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.
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 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 nutrition requirement of buffaloes (ICAR, 2013). The experiment was conducted 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 Tyrell 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.

| 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-esterifies 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 (µmol/l)    | T1 (control)        | 172     | 166      | 170      | 169      | 3.1      | 0.95     | 0.79     | 1.0      |
|                  | T2                  | 170     | 164      | 168      | 167      |          |          |          |          |
|                  | T3                  | 171     | 168      | 166      | 168      |          |          |          |          |
|                  | T4                  | 176     | 171      | 169      | 172      |          |          |          |          |
|                  | Mean                | 172     | 167      | 168      |          |          |          |          |          |
| BHBA (µmol/l)    | T1 (control)        | 337     | 336      | 336      | 337      | 9.8      | 0.99     | 0.99     | 1.0      |
|                  | T2                  | 337     | 336      | 335      | 336      |          |          |          |          |
|                  | T3                  | 343     | 341      | 343      | 342      |          |          |          |          |
|                  | T4                  | 345     | 342      | 340      | 342      |          |          |          |          |
|                  | Mean                | 340.6   | 338.9    | 338.5    |          |          |          |          |          |
| Insulin (µ IU/ml)| T1 (control)        | 10.21   | 10.61    | 9.89     | 10.24    | 0.31     | 0.22     | 0.62     | 0.98     |
|                  | T2                  | 11.75   | 12.87    | 11.46    | 12.03    |          |          |          |          |
|                  | T3                  | 11.38   | 10.95    | 10.44    | 10.92    |          |          |          |          |
|                  | T4                  | 11.84   | 11.28    | 11.15    | 11.42    |          |          |          |          |
|                  | Mean                | 11.30   | 11.43    | 10.74    |          |          |          |          |          |
| IGF-1 (ng/ml)    | T1 (control)        | 121.7   | 123.2    | 122.0    | 122.3    | 10.8     | 0.99     | 0.99     | 1.0      |
|                  | T2                  | 112.2   | 114.0    | 113.2    | 113.1    |          |          |          |          |
|                  | T3                  | 114.3   | 115.6    | 114.3    | 114.7    |          |          |          |          |
|                  | T4                  | 113.3   | 114.5    | 115.0    | 114.2    |          |          |          |          |
|                  | Mean                | 115.4   | 116.8    | 116.1    |          |          |          |          |          |
| T3 (nmol/L)      | T1 (control)        | 1.86    | 1.87     | 1.82     | 1.85     | 0.04     | 0.75     | 0.86     | 0.99     |
|                  | T2                  | 1.81    | 1.73     | 1.68     | 1.74     |          |          |          |          |
|                  | T3                  | 1.84    | 1.80     | 1.82     | 1.82     |          |          |          |          |
|                  | T4                  | 1.78    | 1.88     | 1.79     | 1.82     |          |          |          |          |
