Supplemental chromium in cold-stressed buffalo calves (*Bubalus bubalis*): effects on growth performance, nutrient utilization and cell-mediated and humoral immune response

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**ABSTRACT**

Various stressors significantly increase urinary excretion of chromium (Cr), suggesting that Cr may be physiologically linked to the responses to control stress. The aim of this study was to determine the physiological responses of buffalo calves to increased Cr supply under low ambient temperature. In a randomized complete block design, twenty-four Murrah buffalo calves were assigned to 4 treatments for a period of 120 days. Treatments included either no supplemental Cr (control), 0.5 mg of supplemental Cr/kg DM, 1.0 mg of supplemental Cr/kg DM, or 1.5 mg of supplemental Cr/kg DM. Buffalo calves were monitored daily for physiological variables, dry matter intake (DMI) and fortnightly for body mass change. Blood samples were collected at fortnightly intervals and analyzed for the biomarkers of immunity and plasma trace mineral concentration. At the end of the 120 day experimental period, a 7 day metabolic trial was conducted. The average temperature-humidity index (THI) and relative humidity (RH) during the study were 58.10 units and 52.0% respectively. Physiological variables, DMI and growth performance did not differ among all treatments. 1.5 mg Cr/kg DM increased B and T-cell proliferation, neutrophil phagocytic activity and ferric reducing antioxidant power (FRAP) value, whereas plasma total immunoglobulin (TIg) and immunoglobulin G (IgG)
concentrations were the highest in the 1.0 and 1.5 mg Cr/kg DM group. Nutrient digestibility, nitrogen (N) metabolism and trace mineral bioavailability did not differ between treatments, while the bioavailability of Cr showed a positive correlation with supplemental Cr level. The results suggest that in cold conditions, increased Cr supply can improve immune response without affecting physiological response, growth performance and nutrient utilization in buffalo calves.

Key words: buffalo calves, chromium, growth performance, immunity, cold-stressed

Introduction

Successful livestock production requires applying strategies that optimize the use of the environment and available nutrient sources in order to capitalize on the livestock’s production potential. Changes in environmental temperature below and above the thermal comfort zone reduce animal performance (BLAHOVA et al., 2007). The best milk breeds of buffaloes are essentially of the riverine type, and are mostly confined to the northern part of India (MARAI and HAEEB, 2010). The cold climate in the northern region of the country is quite harsh and stressful because of the low ambient temperature and it is responsible for great economic losses due to the reduced performance of buffaloes (SINGH et al., 2014). Exposure of buffaloes to low ambient temperatures evokes a series of drastic changes in biological functions that include alteration in feed intake, efficiency and utilization, disturbances in metabolism of water, protein, energy and mineral balances and blood metabolites (MARAI and HAEEB, 2010). Cold stress also suppresses nonspecific immunity, alters antibody titer and modulates cell mediated immunity (MATSUMOTO and HUANG, 2000). It is, therefore essential to protect the buffaloes from cold stress to obtain optimum performance as per their genetic potential.

To minimize the adverse effect of environmental stress, various supplements and additives are used in dairy animal rations. Among these, Cr supplementation is one. The primary role of Cr in the metabolism is to potentiate the action of insulin through its presence in an organometallic molecule, the glucose tolerance factor (GTF) (SAHIN et al., 2002a). In domestic animals, Cr has been recognized as a new essential trace mineral and it has been suggested to alleviate stress associated effects (NRC, 1997). Supplemental Cr improves immune function by enhancing serum immunoglobulin production, antibody titer to antigens, or by reducing serum cortisol concentration and modulating inflammatory response (WEISS and SPEARS, 2005). There is evidence in humans and laboratory animals that various stressors significantly increase urinary excretion of Cr, suggesting that Cr may be physiologically linked to the responses to or control of stress (BUNTING et al., 2000). Due to increased urinary losses of Cr during environmental stress, Cr needs to be supplemented during cold stress. Dietary supplementations of Cr alleviated the detrimental effects of heat stress (SAHIN et al., 2005) and cold stress (SAHIN et al., 2002a) in poultry studies.
To the best of our knowledge, there have been no experimental studies regarding the effects of Cr supplementation on performance, nutrient utilization and immune response in cold-stressed dairy animals. Hence, the present study was designed to investigate the possible effects of Cr supplementation growth performance, nutrient digestibility, mineral bioavailability and immune response on buffalo calves during the cold season.

