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Effect of Methionine Supplemented Feed on Nili-Ravi Buffalo Milk Yield and Composition

Nasir Ali Tauqir[1]*, Asim Faraz[2], Abdul Waheed[2], Ayman Balla Mustafa[3], Irfan Shahzad Sheikh[4], Michela Pugliese[5] and Shahid Nazir[6]

[1]*Department of Animal Nutrition, The Islamia University of Bahawalpur, Pakistan*
[2]*Department of Livestock and Poultry Production, Bahauddin Zakariya University, Multan*
[3]*Therapeutic Nutrition Department, Faculty of Nursing and Health Sciences, Misurata University, P.O. Box: 2478, Misurata, Libya*
[4]*Centre for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta, Pakistan*
[5]*Department of Veterinary Sciences, University of Messina, Via Umberto Palatucci–98168 Messina, Italy*
[6]*Department of Animal Science, University of Sargodha, Pakistan*

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Key words
Methionine supplementation, Nili Ravi Buffaloes, Blood metabolites Milk Production, Milk composition

ABSTRACT

The objective of the study was to examine the effect of rumen protected methionine supplementation on milk production and its composition in lactating Nili-Ravi buffaloes. Sixteen early lactating nili-ravi buffaloes were divided into four groups according to Randomized Complete Block Design for this study. Four experimental diets were formulated supplementing 0, 15, 25 and 35g of methionine/ animal/ day. The experimental period was of 56 days out of which 10 days were as adaptation period. Daily feed intake and morning-evening milk yield of each animal was recorded. Body weight of the animals was recorded at the start of experiment and fortnightly thereafter. Total collection of urine and feces were performed fortnightly to determine nutrient digestion and nitrogen balance. Blood samples were collected through jugular vein two hours post feeding and were analysed for blood triglycerides concentration, total proteins and blood urea nitrogen according to standard procedures. Nutrient intake, nutrient digestion, nitrogen balance, milk production, milk fat, total solids in milk and solid not fat did not show any treatment effect. However, milk protein percentage was the highest (3.42% and 3.41%) in buffaloes fed diets containing 35 and 25 g/d methionine followed by (3.26% and 3.17%) those fed 15 g of methionine and control diets, respectively. Milk protein percentage was found to be sensitive to methionine supplementation, because increase in milk protein percentage was observed just after first week of supplementation. Similarly, blood urea nitrogen, triglycerides and total proteins were significantly affected by methionine supplementation. Blood urea nitrogen was lower in buffaloes fed ration containing higher level of methionine. The highest triglycerides (15.40 mg/dl) were observed in buffaloes fed ration containing higher amount of methionine as compared to those fed rations containing lower amount of methionine. Total protein was also higher (8.27 g/dl) in animals fed ration containing higher level of methionine while it was similar in supplementation groups. Although the results were comparatively better when buffaloes were raised on supplemented ratios but due to shorter study period and use of well-fed and healthy animals (not deficient in methionine) the results of the lactation performance were not very obvious as were expected.

INTRODUCTION

In ruminants, like other animals, the requirements for protein are actually the prerequisites for a certain amount and balance of amino acids. But the quality of dietary protein lacks the due consideration in ruminants because rumen microorganisms alter dietary protein qualitatively and quantitatively. Hence, the amino acids for absorption in small intestine of ruminants are made available from microbial proteins, ruminally undegradable proteins (RUP) and endogenous proteins, collectively called as metabolizable proteins (NRC, 2001). In high producing animals only metabolizable protein (MP) cannot fulfill the requirements for amino acids. So, to meet the requirements of amino acids and to efficiently utilize MP, the high producing animals should be offered with a post ruminal supply of amino acids. To achieve this, one of the ways is to feed higher levels of protein which escapes escapes rumen fermentation. However, high RUP has also been reported

* Corresponding author: tauqir041@hotmail.com
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to restrict flow of MP to small intestine by limiting the ruminally available nitrogen (Clark *et al*., 1992).

Supplementing rumen protected amino acids has also captivated the attention of dairy nutritionists by virtue of their favorable effects on milk yield and its composition (Stern *et al*., 1994). Diets which are balanced for amino acids can improve milk yield and even milk components (Noftsger *et al*., 2005). For this purpose, it is preferred that the amino acids being supplied should be the most limiting for the milk yield and milk components (Chalupa, 1975; Clark, 1975).