|                  | Mean                | 1.82    | 1.82     | 1.78     | 1.81     |          |          |          |          |
| T4 (nmol/L)      | T1 (control)        | 32.31   | 34.42    | 33.26    | 33.33    | 1.09     | 0.61     | 0.97     | 0.99     |
|                  | T2                  | 31.60   | 32.14    | 32.36    | 32.03    |          |          |          |          |
|                  | T3                  | 31.66   | 30.18    | 28.92    | 30.25    |          |          |          |          |
|                  | T4                  | 34.09   | 34.46    | 33.89    | 34.15    |          |          |          |          |
|                  | Mean                | 32.42   | 32.80    | 32.11    |          |          |          |          |          |
| Cortisol (nmol/L)| T1 (control)        | 54.23   | 53.11    | 52.08    | 53.14    | 1.02     | 0.31     | 0.03     | 0.19     |
|                  | T2                  | 52.37   | 55.12    | 53.08    | 53.52    |          |          |          |          |
|                  | T3                  | 57.95   | 50.24    | 48.82    | 52.33    |          |          |          |          |
|                  | T4                  | 58.78   | 44.02    | 43.04    | 48.61    |          |          |          |          |
|                  | Mean                | 55.83$^B$ | 50.62$^A$ | 49.26$^A$ |          |          |          |          |          |
| Estradiol (pg/ml)| T1 (control)        | 7.86±1.86 | 7.30±1.66 | 7.21     | 7.46     | 0.50     | 0.43     | 0.83     | 0.99     |
|                  | T2                  | 5.86    | 6.22     | 6.24     | 6.11     |          |          |          |          |
|                  | T3                  | 6.15    | 7.17     | 7.33     | 6.88     |          |          |          |          |
|                  | T4                  | 7.26    | 8.95     | 9.03     | 8.41     |          |          |          |          |
|                  | Mean                | 6.78    | 7.41     | 7.45     |          |          |          |          |          |
| Progestrone (ng/ml)| T1 (control)      | 3.44    | 5.28±0.65 | 5.35±0.65 | 4.69±0.42 | 0.19     | 0.01     | 0.02     | 0.85     |
|                  | T2                  | 1.92    | 2.08     | 3.12     | 2.37$^a$ |          |          |          |          |
|                  | T3                  | 3.08    | 2.99     | 3.84     | 3.30$^a$ |          |          |          |          |
|                  | T4                  | 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 T 3 fed basal diet + 5 mg Cr and in group T 4 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) | T1 (control) | 59.77 | 54.51  | 56.66  | 56.98  | 0.63  | 0.35  | 0.84  | 0.65 |
|             | T2         | 57.62 | 59.76  | 58.98  | 58.79  |       |       |       |      |
|             | T3         | 57.38 | 55.98  | 54.50  | 55.95  |       |       |       |      |
|             | T4         | 57.62 | 58.80  | 59.18  | 58.53  |       |       |       |      |
| Mean        | 58.10      | 57.26 | 57.33  |         |        |       |       |       |      |
| Serum Cr (mg/l) | T1 (control) | 0.59  | 0.59   | 0.61   | 0.60a  | 0.01a | 0.01a | 0.01a | 0.02 |
|             | T2         | 0.55  | 0.57   | 0.53   | 0.55a  |       |       |       |      |
|             | T3         | 0.56  | 0.81   | 0.79   | 0.72b  |       |       |       |      |
|             | T4         | 0.56  | 0.80   | 0.81   | 0.72b  |       |       |       |      |
| Mean        | 0.56A      | 0.69B | 0.68B  |         |        |       |       |       |      |