Materials and methods

The research was approved by the Institutional Animal Ethics Committee (IAEC), constituted as per Article number 13 of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental design, calves and treatments. Twenty-four Murrah buffalo calves (Bubalus bubalis) were divided randomly into four groups of six calves each. The buffalo calves were individually weighed and blocked according to body mass (174 ± 4 kg) and age (10-13 months) before being assigned to treatments of: 1) a nutritionally adequate diet with no supplemental Cr (control), 2) control diet with 0.5 mg of supplemental Cr/kg DM, 3) control diet with 1.0 mg of supplemental Cr/kg DM, or control diet with 1.5 mg of supplemental Cr/kg DM as CrCl₃·6H₂O (Loba Chemie Pvt. Ltd.). To ensure that each buffalo calf consumed the calculated amount of Cr, the CrCl₃ was mixed with 50 g concentrate mixture, and scattered onto a small amount of green fodder before offering the diet. The treatment period continued for 120 days during the cold season. The calves were fed twice daily at 10.00 h and 18.00 h and their nutrient requirements were met by feeding concentrate mixture, berseem fodder and wheat straw (KEARL, 1982). The buffalo calves were individually tied with rope in separate well-ventilated concrete floor pens. Deworming of all the buffalo calves was undertaken before the start of the experiment. To study the effect of Cr supplementation on nutrient digestibility, N balance and mineral metabolism, a metabolic trial with a 4 day adaptation period followed by a 7 day collection period was conducted at the end of the study.

Chemical analyses. Samples of the feed offered, ort left and feces were analyzed for DM (ID no. 973.18c), crude protein (ID no. 4.2.08), ether extract (ID no. 920.85) and ash (ID no. 923.03) content, as per the procedure described by AOAC (1995). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined by the methods of VAN SOEST et al. (1991). Concentrations of Cr (HALDAR et al., 2009), Cu, Zn and Fe in feed, refuse left, feces, and urine were estimated using an atomic absorption spectrophotometer (Model Z-5000, Polarized Zeeman Atomic Absorption Spectrophotometer, Hitachi High-Technologies Corporation, Tokyo, Japan). The composition of the basal diet fed during the experimental period is presented in Table 1.
Table 1. Ingredients and chemical compositions of experimental diet

<table>
<thead>
<tr>
<th>Composition</th>
<th>Basal diet</th>
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<tbody>
<tr>
<td><strong>Basal diet</strong></td>
<td></td>
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<tr>
<td>Berseem fodder</td>
<td>19.8</td>
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<tr>
<td>Wheat straw</td>
<td>20.2</td>
</tr>
<tr>
<td>Ground yellow maize</td>
<td>23.0</td>
</tr>
<tr>
<td>Groundnut cake</td>
<td>15.7</td>
</tr>
<tr>
<td>De-oiled mustard cake</td>
<td>5.3</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>6.4</td>
</tr>
<tr>
<td>Rice bran</td>
<td>8.3</td>
</tr>
<tr>
<td>Salt</td>
<td>0.3</td>
</tr>
<tr>
<td>Trace minerals and vitamins premix&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0</td>
</tr>
<tr>
<td>CrCl&lt;sub&gt;3&lt;/sub&gt;·6H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>Variable&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Chemical composition (g/kg DM)</th>
<th></th>
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<tbody>
<tr>
<td>Dry matter</td>
<td>764</td>
</tr>
<tr>
<td>Organic matter</td>
<td>902</td>
</tr>
<tr>
<td>Crude protein</td>
<td>178</td>
</tr>
<tr>
<td>Neutral detergent fibre</td>
<td>394</td>
</tr>
<tr>
<td>Acid detergent fibre</td>
<td>263</td>
</tr>
<tr>
<td>Ash</td>
<td>98</td>
</tr>
<tr>
<td>Chromium (mg/kg DM)</td>
<td>0.20</td>
</tr>
<tr>
<td>Copper (mg/kg DM)</td>
<td>22.81</td>
</tr>
<tr>
<td>Zinc (mg/kg DM)</td>
<td>46.61</td>
</tr>
<tr>
<td>Iron (mg/kg DM)</td>
<td>298.12</td>
</tr>
</tbody>
</table>

