



Effect of molasses based multi-nutrients and chromium supplementation on milk quality and serum biochemistry of mid and late lactating Murrah buffaloes (*Bubalus bubalis*)

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ABSTRACT

Lactating Murrah buffaloes (28) were divided into 4 groups of 7 each to study the effect of supplement molasses based multi-nutrient containing chromium picolinate on milk quality and serum biochemistry. Basal diet comprising wheat straw, maize green and concentrate mixture were fed to all the groups. In addition to basal diet, the animals were fed 250 g molasses based multi-nutrient supplement (MMS-1), 5 mg Cr-picolinate and MMS-2 (MMS + 5 mg Cr picolinate) in groups T₂, T₃ and T₄, respectively. Daily milk yield and monthly milk composition were recorded. Blood samples were collected from the jugular vein on days 0, 90 and 180 of experimental feeding for the estimation of serum metabolite profile, concentrations of insulin, non-esterified fatty acid (NEFA), beta-hydroxybutyric acid (BHBA), T₃, T₄, IGF-1, cortisol, estradiol, progesterone and Cr levels. Results have revealed that the serum Cr concentration increased in Cr supplemented groups; however, the milk Cr concentration was comparable among all the groups. Hematological parameters were statistically comparable among 4 groups except that RBC concentration was higher in group T₂. Fat corrected milk (FCM), solid corrected milk (SCM), energy corrected milk (ECM) yields and milk energy contents were significantly higher in MMS supplement groups. Supplementation of MMS and Cr-picolinate had no effect on serum estradiol, NEFA, BHBA, T₃, T₄, cortisol and IGF-1, however, the concentration of progesterone was significantly lower in all supplemented groups. From the results, it may be deduced that the supplementation of Cr has no adverse effect on FCM yield, however, supplementation of MMS improved FCM yield by 28%. No synergistic effect of supplementation of Cr and MMS on milk composition (fat, protein, SNF, TS and lactose) was observed in lactating Murrah buffaloes.

Keywords: Cr-picolinate, FCM, Lactating murrah buffaloes, Milk composition, MMS, NEFA

In India, energy and protein demands of buffaloes are being mainly met by feeding them low-quality roughages, agricultural crop-residues and industrial by-products which contain high levels of lingo-cellulosic materials, low levels of fermentable carbohydrate and protein. To increase the productivity of buffaloes, supplementation of nutrients, which can improve the utilization of poor-quality roughages and fulfill the deficiency of nutrients, are essential as the feed utilization can be increased by supplementation of critical nutrients in ration. Urea molasses mineral supplement (MMS) is an alternative feed resource that has been advocated as a solution to mitigate protein and energy deficiency in ruminants especially during dry season (Aye 2012). Supplementation of MMS to buffaloes fed straw based diet has increased the growth and supported moderate milk production (Sahoo *et al.* 2004) One major cause of

reduced production and impaired reproduction is minerals deficiency (Khan *et al.* 2007). Supplementation of trace minerals is one way of tackling this problem.

Cr 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 (Anderson 1987). Feeding Cr to dairy cows during prepartum and postpartum had consistently increased milk yield of cows during early lactation (McNamara *et al.* 2005). The positive influence of Cr on milk production has been attributed to its effects on energy metabolism reflected through decreased mobilization of NEFA from adipose tissue and increased insulin sensitivity (Sumner *et al.* 2007). Cr supplementation in mid and late lactation may be particularly beneficial as it has role in glucose metabolism. Previous studies have reported that Cr supplemented during gestation and early lactation has increased milk production (Hayirli *et al.* 2001), NEFA concentration (Bryan *et al.* 2004), improved fertility (Yang *et al.* 1996) but none of the research pertaining to Cr supplementation during mid and late lactation in buffaloes

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has been conducted so far. Although several investigations were conducted to evaluate the effect of Cr and molasses-based supplement separately, apparently there is no report that studied the effect of Cr in combination with molasses on milk yield, its composition and biochemical profile. Therefore, this experiment was conducted to evaluate the performance of mid and late lactating Murrah buffaloes fed multi nutrients molasses supplement with or without addition of Cr.