Methionine has been considered as the most limiting essential amino acid for dairy animals (NRC, 2001; Casper and Schingoethe, 1988). It has been observed that difficulties exist to fulfill the dietary needs of methionine due to its deficiency in available feed ingredients. However, when this insufficiency is revamped in an appropriate amount, a new protein molecule can be synthesized resulting in efficient utilization of MP and reduction of surplus amino acid nitrogen.

The free form of dietary methionine is degraded in rumen and an insufficient amount of methionine reaches duodenum. This highlights the significance of supplementing rumen-protected methionine in order to ensure a sufficient supply of methionine for optimum milk production and milk protein synthesis (Blum *et al*., 1999). Favorable effects of protected methionine supplementation on milk yield and composition have been reported in dairy animals (Illg *et al*., 1987; Armentano *et al*., 1993). Methionine supplementation gave better results after parturition, when supply of macro- and micro- nutrients have been jeopardized due to reduced dry matter intake.

Although Sufficient scientific information is available about productive effects of methionine supplementation in exotic dairy cows but the date is inadequate on buffalo milk yield and composition. Yet, the information obtained from temperate dairy cows may not be applicable directly to buffaloes being diverge in physiological aspects and also reared under different environmental conditions and feeding regimes. Present study was planned to examine the effect of protected methionine supplementation on milk production and its composition in lactating buffaloes.

MATERIALS AND METHODS

Experimental animals and feed

Sixteen lactating buffaloes of similar body weight (601.46±17.76kg), age (4-5 years), stage of lactation (2nd and 3rd) and milk production (9.08±2.33liters) were randomly divided into four groups of 5 buffaloes each. Four experimental diets serving as control (OM), low methionine (LM), medium methionine (MM) and high methionine (HM) by means of 0, 15, 25 and 35 g of methionine/animal/day respectively, were formulated.

Methionine source

Metasmart, source of methionine developed by Adisseo® is the isopropyl ester of hydroxymethyl butanoic acid (HMBI). This patented product has distinctive characteristics of absorption across the rumen wall, providing 50% methionine bioavailability. The ester side of the molecule enables rumen wall absorption of HMBI into the animal's bloodstream. There, HMBI releases its HMB, which can then be converted into methionine in the liver. The remaining HMBI in the rumen, approximately 50%, is hydrolyzed into HMB. The HMB is then used as a substrate by rumen micro-organisms and stimulates rumen fermentation (Adissio® product fact sheet).

Housing and management

Experimental period was lasted for 70 days including 14 days of adaptation period while every alternative week was served as collection period. The animals had round the clock access to clean and fresh water. Compound feed was formulated from locally available ingredients. Ingredients and nutrient composition is presented in Table I. Feed was offered individually and Metasmart® was added in ration at the time of feeding.

Table I. Ingredients and chemical composition of experimental ration.

Ingredients	Inclusion level, %
Trifolium alexandrinum	62.5
Wheat straw	15.0
Corn grains	3.75
Wheat bran	1.50
Rice polishing	1.87
Corn gluten 30 %	5.63
Canola meal	4.87
Cotton seed cake	2.63
Molasses	1.87
Mineral mixture	0.38
Chemical composition (%)	
Dry matter	46.2
Crude protein	17.15
Crude fiber	23.0
Neutral detergent fiber	48.0
Acid detergent fiber	33.0
Methionine from the diet	0.24
Lysine	0.56
Ether extract	3.15
Ash	7.00

Data collection

Daily milk yield of each animal was recorded. Milk samples were collected at two consecutive milkings and were analyzed for milk fat (AOAC, 1990), total solids (TS), and solids not fat by the difference between TS and milk fat (Khan *et al*., 2005). Fat corrected milk (FCM; 4 % fat) was calculated as described by Tyrrel and Reid (1965) using equation milk (kg/day) × (44.01 × milk fat % + 163.56)/339.60. Total nitrogen in milk was estimated by Kjeldhal method (AOAC, 1990). Retained N (g/d) was calculated as N Intake – (Fecal N + Urinary N + Milk N), Gross N efficiency (%) was calculated as milk N/N intake × 100 (St-Pierre and Sylvester, 2005) while environmental N load was calculated as kg of fecal N + kg of urinary N/g of N milk.