<sup>a</sup>Premix composition per kilogram: vitamin A, 500,000 IU; vitamin D3, 10,000 IU; vitamin E, 100 mg; Ca, 190,000; P, 90,000; Na, 50,000; Cu, 300 mg; Fe, 3,000 mg; Mn, 2,000 mg; I, 100 mg; Co, 100 mg; Se, 1 mg; Mg, 19,000 mg; BHT antioxidant, 3,000 mg.  
<sup>b</sup>Supplemental Cr diets (0.5, 1.0 and 1.5 ppm Cr) were obtained by supplementing Cr with 0.5, 1.0 and 1.5 mg/kg DM in basal diet.

**Biochemical procedures.** Blood samples were withdrawn in heparinized vacuutainer tubes (Becton Drive, Franklin Lakes, NJ, USA) by jugular venipuncture on days 0, 15, 30, 45, 60, 75, 90, 105 and 120 post-Cr supplementation. Collected samples of blood were analyzed for B and T-cell proliferation, neutrophil phagocytic activity, plasma TlG, IgG, ferric reducing antioxidant power (FRAP) assay and plasma Cr, Cu, Zn and Fe concentration. The colorimetric MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay described by MOSMANN (1983) was used to determined lymphocyte proliferation. The mitogens chosen to stimulate T and B-cells were concanavalin A (Con A) and lipopolysaccharide (LPS) respectively. Neutrophil phagocytic activity was determined by semi-quantitative microscopic nitroblue tetrazolium (NBT, Sigma-Aldrich, St Louis,
MO, USA) assay as described by CHAI et al. (2005). TiG and IgG in the plasma samples were estimated by the Zn turbidity method (McEVAN and FISHER, 1970) and a “Bovine IgG ELISA kit” (Cusabio Biotech Co., Ltd. China) respectively. Total antioxidant activity was measured by FRAP stoichiometric assay (BENZIE and STRAIN, 1999). Plasma content of Cr, Cu, Zn and Fe were estimated using an atomic absorption spectrophotometer (Model Z-5000, Polarized Zeeman Atomic Absorption Spectrophotometer, Hitachi High-Technologies Corporation, Tokyo, Japan). The buffalo calves were monitored daily for physiological variables, dry matter intake (DMI) and fortnightly for body mass change.

Climatic data and physiological response. Dry bulb temperature (Cdb) and wet bulb temperature (Cwb) (Zeal, UK) were recorded in degrees Celsius at 07.30h and 14.30h every day to calculate the temperature humidity index (THI) according to the following formula (McDOWELL et al., 1976):

\[
\text{THI} = 0.72 \times (\text{Cdb} + \text{Cwb}) + 40.6
\]

Daily relative humidity (RH) data were obtained by the difference between Cdb and Cwb. The physiological response of the experimental buffalo calves was determined daily by recording respiration rate (RR), pulse rate (PR) and rectal temperature (RT).

Statistical analyses. The data were subjected to the multivariate analysis of general linear model (GLM) procedure of the Statistical Software Package (SPSS for Windows, V19.0; SPSS Inc., Chicago, IL, USA). To estimate the effect of treatment and time period, and their interaction, the following model was used:

\[
Y_{ijk} = \mu + Ti + Dj + (T \times D)ij + e_{ijk}
\]

Where: \(Y_{ijk}\) is the dependent variable, \(\mu\) the overall mean of the population, \(Ti\) the mean effect of the \(ith\) treatments, \(Dj\) the mean effect of the day of sampling with day as a repeated factor (j = 0, 15, 30, 45, 60, 75, 90, 105, and 120 days of dietary treatment), \((T \times D)ij\) the effect of the interaction between the effects of treatment, group and day of sampling, and \(e_{ijk}\) the unexplained residual element assumed to be independent and normally distributed. An individual animal was used as the experimental unit for all data. The pair-wise comparison of means was carried out using “Tukey’s honest significant difference (HSD) test”. Significance was determined at \(P<0.05\) and the values are presented in the tables.

