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Effect of Molasses Based Multi-nutrient Supplement Containing Chromium on Nutrient Utilization, Milk Yield, Microbial Protein Flow and Antioxidant Status of Lactating Murrah Buffaloes

A.K. Patil*, A.K. Verma, Putan Singh and Asit Das

ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh-243122, India

ABSTRACT

The present experiment was conducted to study the effect of supplement of molasses based multi-nutrients containing chromium picolinate on nutrient utilization, milk yield, microbial protein flow and antioxidant status of lactating Murrah buffaloes. Twenty one lactating buffaloes were equally divided into three groups (n=7) on the basis of body weight (560±10.0 kg) and milk yield. Basal diet consisting of wheat straw, maize green and concentrate mixture was fed to all the groups. In addition of basal diet, the animals of group T_2 were fed 250 g molasses based multi-nutrients supplement (MMS-1) and the animals in group T_3 fed with 250 g MMS plus 5 mg Chromium Picolinate (MMS-2). The digestibility of the nutrients remained similar (P>0.05) among all the groups. Dietary MMS supplementation tended to improve milk yield by 18 and 16% in groups T_2 and T_3, respectively. The activities of superoxide dismutase, catalase and glutathione peroxidase were significantly (P<0.05) higher in both the supplemented groups, however, the mean value of glutathione reductase was similar in all three groups. It was observed that MMS supplementation did not have any significant effect on urinary excretion of allantoin, uric acid, purine derivative, creatinine and PDC index (mmol/l). From the results it can be concluded that supplementation of molasses based multi-nutrients (250 g/buffalo/day) improved milk yield by 18 (T_2) and 16 (T_3) percent and had better antioxidant status with comparable intake and digestibility of nutrients in lactating Murrah buffaloes.

Keywords: Cr-Picolinate, Milk Yield, Molasses, Multi nutrient supplementation, Murrah buffaloes, Nutrient utilization, Purine derivative index

INTRODUCTION

In our country, the buffalo mainly thrives on cereal straws that are highly lignified and have lower content of fermentable energy and protein, minerals and vitamins. Poor performance of buffaloes are mainly due to irregular and inadequate availability of quality feedstuff and imbalance of nutrients. Considering the availability and price of concentrate mixture, resource poor farmers can hardly afford them. Use of NPN substances like urea was tried earlier to replace the costly sources of proteins in ruminant diet. Feeding of molasses and urea is a suitable way of supplying fermentable energy and degradable protein, respectively. Urea molasses mineral supplement is an alternative feed resource that has been advocated as a panacea to protein and energy deficiency in ruminants especially during dry season (Aye, 2012). Number of on-station trials have been conducted in India and assessed the animal response and economic benefits of using UMMB (Srinivas and Gupta, 1997; Misra and Reddy, 2004) and results revealed that 30 to 40% of concentrate allowances could be reduced by feeding UMMB without any loss of milk production (Singh and Singh, 2003; Misra and Reddy, 2004). Mineral deficiency in grazing ruminants has been reported by several authors (Gowda *et al.,* 2004; Khan *et al.,* 2007) and supplementation is one way of tackling this problem. In an earlier study chromium was supplemented as it regulates carbohydrate metabolism as a structural component of glucose tolerance factor (GTF) (Mertz, 1993) which increases the absorption of glucose from circulation into peripheral tissues. In India, studies on supplementation of molasses based multi-nutrient (MMS) containing chromium for buffaloes and its effect on nutrients utilization, lactation performance and antioxidant status are limited. Hence, the present study

*Corresponding author: Email : ashokdrpatil@gmail.com

was conducted to find out the effect of MMS and chromium picolinate on nutrients utilization, milk yield, microbial protein flow and antioxidant status of lactating Murrah buffaloes.

MATERIALS AND METHODS

Twenty one healthy dairy buffaloes were selected from Cattle and Buffalo Farm in LPM section of ICAR-Indian Veterinary Research Institute, Izatnagar and were housed in a separate shed of having provision of both open and close space. Prior to the experimental feeding, all animals were dewormed with albendazole at 5 mg/kg body weight. Proper health management and sanitation conditions were maintained throughout the experimental period.

