needs of livestock for amino acids. The objective of this article is to review all important studies on RPP, RPL and RPM and their effects on dry matter intake (DMI), digestibility and production performance of cows and sheep. Protein is an important limiting nutrient in ruminants. It contains two fractions: RDP and RUP/RPP. The rumen microbes breakdown RDP to small peptide, AA and ammonia. These, in turn, can be used for synthesis of microbial protein. The rate of microbial protein synthesis is limited by the rate of passage of feed from the rumen. Therefore, supplementation of RDP, RUP or AA is considered important to satisfy animal's requirements. This is especially true for animals fed on poor quality forages. Supplementation of quality protein or rumen protected amino acids (RPAA) particularly Met and Lys results in increased DMI and digestibility in cattle and sheep. It also increases growth performance, reproductive efficiency and milk yield. It may have some negative effects if Met is supplied at levels substantially in excess of calculated intestinally absorbable requirements, either alone or together with Lys. Supplementation of Met may be useful in rations with low protein content fed to early lactating cows in order to prevent negative long term effects. In conclusion, supplementation of ruminally protected proteins and amino acids improved feed consumption, digestion and performance of ruminant animals fed low quality forages.

Key Words: Rumen protected amino acids; Intake; Digestibility; Growth; Milk yield

Abbreviation key: AA=Amino acid, ADF=Acid detergent fiber, CP=Crude protein, DMI=Dry matter intake, Lys=Lysine, Met=Methionine, N=Nitrogen, NDF=Neutral detergent fiber, RDP=Rumen degradable protein, RPAA=Rumen protected amino acid, RPP=Rumen protected protein, RPL=Rumen protected lysine, RPM= Rumen protected methionine, RPLM=Rumen protected lysine and methionine, RUP=Rumen undegradable protein, Suppl=Supplement, TDN=Total digestible nutrients.

INTRODUCTION

Protein is an important limiting nutrient in ruminant animals fed low quality forages. It becomes necessary when animal attains its optimum growth or peak production. This is because nutrient requirements of ruminants vary according to the physiological state like growth, lactation and pregnancy. Ruminant animals fed on poor quality forages with inadequate protein showed better performance with supplementation of quality protein or RPAA particularly Met and Lys. It has two fractions: RDP and RUP/RPP. The rumen microbes breakdown the degradable protein to small peptide, AA and ammonia, which in turn, can be used by the microbes for synthesis of microbial

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protein. Its synthesis is limited by the rate of passage of feed from the rumen. Therefore, supplementation of RDP, RUP or AA is considered important to satisfy animal's requirements.

Supplementation of RPM increases the proportion of dietary AA that is absorbed from the intestine (Archibeque *et al.*, 2002). They said that the absorbed Met meets a critical limitation and improves the overall use of N in the diet. There is more potential to produce profit, while minimizing undesirable environmental impacts through modification of urea kinetics.

To optimize the efficiency of utilization RPP/RUP, an optimum ratio of the RPP and RUP is essential (NRC, 2001). Under routine farm conditions, protein entering the small intestine is not sufficient to meet the production requirements of the animals. The following two requisites are important to support the utilization of RPPA: (a) the requirements must be able to hydrolyze the RUP in the small intestine and (b) the RUP must provide the required AA profile to the animal (Sarwar & Hassan, 2001). One of the basic goals of feeding proteins to ruminants is to provide adequate amounts of RDP to optimize the microbial protein synthesis and also to supply additional RUP required to optimize the absorbable AA flowing to the small intestine. Lysine is typically considered the most limiting AA for milk production. Experiments in dairy animals revealed that supplementation of L-lysine-HCl along with steam flakes corn rations increased microbial protein synthesis and flow of AA to the duodenum (Bernard et al., 2004).

It is estimated that fairly large amount of RPM fed to ruminants enters the small intestine. Koening *et al.* (2002) reported that in lactating cows, liquid analog of Met fed along concentrate ration escaped rumen degradation (a) of 39.5% irrespective of dose and the analog that escaped rumen degradation was likely absorbed and metabolized as in Met. Berthiaume *et al.* (2001) reported that addition of RPM in the diet of cows increased the duodenal flux of Met from rumen to small intestine leading to higher apparent digestibility of Met in the small intestine. Sixty percent of RPM by passed the rumen and 82% of that disappeared from small intestine. Arterial plasma Met concentration increased with RPM (45 vs 18 uM), while total AA concentration decreased. Feeding RPM resulted in higher concentration of urea-N and glucose in arterial plasma.

