Pakistan J. Zool., vol. 48(2), pp. 359-363, 2016.

Effect of Supplementation of Rumen Protected Lysine and Methionine on Production Performance, Milk and Blood Parameters of Early Lactating Nili-Ravi Buffaloes

Saeed Ahmed[1,*] Madiha Gohar,[1] Anjum Khalique,[1] Nisar Ahmad,[2] Faisal Shahzad,[3] Burhan-e-Azam,[4] Abdur Rahman[1] and Muhammad Irfan Khan[1]

[1]*Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore*
[2]*Department of Livestock Production, University of Veterinary and Animal Sciences, Lahore*
[3]*Faculty of Veterinary Sciences, Islamia University, Bahawalpur*
[4]*Buffalo Research Institute, Pattoki, Pakistan*

ABSTRACT

Milk production potential of buffaloes can be exploited through fulfilling nutritional requirements in terms of protein and energy. Two essential amino acids lysine (Lys) and methionine (Met) are limiting amino acids for optimum milk production in early lactation of these animals and are derived from dietary source. The objective of the present study was to analyze the effect of rumen protected lysine and methionine on the production performance of early lactating Nili-Ravi buffaloes. In total, (n=20) lactating buffaloes with same weight and parity were divided into four groups (A, B, C and D) for 60 days trial. The groups A, B and C received basal diet+Lysine 40g + Methionine 14g, basal diet + Lysine 30g+ Methionine 10g and basal diet + 20g Lysine + 7g Methionine, respectively. Whereas group D (control group) received basal diet without lysine and methionine supplementation. Milk production, however, was significantly (P<.05) greater in groups A and B as compared with the C and D. Milk protein, total solids, solid not fat and lactose were significantly greater (P<.05) in group C than A, B and D. Blood glucose level was greater in group C as compared to all other groups. Whereas, blood urea nitrogen was significantly greater in groups A and B as compared to rest of all other groups. It can be concluded that methionine and lysine supplementation had a positive effect on milk yield and milk composition (milk protein, solid not fat, total solids and lactose). It is suggested that for the optimum production performance in early lactating Nili Ravi buffalos, lysine and methionine supplementation can be a part of their ration.

Article Information
Received 6 April 2015
Revised 11 June 2015
Accepted 6 September 2015
Available online 1 March 2016

Authors' Contributions
SA, MG, conducted the experiments. BA helped in experimental work. AK supervised the work. NA, AR and MIK wrote the article. FS helped in statistical analysis.

Key words
Lysine, methionine, milk production, Nili-Ravi buffaloes.

INTRODUCTION

Genetic potential of an animal determines how much an animal has ability to produce milk and it can be exploited through fulfilling nutritional requirement of animal in terms of protein and energy. Dietary protein requirements of the animal depend upon the microbial population in the digestive system. Ruminal microorganism alters the quality and quantity of dietary protein before it reaches in small intestine. The protein absorbed in small intestine is named as metabolizable protein which has little resemblance to quantity and quality of protein provided by diet. Metabolizable protein is efficiently utilized when there is balance of amino acids which are required for maintenance of animals and milk production. Increased milk production and DM intake requires the highest intake of crude protein in diet. Two essential amino acids lysine (Lys) and methionine (Met) are limiting amino acids for milk protein and milk production (NRC, 2001).

Lys and Met are found in low concentration in feed protein and it cannot fulfill the animal requirement, secondly microbial protein is also insufficient to meet the animal requirements. Lys and Met has been identified as potentially limiting AA for milk protein synthesis (Kholif and Ebeid, 2009). So protected Lys and Met are added in feed to fulfill the deficiency of these amino acids. Apart from milk production they are also helpful in reducing the metabolic disorders and also have positive effect on energy balance and reproductive performances.

Nutrient requirement of dairy animal must be fulfilled from dietary sources for maximum production and optimum reproductive performance. During early lactation, energy as well as amino acid requirements for maintenance and milk production exceeds the amount of energy and amino acids obtained from the dietary sources. High energy requirements at the onset of lactation result in a negative energy balance, which adversely affects health and over all milk production of the animal (Riest *et al.*, 2002). Keeping in view the above facts, the present study was conducted to explore the effect of rumen protected lysine and methionine supplementation on production performance, milk and blood parameters of early lactating Nili Ravi buffaloes.