Animals were divided into 3 groups of 7 each on the basis of milk yield and body weight (560±10.0 kg) following randomized block design (RBD). All animals were supplied with green forages (5 kg DM/d), wheat straw *ad lib* and concentrate mixture as to meet nutrients requirement of animals (ICAR, 2013). Feeding regimens of experimental buffaloes were similar in all the groups except in the treatment groups where diets were additionally supplemented with 250 g MMS-1 and MMS-2 in T_2 or T_3 groups, respectively. The physical composition of MMS-1 and MMS-2 was same except addition of 2 g chromium picolinate per 100 kg of MMS-2 supplement. Ingredient composition of MMS was molasses (40%), urea (5%), deoiled mahua seed cake (10%),wheat bran (20%), crushed maize (20%), mineral mixture (4%) and salt (1%). Chemical composition of the diet fed to the animals is presented in Table 1. All the diets were isonitrogeneous. The milking was performed twice daily at 5 AM and 4 PM and milk yield (MY) was recorded daily at each milking using an electronic digital balance. The experiment was continued for 210 days, after 90 days of experimental feeding, a digestion trial of 6 days collection period was conducted to determine intake and digestibility of nutrients in different groups. The quantity of feed offered, residue and faeces voided was recorded. Representative samples of feed, residue and faecal samples were collected, preserved and analyzed for their proximate principles (AOAC, 2005) and fibre fractions (Van Soest *et al.,* 1991). During experimental period, daily feed offered and residue leftover was recorded to determine voluntary feed intake at fortnightly intervals. The urine samples (10 ml) were collected after 4-6 h of feeding during the digestibility trial from each animal, the sample of each animal was pooled and kept in a vial containing 20% H_2SO_4 to keep the pH below 3 and analyzed for allantoin (Young and Conway 1942), uric acid and creatinine using commercial kit. Serum was

Table 1. Chemical (% DM basis) composition of feed ingredients

Particulars	Concentrate mix.	Maize green	Wheat straw	MMS
Dry matter (DM)	93.39	21.8	93.08	88.96
Organic matter (OM)	93.09	91.19	93.49	81.59
Crude protein (CP)	17.98	5.96	2.94	23.13
Ether extract (EE)	2.50	1.42	1.35	0.54
Total ash (TA)	6.91	8.81	6.51	18.41
Acid detergent fibre (ADF)	8.38	41.78	51.24	18.86
Neutral detergent fibre (NDF)	38.83	49.43	79.81	35.73
Hemicellulose	30.45	7.65	28.57	25.83
Cellulose	8.35	41.96	51.05	6.06
Calcium (Ca)	0.61	0.43	0.27	0.72
Phosphorus (P)	0.56	0.25	0.08	0.68
Chromium (ppm)	0.87	1.53	0.42	0.93

analysed after thawing for various antioxidant enzymes following standard protocol using commercial diagnostic kit.

Statistical analysis

Statistical analysis: Data pertaining to feed intake, intake and utilization of nutrients were subjected to one way ANOVA. Data pertaining to serum antioxidant profile were subjected to two-way ANOVA Treatment means were separated by Duncan's multiple range test and the differences were considered to be significant at P<0.05. All analysis were performed using statistical package SPSS (20.0).

RESULTS AND DISCUSSION

Results indicated that the voluntary dry matter intake at fortnightly intervals did not vary significantly among three groups. Similar intake of DM and digestibility of nutrients is suggestive of no positive effects of molasses based multinutrient supplements on palatability (Table 2). Earlier finding showed that the sudden introduction of urea into the diet sometimes was associated with palatability problems (Koster *et al.,* 1997). However, in the present experiment MMS-1 and MMS-2 plus were introduced gradually over a period of 25 days that allowed the animals to be adapted smoothly.