Milk CP contents were calculated by multiplying percentage of nitrogen with 6.38. Urine and feces were sampled out of collected by total collection method to determine nitrogen balance. Blood samples were collected from jugular vein and were tested for blood triglycerides, total proteins and blood urea nitrogen (Broderick and Kang, 1980).

Chemical analysis

The proximate analysis of feed and fodder offered, refusals and fecal samples were accomplished according to the methods described by AOAC (1990).

Statistical analysis

The data were analyzed by analysis of variance technique under Randomized Complete Block Design using $Y_{ij} = \mu + \beta_k + \tau_j + \varepsilon_{ijk}$. Where, μ was overall mean, β_k and τ_j were the effects of block and treatment (4 treatments) respectively and ε_{ijk} was difference within treatment means (error term). Tukey's significant difference test was applied to compare the means (Steel *et al*., 1996).

RESULTS

Nutrient intake, digestibility and body weight

Dry matter intake ranged from 14.24 to 15.17, however it was higher in buffaloes reared on 25g methionine (MM) ration followed by those fed no, 35 and 15g methionine. Dry matter digestibility was the highest (62.03%) in buffaloes fed HM ration containing 35g methionine followed by those fed LM (55.866 %), MM (54.891 %) and OM (52.529 %) rations, respectively. Similarly, body weight gain ranged from 31.00 to 40.00 kg during trial period. Although the results of nutrient intake, digestibility and body weight were better numerically yet same were non-significant statistically (Table II). A similar trend was observed in buffaloes fed all experimental rations for CP, CF and EE digestibility.

Milk yield and its composition

Milk yield and 4%FCM ranged from 9.05 to 9.31 kg/day and 13.26 to 14.33 kg/day, respectively (Table III). However, the difference was non-significant ($p > 0.05$). Significantly higher milk protein (3.42% and 3.41%) was recorded in buffaloes reared on HM and MM treatments, while, it was lower (3.26% and 3.17%) in those reared on LM and control (0M) treatments, respectively. The results of milk fat, total solids and solids not-fat did not show any treatment effect (Table III).

Blood parameters

Significantly highest (15.40 mg/dl) triglyceride concentration was observed in buffaloes fed HM ration followed by those fed MM (14.48 mg/dl), OM (13.37 mg/dl) and LM (13.31 mg/dl) rations, respectively (Table IV). A linear and quadratic trend was observed to depict that if the study period is extended, the results would be more cogent and transparent. The highest (8.27 g/dl) total blood protein was observed in buffaloes fed HM ration followed by those fed LM (8.20 g/dl), MM (7.93 g/dl) and OM (7.41 g/dl) rations, respectively (Table IV). A curvilinear regression was observed with respect to blood proteins exhibited that in continuation of treatment for prolonged study period may anticipate consistent results.

Nitrogen balance

Nitrogen intake ranged from 415 to 390.09 g/day in buffaloes reared on all experimental rations but the difference was non-significant. However, the cubic regression was positive at $p=0.051$. The highest fecal nitrogen was observed in buffaloes fed MM ration (136.09 g/d) followed by those reared on LM (115.92 g/d), OM (115.92 g/d) and HM (105.26 g/d) treatments. Similarly, the highest (122.07 g/day) urinary nitrogen was observed in buffaloes fed MM ration followed by those fed HM (110.47g/day), LM (99.74 g/day) and OM (89.46 g/day) respectively, however, it was not statistically supported. It was observed that the methionine level in the rations and the excretion were parallel.

Significantly highest (35.05 mg/d l) BUN was observed in buffaloes fed LM ration followed by those fed MM (31.19 mg/dl), OM (28.32 mg/dl) and HM (26.24 mg/dl), respectively (Table V). Methionine supplementation in the rations of buffalo posed curvilinear response with respect to BUN.

The highest (49.83 g/day) milk nitrogen was observed in buffaloes fed MM ration followed by those fed HM (49.82g/day), LM (45.76 g/day) and OM (44.89 g/day), respectively. The results were slightly positive at $p=0.052$ representing that these results might have been more obvious if the study period was extended.

Table II. Effect of methionine supplemented diet on nutrient intake, nutrient digestibility and body weight gain in buffaloes.