* Corresponding author: saeed.ahmed@uvas.edu.pk
0030-9923/2016/0002-0359 $ 8.00/0

MATERIALS AND METHODS

The present study was conducted at Livestock Experimental Station, Buffalo Research Institute (BRI) Pattoki. Twenty lactating buffaloes of the same parity and similar body weight were selected. Buffaloes were divided into four groups A, B, C and D. Each group consisted of 5 buffaloes. Group A, B and C were treatment groups and D was control group. The group A, B and C received basal diet + 40g Lys + 14g Met, basal diet +30g Lys +10g Met, and basal diet + 20g Lys + 7g Met, respectively. Whereas group D (control group) received basal diet without lysine and methionine supplementation during the trial period of 60 days. The animals were given adjustment period of first 15 days and remaining 45 days were for data collection. All animals were weighed before the start of experiment. The animals were provided with corn silage, wheat straw according to NRC (2001) requirements and concentrate ration according to milk production. Ingredient composition of concentrate ration was maize, 8%; cotton seed cake, 22; rape seed cake, 3; wheat bran, 32; maize gluten, 20; molasses, 14; mineral mixture, 1%, respectively. Chemical composition crude protein, 18.0; TDN, 76.0; ether extract, 2.90; crude fiber, 7.11; dry matter, 88.0; phosphorous, 0.29 and ash 1.60%, respectively. Protected Lys and Met were supplemented by mixing in concentrate ration. Fresh water was provided at *ad libitum* for 24 h daily. Vaccination and deworming of all animals was done before the initiation of the experiment. Individual feeding of all experimental animals were done. The data were collected for the following parameters.

Dry matter intake (DMI) was determined regularly on daily basis. Data on daily milk production (morning and evening) were also collected. Milk samples were collected on weekly basis and were analyzed for milk composition e.g. milk fat, milk protein, solid not fat (SNF), milk lactose and total solids by the method as described by AOAC (1999). Blood samples from the jugular vein were collected on monthly basis using non heparin vacutainers and serum was separated by centrifugation at 3000 rpm/ 15 minutes.

Feed efficiency= Milk produced (kg) / DMI (kg)

Statistical analysis

The data were analyzed through analysis of variance (ANOVA) technique Steel *et al.* (1997). The difference among the means was tested through DM range test (Duncan, 1955).

Table I.- Effect of amino acid supplementation on milk production of early lactating Nili-Ravi buffaloes.

Groups	No. of observations	Dry matter intake (kg/day)	Milk production (kg/day)
A	300	16.6±0.02	8.83± 10[b]
B	300	16.8±0.02	8.76±0.07[b]
C	300	16.4±0.02	7.27±0.12[a]
D	300	16.3±0.02	7.17±0.13[a]
P-value		0.440	0.001

Superscripts with different alphabets indicate significant differences (P<0.05)

A, basal diet + 40 g Lys + 14g Met; B, basal diet + 30 g Lys + 10 g Met; C, basal diet + 20 g Lys + 7 g Met; D, basal diet (silaent concentrations)

RESULTS AND DISCUSSION

Dry matter intake

Mean values of DM intake were observed as 16.6±0.02, 16.8±0.02, 16.4±0.02 and16.3±0.02 kg/day in the groups A, B, C and D, respectively (Table I). The highest DM intake was recorded in group B as 16.8±0.02 and the lowest in group D as 16.3±0.02. Statistically the difference of DM intake among the experimental groups was non-significant (P>.05). Results of the present study are in line with the findings of Lara *et al.* (2006) who fed different levels of methionine to multiparous dairy cows showing no effect on DM intake. Colin-Schoellen *et al.* (1995) studied interaction of Lys and Met through supplementation in feed and concluded that there was no effect of Lys and Met supplementation on DM intake. Similar observations were also recorded by Lee *et al.* (2012) who supplemented Holstein cows with rumen protected Lys and Met and concluded that there was no effect of supplementation of Lys and Met on DM intake. Results of the present experiment were not in line with the findings of Davidson *et al.* (2008**)** who supplemented methionine in feed to Holstein cows. There was increase in DM intake in treatment groups as compared to non-treated. This variation in result might be due to change of specie.