MATERIALS AND METHODS

Selection of animals, experimental design and dietary treatments: Healthy mid lactating Murrah buffaloes (28) were selected in the subtropical region (Indian Veterinary Research Institute, Bareilly) of India and divided into 4 groups (n=7) on the basis of on the basis of milk yield and body weight (560±10.0 kg). All the experimental procedure was in compliance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, India) for the care and use of animal for scientific purposes.

All animals were supplied with green forages (5 kg DM/d), wheat straw *ad lib.* and concentrate mixture as per milk production requirement of animals (ICAR, 2013). Feeding regimen of experimental buffaloes was similar in all the groups except in the treatment groups where diet was additionally supplemented with 250 g MMS-1, 5 mg Cr Picolinate and 250 g MMS-2 plus 5 mg Cr Picolinate in T₂, T₃ and T₄ groups, respectively. The physical composition of MMS-1 and MMS-2 was similar except addition of 5 mg chromium picolinate per 250 g in MMS-2 supplement. Ingredients 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 made iso-nitrogenous and were formulated to meet the nutrients requirement of buffaloes (ICAR, 2013). The experiment was continued for 210 days and milk composition was evaluated at monthly intervals. Serum concentrations of hormones and metabolites were determined by using various diagnostic kits. Cr contents of milk and serum samples were analyzed by Atomic Absorption Spectrophotometer (Model 4141, ECI Ltd., India). Fat corrected milk yield was calculated using the formula of Rice *et al.* (1970) in which milk yield was adjusted to 6% FCM (kg) = (0.308 × M) + (11.54 × F × M)/10 and milk energy content was calculated according to method of Tyrrell and Reid (1965). Milk fat, Protein, total solid, solid not fat and lactose were measured in fresh milk by using Ultrasonic Milk Analyser (Master LM2, Bulgaria).

Statistical analysis: Data pertaining to milk composition, hematology and biochemical parameters were subjected to statistical analysis following general linear model (GLM)-univariate or multivariate analysis to separate the effect of treatment, day of sampling and their interaction. Significance was declared at P<0.05 and P<0.01 levels.

Table 1. Chemical (% DM basis) composition of concentrate mixture, green fodder (maize), wheat straw and molasses based multi-nutrient supplement

Particular	Concentrate mix.	Green fodder	Wheat straw	MMS
Dry matter (DM)	93.39	21.80	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

Significant differences were separated using Duncan's test. Treatment means were presented along with standard errors of the mean (SEM). All analysis were performed using statistical package SPSS (20.0).

RESULTS AND DISCUSSION

Effect of Cr and MMS supplementation on serum biochemical profile: Circulating concentrations of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) measure the success of adaptation to negative energy balance. An elevated level of serum NEFA is one of the indicators of negative energy balance (NEB) in postpartum dairy cattle (Bell *et al.* 1995). Other indicators of NEB are an increased plasma concentration of BHBA (Bell *et al.* 1995), decreased plasma glucose (Vazquez-Anon *et al.* 1994) and decreased amount of insulin and insulin-like growth factor-1 (IGF-1) (Butler *et al.* 2003).

In the present study, serum concentrations of NEFA were comparable among all the dietary treatments (Table 2). Similarly, interaction between treatment and periods on serum NEFA concentration was found comparable among all the groups. Drackely *et al.* (2001) reported that normal values of NEFA in positive energy balance were less than 200 μmol/litre. Values more than 700 μmol/litre for more than 7-d post calving indicate severe negative energy balance causing severe health problems. In the present study, the NEFA level ranged from 167 to 172 μmol/litre, which indicates that animals were in positive energy balance (Drackely *et al.* 2001). In agreement to present study, Hayirli *et al.* (2001) and Smith *et al.* (2005) reported that no changes in the blood metabolites were observed when cows were supplemented with Cr. Conversely, reduced NEFA concentration was observed in response to Cr supplementation (Bryan *et al.* 2004).