Table 2. Intake and digestibility of nutrients and urinary excretion of purine derivatives in different groups

Particulars	Groups			SEM	P-Value
	T_1(control)	T_2	T_3		
Body weight (kg)	572.5±15.9	559.6±15.1	568.2±11.9	6.90	0.859
DMI (kg/d)	12.78±0.01	13.25±0.14	13.00±0.21	0.094	0.111
DOMI (kg/d)	8.49±0.15	8.83±0.17	9.00±0.18	0.106	0.134
CPI (g/d)	1122^{a}±1.21	1193^{c}±5.22	1154^{b}±5.90	8.14	0.001
CPI (g/kg $W^{0.75}$/d)	9.59^{a}±0.01	10.37^{c}±0.05	9.91^{b}±0.05	0.088	0.001
DCPI (g/d)	642.7±11.65	690.7±27.11	686.5±4.38	10.88	0.134
DCPI (g/kg $W^{0.75}$/d)	5.49±0.10	6.00±0.24	5.90±0.04	0.099	0.073
TDN (kg/d)	8.69±0.16	9.05±0.18	9.21±0.19	0.110	0.145
TDN (g/kg $W^{0.75}$/d)	74.26±1.33	78.63±1.55	79.15±1.66	1.01	0.081
Nutrient digestibility (%)					
DM	70.07±1.35	70.33±1.01	72.97±0.41	0.704	0.062
OM	71.83±1.28	72.03±1.02	74.91±0.44	0.645	0.068
CP	57.26±1.08	57.86±2.12	59.52±0.65	0.657	0.353
EE	73.67±3.18	75.38±3.35	75.76±0.54	1.456	0.844
NDF	58.34±2.31	59.13±2.25	61.79±0.83	1.101	0.439
ADF	56.08±1.86	56.63±2.32	58.50±1.02	1.008	0.626
Cellulose	60.53±2.04	60.61±2.44	62.95±1.31	1.102	0.630
HC	64.49±3.31	65.33±2.56	69.22±1.09	1.443	0.388
Urinary excretion (mmol/l)					
Allantoin	3.78±0.625	3.90±0.373	3.75±0.421	0.26	0.972
Uric acid	0.28±0.011	0.25±0.017	0.29±0.012	0.01	0.281
Purine derivatives	4.05±0.63	4.15±0.37	4.03±0.42	0.26	0.982
Creatinine	10.95±0.98	11.85±1.02	11.53±1.07	0.56	0.823
PDC index	42.75±4.29	40.49±1.79	41.02±3.77	1.86	0.890

[abc] Mean values bearing different superscripts varies significantly (P<0.05), (P<0.01)

In agreement to present study, Hosmami *et al.* (1998) observed that there was non-significant increase in intake of DM and TDN due to block feeding in buffaloes while intake of digestible crude protein (DCP) was significantly higher (P>0.01) in UMMB fed group over control group. On the contrary, Tripathi *et al.* (2006) reported that the dry matter intake improved (P<0.05) both in cows (14.13%) and buffaloes (22.76%) as a result of UMMB supplementation, which was reflected in improved (P<0.05) milk yield in cows (31.58%) and buffaloes (35.18%).

Most notable effect of MMS supplementation that we observed in this experiment was, increased intake of N. Intake of CP (g/d and g/kgW$^{0.75}$/d) was higher (P<0.05) in both T_2 and T_3 than control. The apparent digestibility of DM, OM, CP, EE, NDF, ADF and cellulose was not statistically different in 3 groups (Table 2). Similarly, Sahoo *et al.* (2004) reported that the digestibility of DM, OM, total carbohydrate, NDF, ADF, hemicellulose and cellulose in all the dietary groups (fed urea molasses liquid diet) were comparable. Contrary to this finding, Choubey *et al.* (2015) observed that the digestibility of various nutrients was significantly increased in UMMB fed groups, However, such supplements are more effective when the basal ration contained less CP. In the present study, the animals were fed as per the requirement for maintenances and production. Similarly, Sadri *et al.* (2009) reported no change in DMI and DMI as percent in body weight by supplementation (0.8 mg/kgW$^{0.75}$) of dietary Cr to periparturient cows. Report in similar lines is available in lambs (Depew *et al.* 1998).