Factors	Methionine supplementation (g/animal/day)				SE	Linear	Quadratic	Cubic
	0	15	25	35				
Dry matter intake (Kg)	15.13	14.24	15.17	14.68	0.166	0.776	0.556	0.050
Dry matter digestibility (%)	52.52	55.86	54.89	62.03	2.183	0.361	0.451	0.638
Crude protein intake (Kg)	1.93	1.63	1.94	1.78	0.057	0.778	0.553	0.050
Crude protein digestibility (%)	58.85	62.45	61.93	65.90	2.319	0.339	0.969	0.686
Body weight gain (Kg)	34.25	30.75	40.00	31.00	4.791	0.991	0.779	0.483

SE, Standard Error; Means bearing similar letters in the rows are non-significant at ($p<0.05$).

Table III. Effect of methionine supplemented diet on milk production and composition in buffaloes fed different levels of methionine supplemented diets.

Factors	Methionine supplementation (g/animal/day)				SE	Linear	Quadratic	Cubic
	0	15	25	35				
Milk yield (kg)	9.05	8.96	9.33	9.31	0.185	0.498	0.933	0.611
4% FCM (kg)	13.26	12.92	14.33	14.22	0.265	0.096	0.834	0.194
Milk protein (%)	3.17[b]	3.26[ab]	3.41[a]	3.42 [a]	0.024	0.001	0.455	0.376
Milk Protein (g/d)	286	291	317	317	6.227	0.052	0.826	0.421
Milk fat (%)	7.10	6.96	7.58	7.57	0.146	0.148	0.820	0.309
Milk fat (g/d)	642	622	706	699	14.354	0.071	0.819	0.156
Total solids (%)	14.82	14.87	15.52	15.69	0.183	0.070	0.873	0.515
Total solids (g/d)	1342	1331	1447	1454	26.335	0.078	0.867	0.337
Solid not fat (%)	7.73	7.92	7.95	8.12	0.048	0.016	0.940	0.493
Solid not fat (g/d)	699	708	740	755	14.106	0.138	0.927	0.760

SE, Standard Error; Means bearing similar letters in the rows are non-significant at ($p<0.05$).

Table IV. Effect of methionine supplemented diet on triglycerides, total blood protein, blood urea nitrogen in buffaloes fed different levels of methionine supplemented diets.

Factors	Methionine supplementation (g/animal/day)				SE	Linear	Quadratic	Cubic
	0	15	25	35				
Triglycerides (mg/dl)	13.37[c]	13.31[c]	14.48[b]	15.40[a]	0.087	0.003	0.00	0.376
Total proteins (g/dl)	7.41[b]	8.20[a]	7.93[a]	8.27[a]	0.052	0.000	0.056	0.004
Blood urea nitrogen (mg/dl)	28.32[c]	35.05[a]	31.19[b]	26.24[d]	0.098	0.000	0.00	0.00

SE, Standard Error; Means bearing similar letters in the rows are non-significant at ($p<0.05$).

Table V. Effect of methionine supplemented diet on nitrogen balance, gross nitrogen and environmental nitrogen load in buffaloes fed different levels of methionine supplemented diets.

Factors	Methionine supplementation (g/animal/day)				SE	Linear	Quadratic	Cubic
	0	15	25	35				
Intake N (g/d)	415.40	390.09	414.45	402.11	4.538	0.778	0.553	0.051
Fecal N (g/d)	115.92	115.92	136.09	105.26	7.159	0.339	0.969	0.686
Urinary N (g/d)	89.46	99.74	122.07	110.47	5.742	0.122	0.360	0.388
Milk (g/d) N	44.89	45.76	49.83	49.82	0.976	0.052	0.826	0.421
Retained N (g/d)	165.13	128.67	106.47	136.57	9.488	0.550	0.375	0.922
Gross N efficiency (%)	10.82	11.71	12.05	12.44	0.276	0.065	0.703	0.789
Environmental N load	0.12	0.12	0.14	0.11	0.007	0.407	1.000	0.595

SE, Standard Error; Means bearing similar letters in the rows are non-significant at ($p<0.05$).

The highest nitrogen was retained by the buffaloes fed OM (165 g/day) ration followed by those fed HM (136.57 g/day), LM (128.67 g/day) and MM (106.47 g/day) rations. The highest (12.44 %) gross nitrogen efficiency was observed in buffaloes fed HM ration followed by those fed MM (12.05 %), LM (11.71 %) and OM (10.82 %) rations. The highest (0.14) environmental nitrogen load was observed in buffaloes fed MM ration followed by those fed LM (0.12), OM (0.12) and HM (0.11) rations (Table V). However, the results did not show any treatment effect.