Milk production

Mean values of milk production were recorded as 8.83± 10, 8.76±0.07, 7.27±0.12 and 7.17±0.13 kg/day for the groups A, B, C and D, respectively (Table I). The highest value was observed in the group A (8.83± 10) and B (8.76±0.07) and the lowest in groups C and D. Milk production of groups A and B was significantly (P<0.05) greater than C and control, while difference of milk production was non-significant (P>0.05) between C and

Table II.- Effect of amino acid supplementation of milk produced by early lactating Nili-Ravi buffaloes.

Parameters	No. of observations	Groups				P- value
		A (40:14)	B (30:10)	C (20:07)	D (control)	
Milk protein	35	3.75±0.04^{ab}	3.81±0.05^{ab}	3.86±0.26^{b}	3.71±0.07 ^{a}	0.050
Milk fat	35	6.07±0.03	6.50±0.23	6.50±0.18	6.13±0.24	0.359
Total solids	35	16.27±0.30^{ab}	16.63±0.32 ^{a}	16.41±0.23 ^{b}	16.27±0.30 ^{ab}	0.020
SNF	35	9.65±.077^{ab}	9.78±0.10^{ab}	9.91±0.07 ^{a}	9.51±0.16 ^{b}	0.042
Lactose	35	5.16±0.08	5.16±0.06	5.22±0.04	5.12±0.98	0.063

Superscripts with different alphabets indicate significant differences (P<0.05)
For abbreviation and details of different groups, see Table I.

D groups. In the present study, Lys and Met supplementation in feed of Nili Ravi buffaloes had positive effects (P<0.05) on milk production. Results of the present experiment are in line to Cabrita *et al.* (2011) who studied the effect of these amino acids on lactating animals, fed various protein sources. Corn silage with mixture of Lys and Met increased milk production without changing milk fat content. Roger *et al.* (1987) supplemented cows with encapsulated rumen protected Lys and Met and observed the effect of Lys and Met on production performance. It was concluded that Lys and Met supplementation in feed of dairy animal resulted in higher milk production. Results of milk production were also in line with the findings of Overton *et al.* (1998), Leonardic *et al.* (2003) and Wang *et al.* (2010) who concluded that the supplementation of Lys and Met to the diets of lactating cows had significant effect on milk production.

Milk composition

Mean values for milk protein were found as 3.75±0.04, 3.81±0.05, 3.86±0.26 and 3.71±0.07% for the groups A, B, C and D, respectively (Table II). The highest value for milk protein was observed in group C (3.86±0.26) and the lowest in group D (3.71±0.07). Difference of milk protein in groups A and B was non-significant (P>.05). There was a significant difference (P<.05) between groups C and D. Similar observations were also recorded by Socham *et al.* (2005) who determined the effect of Met and lysine on production performance of postpartum dairy cows and concluded that supplementation of Met and Lys has increased milk protein. Mean values for milk fat were observed as 6.07±0.03, 6.50±0.23, 6.50±0.18 and 6.13±0.24 % for the groups A, B, C and D, respectively. The highest milk fat was observed in group B and C as 6.50±0.23, 6.50±0.18%, respectively. Difference of the milk fat was non-significant (P>.05) between treatment and control groups. Similar observations were also recorded by Bateman *et al.* (1999) who reported that milk composition remained unchanged especially fat contents, when rumen protected Lys and Met was added to the diets of lactating cows. Mean values for total solids were observed as 16.27±0.30, 16.63±0.32, 16.41±0.23 and 16.27±0.30% for the groups A, B, C and D, respectively. Mean value of treatments was significant different (P<.05) among all treatments A, B, C and D group. The value of total solids was significantly (P<.05) higher in the group B than rest of all other groups. Similar observations were recorded by Girard *et al.* (2005) who concluded that cows supplemented only Met had more total solids in milk than the other groups. Mean values for SNF were recorded as 9.65±.077, 9.78±0.10, 9.91±0.07 and 9.51±0.16% of the groups A, B, C and D, respectively (Table II). Difference of SNF was significant (P>.05) among all treatment groups. The highest value for SNF was noticed as 9.91±0.07% in group C as compared to other treatments. Similar observations were also recorded by Titi *et al.* (2013) who reported that Met supplementation to dairy cow enhanced SNF and total solids as compared to control group. Mean values for milk lactose were found as 5.16±0.08, 5.16±0.06, 5.22±0.04 and 5.12±0.98% for the groups A, B, C and D respectively. Difference of milk lactose was non-significant (P>.05) among the treated and control groups. Similar observations were also recorded by Robinson *et al.* (1999) who reported that rumen protected Lys and Met has no influence on milk lactose percentage in dairy cows.