Urinary excretion of allantoin, uric acid, creatinine and purine derivative index were similar and did not differ significantly among the groups (Table 2). It is thus evident that the MMS-1 and MMS-2 supplementation had no significant effect on microbial protein synthesis. This corresponds well with our data that showed similar dry matter intake, digestible organic matter intake, OM digestibility and TDN intake among the animals of different groups (Table 2). Similar results were also observed by Choubey *et al.* (2015). Urinary excretion of purine derivative is used as a marker of microbial protein synthesis in ruminants. However, excretion of PD in buffaloes is rather less as compared to other species so that PD is not considered to be an

Table 3. Fortnightly mean milk yield (kg/d) of lactating Murrah buffaloes in different groups

Fortnights	Groups			SEM	P-Value
	T_1(control)	T_2	T_3		
1st	7.87±0.57	8.10±0.42	7.86±0.59	0.29	0.94
2nd	7.69±0.62	8.84±0.49	8.05±0.92	0.38	0.47
3rd	7.26±0.56	8.56±0.60	7.38±1.16	0.45	0.44
4th	7.19±0.57	8.28±0.52	8.07±1.24	0.45	0.59
5th	6.60±0.63	7.86±0.61	7.57±1.28	0.48	0.54
6th	6.42±0.63	7.73±0.57	7.17±1.31	0.48	0.55
7th	5.51±0.64	6.99±0.58	6.51±1.37	0.50	0.48
8th	5.15±0.80	6.34±0.46	6.44±1.16	0.47	0.47
9th	4.94±0.57	5.98±0.46	6.07±1.32	0.46	0.56
10th	4.36±0.57	5.44±0.42	6.20±1.09	0.42	0.22
11th	4.31±0.68	5.47±0.45	6.14±0.95	0.41	0.28
12th	4.26±0.50	5.57±0.35	5.30±0.67	0.31	0.18
13th	4.27±0.43	4.34±0.43	4.69±0.36	0.23	0.76
14th	3.60±0.49	4.18±0.47	4.92±0.35	0.27	0.16
Total mean yield	5.67±0.51	6.69±0.43	6.58±0.93	0.36	0.46

effective marker of microbial protein synthesis in buffaloes (Wanapat *et al.,* 2012). While, our objective was to generate data on excretion pattern of PD in buffaloes that would supplement the small pool of data available in this species reported earlier by Dipu *et al.* (2006).

Daily milk production per buffalo was recorded and presented in Table-3. It revealed that overall mean milk production was 5.67, 6.69 and 6.58 kg/day for groups T_1, T_2 and T_3, respectively. The data showed that milk production increased by 1.02 and 0.91 kg/cow/d in groups T_2 and T_3 over control, which was 18 and 16

Table 4. Mean antioxidant status of lactating Murrah buffaloes in different groups

Particulars	Groups	0 Day	90 Day	180 Day	Mean	SEM	T	P	T*P
SOD (u/l)	T1 (control)	33.83 ±5.10	31.64 ±4.08	32.23 ±2.83	32.57[a] ±2.21	1.02	0.04	0.36	0.55
	T2	34.95 ±2.60	42.33 ±1.87	40.04 ±1.22	39.11[b] ±1.34				
	T3	32.44 ±3.21	37.20 ±2.89	37.94 ±1.94	35.86[ab] ±1.60				
	Mean	33.74 ±2.04	37.05 ±2.02	36.74 ±1.43					
Catalase (nmol/min/ml)	T1 (control)	44.26 ±6.06	43.33 ±5.66	49.97 ±1.88	45.85[a] ±2.74	1.59	0.02	0.40	0.85
	T2	48.19 ±8.77	55.68 ±3.22	56.77 ±1.47	53.55[ab] ±3.09				
	T3	55.37 ±4.53	58.26 ±4.50	57.08 ±1.97	56.90[b] ±2.09				
	Mean	49.27 ±3.78	52.42 ±3.00	54.61 ±1.30					
GPx (nmol/ min/ml)	T1 (control)	12.44 ±0.86	12.35 ±0.84	12.16 ±1.31	12.32[a] ±0.55	0.39	0.01	0.01	0.12
	T2	12.67 ±1.65	17.42 0.82	16.71 ±1.40	15.60[b] ±0.91				
	T3	12.23 ±1.48	17.50 ±0.82	17.07 ±1.03	15.60[b] ±0.88				
	Mean	12.45[A] ±0.73	15.76[B] ±0.78	15.31[B] ±0.90					
Glutathione reductase (nmol/ min/ml)	T1 (control)	140 ±13.35	133 ±11.98	135 ±10.76	136 ±6.51	3.21	0.97	0.93	0.97
	T2	132 ±13.35	133 ±6.27	139 ±2.97	135 ±4.71				
	T3	132 ±6.54	134 ±7.17	135 ±8.69	134 ±4.04				
	Mean	135 ±6.24	133 ±4.73	136 ±4.40					