Results

Climatic and physiological variable. The average THI during the experimental period of 120 days decreased with the decrease in environmental temperature and was lowest during the month of January (Fig. 1). In contrast to THI, RH % was found to be highest during the month of January. The calculated THI and RH % averaged 58.10 units and 52.00 %, respectively. Supplementation of different levels of inorganic Cr in the diet
of cold-stressed buffalo calves did not show any significant effect on RR, PR and RT (Table 2).

Table 2. Effect of Cr supplementation on growth performance, DMI and physiological variables

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Supplemental Cr (mg/kg DM)</th>
<th>SEM</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of buffalo calves</td>
<td>0</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Days in trial</td>
<td>120</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Initial body mass (kg)</td>
<td>170.46</td>
<td>171.27</td>
<td>169.65</td>
</tr>
<tr>
<td>Final body mass (kg)</td>
<td>225.17</td>
<td>227.35</td>
<td>224.38</td>
</tr>
<tr>
<td>Gain (kg)</td>
<td>54.71</td>
<td>56.08</td>
<td>54.73</td>
</tr>
<tr>
<td>Average daily gain (kg/day)</td>
<td>0.455</td>
<td>0.467</td>
<td>0.456</td>
</tr>
<tr>
<td>Dry matter intake (kg/100 body mass)</td>
<td>2.54</td>
<td>2.50</td>
<td>2.59</td>
</tr>
<tr>
<td>Feed conversion ratio</td>
<td>12.02</td>
<td>11.80</td>
<td>11.91</td>
</tr>
<tr>
<td>Respiration rate (breaths/min)</td>
<td>16.23</td>
<td>15.82</td>
<td>16.76</td>
</tr>
<tr>
<td>Pulse rate (min⁻¹)</td>
<td>57.10</td>
<td>59.01</td>
<td>56.87</td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
<td>38.17</td>
<td>38.13</td>
<td>38.17</td>
</tr>
</tbody>
</table>

SEM = Standard error of mean, G×P = Interaction effect of Cr and winter, NS = Non significant (P>0.05).

**Growth performance, nutrient digestibility and mineral bioavailability.** The average values of all measurements taken for growth performance, DMI, feed conversion ratio, nutrient digestibility and N and trace mineral metabolism during the 120 day experimental period are depicted in Tables 2, 3 and 4. Statistical analysis revealed no distinct effect of dietary Cr supplementation on DMI, body mass gain and the efficiency of feed conversion in buffalo calves during the cold season. The interaction effect between Cr treatment and cold was also non-significant (P<0.05) for growth performance, DMI and feed conversion ratio. Digestibility of nutrients and N metabolism were not altered by additional dietary Cr supplementation. Diets with different Cr levels did not affect metabolism and plasma concentrations of Cu, Zn and Fe. However, the intake, excretion and plasma concentration of Cr showed a positive correlation (P<0.05) with supplemental Cr levels, and was found to be the highest in the buffalo calves fed 1.5 mg Cr/kg DM.

**Cell mediated and humoral immunity.** The results concerning changes in the mitogenic response of lymphocyte, neutrophil phagocytic activity, plasma TlG, IgG concentrations and FRAP value are shown in Table 5 and Figs. 2, 3, 4 and 5. The cell mediated immune
response of cold-stressed buffalo calves was assessed by estimation of the proliferation of B and T-cells and neutrophil phagocytic activity. However, the humoral immune response was determined by estimation of plasma TIg, IgG concentrations and FRAPS value. 1.5 mg Cr/kg DM supplementation significantly increased (P<0.05) B and T cell blastogenic response, as compared to the buffalo calves fed on a basal diet and a diet supplemented with 0.5 and 1.0 mg Cr/kg DM (Fig. 2). Accordingly, increased (P<0.05) neutrophil phagocytic activity was observed in the buffalo calves fed the 1.5 mg Cr/kg DM diet (Fig. 3). However, calves fed diets which contained 1.0 and 1.5 mg Cr/kg DM had higher (P<0.05) plasma concentrations of TIg and IgG (Fig. 4). The total antioxidant activity (FRAP value) was highest (P<0.05) in buffalo calves supplemented with 1.5 mg Cr/kg DM (Fig. 5).