DISCUSSION

Nutrient intake, digestibility and body weight

The supplementation of methionine did not affect the nutrient intakes, digestibility and body weight in experimental animals. The results of the current study have supported the findings of Armentano *et al.* (1996) who supplemented high protein alfalfa and heated soybeans diets with methionine in the diets of lactating cows and did not find any difference of feed intakes. Similar findings were reported by Bertrand *et al.* (1998) who conducted research to study the influence of protected amino acids on milk production and composition of Jersey cows. They correspondingly reported that DMI was not affected by supplementation of protected amino acids. Similarly, Noftsger and St-Pierre (2003) studied the effect of highly digestible rumen protected protein to improve nitrogen utilization for milk production in dairy cows. It was revealed that methionine supplementation had no effect on DMI. The findings of the current study were consistent with the findings of Overton *et al.* (1995) and Leonardi *et al.* (2003) who added bypass methionine in the diets of lactating cows with two levels of crude proteins and did not find any difference in DMI. Bateman *et al.* (1999), Ouellet *et al.* (2003), Rogers *et al.* (1989), and Guillaume *et al.* (1991) also reported that nutrient digestibilities were not affected by supplementation of rumen-protected methionine. Ordway *et al.* (2009) studied effect of providing two forms of supplemental methionine to pre-parturient dairy cows on feed intake and lactational performance and reported that DMI was not affected by supplementation.

In contrast, Polan *et al.* (1991) revealed that during early lactation the cows consuming rumen protected methionine showed depression in DMI in corn gluten meal diets, when compared with soy bean based diets. However, this depression in intake was found to be reduced by supplementing lysine. They further found that feeding 15g of protected methionine increased methionine intake nearly by 20% in corn gluten diets. Watanabe *et al.* (2006) fed supplemented fat coated rumen protected methionine to dairy cows and studied the effects on their performance when methionine deficient diet was fed. They reported that the control cows had a greater DM intake as compared to treated cows. This could be due to more intakes of alfalfa hay, corn silage and concentrate. Subsequently, the nutrient intake was greater for the control cows. They further reported that the reason for lower DM intake of cows consuming protected amino acids was not known.

Milk production and composition

Armentano *et al.* (1996) supplemented high protein diets with methionine in alfalfa and heated soybeans and studied the response of dairy cows depicting the fact that methionine was not the limiting factor in terms of milk production. Corresponding findings were described by Casper and Schingoethe (1988) who reported that cows fed corn silage and barley based total mixed diet supplemented with rumen protected methionine did not increase milk yield but increased the milk protein percentages. They determined that methionine increased mammary nutrient syntheses, but it was not first limiting factor in milk production. Bertrand *et al.* (1998) found a slight decrease or no effect on milk yield when protected amino acids were fed to cows. They attributed that such results were received due to the decrease in DMI. However, Varvikko *et al.* (1999) found that supplementing protected methionine had no effect on milk production. They further stated that such results were noted because the animals might not be deficient in methionine.

Leonardi *et al.* (2003) added bypass methionine in the diets of lactating cows at two levels of crude proteins and found no effect of protected methionine supplementation on milk yield of cows. Such results might be due to deficiency of lysine in the diets they used, so methionine may not be the first limiting amino acid in this case. The results of present study have supported the findings of Bateman *et al.* (1999) and Overton *et al.* (1995) who reported that milk yield was not affected by amino acid supplementation in the diet of lactating cows.

In contrast, St-Pierre and Sylvester (2005) reported that 2-hydroxy-4-(methioninehylthio) butanoic acid (HMB) or its isopropyl ester (HMBi) added in the corn silage diets show a substantial increase in milk yield. Cows supplemented with isopropyl ester of methionine yielded 2.9 kg more milk compared with the control whereas, cows supplemented with both HMB and HMBi produced 0.9 kg/d more milk ($p>0.05$) than cows supplemented only with HMBi. It was opined that the cows they used were in early lactation which responded swiftly to the supplementation. Moreover, the control diet was short in calculated methionine quantity (1.80% of MP). Thus, the substantial responses in milk yield and milk composition they observed from HMBi addition was consistent with that as estimated from the metabolizable methionine source under the conditions of their study. Pruekvimolphan and Grummer (2001) reported an increase in 4%FCM yield while working with the supplementation of rumen-protected methionine to feather meal-based diet in dairy cows. The addition of ruminally protected methionine increased both milk yield (38.1 kg/d) and 4% FCM (39.3%) yield. It was supposed that the feather meal-based diet they used might have been deficient in methionine. Hence, methionine supplementation adequated this deficiency which resulted in increase in milk yield. In the present study, milk protein percentage was improved significantly.