The objective of the present review article was to collect important studies on RPP, RPL and RPM and their effects on DMI, digestibility and production performance of cows and sheep.

Dry Matter Intake and Digestibility

Cattle. Under normal farm conditions, feeding hay and silage with limited amount of dietary protein, the microbial synthesis in the rumen and their hydrolysis in small intestine are sufficient for maintenance and limited growth or milk production. Supplementation in the rumen of RPP/RPAA support high production levels (Armentano *et al.*, 1993). Intestinal bioavailability of Met in the protected form ranges

from 22 to 32% (Koening & Rode, 2001). There are variable effects on DMI when different levels of RPM and RPL were supplemented with the basal diets of animals. In a trial with finishing calves, no response to supplemental Met alone suggest that synergistic effect of Met and Lys is responsible for the improved performance of ruminants (Klemesrud *et al.*, 2000). In an effort to measure the ruminal effects of RPM in lactating cows, apparent digestibility of OM and NDF were higher for the diets supplemented with RPM than control diet (Noftsger *et al.*, 2005). However, the rumen volatile fatty acids profile, ammonia concentration and bacterial N entering omasum were not affected.

The supplementation of RPM and Lys in basal ration of pre-partum and post-partum dairy cows containing 18.5 and 16% CP showed no increase in DMI (Socha *et al.*, 2005). Contarary to this, Piepenbrink *et al.* (1996) reported that addition of these RPAA, the DMI of the ration containing 18% CP was higher compared to ration containing 14% CP. Supplementation of RPP Met @ 2% of basal ration of multiparous cows did not affect the DMI (Armentano *et al.*, 1997). However, they indicated that the threshold response to supplementation RPP is when forage contains approximately 7% CP or less, indicating that RUP/RPAA will be beneficial when animals are fed on average or poor quality forages.

Supplementation of RPM @ 10 g/d to the Holstein steers maintained on the grain feed gained @ 12% faster during 98 d of the trials as a direct response to the cubic effect of RPM on DMI. Results suggest a cost effective advantage for replacing 50% of soyabean meal N with urea to meet the AA requirements of beef animals (Hussein & Berger, 1995). Socha *et al.* (2005) reported improved intestinal amino acid supply in pre-partum and post-partum cows but no effect on body weights of cows receiving diets containing 18.5 and 16% CP supplemented with RPM and Lys.

Sheep. The digestibility of DM and CP increased when RUP content of the diets of ruminants increased (Haddad et al., 2005). Use of fish and blood meal (both are RUP) in diets of goats and weathers at 12 and 15% levels revealed that ruminal organic matter and N digestibility were greater for lower level and decreased linearly with increasing fish meal levels (Soto Navarro et al., 2006). They also reported that duodenal flow of microbial and non microbial N was greater for 15% protein with increasing fish meal levels. Increasing magnitude of duodenal N flow as dietary levels of fish meal increased due to increasing extent of ruminal N recycling as level of fish meal and ration of intake of ruminally degraded N to TDN decreased (Van Soest, 1994). Fahmy et al. (1992) determined increased digestibility of NDF in lambs with soyabean meal in roughage based diets as compared to fish meal and corn gluten meal. They hypothesized that soyabean meal enhances rumen microbial growth and provides high quality AA in the intestine. Post ruminal digestibility of RUP and AA balance can be more important than total RUP supplementation (Noftsger & St-

Table I. Effects of feeding varying levels of rumen protected amino acids on dry matter intake, observed in various studies

Diet	DM intake (kg d ⁻¹)		Reference
	Contr	ol AA suppl	
Control, 13.7g RPL	23.8	24.1	Johnson et al. (2007)
Control, 1.5g/d RPM	15.9	15.9	Berthiaume et al. (2006)
Control, 18g RPM	23.2	24.5	Girard et al. (2005)
Control, RPL 0.1% DM	19.9	20.5	Noftsger et al. (2005)
Control, 10g/d RPL	15.3	15.4	Bernard et al. (2004)
Control RPM 91%, 104%	23.2	23.6	Noftsger and St-
of requirement			Pierre (2003)
Control, 24g/d RPL	18.7	17.8	Misciattelli et al. (2003)
Control, RPM 0.08% of	23.8	25.3	Pruekvimolphan and
the DM			Grummer (2001)
Control, 100g RPLM	27.8	27.3	Liu et al. (2000)
Control, RPM 20g/d	23.2	24.1	Overtoon et al. (1998)

