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EFFECT OF FEEDING MICROENCAPSULATED RUMEN PROTECTED METHIONINE ON MILK YIELD AND MILK COMPOSITION IN CROSSBRED COWS

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Abstract: A study was conducted to assess the effect of supplementing microencapsulated rumen protected methionine on milk yield, fat and SNF contents in Holstein Friesian crossbred cows. Thirty Holstein Friesian crossbred cows (*Bos taurus* × *Bos indicus*) in early lactation were selected and divided into three groups (10 each) on the basis of most probable production ability (MPPA) and lactation number. Animals in control group (G-1) were fed chopped wheat straw, chaffed green maize fodder and concentrate mixture as per the requirements. However, animals in second supplemented group (G-2) were fed same ration as control group plus 30 g multi-layer micro-encapsulated methionine which was prepared in the laboratory. Animals in third group (G-3) were fed same ration as control group and supplemented 30.5 g of RPM (Rumen Protected Methionine) which was purchased from the local market. The experiment was continued for 29 days and individual milk yield from these animals were recorded daily before and after the treatment. Fat and SNF (Solid not fat) content of milk were also analyzed at weekly interval in the pooled milk sample of each group before and after the treatment. The difference in milk yield before and after treatment in control group (G-1) was 9 g/day, whereas in group supplemented with triple-layer microencapsulated methionine (G-2) was significantly increased to 284 g/day ($P<0.000$). Similarly, Milk yield in the treatment group supplemented with commercially available rumen protected methionine (G-3) was 207 g/day ($P<0.000$) when compared to control group. The average milk fat per cent was significantly higher ($P<0.032$) by 0.140 in group fed with multi-layer microencapsulated methionine (G-2) and 0.060 in group fed commercial rumen protected methionine (G-3) when compared to control group (G-1 - 0.04) during the trial period. Average SNF per cent (solid not fat) was significantly higher ($P<0.042$) in group fed with multi-layer microencapsulated methionine (G-2 0.180) and commercial rumen protected methionine (G-3, 0.280) group when compared to in control group (G1, 0.080). Net return over feed cost of milk yield, fat and SNF per animal per day over control were Rs.55.20 and 48.65 in treatment groups G-2 and G-3, respectively, indicating that it was higher by Rs. 6.55 in multi-layer microencapsulated methionine group (G-2). Therefore, from the present study, it could be concluded that feeding multi-layer microencapsulated methionine to high yielding lactating cows during early lactation was cost effective and beneficial to the farmer.

Keywords: Crossbred cows, Micro-encaptulated methionine, Milk yield, Milk composition**.**

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Introduction

Today, protein quality and price are major concerns under Indian animal feeding systems. Ruminants need the same amino acids at tissue level and have similar protein metabolism as compared to non-ruminants (ARC, 1980). However, in ruminants the amount and profile of amino acids available at the site of absorption does not relate to amino acids in the feed ration, but totally depends on the complex protein degradation in the rumen. In the rumen, crude protein from the feed is degraded to a mixture of amino acids, ammonia and peptides for the synthesis of microbial protein, which supplies most of the amino acids in small intestine (Iburg and Lebzien, 2000). Dietary protein requirement for the host animal are complex, because rumen microbes alter the amount and quality of ingested protein before it reaches the small intestine to be absorbed by the animal. Therefore, the protein available for absorption in the animals' small intestine, also classified as metabolizable protein (MP), which is true protein digested post-ruminally (NRC, 2001). Microbial protein serves as the major source of absorbable amino acids to the small intestine for ruminants. Metabolizable protein reflects predominantly the combination of microbial protein, rumen undegradable protein (RUP), and endogenous protein (NRC, 2001). The metabolizable amino acid profiles depend upon various factors that affect protein degradation in the rumen and microbial protein synthesis. But amino acids supplied to the small intestine from rumen microbial protein are limiting, because microbial protein does not supply sufficient amounts to satisfy animals' specific amino acids needs to achieve a certain level of performance. Methionine and lysine seems to be the two amino acids limiting for growth in microbial protein (Komarek *et al*., 1983). Ruminants have the specific metabolic requirements of amino acids for their optimum growth, maintenance and productive functions. Amino acids are uniquely essential as precursors for protein synthesis. They also serve for the synthesis of other nitrogen containing metabolites as precursors for gluconeogenesis, and as a source of metabolic energy when oxidized to CO_2.