In present study the values of BHBA were also comparable among different treatment groups. The comparable BHBA and its relatively low concentration is further indicative of positive energy balance. Our results are in agreement with Hayirli *et al.* (2001) and Bryan *et*

Table 2. Mean serum metabolite and hormonal profile of different groups

Particular	Treatment#	Periods							
		0 Day	90 Day	180 Day	Mean	SEM	T	P	T*P
NEFA ($\mu\text{mol/l}$)	T ₁ (control)	172	166	170	169	3.1	0.95	0.79	1.0
	T ₂	170	164	168	167				
	T ₃	171	168	166	168				
	T ₄	176	171	169	172				
	Mean	172	167	168					
BHBA ($\mu\text{mol/l}$)	T ₁ (control)	337	336	336	337	9.8	0.99	0.99	1.0
	T ₂	337	336	335	336				
	T ₃	343	341	343	342				
	T ₄	345	342	340	342				
	Mean	340.6	338.9	338.5					
Insulin ($\mu\text{ IU/ml}$)	T ₁ (control)	10.21	10.61	9.89	10.24	0.31	0.22	0.62	0.98
	T ₂	11.75	12.87	11.46	12.03				
	T ₃	11.38	10.95	10.44	10.92				
	T ₄	11.84	11.28	11.15	11.42				
	Mean	11.30	11.43	10.74					
IGF-1 (ng/ml)	T ₁ (control)	121.7	123.2	122.0	122.3	10.8	0.99	0.99	1.0
	T ₂	112.2	114.0	113.2	113.1				
	T ₃	114.3	115.6	114.3	114.7				
	T ₄	113.3	114.5	115.0	114.2				
	Mean	115.4	116.8	116.1					
T ₃ (nmol/L)	T ₁ (control)	1.86	1.87	1.82	1.85	0.04	0.75	0.86	0.99
	T ₂	1.81	1.73	1.68	1.74				
	T ₃	1.84	1.80	1.82	1.82				
	T ₄	1.78	1.88	1.79	1.82				
	Mean	1.82	1.82	1.78	1.81				
T ₄ (nmol/L)	T ₁ (control)	32.31	34.42	33.26	33.33	1.09	0.61	0.97	0.99
	T ₂	31.60	32.14	32.36	32.03				
	T ₃	31.66	30.18	28.92	30.25				
	T ₄	34.09	34.46	33.89	34.15				
	Mean	32.42	32.80	32.11					
Cortisol (nmol/l)	T ₁ (control)	54.23	53.11	52.08	53.14	1.02	0.31	0.03	0.19
	T ₂	52.37	55.12	53.08	53.52				
	T ₃	57.95	50.24	48.82	52.33				
	T ₄	58.78	44.02	43.04	48.61				
	Mean	55.83 ^B	50.62 ^A	49.26 ^A					
Estradiol (pg/ml)	T ₁ (control)	7.86 \pm 1.86	7.30 \pm 1.66	7.21	7.46	0.50	0.43	0.83	0.99
	T ₂	5.86	6.22	6.24	6.11				
	T ₃	6.15	7.17	7.33	6.88				
	T ₄	7.26	8.95	9.03	8.41				
	Mean	6.78	7.41	7.45					
Progesterone (ng/ml)	T ₁ (control)	3.44	5.28 \pm 0.65	5.35 \pm 0.65	4.69 ^b \pm 0.42	0.19	0.01	0.02	0.85
	T ₂	1.92	2.08	3.12	2.37 ^a				
	T ₃	3.08	2.99	3.84	3.30 ^a				
	T ₄	1.96	3.01	3.68	2.88 ^a				
	Mean	2.60 ^A	3.34 ^A	4.0 ^B					

^{AB}Mean values with different superscripts within a row differ significantly ($P < 0.05$). #Animals in group T₁ fed a basal diet only; in group T₂ fed basal diet + MMS-1; in group T₃ fed basal diet + 5 mg Cr and in group T₄ fed basal diet + MMS-2. SEM standard error of mean.

al. (2004) who reported that postpartum concentrations of plasma BHBA were not affected by Cr supplementation.