Table II. Effects of feeding varying levels of rumen protected amino acids on body weight, observed in various studies

Diet	Body weig	ght gain (kg)	Reference
	Control	AA suppl	
Control, 1.5 g/d RPM	7.3	-9.4	Berthiaume et al. (2006)
Control, 24 g/d RPL	0.11kg/d	0.30kg/d	Misciattelli et al. (2003)
Control, RPLM 113.7 g/d	0.35kg/d	0.30kg/d	Robinson et al. (1998)
Control, RPLM 139 g/d	5.9	0.4	Piepenbrink et al. (1996)
Control, RPLM 70 g/d	1.1kg/d	1.7kg/d	Rogers et al. (1987)

Pierre, 2003).

Growth Performance

Cattle. Growing cattle fed diets low in RUP would benefit from the supplementation with limiting AA. Supplementation with Met, the first limiting AA, in diets with insufficient bypass protein improved N retention (Greenwood & Titgemeyer, 2000). However, Klemesrud *et al.* (2000) concluded that supplementation of metabolizable Lys rather than Met in finishing calves ration was responsible for improved performance.

The beef cows consuming low quality forages with inadequate supply of metabolizable AA may limit protein accretion during pregnancy. Supplementation of a combination of urea and 5 g/d of RPM improved N retention and protein accretion during late pregnancy (Waterman *et al.*, 2007). Methionine supplementation decrased daily urine N excretion and increased both the amount of N retained and the percentage of N digested that was retained by beef steers fed tall fescue hay. It is evident that supplemented Met can meet a specific dietary limitation by increasing the amount of N retention by the steers (Archibeque *et al.*, 2002).

Sheep. Sheep can thrive well on all agro climatic conditions and can subsist on sparse vegetation (Habib *et al.*, 2001). Fast growing sheep have protein requirements that exceed the amount provided by bacteria (ARC, 1998). The new protein feeding system for small ruminants emphasize on maximizing microbial protein supply to the intestine and completing it with dietary protein that escapes undegraded

from the rumen (Habib et al., 2001). Ruminants do not usually have dietary requirement of essential AA. However, when rumen microbial protein synthesis is limited or AA requirements are not met, the animal suffers due to deficiency (NRC, 1985). The quantity and quality of AA reaching the small intestine is influenced by microbial protein synthesis and supplemental protein source escaping the rumen (Titgemeyer et al., 1988). Sheep fed Lucerne and wheaten chaffs and infused abomassaly with casein and Met revealed that beneficial effect of the infused protein/AA were observed with Lucerne chaff only. This suggests that wool production was greater in response to sulphur containing AA/casein when basal diets were of high quality (Dove & Roberds, 1974). Supplemental RUP increases feed intake and body growth rate of sheep (Hassan & Bryant, 1986). Different RUP are high in essential AA, which flow to the small intestine thus improves performance of the animal (Blauwiekel et al., 1992).

Can *et al.* (2004) reported that male lambs fed rations containing 16% CP+5% RUP increased DMI and feed efficiency. This finding is contrary to the report of Hussein and Jordan (1991). Inclusion of RUP in low quality roughage based diets increased the DMI, ME and CP intake in small ruminants maintained at neutral and high ambient temperatures (Ponnampalam *et al.*, 2003). The DMI is significantly increased when RUP in the diet is sufficient. Haddad *et al.* (2001) determined the effect of optimum dietary CP in finishing rations of lambs. They used to 10 to 18% CP in the diets and found increased intake of dry matter and CP with increasing level of protein in the diets.

The growth performance of lambs was improved by the supplementation of RUP in the diets (Habib *et al.*, 2001). Growing Awassi lambs may require more Met for the last stage of finishing (Abdelrahman *et al.*, 2003). These results were confirmed in a later study by Ponnampalam *et al.* (2006), who reported that fish meal resulted in better growth rates and feed efficiency as compared to canola meal and soybean meal. Contrary to the above reportes, Fahmy *et al.* (1992) observed that average daily gains of sheep were better with roughage based diets supplemented with soybean meal than those fish meal or corn gluten meal.