Fig. 1. Fortnightly mean values of THI and RH during the 120 day course of the experiment. Values are the means, and vertical lines represent the SE, * = fortnight

Fig. 2. The effect of chromium supplementation on lymphocyte proliferation index. Means with asterisks (*, **) differ significantly (*P<0.05 and **P<0.01). Values are the means and vertical lines represent the SE.
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Fig. 3. The effect of chromium supplementation on neutrophil phagocytic activity. Means with an asterisk (*) differ significantly (P<0.05). Values are the means and vertical lines represent the SE.

Fig. 4. The effect of chromium supplementation on immunoglobulin concentration. Means with asterisks (*, **) differ significantly (*P<0.05 and **P<0.01). Values are the means and vertical lines represent the SE.
Discussion

THI combines temperature and humidity in a single value and has been widely used to quantify environmental stress in dairy animals (CORREA-CALDERON et al., 2004). In the present study, THI and RH observed during the 120 day test period are considered within the normal physiological range of 35-72% (KOHLI et al., 2014). Therefore, variations in THI and RH did not exert any effect on the physiological variables, as shown by the normal RR, PR and RT in the four groups. The lack of effect of supplemental Cr on RR, PR, and RT might be attributed to the fact that the calves were not exposed to such a low ambient temperature that could alter these physiological variables. The findings of the present study on physiological variables are consistent with previous findings by KUMAR et al. (2013), who also found no effect of Cr supplementation on the physiological responses of heat-stressed buffalo calves. DEKA (2014) conducted a feeding trial in Murrah buffaloes to study the effect of inorganic Cr supplementation on immune-endocrine parameters, nutrient utilization and productive performance in similar agro climatic conditions. She found no effect of feeding diets supplemented with 0.5, 1.0 and 1.5 mg Cr as Cr-chloride/ kg DM on PR, RR, and RT. ANQIANG et al. (2009) also did not report any differences in PR, RR, and RT in inorganic Cr supplemented, transportation stressed calves.

**Growth performance and mineral bioavailability.** The variation in ambient temperature, THI, RH and supplemental levels of Cr during the cold season did not exert any effect on the DMI, growth performance and metabolism of N and Cu, Zn and Fe. Information on this aspect of how supplementation of Cr affects performance in buffalo
calves during the cold season is lacking. However, in other species findings regarding the effect of Cr supplementation on animal performance in response to the cold season are variable. FRANK et al. (2003) reported increased feed intake and reduced feed:gain ratio in pigs housed at low temperatures compared to normal temperature. However, no effect of Cr supplementation on nutrient intake under optimum ambient temperatures was reported in periparturient Murrah buffaloes (DEKA et al., 2014) and Holstein calves (DEPEW et al., 1996). KRAIDEES et al. (2009) in transportation stressed calves, and KUMAR et al. (2015) also ascribed no effect of Cr supplementation on growth performance in heat-stressed buffalo calves. Accordingly, no difference in average daily gain (ADG), DMI, and gain:feed ratio in calves following dietary supplementation of 1.0 mg Cr/kg DM was reported by GENTRY et al. (1999). However, the beneficial effects on the performance of calves of supplemental trivalent Cr as Cr-yeast, chelated-Cr or Cr-nicotinate during a stressful period have been recorded by various workers (KRAIDEES et al., 2009). We failed to observe any beneficial effects of supplemental Cr in cold-stressed buffalo calves. This discrepancy may be attributed to the physiological differences between these species, and the fact that the pooled Cr in the body of these buffalo calves was not depleted as a result of the low ambient temperature.