Thus, to increase the total amino acids supply to small intestine, amino acids must be supplied in protected form, or increasing the proportion of rumen undegradable protein. Generally, cows have relatively high metabolizable protein requirement for their rapid and efficient gains. The efficiency of metabolizable protein used by ruminants highly depends on the profile of absorbable amino acids, because the deficiency of any single amino acid can limit the use of other amino acids that are in adequate supply (Cole and Van Lunen, 1994). A dietary deficiency of essential amino acids will cause milch cows to use metabolizable protein less efficiently. Forage diets for milch cows are often deficient in metabolizable protein (Wilkerson *et al*., 1993). To meet the animal metabolizable protein requirement, protected amino acids are supplemented. Rumen-protected amino acids supplementation may correct amino acids deficiencies without increasing the quantity of metabolizable protein. Alternately, it may decrease protein levels without affecting the levels of performance

Methionine and lysine is the most limiting essential amino acids for cows (Richardson and Hatfield, 1978), which may be partially responsible for the inferior performance of cows consuming forage based diets. Methionine and lysine as the first two limiting amino acids in most dietary situation (NRC, 2001; Doepel *et al*., 2004; Lapierre *et al*.,2006; Benefield *et al*., 2009). Lysine and methionine are considered to be co-limiting amino acid required for optimum growth and milk synthesis in dairy cattle (Nichols *et al*., 1998; Socha *et al*., 2005). This inferior performance may likely results from the low availability of lysine and methionine from rumen undegradable protein. This may be insufficient to meet the requirement of these amino acids for proper growth and deficiency of these amino acids may result in inefficient use of dietary protein for protein deposition. Methionine has many functions in the body in addition to its direct function in protein synthesis, Methionine can be converted to S-adenosylmethionine (SAM), which is a methyl group donor for many trans methylation reactions in the body (Loest *et al*., 2002). Methionine also has an important function in the synthesis of cysteine via homocysteine, which is a product of SAM-dependent methylation reactions and a critical branch point between the trans-sulfuration to cysteine and remethylation to methionine. In addition, methionine is the source of methyl donor *S*-adenosyl methionine, the metabolite that provides methyl groups in a variety of reactions including the de novo synthesis of choline from phosphatidylethanolamine. (Lobley *et al*., 1996). Although an increase in milk production, milk protein or milk fat in high producing dairy cattle fed rumen protected methionine has been reported, however the methods of protection has not been clearly understood. Therefore, the present study has been planned to assess the effect of supplemental rumen protected methionine after preparation at laboratory and then evaluating it against easily available rumen protected product in the market.

Materials and Method

Micro-Encapsulation of Methionine

The machine used for microencapsulation was Glatt GPCG 1.1 from New Jersey, USA. Powdered methionine was converted into granules with Fluid Bed Technology to improve flowability, dispensability, uniformity and reduce dustiness of the product. First, the product went three layers of coating in the machine. First two layers were coated with hydrogenated vegetable oil - a blend of palm oil with hydrogenated vegetable oil with a melting temperature of over 90^{o}C to overcome the melting temperature. The third layer of coating was done with organo-silicates which gave the molecule the property of slow release. In the process of encapsulation, a bed of the coating material was created in the machine, methionine granules which were to be coated were then injected with a flow of air. The methionine granules had to move through the matrix and were thrown towards upper side by the air pressure from below. This made the methionine granules coat with hydrogenated fat and then dried by air movement. Triple layer microencapsulated methionine had 50% encapsulation was thought to be efficient rumen bypass. The stability of the product in the rumen was > 80% which ensures high availability of methionine in the small intestine and thus providing a continual supply of methionine to the milk protein producing cells.

By the use of a dissolution test the release rate protected methionine was tested and analyzed. Rumen liquor was used in the apparatus for testing the dissolution of methionine after adjustment of pH. After 8 hours, samples were collected from the chambers and were evaluated for methionine content by HPLC method.

Animal Feeding Trial

Thirty Holstein Friesian crossbred early lactating cows (*Bos taurus* × *Bos indicus*) were selected and divided into three homogenous groups (10 each) on the basis of most probable production ability (MPPA) and lactation number at Vishal farms, Moonak Village, Sangram District in Punjab. Animals in control group (G-1) were fed chopped wheat straw (particle size: 1.5 -2.0 cm), chaffed green maize fodder (particle size: 2.0 -2.5 cm) and concentrate mixture as per requirements (NRC, 2001). However, animals in second supplemented group (G-2) were fed same ration as control group plus 30 g multi-layer micro-encapsulated methionine which was prepared in the laboratory. Animals in third supplemented group (G-3) were fed same ration as control group plus 30.5 g RPM (Rumen Protected Methionine) which was purchased from the local market. The experiment continued for 29 days.