Mean values with different superscripts within column differ significantly (P<0.01). Mean values with different superscripts within a row differ significantly (P<0.01).

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 |
|-----------------------|-------|-------|-------|-------|-------|-------|-------|--------|-------|-------|-------|-----|
| FCM (kg/d)            |       |       |       |       |       |       |       |        |       |       |       |     |
| T1 (control)          | 8.13  | 7.05  | 6.14  | 4.53  | 3.69  | 3.12  | 2.77  | 5.03±a | 0.06  | 0.18  | 0.01  | 0.01 |
| T2                    | 9.21  | 9.16  | 8.38  | 6.33  | 4.95  | 4.54  | 3.13  | 6.50±b | 0.07  |       |       |      |
| T3                    | 8.60  | 7.77  | 7.26  | 5.63  | 4.44  | 3.60  | 2.80  | 5.69±b | 0.07  |       |       |      |
| T4                    | 8.38  | 8.56  | 7.66  | 6.36  | 5.93  | 4.90  | 3.59  | 6.46±b | 0.07  |       |       |      |
| Mean                  | 8.59D | 8.12D | 7.35D | 5.69C | 4.71BC| 4.01AB| 3.05A |        |       |       |       |     |
| SCM (kg/d)            |       |       |       |       |       |       |       |        |       |       |       |     |
| T1 (control)          | 13.30 | 12.16 | 11.14 | 9.10  | 8.12  | 6.84  | 6.08  | 9.55a  | 0.21  | 0.01  | 0.01  | 1.00 |
| T2                    | 14.43 | 14.38 | 13.63 | 11.56 | 10.00 | 9.17  | 7.07  | 11.46b |       |       |       |      |
| T3                    | 13.73 | 12.98 | 12.37 | 10.70 | 9.27  | 7.89  | 6.51  | 10.49ab|       |       |       |      |
| T4                    | 13.54 | 13.36 | 12.42 | 11.06 | 10.30 | 9.20  | 7.01  | 11.01b |       |       |       |      |
| Mean                  | 13.76D| 13.22D| 12.39D| 10.59C| 9.39BC| 8.24AB| 6.65A |        |       |       |       |     |
| ECM (kg/d)            |       |       |       |       |       |       |       |        |       |       |       |     |
| T1 (control)          | 14.75 | 13.41 | 12.37 | 10.03 | 8.91  | 8.05  | 7.49  | 10.72a | 0.22  | 0.01  | 0.01  | 1.00 |
| T2                    | 16.07 | 15.88 | 15.08 | 12.68 | 11.01 | 10.46 | 8.22  | 12.77b |       |       |       |      |
| T3                    | 15.25 | 14.40 | 13.77 | 11.90 | 10.25 | 8.76  | 7.22  | 11.65ab|       |       |       |      |
| T4                    | 14.79 | 14.61 | 13.74 | 12.10 | 11.77 | 10.72 | 9.03  | 12.40b |       |       |       |      |
| Mean                  | 15.23D| 14.57D| 13.74D| 11.66C| 10.44BC| 9.45AB| 7.95A |        |       |       |       |     |
| NEL (Mcal/kg)         |       |       |       |       |       |       |       |        |       |       |       |     |
| T1 (control)          | 1.14  | 1.11  | 1.14  | 1.12  | 1.15  | 1.13  | 1.13  | 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  | 1.13  | 1.14   |       |       |       |      |
| ME (Mcal/kg)          |       |       |       |       |       |       |       |        |       |       |       |     |
| T1 (control)          | 9.97  | 9.12  | 8.36  | 6.83  | 6.09  | 5.13  | 4.56  | 7.17a  | 0.16  | 0.01  | 0.01  | 1.00 |
| T2                    | 10.82 | 10.79 | 10.23 | 8.67  | 7.50  | 6.88  | 5.30  | 8.60b  |       |       |       |      |
| T3                    | 10.30 | 9.74  | 9.28  | 8.03  | 6.95  | 5.92  | 4.89  | 7.87ab |       |       |       |      |
| T4                    | 10.16 | 10.02 | 9.32  | 8.30  | 7.72  | 6.90  | 5.26  | 8.26b  |       |       |       |      |
| Mean                  | 10.32D| 9.91D | 9.29D | 7.94C | 7.04BC| 6.18AB| 4.99A |        |       |       |       |     |

#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   |
|---------------------|-------|--------|--------|--------|---------|---------|---------|---------|--------|-------|--------|--------|-------|
| **Fat**             |       |        |        |        |         |         |         |         |        |       |        |        |       |
| 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    |        |       |        |        |       |
| **Solid not fat**   |       |        |        |        |         |         |         |         |        |       |        |        |       |
| 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    |        |       |        |        |       |
| **Total solid**     |       |        |        |        |         |         |         |         |        |       |        |        |       |
| 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   |        |       |        |        |       |
| **Total protein**   |       |        |        |        |         |         |         |         |        |       |        |        |       |
| 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    |        |       |        |        |       |
| **Lactose**         |       |        |        |        |         |         |         |         |        |       |        |        |       |
| 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  | 5.32   | 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   | 7.17    | 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 UMMB 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 adrenocorticotropic 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 (T3 and T4). 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 µg/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 NEFA: 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, T2, T3 and T4, 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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REFERENCES