Milk Production and Composition

Cattle. Supplementation of diet with Met and Met+Lys had no effect on milk, true protein and fat content in early lactating cows (Bertrand *et al.*, 1998; Socha *et al.*, 2005). There was slight increase of milk production by dairy cows in early or mid lactation with supplementation of RPM and Lys in basal rations with two levels of protein but milk protein was significantly increased (Armentano *et al.*, 1993). Polan *et al.* (1991) reported that feeding of these AA to dairy cows with basal rations containing corn silage, ground corn with soyabean meal and corn gluten meal, fat corrected milk and milk protein yield were greater during early, mid and total lactation periods. Supplementation of RPM and Lys to cows fed 18 and 14% CP containing diets yielded more milk, total N and protein-N with protein rich

Diet	M	ilk yield	Milk protein (kg d ⁻¹)		Milk fat (kg d ⁻¹)		Reference
	Control	AA Suppl	Control	AA Suppl	Control	AA Suppl	
Control, 13.7g RPL	40.1	41.2	1.23	1.27	1.42	1.45	Johnson et al. (2007)
Control, 1.5g/d RPM	27.7	29.4	0.849	1.024	0.945	0.898	Berthiaume et al. (2006)
Control, 18g RPM	33.9	34.1	1.315	1.163	1.25	1.301	Girard et al. (2005)
Control, RPL 0.1% DM	38.5	38	1.12	1.12	1.29	1.26	Noftsger et al. (2005)
RPM 91%, 104% of requirement	42.9	46.6	1.28	1.44	1.57	1.71	Noftsger and St-Pierre (2003)
Control, 24g/d RPL	32.8	33.7	1.015	1.059	1.144	1.035	Misciattelli et al. (2003)
Control, RPM 0.08% of the DM	36.4	38.1	1.06	1.14	1.34	1.40	Pruekvimolphan and Grummer (2001)
Control, 100g RPLM	32.8	32.8	1.06	1.07	1.20	1.18	Liu et al. (2000)
Control, 3.3 RPM mg/kg DM	34.8	36.9	1.10	1.18	1.55	1.62	Tom overtoon (1999)
Control, RPLM 113.7g/d	33.85	33.92	1.09	1.10	1.29	1.30	Robinson et al. (1998)
Control, RPLM 139g/d	27.3	25.7	0.94	0.89	1.04	0.99	Piepenbrink et al. (1996)
Control, RPLM 55g/d	25.3	26.3	0.80	0.85	0.92	0.92	Donkin et al. (1989)

Table III. Effects of feeding varying levels of rumen protected amino acids on milk yield and its composition, observed in various studies (Summary)

diets (Piepenbrink *et al.*, 1996). Supplementation of rations of pre-partum and post-partum cows with RPM and Lys yielded more milk (Chapoutot *et al.*, 1992; Schwab *et al.*, 1995; Socha *et al.*, 2005).

Supplementation of 0.52% Met and 1.03% Lys to an alfalfa hay-concentrate based diet (1:1 ratio) may supply the ruminal microorganisms an optimal level of Met and Lys to improve ruminal fermentation and post-ruminal supply of metabolizable AA in the continuous culture system. Results of in vivo study confirmed the findings obtained from the in vitro study and indicate that positive responses of Met and Lys in vivo should be expected and profitable if correctly timed and supplemented at the proper concentrations. Data of the in vivo study suggested that supplementing free Met and Lys to Holstein cows in late lactation altered ruminal fermentation and post-ruminal supplies of metabolizable AA, but these impacts may have favoured energy partitioning to body tissue rather than elicit significant responses of milk production and milk contents. This is because (1) cows were in late lactation, (2) a short experimental period was utilized and (3) a less than optimal combination of Met and Lys was used in this study. Based on numerically greater milk efficiency and body weight gain observed in the treatment group when cows were in late lactation with positive energy balance, improvements in lactational responses should be expected when the ideal concentrations of Met and Lys are supplemented to cows in peak lactation (Chung, 2003).