The concentrate mixture contained maize 33%, groundnut cake 21%, mustard cake 12%, wheat bran 20%, deoiled rice bran 11%, mineral mixture 2% and common salt 1%. Chopped green maize forage was fed separately, whereas wheat straw and concentrate mixture were mixed before feeding and fed as per weekly calculated requirements (NRC, 2001) of each cow. The concentrate mixture was offered two times a day in equal parts at the time of milking i.e. 05:00 and 18:00 h. Micro-encapsulated rumen protected methionine’s were fed through concentrate mixture at one time i.e. 05:00 h. Fresh chopped green maize forage was fed at 10:00 and 19:00

h in addition to wheat straw, which was offered at 05:00 h. Left over, if any, was weighed next morning. Milk yield of the individual cows were recorded twice daily for 29 days for the three groups. Milk samples were collected weekly from each cow of the same group and pooled in proportion to the milk yield of individual cow of same group and representative samples of three groups were analyzed for its composition (fat and SNF) using pre-calibrated milk analyzer (LactoStar, FUNKE GERBER, Article No 3510, Berlin).

Economics of feeding mirco-encapsulated and protected methionine was calculated by taking cost of protected methionine and feed cost. Average daily milk yield, Fat and SNF percentage were calculated before the start of the treatment and 29 days after the trial. Data on change in average daily milk yield, fat and SNF percentage were calculated for each group and analyzed using one-way ANOVA and Duncan's multiple range test with the help of SPSS software version 20 for testing the significance of the data between three groups.

Result and Discussion

Dissolution Profile of Rumen Protected Products

Dissolution profile of microencapsulated methionine and rumen protected methionine purchased from local market is presented in the table 1. The per cent methionine protected from the rumen degradation was 97 and 95.5, respectively for microencapsulated methionine and rumen protected methionine purchased from local market.

Table 1: Dissolution profile of Rumen protected products

Product	Release of methionine in rumen liquor	Methionine bypass the rumen
Multi-layer microencapsulated Methionine prepared in lab	3%	97%
Commercial rumen protected methionine purchased from local market	4.5%	95.5%

Milk Yield, Fat and SNF Contents

The change in milk yield, milk fat and solids-not fat contents of dairy cows fed control diet, multi- layer microencapsulated methionine and rumen protected methionine supplemented group are presented in the table 2

Table 2 The Change in milk yield, milk fat and solids-not fat contents of dairy cows

S. No	Treatment group	Average change in milk yield*	Average change in milk fat**	Average change in Solid-not fat content**
1	Control (G1)	$0.009^b \pm 0.034$	$0.040^b \pm 0.024$	$0.080^b \pm 0.037$

2	Multi-layer microencapsulated methionine (G2)	0.284[a] ± 0.037	0.140[a] ± 0.025	0.180[ab] ± 0.049
3	Rumen protected methionine from local market (G3)	0.207[a] ± 0.031	0.060[ab] ± 0.025	0.280[a] ± 0.058
P value		0.000	0.032	0.042

* - Average of ten cows with 29 days of milk yield data
** - Group average of pooled fat and SNF content of cows for five week

The difference in milk yield before and after treatment in control group (G-1) was 9 g /day, whereas in group supplemented with multi-layer microencapsulated methionine (G-2) was significantly higher by 284 g/day (P<0.000). Milk yield in the treatment group supplemented with commercially available rumen protected methionine (G-3) was also significantly higher (207 g/day, P<0.000) when compared to control group. Average change in milk yield in the group supplemented with multi-layer microencapsulated methionine (G-2) was 37% higher than the group supplemented commercial rumen protected methionine (G-3). The major reason for higher milk production in treatment groups may be improved amino acids efficiency on supplementation of rumen protected methionine. The significant improvement in milk production on supplementation of rumen protected methionine and lysine is in line with the findings of many other researchers. Bach and Marshall (2000) demonstrated an increase in milk yield on supplementing rumen protected methionine to lactating Holsteins cows (45.9 vs 47.7 kg/day). Similarly, Broderick *et al*. (2009) reported that supplementation of rumen protected methionine and lysine enhanced the milk yield in crossbred cows (41.5 kg/day) to that of control (39.4 kg /day). Similar results of increased (18.95 vs. 21.55 kg d -1) milk yield on supplementation of rumen protected methionine and lysine was reported by Yang *et al.* (2010). In high-forage cattle diets, methionine has been identified as the first limiting amino acid. However, methionine is more than just an amino acid. It's one of the few amino acids that participates in a wide variety of metabolic pathways. It can serve as a precursor to other amino acids, like cysteine, another sulphur-containing amino acid. With Indian conditions, where dairy animals are fed with lots of forage, this trial proved that milk yield can also be improved by supplementing rumen protected methionine.