[ab]Mean values bearing different superscripts within a column varies significantly (P<0.05), (P<0.01); [AB]Mean values bearing different superscripts within a row varies significantly (P<0.01)

% per cent more in comparison to control. Similarly, Tanwar (2013) observed that there was an increase of 1.02 l (13.21 percent) milk in UMMB supplemented group. Upreti *et al.* (2010) also observed that average total daily milk production per animal increased by 17.7% (i.e. 1.1 l/day). Our results also corroborated well with the finding of Singh and Singh (2003), Sahoo *et al.* (2009) and Avila (2006). Chromium supplemented group had comparable milk yield with MMS-1 group. Similarly Cr supplementation from either CrPic (Peterson, 2000) or CrMet (Bryan *et al.,* 2004) did not affected milk production in grazing dairy cows where forage was the major source of energy. Contrary to this Deka *et al.* (2015) observed significant increase in milk yield when buffaloes diets were supplemented with 1.0 and 1.5 mg Cr/kg DM.

The results revealed that the chromium supplementation with MMS did not affect the milk yield in mid and late lactation, however, previous researchers have found higher milk yield in chromium supplemented groups in early lactating animals. Most of this difference could be accounted due to the stage of lactation. Most of the lipolytic effect of supplementary chromium is observed during early lactation because of high demand of glucose for the synthesis of milk. Thus, it seems that supplementation of chromium during mid and late lactation may not be beneficial.

Nutritional deficiencies and adverse climatic conditions generally increase the production of free radicals, leading to oxidative stress (Elsayed, 2001; Saleh *et al.,* 2008) which has a negative impact on, live weight gain production and health status of animals. The activities of SOD, catalase and glutathione peroxidase were significantly ($P<0.05$) higher in supplemented groups (T_2 and T_3) than control group (Table 4). The antioxidant status of supplemented group was better in comparison with control group. The animals of group T_2 and T_3 whose diets were supplemented with MMS-1 and MMS-2 had comparable anti-oxidation status indicating that supplementation of chromium may not be beneficial in relation of antioxidant capacity of animal. The better antioxidant status of animals in both the groups is due to only superposing of trace minerals in form of MMS. Similarly, Zhang *et al.* (2014) reported that there was no change in total antioxidant capacity in Cr supplemented group. Similar results were also reported by Parashuramulu *et al.* (2015), where the catalase activity was higher in 80 ppm Zn supplemented calves. Previous studies reported that the supplementation of trace minerals and vitamin (vitamin E and copper) affect the oxidant-antioxidant balance in dairy cows since Cu is a component of SOD and ceruloplasmin (Underwood and Suttle, 1999). Shinde *et al.* (2008) found that supplementation of vitamin E and Se improved the antioxidant status in terms of erythrocyte GSH-Px activity in buffalo calves, as compared to the unsupplemented group. Our results corroborated well with those who reported that micronutrients such as Zn, Cu, Fe and Mn improved the efficiency of antioxidant system (Nagalakshmi *et al.,* 2009).

CONCLUSIONS

From the results it can be concluded that supplementation of molasses based multi-nutrients (250 g/buffalo/day) improved milk yield by 18 (T_2) and 16 (T_3) percent and had better antioxidant status with comparable intake and digestibility of nutrients in lactating Murrah buffaloes.

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