Blood glucose

Blood glucose values were found as 36.85± 2.85, 37.40±3.17, 37.80±5.5 and 37.9.64±1.28 mg/dl for the groups A, B, C and D, respectively (Table II). The blood glucose level was non-significantly (P>.05) different among treatment and control groups. Similar observations were also recorded by Colin-Schoellen *et al.* (1995) who studied the interactions between supplementation with ruminally protected Met and lysine and the nature of protein or energy concentration of the

diet, concluded that there is no effect on blood glucose level.

Blood urea niterogen

Blood urea nitrogen values were found as 15.75±2.48, 15.41±3.30, 15.58±2.58 and 15.42±2.27 mg/dl for the groups A, B, C and D, respectively (Table III). Blood urea nitrogen level was non-significantly (P>.05) different among all the groups. These results are in line with the findings of Movaliya *et al.* (2013) who reported that Lys and Met supplementation to the Jaffarabadi heifers had no significant effect on blood urea nitrogen levels as compared to control group.

Table III.- Effect of amino acids supplement on blood glucose and blood urea nitrogen of early lactating Nili-Ravi buffaloes.

Groups	No. of observations	Blood glucose (mg/dL)	Blood urea (mg/dL)
A	15	36.85± 2.85	15.90±2.48[b]
B	15	37.40±3.17	15.82±3.30[b]
C	15	37.80±5.5	15.58±2.58[a]
D	15	37.90±1.28	15.42±2.27[a]
P-value		0.10	0.001

Superscripts with different alphabets indicate significant differences (P<0.05).
For details of different groups, See Table I.

CONCLUSION

It was concluded that dietary supplementation of Lys and Met in lactating Nili Ravi buffaloes have pronounced impact on some milk components and on milk production. There is no effect of Lys and Met supplementation on dry matter intake. From the present study, it is recommended that optimum supplemented concentration of Lys and Met in feed should be determined in large scale study and it can be supplemented to increase milk production in Nili Ravi buffaloes.

REFERENCES

AOAC 1999. *Official method of analysis of AOAC* Intl. 16th Edition, Association of Official Analytical Chemists, Maryland.

Bateman, H.G.I., Spain, J.N., Kerley, M.S., Belyea, R.L. and Marshall, R.T., 1999. Evaluation of ruminally protected methionine and lysine or blood meal and fish meal as protein sources for lactating Holsteins. *J. Dairy Sci.*, **82**: 2115–2120.

Cabrita, A.R.J., Dewhurst, R.J., Melo, D.S.P., Moorby, J.M. and Fonseca, A.J.M., 2011. Effects of dietary protein concentration and balance of absorbable amino acids on productive responses of dairy cows fed corn silage-based diets. *J. Dairy Sci*., 94: 4446-4456.

Colin-Schoellen, O., Laurent, F., Vignon, B., Robert, J.C. and Sloan, B., 1995. Effect of ruminaly protected lysine and methionine with protein or energy level in diets of cows. *J. Dairy Sci.*, **78**: 2807-2818.

Davidson, S., Hopkins, B.A., Odle, J., Brownie, C., Fellner, V. and Whitlow, L.W., 2008. Supplementing limited methionine diets with rumen-protected methionine, betaine, and choline in early lactation Holstein cows. *J. Dairy Sci*., **91**:1552–1559.