Dietary Cr supplementation did not have any effect on nutrient digestibility and balance of N, Cu, Zn and Fe. However, Cr balance showed a positive correlation with supplemental Cr levels. The results obtained in the present study are in agreement with the findings of KITCHALONG et al. (1995), who also did not report any effect of Cr supplementation on N balance. However, HALDAR et al. (2009) stated that supplementation of inorganic Cr in goats had a positive effect on N retention. In our research, the lack of effect of Cr supplementation on the metabolism of Cu, Zn and Fe agrees with previously reported findings (CHANG et al., 1995). Similarly, rats fed with Cr, at 5 mg/kg of their diet, did not show any change in plasma concentrations of Cu, Zn, and Fe (ANDERSON et al., 1997). In contrast to the findings of the present study, a higher dietary intake of Cu, Zn and Fe (BISWAS et al., 2006) and a dose-dependent linear decrease in the apparent absorption of Zn were observed in animals fed inorganic Cr (UNDERWOOD and SUTTLE, 1999). Increased intake, excretion, and retention of Cr by buffalo calves fed diets supplemented with different levels of Cr is in agreement with the findings of SCHRAUZER et al. (1986). The apparent absorption coefficient of Cr from inorganic trivalent sources ranged between 4 to 10 %, compared to 10 to 25 % for organic sources of Cr (UNDERWOOD, 1977). SPEARS (1999) also reported a dose dependent increase in tissue Cr concentrations in slaughter studies. Blood Cr concentration might to a certain extent reflect the intake of this element, but in cases of excessive Cr intake, it is inappropriate to use the blood Cr concentration as an indicator of Cr status in animals (UNDERWOOD, 1977).
Cell mediated and humoral immunity. Higher cell mediated immunity was obtained with inorganic Cr supplementation in the current study, which is consistent with the findings of KUMAR et al. (2015) in heat-stressed buffalo calves. DEKA et al. (2014) showed that periparturient Murrah buffaloes supplemented with 1.5 mg inorganic Cr/kg DM had higher B and T-cell proliferation. CHANG et al. (1994) observed 70% increased lymphocyte blastogenesis in response to Con A in calves receiving 1.0 ppm supplemental chelated-Cr. LIEN et al. (2005) used Escherichia coli LPS as a stress inducing agent in weanling pigs with supplementation of 0.2 mg Cr-propionate/kg DM, and found a higher lymphocyte blastogenic response. An increased blastogenic response of lymphocytes in Cr supplemented animals was also reported by CHANG et al. (1994). In contrast to the present study, VAN HEUGTEN and SPEARS (1997) did not ascribe any effect of supplemental Cr on cell mediated and humoral immune response in calves.

Increased neutrophil phagocytic activity in the buffalo calves fed a supplement of 1.5 mg Cr/kg DM is in accordance with the findings of DEKA et al. (2014) who also reported increased neutrophil phagocytic activity in buffaloes fed 1.5 mg Cr/kg DM periparturient Murrah. A similar result was also reported by KUMAR et al. (2015), who found that the addition of inorganic Cr to heat-stressed buffalo calves influenced neutrophil phagocytic activity. Increased neutrophil phagocytic activity was also reported in Cr supplemented transition cattle (KAFILZADEH et al., 2012). However, no effect of dietary Cr supplementation on peripheral neutrophil phagocytic activity was reported by HALDAR et al. (2009).

The increase in humoral immune response in the 1.0 and 1.5 mg Cr/kg DM supplemented groups is consistent with the findings of KUMAR et al. (2015) and DEKA et al. (2014) who reported higher levels of TIg and IgG in Cr supplemented periparturient Murrah buffaloes and Murrah buffaloes calves, respectively. WANG et al. (2007) stated that pigs supplemented with 200 μg/kg Cr as Cr- Nano had 13.2% and 20.6% higher serum concentrations of IgM and IgG. Increased serum total IgG and IgM levels in high Cr-yeast supplemented calves following transport stress was also reported by MOONSIESHAGEER and MOWAT (1993). In contrast to the present study, KEGLEY et al. (1997) ascribed no change in total IgM in both high-Cr yeast and Cr-nicotinate supplemented transport stressed calves.