The average milk fat per cent was significantly higher (0.140, P<0.032) in group fed with multi-layer microencapsulated methionine (G-2) as compared to control group (0.04, G-1). The average milk fat per cent was 0.060 in group fed commercial rumen protected methionine (G-

3) during the trial period. Average change in milk fat per cent in group fed with multi-layer microencapsulated methionine as higher by 250% against control (G-1) and 133% against commercial product (G-3). The average SNF (solid not fat) was significantly higher (0.180, (P<0.042) in group fed with multi-layer microencapsulated methionine (G-2) and in group fed commercial rumen protected methionine (0.280, G-3) when compared to control group (0.080, G1). Average SNF per cent in group fed with multi-layer microencapsulated methionine (G-2) was higher by 125% against control (G-1) and 37% lower against commercial product (G-3). Reported effects of methionine and lysine supplementation on milk fat percentage and yield have been inconsistent. Socha *et al.* (2008) reported that duodenal infusion of methionine increased milk fat percentage and yield in cows during early lactation (~15 week of lactation), but not during peak (~5 week of lactation) or mid lactation (~21 week of lactation). Misciatteilli and Sejrsen (2003) also concluded that early-lactation cows fed rumen protected methionine had increased milk fat percentage when compared with control cows; however, supplementation did not affect milk fat yield.

ECONOMICS OF FEEDING RUMEN PROTECTED METHIONINE

The economics of feeding rumen protected methionine to lactating cows throughout the trial period (29 days) has been shown in Table 4.3. The cost of rumen protected methionine was Rs 400 and Rs 450 per kg, respectively for multi-layered microencapsulated methionine (based on rough cost of production) and commercial rumen protected methionine. the additional cost of supplementation for the trial period was Rs 3480 and Rs 3980.25, respectively for multi-layered microencapsulated methionine (based on rough cost of production) and commercial rumen protected methionine. Net return over feed cost on milk yield, fat and SNF per animal per day over control group (G-1) was Rs.55.20 (G-2) and 48.65 (G-3) in treatment groups, respectively, indicating that it was higher by Rs. 6.55 in multi-layer microencapsulated methionine supplemented group. The net return in G-2 was markedly higher by 13.5% than that of the G-3 group. As a result, additional profit in 29 days from 10 cows on supplementation of multilayer microencapsulated methionine was Rs. 1900. The cost benefit ration for G-2 was calculated to be 4.6, whereas for G-3 was calculated as 3.5.

Table 3: Economics of feeding Rumen protected methionine in dairy cows

Attribute	**G2**	**G3**
Milk yield and gross income		
Average change in Milk yield (kg/d/cow)	0.284	0.207

Average change in Milk fat %	0.14	0.06
Average change Milk SNF %	0.18	0.28
Additional milk yield in 29 days by 10 animals (kg)	82.36	60.03
Additional milk fat in 29 days by 10 animals (kg)	40.6	17.4
Additional milk SNF in 29 days by 10 animals (kg)	52.2	81.2
Market price of milk (@ Rs 25/kg)	2059	1501
Market price of Milk fat (@ 230/kg)	9338	4002
Market price of SNF (@155/kg)	8091	12586
Total additional income on milk	19488	18089
Expense		
Total prepared multi-layer micro-encapsulated methionine fed in kg (@ 30 g/cow/day for 29 days) and locally purchased rumen protected methionine fed in kg (@ 30.5g/cow/day for 29 days)	8.70	8.85
Investment in feeding Rumen protected methionine (Rs 400/kg Multilayer microencapsulated product and Rs 450/kg commercial product)	3480	3980.25
Profit		
Net return in 29 days from 10 cows (Rs.)	16008	14108.5
Net return (Rs./cow/day)	55.20	48.65
Benefit cost ratio	4.6	3.5

Conclusions: Therefore, from the results of the present study, it could be concluded that the feeding multi-layer microencapsulated rumen protected methionine to high yielding lactating cows during early lactation was cost effective and beneficial in terms of milk yield and milk constituents.

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