Cr enhances the action of insulin by increasing insulin binding with receptor, phosphorylation, and protein kinase

activity that results in decreased insulin resistance. The mean concentration of serum insulin was comparable among all groups. The periodic changes in the serum insulin concentration were also non-significant. Similarly, Peterson

Table 3. Mean milk and blood chromium content in different groups

Particular	Treatment#	0 Day	90 Day	180 Day	Mean	SEM	T	P	T×P
Milk Cr (µg/l)	T ₁ (control)	59.77	54.51	56.66	56.98	0.63	0.35	0.84	0.65
	T ₂	57.62	59.76	58.98	58.79				
	T ₃	57.38	55.98	54.50	55.95				
	T ₄	57.62	58.80	59.18	58.53				
	Mean	58.10	57.26	57.33					
Serum Cr (mg/l)	T ₁ (control)	0.59	0.59	0.61	0.60 ^a	0.01	0.01	0.01	0.02
	T ₂	0.55	0.57	0.53	0.55 ^a				
	T ₃	0.56	0.81	0.79	0.72 ^b				
	T ₄	0.56	0.80	0.81	0.72 ^b				
	Mean	0.56 ^A	0.69 ^B	0.68 ^B					

^{a,b}Mean values with different superscripts within column differ significantly ($P < 0.01$). ^{A,B}Mean values with different superscripts within a row differ significantly ($P < 0.01$). #Animals in group T₁ fed a basal diet only; in group T₂ fed basal diet + MMS-1; in group T₃ fed basal diet + 5 mg Cr and in group T₄ fed basal diet + MMS-2. SEM standard error of mean.

Table 4. Mean values of milk constituents at different intervals in different groups

Particular/ Treatment	1st M	2nd M	3rd M	4th M	5th M	6th M	7th M	Mean	SEM	T	P	T×P
<i>FCM (kg/d)</i>												
T1 (control)	8.13	7.05	6.14	4.53	3.69	3.12	2.77	5.03 ^a ±0.67	0.18	0.01	0.01	1.00
T2	9.21	9.16	8.38	6.33	4.95	4.54	3.13	6.50 ^b ±0.87				
T3	8.60	7.77	7.26	5.63	4.44	3.60	2.80	5.69 ^{ab} ±0.76				
T4	8.38	8.56	7.66	6.36	5.93	4.90	3.59	6.46 ^b ±0.93				
Mean	8.59 ^D	8.12 ^D	7.35 ^D	5.69 ^C	4.71 ^{BC}	4.01 ^{AB}	3.05 ^A					
<i>SCM (kg/d)</i>												
T1 (control)	13.30	12.16	11.14	9.10	8.12	6.84	6.08	9.55 ^a	0.21	0.01	0.01	1.00
T2	14.43	14.38	13.63	11.56	10.00	9.17	7.07	11.46 ^b				
T3	13.73	12.98	12.37	10.70	9.27	7.89	6.51	10.49 ^{ab}				
T4	13.54	13.36	12.42	11.06	10.30	9.20	7.01	11.01 ^b				
Mean	13.76 ^D	13.22 ^D	12.39 ^D	10.59 ^C	9.39 ^{BC}	8.24 ^{AB}	6.65 ^A					
<i>ECM (kg/d)</i>												
T1 (control)	14.75	13.41	12.37	10.03	8.91	8.05	7.49	10.72 ^a	0.22	0.01	0.01	1.00
T2	16.07	15.88	15.08	12.68	11.01	10.46	8.22	12.77 ^b				
T3	15.25	14.40	13.77	11.90	10.25	8.76	7.22	11.65 ^{ab}				
T4	14.79	14.61	13.74	12.10	11.77	10.72	9.03	12.40 ^b				
Mean	15.23 ^D	14.57 ^D	13.74 ^D	11.66 ^C	10.44 ^{BC}	9.45 ^{AB}	7.95 ^A					
<i>NEL (Mcal/kg)</i>												
T1 (control)	1.14	1.11	1.14	1.12	1.15	1.13	1.15	1.13	0.01	0.06	0.97	1.00
T2	1.14	1.14	1.18	1.15	1.17	1.14	1.16	1.11				
T3	1.13	1.13	1.12	1.10	1.11	1.12	1.12	1.12				
T4	1.13	1.15	1.13	1.13	1.17	1.15	1.13	1.14				
Mean	1.13	1.13	1.14	1.13	1.15	1.13	1.14					
<i>ME (Mcal/kg)</i>												
T1 (control)	9.97	9.12	8.36	6.83	6.09	5.13	4.56	7.17 ^a	0.16	0.01	0.01	1.00
T2	10.82	10.79	10.23	8.67	7.50	6.88	5.30	8.60 ^b				
T3	10.30	9.74	9.28	8.03	6.95	5.92	4.89	7.87 ^{ab}				
T4	10.16	10.02	9.32	8.30	7.72	6.90	5.26	8.26 ^b				
Mean	10.32 ^D	9.91 ^D	9.29 ^D	7.94 ^C	7.04 ^{BC}	6.18 ^{AB}	4.99 ^A					

#M=months.