In the present study, Cr supplementation increased FRAP values, with the group receiving 1.5 mg Cr/kg DM recording the highest. Information on how Cr supplementation alters the antioxidant status in dairy animals during cold season is not available. In a summer season study, KUMAR et al. (2015) reported increased antioxidant status in Murrah buffalo calves fed a diet supplemented with different levels of Cr as Cr-chloride. PERAI et al. (2014) conducted a study on the effects of supplemental vitamin C and Cr-chloride on the metabolic and hormonal responses, antioxidant status, and tonic immobility.
reactions of transported broiler chickens. They both reported that, either alone or in combination, Cr increased the FRAP value before transport. It has been well-established that Cr potentiates insulin metabolism, which alters lipid peroxidation and therefore Cr is postulated to function as an antioxidant (PREUSS et al., 1997). Thus, concurrent use of Cr during stress conditions enhances antioxidant capacity (SAHIN et al., 2002a). In contrary, other reports have indicated that supplemental Cr significantly decreased antioxidant levels (ANNE-MARIE et al., 2012).

It is not clear how the mechanism of immunomodulation and antioxidant status is induced by dietary Cr supplementation. The increased immune response in the present experiment might be due to the regulation of Cr containing specific enzymes, required for B and T cell proliferation and immunoglobulin production (FIELDEN and ROTILIO, 1984). Reduced cortisol concentration in Cr supplemented animals during stress can be correlated with improved immune response and antioxidant status (KUMAR et al., 2015). Increased cortisol concentration during stress is believed to be responsible for immunosuppression by inhibiting lymphocyte proliferation, lymphocyte activating factor and T-cell growth factor production (MUNCK et al., 1984).

Conclusions

The dietary addition of inorganic Cr in cold-stressed Murrah buffalo calves did not have any impact on physiological variables, nutrient utilization, growth performance and metabolism of N and Cu, Zn and Fe. However, the bioavailability of Cr showed a positive correlation with the supplemental level of Cr. Cr supplementation in buffalo calves reared under low ambient temperatures increased immunity and antioxidant status, which was evident from the increased lymphocyte proliferation, neutrophil phagocytic activity, plasma immunoglobulin and total antioxidant status.

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SAŽETAK
Različiti stresori značajno povećavaju izlučivanje kroma (Cr) mokraćom što naznačuje da Cr može biti fiziološki vezan za odgovor na stres. Cilj je ovog rada bio odrediti fiziološke odgovore u bivlove teladi na povećani dodatak Cr u uvjetima niske temperature okoliša. Prema metodi slučajnog odabira skupina, 24 Murrah bivolska teleta bila su raspodijeljena u četiri skupine koje su bile različito tretirane u razdoblju od 120 dana. Životinjama jedne skupine dodavano je 0,5 mg Cr/kg suhe tvari, druge skupine 1,0 mg Cr/kg suhe tvari, a treće skupine 1,5 mg Cr/kg suhe tvari, dok kontrolnoj skupini Cr nije bio dodavan. U teladi su dnevno bile promatrane fiziološke varijable, uzimanje suhe tvari te svaka dva tjedna promjena tjelesne mase. Uzorci krvi bili su uzimani u razmacima od 14 dana i pretraženi na biomarkere imunosti i koncentraciju minerala u tragovima u plazmi. Na kraju pokusnog razdoblja od 120 dana proveden je sedmodnevni metabolitski pokušaj. Prosječni indeks temperature i vlažnosti tijekom istraživanja iznosio je 58,10 jedinica dok je relativna vlažnost bila 52,0 %. Fiziološke varijable, uzimanje suhe tvari i prirast reducirajućeg željeza nisu se razlikovali među skupinama. Količina od 1,5 mg Cr/kg suhe tvari povećala je proliferaciju B- i T-limfocita, fagocitoznou aktivnost neutrofila i vrijednost antioksidacijske sposobnosti, dok su koncentracije ukupnih imunoglobulina plazme i imunoglobulina G (IgG) bile najveće u skupinama koje su dobivale 1,0 odnosno 1,5 mg Cr/kg suhe tvari. Nutritivna probavljivost, metabolizam dušika (N) i biološka raspoloživost minerala u tragovima nisu se razlikovali među skupinama. Biološka raspoloživost Cr bila je u korelaciji s razinom njegovog dodatka. Rezultati upućuju na zaključak da povećani dodatak Cr u hladnoći može poboljšati imunosni odgovor bez utjecaja na fiziološki odgovor, prirast i hranidbenu iskoristivost u bivlove teladi.

Ključne riječi: bivol, telad, krom, prirast, imunost, stres, hladnoća