Four multiparous late-lactation cows were fed a basal ration designed to be co-limiting in intestinally absorbable supplies of Met and Lys. Cows were supplemented with no AA, Lys by abomasal infusion to 140% of the calculated intestinally absorbable requirement, Met by abomasal infusion to 140% of requirement, or both AA. Results show that negative effects on performance of lactating dairy cows can occur if Met is supplied at levels substantially in excess of calculated intestinally absorbable requirements, either alone or together with lysine (Robinson *et al.*, 2000).

Feeding RPM resulted in higher concentration of urea N and glucose in arterial plasma. Milk production and composition were, however, not affected (Piepenbrink *et al.*,

1998; Berthiaume *et al.*, 2000). In later reports (Misciattelli *et al.*, 2003; Rulquin *et al.*, 2006), supplementation with RPM led to increase in milk fat and protein contents, respectively. They also observed that supplementation with RPL or Met numerically increased protein yield comparable to values reported in literature, but the treatment effects were not statistically significant. Efficiency of use of N for milk production was higher on feeding higher digestible RUP. Milk production, milk protein productions were not significantly different in supplemented group. However, RPM resulted in maximal milk and protein production along with maximum N efficiency by cows in production trials. This indicates that post-ruminal digestibility of RUP and AA balance can be more important than total RUP supplementation.

CP No interactions between levels and supplementation of Met in cows were observed for milk production and its composition. Met supplementation did not affect N excretion in urine, feces and milk (Leonardi et al., 2003). The effects of a dietary supplement with calcium salts of fatty acids and Met hydroxyl analogue increased milk yield, milk lactose production and blood cholesterol concentration but did not improve reproductive performance, except in first lactation cows (Fahey et al., 2002). Holstein cows supplemented with 0, 30 and 60 g/day of slowly degraded RPM revealed higher plasma Met concentration with increasing dietary levels (Bach & Stern, 2000). Supplementary RUP had little effect on milk yield, milk protein content when crude protein content of the pastures were lower (Casals et al., 1999). Girard and Matte (2005) reported beneficial effects of vitamin B_{12} injection to lactating cows fed dietary supplements of folic acid and RPM. There was increased energy corrected milk yield, milk yield of solids, fat and lactose.

Milk yield and its composition were not affected when cows were fed with or without RPM and Lys (Liu *et al.*, 2000; Berthiaume *et al.*, 2001). Net mammary intake of Met did not change with the addition of RPM. However, mammary extraction of Met decreased in a linear fashion in response to increased arterial inflow (Berthiaume *et al.*, 2006). With silage diets, the supply of Met and Lys to duodenum is likely to be of low value because the rates of microbial protein synthesis in the rumen may be limiting for milk production (Chamberlain *et al.*, 1986). Supplementation of RPM in feeds containing low level of RUP based on meat-bone meal and hydrolyzed feather meal enhanced milk, milk protein and milk fat contents. The results indicate that feeding hydrolyzed feather meal may not alleviate Met deficiency in lactating dairy cows (Preukvimolphan & Grummer, 2001).

Feeding RPM and blood meal did not increase the milk yield but it increased the composition (Chung Wen *et al.*, 2005). The dietary supplementation of RPL and Met improved the lactation performance of dairy cows (Watanabe *et al.*, 2006). The D, L-Met and Met hydroxyl analogue performed similarly in promoting milk yield, milk fat and protein, linear somatic cell count, animal body condition and days to first service in dairy cows (Watanabe *et al.*, 2006). Supplementation of Met showed best results in those animals fed low protein diets during early lactation (Krober *et al.*, 2000; Lara *et al.*, 2006).

When Met and Lys were top-dressed on diets fed to non-lactating cows, no change in total tract N digestion were observed. No changes in microbial protein production or ruminal fermentation were observed. Adding Met and Lys did not change production or efficiency of production of milk or milk components in late lactating cows. These data indicate that providing supplemental Met and Lys during late lactation does not significantly improve the protein status of the cows and therefore may not improve milk production (Chung *et al.*, 2006).

CONCLUSION

Increasing RUP in the diet increased the duodenal flow of amino acid in small intestine. Post-ruminal supply of protein or rumen protected amino acids wass more important as compares to diets high in RUP value. Rumen protected amino acids must be provided in ruminant diet as they will be available in the small intestine for complete absorption. Among rumen protected amino acids, Met and Lys were limiting as their deficiency limited the milk production and growth of ruminant animals. Supplementation of these amino acids was especially useful in rations with particularly low protein content.

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