In the present study milk, fat and SNF did not show significant differences among treatments. Misciattelli *et al*. (2003) reported increased milk fat by 2.4 g/kg of milk when dairy cows were reared on rations supplemented with methionine. The possible reason for this increase may be that the methionine is involved for synthesis of serum lipoproteins and as methyl group donor it is involved in synthesis of choline and phosphocholine and these two compounds are constituents of lipoproteins which take part in the transfer of lipids and supply fatty acids to the udder cells.

Higher level of methionine (35g/d) offered to the lactating buffaloes resulted in significantly highest milk protein percentage. Pruekvimolphan and Grummer (2001) reported similar findings while working with the supplementation of rumen-protected methionine to meat and bone meal-based diet in dairy cows. Milk protein percentage and yield were significantly higher averaging 2.99 % and 1.04 kg/d, respectively. Meat and bone meal is considered low in methionine compared with the amino acid profile of ruminal bacteria and milk. Therefore, they attributed this increase in milk protein to the fact that a more favorable amino acid profile may have been absorbed from the duodenum when rumen protected methionine was added. Yanxia *et al*. (2008) supplemented 25 g of methionine per day per animal and reported significant increase ($p<0.05$) in milk protein percentage from 3.02% to 3.08% in dairy animals. They attributed these findings to the difference caused by the efficacy of protection scheme of methionine, the status and amount of methionine and other amino acids in cows. In contrast, Bateman *et al*. (1999) found that in lactating Holstein fed blood and fish meal diets supplemented with ruminally protected methionine had no effect on milk protein percentage averaging 3.1% and milk protein yield was also not affected. The methionine was used at the rate of 10 g/d and all the diets exhibited similar results in terms of milk protein percentage and protein yields. They attributed such findings to the fact that the cows consumed higher DMI than planned and the diets they used contained higher crude protein (18%), therefore protein was not a limiting nutrient for milk production. Hence, the high concentration of protein intake resulted in masking the effect of any of the treatments. The study on combination of protein supplements and corn distillers' grains supplemented with and without protected amino acids in lactating cows by Liu *et al*. (2000) revealed that the milk protein percentages tended to be higher when cows were fed rumen protected methionine. They also found that the milk protein yield was unaffected by diets. The marginal increase of milk protein content supported the hypothesis that milk protein percentage is more sensitive to rumen protected methionine supplementation than either milk yield or milk protein yield in mid lactation. Adding rumen protected methionine in diets supplemented with folic acid and vitamin B12 tends to increase milk protein percentage and (+0.13 g/100g) milk protein yield (Preynat *et al*., 2009). It could be presumed that in our study buffaloes were multifarious and their diets were not deficient in methionine hence the results were not clearly noticeable. The Understanding of ruminant nutrition supports the theory that requirement for amino acids is decreased and efficiency to use amino acids for protein synthesis is amplified when necessary amino acids are provided in the proportions as required by the animal (NRC, 2001). It was further justified that in conditions where the quantity of one amino acid restricts protein synthesis by the mammary glands, alimentary supplementation of that particular amino acids in a metabolizable form would increase the profile of absorbed amino acids, ensuing additional protein synthesis. According to our observations, the immediate increase in milk protein percentage with the feeding of MetaSmart supports this conceptual context. However, the extensive studies with prolonged trial periods may produce more interpretable results.

Blood parameters

Triglycerides, total proteins and blood urea nitrogen showed significant variations across all treatments. The present findings have supported the results of Liu *et al*. (2000). They attributed such results to the increase in plasma concentration of methionine. This increase in plasma methionine may have been involved in synthesis of serum lipoproteins and methyl donor methionine is involved in synthesis of choline and phosphocholine. These two compounds are constituents of lipoproteins which take part in the transfer of lipids through blood. This could be the probable reason for increased blood triglycerides and supply them to the udder cells (Liu *et al*., 2000). Noftsger *et al*. (2005) comparing three sources of methionine reported an increase in blood triglycerides but found no effect on blood glucose level. They attributed such results to negative energy balance of cows. Moreover, they found no effect of methionine supplementation on plasma methionine concentration in cows. It could be inferred from such findings that most of plasma methionine might have been utilized for apoprotein synthesis, which was then used for synthesis of very low-density lipoproteins (VLDL). And VLDL'S are involved in evacuating triglycerides from liver to peripheral tissues to fulfill or compensate energy demands of animals. In the present study blood urea nitrogen was observed to be minimum at a higher level of methionine supplementation and vice versa. Yanxia *et al*. (2008) reported decrease in BUN when rumen protected methionine was supplemented in the diet of lactating cows. The significantly decreasing trend in BUN and increase in milk protein concentration depicts the fact that most of plasma methionine might have been utilized for compensating blood nitrogen.