Duncan, D.B., 1955. Multiple range and multiple F tests, *Biometrics*, **11**: 1-42.

Girard, C.L., Apierre, H.L., Matte, J.J. and Lobley, G.E., 2005. Effects of dietary supplements of folic acid and rumen-protected methionine on lactational performance and folate metabolism of dairy cows. *J. Dairy Sci.*, **88:** 660-670

Kholif, A.M. and Ebeid, H.M., 2009. Effect of calcium soap supplementation without or with rumen protected methionine and lysine on lactating buffaloes, ration on milk production and composition. *Pakistan J. Zool*., *suppl. ser*., **9**: 697-700.

Lara, A., Mendoza, G.D., Landois, L., Barcena, R., Saanchez, M.T., Rojo, R., Ayala, J. and Vaga, S., 2006. Milk production in Holstein cows using different levels of ruminaly protected methionine. *Livest. Sci*., **105**: 105-108.

Leonardic, S.M. and Armentano, L.E., 2003. Effect of two levels of crude protein and methionine supplementation on performance of dairy cows. *J. Dairy Sci*., **86**: 4033-4042.

Lee, C., Hristov, A.N., Cassidy, T.W., Heyler, K.S., Lapierre, H., Varga, G.A., Veth, M.J., Patton, R.A. and parys, C., 2012. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. *J. Dairy Sci.*, **95**: 6042-6056.

Movaliya, J.K., Dutta, K.S., Padodara, R.J., Bhadaniya, A.R. and Savsani, H.H., 2013. Effect of by pass methionine-lysine supplementation on haematological and blood biochemical parameters of Jaffarabadi heifers. *Vet. World*, **6**: 147-150.

NRC, 2001. *Nutrient requirements of dairy cattles* (7th Rev. Ed.), National Research Council, National Academy Press, Washington D.C. USA.

Overton, T.R., Emmert, L.S. and Clark, J.H., 1998. Effects of source of carbohydrate and protein and rumen protected methionine on performance of cow. *J. Dairy Sci*., **81**: 221-228.

Riest, M., Erdin, D., Von Euw, D., Chilliard, Hammon, Y. and Morel, H.M., 2002. Estimation of energy balance at the individual and herd level using blood and milk traits in high yielding dairy cows. *J. Dairy Sci*., **85**: 3314-3327.

Robinson, H., Chalupa, W., Sniffen, C., Julien, W.E., Sato, H.F., Ujieda, T., Watanabe, K. and Suzuki, H., 1999. Influence of postruminal supplementation of methionine and lysine, isoleucine, or all three amino acids on intake and chewing behavior, ruminal fermentation, and milk and milk component production. *J. Anim. Sci.*, **77**: 2781-2792.

Rogers J.A., Krishnamoorthy U., Sniffen C.J., 1987. Plasma amino acids and milk protein production by cows fed rumen-protected methionine and lysine. *J. Dairy Sci.*, **70**: 789–798.

Socham, T., Putnam, D.E., Garthwaite, B.D., Whitehouse, N.L., Kierstead, N.A., Schwab, C.G., Ducharme, G.A. and Robert, J.C., 2005. Improving intestinal amino acid supply of pre- and postpartum dairy cows with rumen protected methionine and lysine. *J. Dairy Sci*., **88**: 1113-1126.

Steel, R.G.D. and Torrie, J.H., 1997. *Principles and procedures of statistics: A biometrics approach*, 2nd ed. McGraw-Hill, New York.

Titi, H., Azzam, S. and Alnimer, M., 2013. Effect of protected methionine supplementation on milk production and reproduction in first calf heifers. *Archiv. Tierzucht.*, **56**: 225-236.

Wang, Q., Zhang, Y., Yang. C., Xiong, H., Lin, Y., Yao, J., Li, H., Xie, L., Zhao, W. and Yao, Y., 2010. Acetylation of metabolic enzymes coordinates carbon source utilization and metabolic flux. *Science*, **327**: 1004–1007