Anderson R A. 1987. Chromium, pp. 224–25. Trace Elements in Human and Animal Nutrition. (Ed.) W. Mertz. Vol. 1. Academic Press, New York.
Anderson R A. 2003. Chromium and insulin resistance. Nutrition Research Review 16(2): 267–75.
Aye P A. 2012. Production of Gliciridia and Leucaena based multi-nutrient blocks as supplementary ruminant feed resource in South Western Nigeria. Agriculture and Biology Journal of North America 3(5): 213–20
Bell A W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. Journal of Animal Science 73: 2804–19.
Bryan A, Socha M T and Tomlinson D J. 2004. Supplementing intensively grazed late gestation and early lactation dairy cattle with chromium. Journal of Dairy Science 87: 4269–77.
Butler S T, Marr A L, Pelton S H, Radcliff R F, Lacy M C and Butler W R. 2003. Insulin restores GH responsiveness during lactation induced NEBAL in dairy cattle: Effects on expression of IGF-1 and GH receptor 1a. Journal of Endocrinology 176: 205–17.
Chang X B, Mallard A and Mowat D N. 1996. Effects of chromium on health status, blood neutrophil phagocytosis and in vitro lymphocyte blastogenesis of dairy cows. Veterinary Immunology and Immunopathology 52: 37–52.
Deka R S, Mani V, Kumar M, Shiwajirao Z S and Kaur H. 2015. Chromium supplements in the feed for lactating Murrah buffaloes (Bubalus bubalis): Influence on nutrient utilization, lactation performance, and metabolic responses. Biological Trace Element Research 168: 362–71.
Depew C L, Bunting L D, Fernandez J M, Thompson D L and Adkinson R W. 1998. Performance and metabolic responses of young dairy calves fed diets supplemented with chromium triprocyclinate. Journal of Dairy Science 81: 2916–23.
Drackley J K, Overton T R and Douglas G N. 2001. Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. Journal of Dairy Science 84: 100–12.
Gendley M K, Tiwari S P, Dutta G K, Kumari K and Ratre H. 2015. Effect of urea molasses mineral block on hematological and biochemical blood parameters in adult goats. Indian Veterinary Journal 92(5): 98–100.
Hayirli A, Bremmer D R, Bertsie S J, Socha M T and Grummer R R. 2001. Effect of chromium supplementation on production and metabolic parameters in periparturient dairy cows. Journal of Dairy Science 84: 1218–30.
ICAR. 2013. Nutrient Requirements of Animals–Cattle and Buffalo. Indian Council of Agricultural Research, New Delhi.
Kegley E B, Spears J W and Eisemann J H. 1997b. Performance and glucose metabolism in calves fed a chromium-nicotinic acid complex or chromium chloride. Journal of Animal Science 80: 1744–50.
Khan M A S, Chowdhury M A R, Akbar M A and Shamsuddin M. 2007. Urea molasses multinutrient blocks technology Bangladesh experiences. Feed Supplementation Blocks. Urea molasses multinutrient blocks: simple and effective feed supplement technology for ruminant agriculture, FAO, Rome, pp. 7588.

Knesekern G S. 2015. ‘Effects of chromium supplementation on dam performance and progeny growth and development’. M.Sc. Thesis. University of Illinois at Urbana-Champaign.

McNamara J P and Valdez F. 2005. Adipose tissue metabolism and production responses to calcium propionate and chromium propionate. Journal of Dairy Science 88: 2498–07.

Mertz W. 1976. Effects and metabolism of glucose tolerance factor, pp. 365–72. Present Knowledge in Nutrition (4th Ed.) Washington DC. The Nutrition Foundation Inc.

Mertz W. 1993. Chromium in human nutrition: A review. Journal of Nutrition 123: 626–33.

Pechova A, Cech P, Pavlata L and Podhorsky A. 2003. The influence of chromium supplementation on metabolism, performance and reproduction of dairy cows in a herd with increased occurrence of ketosis. Czech Journal of Animal Science 48: 349–58.

Peterson S W. 2000. Effects of chromium picolinate on milk production and plasma insulin concentration in dairy cows. Proceedings of the New Zealand Society of Animal Production 60: 307–10.

Sahoo A, Elangovan A V, Mehra U R and Singh U B. 2004. Catalytic supplementation of urea –molasses on nutritional performance of male buffalo (Bubalus bubalis) calves. Asian-Australasian Journal of Animal Science 17: 621–28.

Smith K L, Waldron M R, Drackley J K, Socha M T and Overton T R. 2005. Performance of dairy cows as affected by prepartum dietary carbohydrate source and supplementation with chromium throughout the transition period. Journal of Dairy Science. 88: 255–63.

Sumner J M, Valdez F and McNamara J P. 2007. Effects of chromium propionate on response to an intravenous glucose tolerance test in growing Holstein heifers. Journal of Dairy Science 90: 3467–74.

Tyrrell H F and Reid J T. 1965. Prediction of the energy value of cow’s milk. Journal of Dairy Science 48: 1215–23.

Underwood E J and Suttle N F. 1999. The Mineral Nutrition of Livestock. 3rd ed. CABI

Vazquez-Anon M, Bertics S, Luck M and Grummer R R. 1994. Peripartum liver triglyceride and plasma metabolites in dairy cows. Journal of Dairy Science 77: 1521–28.

Yang W Z, Mowat D N, Subiyatno A and Liptrap R M. 1996. Effects of chromium supplementationon early lactation performance of Holstein cows. Canadian Journal of Animal Science 76: 221–30.