In contrast, Piepenbrink *et al*. (1996) found a linear increase in BUN of dairy cows fed rumen-protected methionine. There were two explanations offered for these results: either the amino acids were not present in proper ratios for efficient extraction from blood by the mammary glands or they were supplemented in excess. Pruekvimolphan and Grummer (2001) reported an increase in concentration of BUN of cows fed feather meal-based diet supplemented with rumen-protected methionine as compared to the diet with no methionine supplementation. They attributed such findings to the fact that the diet they used having high degradable nitrogen might be in excess to their need for microbial protein synthesis. Another plausible explanation is that feed nitrogen might have been released in the rumen at rates that were uncoupled from carbohydrates fermentation and absorbed across the rumen wall to be transported for excretion. This low concentration of the nonstructural carbohydrates resulted in insufficient amounts of rapidly fermentable carbohydrate available at the same rate of protein fermentation for the microbes to efficiently utilize the nitrogen released from the urea.

Nitrogen balance

Nitrogen intake, fecal N, urinary N, N milk, retention, gross nitrogen efficiency and environmental nitrogen load were not affected by supplementation of protected methionine. St-Pierre and Sylvester (2005) conducted a study on 2-hydroxy-4-(methylthio) butanoic acid and its isopropyl ester when supplemented in the corn silage-based diets and found that nitrogen intake did not differ. It was further explained that diets were iso-nitrogenous hence treatments had no effect on dry matter intake. Estimated fecal N was also not affected by treatments and averaged 204 g/d., however, estimated urinary N was reduced by 17.5 g/d by when supplemented with isopropyl ester. Milk N was significantly increased across all treatments. Thus, a greater proportion of intake N and absorbed N was being partitioned to milk N. Gross N efficiency was increased by 3.1%. Dietary supplementation of isopropyl ester reduced the amount of N excreted per kilogram of milk N produced (environmental N load) from 2.04 to 1.77. They suggested that such results were observed due to adequating the inadequacy of methionine which eventually resulted in better N utilization.

The requirement for amino acids is decreased and efficiency to use amino acids for protein synthesis is amplified when necessary amino acids are absorbed in the proportions as required by the animal (NRC, 2001). In conditions where the quantity of one amino acid restricts protein synthesis by the mammary glands, alimentary supplementation of that amino acids in a metabolizable form would increase the profile of absorbed amino acids, ensuing additional protein synthesis. According to the findings of current study, the immediate increase in milk protein percentage with the feeding of MetaSmart supports this conceptual context.

The results of N intake, fecal N, urinary N, milk N, retained N, gross nitrogen efficiency and environmental nitrogen load was not affected by supplementation of protected methionine. However, nitrogen intake was found to be slightly highest in buffaloes fed OM and MM treatments. This apparent increase in nitrogen intake could be related to the apparent increase in DMI. Similar findings were discussed by St-Pierre and Sylvester (2005) who observed that nitrogen intake did not differ across experimental diets. They further explained that the diets used were isonitrogenous and methionine supplementation had no effect on DMI.

Nitrogen in milk increased with the increase in methionine level. This depicts the fact that an apparently better utilization of nitrogen was observed with the increasing dose of methionine. It was previously (Preynat *et al*., 2009) observed that adding rumen protected methionine in diets supplemented with folic acid and vitamin B12 have no effect on N retention as most of the methionine absorbed was used in formation of milk protein Moreover, it was further discussed that the interaction was non-significant.

CONCLUSION

Performance of lactating buffaloes reared on methionine supplemented rations was comparatively better with positive nitrogen balance and better body gains. It is recommended that extensive studies with prolonged trial period using emaciated, early lactating animals may produce further interpretable results.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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