Table 5. Mean values of milk composition (%) at different intervals in different groups

Particular/ Treatment	0 day	30 day	60 day	90 day	120 day	150 day	180 day	210 day	Mean	SEM	T	P	T×P
<i>Fat</i>													
T1 (control)	7.51	7.84	7.62	7.85	7.76	8.08	7.78	7.99	7.80	0.05	0.08	0.89	0.99
T2	7.71	7.86	7.95	8.27	8.04	8.16	7.86	8.02	7.98				
T3	7.61	7.81	7.77	7.67	7.45	7.49	7.61	7.65	7.63				
T4	7.86	7.94	8.18	7.72	7.77	8.23	7.97	7.76	7.93				
Mean	7.67	7.86	7.88	7.88	7.76	7.98	7.80	7.86					
<i>Solid not fat</i>													
T1 (control)	10.22	10.14	10.08	10.07	10.21	10.14	10.07	10.33	10.16	0.05	0.42	0.31	0.35
T2	10.19	10.11	10.12	10.05	10.22	10.21	10.24	8.66	9.97				
T3	10.11	9.98	10.07	10.16	10.36	10.36	10.08	10.22	10.17				
T4	10.19	10.10	10.12	10.30	10.40	10.19	10.01	9.95	10.15				
Mean	10.17	10.08	10.10	10.14	10.29	10.23	10.10	9.78					
<i>Total solid</i>													
T1 (control)	17.71	17.98	17.70	17.91	17.97	18.22	17.85	18.31	17.96	0.07	0.58	0.64	0.81
T2	18.19	17.97	18.07	18.31	18.26	18.37	18.09	16.68	17.99				
T3	17.99	17.80	17.84	17.84	17.82	17.85	17.70	17.86	17.84				
T4	18.14	18.04	18.30	18.02	18.17	18.42	17.98	17.70	18.10				
Mean	18.01	17.95	17.98	18.02	18.05	18.21	17.90	17.64					
<i>Total protein</i>													
T1 (control)	4.01	3.97	3.81	3.92	3.88	3.88	4.00	3.90	3.92	0.02	0.33	0.72	0.87
T2	3.98	4.03	3.90	3.95	3.88	3.95	3.98	4.03	3.96				
T3	4.06	3.90	3.90	4.02	4.02	4.00	3.94	3.96	3.98				
T4	3.56	3.75	3.72	4.00	3.93	4.00	4.01	3.99	3.86				
Mean	3.90	3.91	3.83	3.97	3.93	3.96	3.98	3.97					
<i>Lactose</i>													
T1 (control)	5.33	5.49	5.53	5.47	5.57	5.44	5.59	5.51	5.49	0.20	0.40	0.41	0.59
T2	5.33	5.23	5.41	5.46	5.57	5.50	5.59	5.49	5.45				
T3	5.41	5.41	5.56	5.46	5.66	5.57	5.59	5.50	5.52				
T4	5.51	5.46	5.50	5.60	5.65	5.47	5.45	5.58	5.53				
Mean	5.40	5.40	5.50	5.49	5.57	5.50	5.56	5.52					

(2000) did not find any significant effect on plasma insulin concentrations in Cr supplemented group. Conversely, Gendley *et al.* (2015) reported significantly higher insulin concentration in UMBB fed group and inferred that supplemented group can better utilize glucose than non supplemented group. These positive effects on animal performance may be related to Cr effects on insulin. Some researchers suggested that Cr enhances the response to insulin receptors by increased sensitivity (Mertz *et al.* 1976), while others propose Cr increases the number of insulin receptors (Anderson, 2003). Similarly, IGF-1, T₃ and T₄ were also statistically similar among the different groups. Negative energy balance during early lactation in dairy cows leads to an altered metabolic state that has major effects on the production of IGF. Low IGF-I are associated with poor fertility and therefore, poor conception rate. In our study, we did not find any significant difference in IGF-1 which indicates that the animals were not in negative energy balance.

The stress relieving effect of Cr has been well studied. Stress factors stimulate the hypothalamus leading to the production of corticotropin releasing factor, which

stimulates the pituitary to produce adrenocorticotrophic hormone, which in turn stimulates the adrenal cortex to increase the production and release of corticosterone. Cortisol is widely used as a marker of “stress” (Chang and Mowat 1992), i.e. cortisol concentrations are greater in stressed animals than in those that are not stressed. Response of Cr supplementation on serum cortisol levels in cattle has been variable. In this study the concentration of cortisol was comparable among the different groups. Correspondingly, Depew *et al.* (1998) and Kegley *et al.* (1997b) did not observe any effect of Cr supplementation on serum cortisol concentration.

The overall mean serum progesterone (P₄) concentration was significantly (P<0.01) higher in control group. Likewise, the mean periodical concentration of progesterone was significantly (P<0.05) higher at 180 days. However, the mean serum estradiol and its periodical concentration was found comparable (P>0.05) among the groups. We have taken animals for study in mid lactation (2–3 months post-calving) and at that time most of the animals were inseminated and were pregnant, so that we cannot make any clear cut conclusion about pregnancy rate

by just considering the level of progesterone and estradiol in serum.

Effect of Cr and MMS supplementation on blood and milk Cr concentration: The effect of Cr supplementation of cattle diets on Cr concentration in milk and blood have received little attention. Cr analysis of milk is challenging task because of extremely low concentration present in it. Further there is potential of Cr contamination during collection, storage and preparation of samples for analysis. As it has been shown in Table 3 the overall mean serum Cr and its periodical means were significantly ($P < 0.01$) higher in Cr supplemented groups (T_3 and T_4). Mean serum Cr increased ($P < 0.05$) significantly after supplementation indicating proper absorption of the element. Similar to present study, Hayirli *et al.* (2001) reported that milk Cr concentration varied between 55.4 and 56.6 $\mu\text{g/L}$ in dairy cows and was not affected by supplementation with Cr methionine. Conversely, Deka *et al.* (2015) reported that the dietary Cr supplementation had significantly increased plasma and milk Cr concentration. Blood Cr concentration might reflect to an extent the intake of this element, but in case of excessive Cr intake, it is inappropriate to use the blood Cr concentration as an indicator of Cr status in animals (Underwood 1999).

Effect of Cr and MMS supplementation on FCM, ECM, SCM and NE_L : The effects of Cr and MMS supplementation on milk parameters are shown in Table 4. As shown, FCM, SCM, ECM and milk energy were significantly ($P < 0.01$) higher in MMS supplemented groups. There was no synergistic effect of Cr and MMS on yield of FCM, SCM, ECM and milk energy. In agreement to present findings, Bryan *et al.* (2004) suggested that there was no effect of Cr supplementation ($P > 0.10$) on milk yield, ECM, 3.5% FCM and milk composition. Conversely, Deka *et al.* (2015) have observed that the supplementation of 1.5 ppm Cr increased the overall average FCM, ECM and SCM content of milk in lactating Murrah buffaloes. The value of net energy of lactation did not differ significantly and were comparable among 4 groups. However, average milk energy differed significantly ($P < 0.01$) as compared to control group, values were higher ($P < 0.01$) by 22.12, 11.63 and 6.8% in groups, T_2 , T_3 and T_4 , respectively.

Milk composition was not affected by Cr supplementation (Table 3 and 5). The composition of milk with regard to Cr supplementation was studied by relatively few research workers (Pechova *et al.* 2003). In most cases, they found no difference between the experimental and control group (Yang *et al.* 1996). Hayirli *et al.* (2001) reported increased fat production and lactose levels in milk after Cr supplementation, which is contrary to present findings. Similar results were also observed by various workers (Kneeskern *et al.* 2015).

From these results, it may be deduced that supplementation of Cr has no effect on FCM yield, however, supplementation of MMS improved FCM yield by 28%. Moreover, milk composition was unaffected by supplementation of either Cr or MMS. Further,

supplementation of Cr and MMS had no significant effect on serum NEFA, BHBA, insulin, IGF-1, cortisol, T3 and T4 concentration.

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