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Effect of Feeding Inorganic Chromium on Growth Performance, Endocrine Variables, and Energy Metabolites in Winter-Exposed Buffalo Calves (*Bubalus bubalis*)

Muneendra Kumar · Harjit Kaur · Amrish Kumar Tyagi · Neelam J. Kewalramani · Veena Mani · Rijusmita Sarma Deka · Vijay Kumar Sharma · Gulab Chandra · Ajay Kumar Dang

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Abstract We investigated the effect of chromium (Cr) supplementation on the growth performance, energy metabolites, and hormonal variation in winter-exposed buffalo calves. Twenty-four female buffalo calves were randomly allotted to four dietary treatments ($n=6$) for a period of 120 days. Feeding regimen was the same in all the groups, except the animals in the four respective groups were additionally supplemented with 0.0, 0.5, 1.0, and 1.5 mg of Cr/kg DM in the form of $CrCl_3.6H_2O$. Calves were monitored daily for physiological variables and dry matter intake (DMI). Blood samples were collected at fortnightly intervals from each buffalo calves to measure concentrations of hormones (insulin, cortisol, and growth hormone), energy metabolites (glucose and non-esterified fatty acids), and plasma mineral levels. After 120 days of feeding trial, buffalo calves fed with Cr had lower ($P<0.05$) circulating plasma concentrations of glucose, insulin, and cortisol hormones, whereas plasma thyroid hormone and non-esterified fatty acids concentrations were found similar ($P>0.05$) among all the treatments. The results suggested that dietary Cr supplementation influenced plasma Cr levels without affecting the plasma concentrations of other trace minerals. However, physiological variables, nutrient intake, and growth performance of buffalo calves did not differ among all treatments ($P>005$). In summary, the current study showed that supplementation of Cr at the level of 1.0 and 1.5 mg of Cr/kg DMI was more effective in improving glucose utilization by increasing potency of insulin hormone and reducing concentration of cortisol hormone. Results also suggested that supplemental Cr also improves blood plasma Cr levels.

Keywords Buffalo calves · Chromium · Growth performance · Energy metabolites · Endocrine variables

M. Kumar (✉)
Animal Nutrition Department, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura 281001, India
e-mail: muneendra82@gmail.com

H. Kaur
Agricultural Extension Division, KAB-I, Pusa, ICAR, New Delhi 110012, India

A. K. Tyagi · N. J. Kewalramani · V. Mani · V. K. Sharma
Dairy Cattle Nutrition Division, National Dairy Research Institute, Karnal 132001, India

R. S. Deka
Krishi Vigyan Kendra, Dhemaji 787059, India

G. Chandra · A. K. Dang
Dairy Cattle Physiology Division, National Dairy Research Institute, Karnal 132001, India

Introduction

Successful livestock production requires applying strategies that optimizes the use of the environment and available nutrient sources in order to capitalize on the livestock's production potential. Cold exposure is one of the stresses that results in a variety of negative effects on productivity of livestock through modified digestive, metabolic, and endocrine functions [1, 2]. An immediate requirement under such unfavorable conditions is to increase or decrease, as necessary, the availability of blood glucose, and is mediated through increased output of hormones [3]. Hormones, particularly from the adrenal and

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thyroid glands, are known to play major roles in thermoregulation and metabolic adjustments [4]. Exposure of animals to cold increased the production of adrenal cortisol [5, 6] which antagonizes synthesis, release and action of insulin hormone, and hence impaired glucose utilization. In domestic animals, chromium (Cr) has been recognized as a newer essential trace mineral [7] and suggested to alleviate stress associated effects [8]. The assumed mechanism of Cr for the action of chromodulin has been described by Vincent [9]. Low ambient temperature-induced cortisol secretion is reduced by Cr supplementation. Cr is an integral component in activating enzymes and is involved in carbohydrate, lipid, and protein metabolism. In order for thyroid gland to produce thyroxine (T_4), it needs the trace element Cr, and it is also an essential component to enzyme function that supports the conversion of T_4 to triiodothyronine (T_3). Cr has an improving effect on insulin binding and increases the number of insulin receptors on the cell surface and sensitivity of pancreatic B cells [10–12]. The purpose of the present study is to summarize information pertaining to the effects of dietary Cr supplementation on growth performance, energy metabolites, and endocrine variables in winter-exposed buffalo calves.

Materials and Methods

Animal Management, Feeding, and Experimental Design

This experiment was conducted at the National Dairy Research Institute, Karnal, India. All protocols approved by the institutional animal ethics committee, constituted as per the article number 13 of the committee for the purpose of control and supervision on experiments on animals rules laid down by the Government of India, were followed. In the present feeding trial, 24 female buffalo (*Bubalus bubalis*) calves were randomly assigned to 4 treatments ($n=6$) on body mass (174±4 kg) and age basis (10 to 13 months). Feeding trial was conducted for 120 days in total length from the months of mid-October to mid-February. The buffalo calves were tied with rope in well ventilated separate concrete floor pens. Deworming of all the buffalo calves was done before the start of the experiment, and animals were let loose daily at 4:00 p.m. for exercise. The nutrient requirements of calves were met by feeding concentrate mixture, berseem fodder, and wheat straw [13]. Experimental buffalo calves either received a basal diet devoid of supplemental Cr (control) or were supplemented with 0.5, 1.0, and 1.5 mg/kg dry matter (DM) of trivalent inorganic Cr in the form of $CrCl_3.6H_2O$ (molecular weight 266.45, minimum assay 97 %; Hi-Media Laboratories Ltd., Mumbai, India). To ensure that each calves consumed the calculated amount of Cr, the $CrCl_3$ was mixed with 50 g concentrate mixture, and scattered onto a small amount of green fodder before offering the diet.

Observation Recorded, Blood Sampling, and Laboratory Analyses

In the present experiment, climatic variables, physiological variables, and DMI were recorded daily. However, buffalo calves were weighed at fortnightly intervals. Peripheral blood samples were collected in heparinized Vacutainer tubes (Becton Drive, Franklin Lakes, NJ, USA) by jugular vein puncture on days 0, 15, 30, 45, 60, 75, 90, 105, and 120 post-Cr supplementation. Immediately after collection, blood samples were centrifuged at 1,200×*g* at 4 °C for 20 min to separate the plasma for the analysis of glucose, non-esterified fatty acids (NEFAs), insulin, cortisol, and T_3 and T_4 hormones and plasma trace minerals (chromium, copper, zinc, iron, and manganese).

Recording of Climatic and Physiological Variables

The daily minimum and maximum temperatures, dry bulb temperature (C_{db}), and wet bulb temperature (C_{wb}) in degree Celsius were recorded at 7.30 a.m. and 2.30 p.m. (Zeal, UK). Relative humidity (RH) values were calculated using a psychometric chart [14]. However, the temperature humidity index (THI) was calculated using the formula [15] $THI = 0.72\times(Cdb+Cwb)+40.6$.

Respiration rate (RR) of the animals was recorded by observing flank movement, and one inward and outward movement was counted as one respiration and recorded/minute. To record the pulse rate (PR), coccygeal artery was palpated and recorded/minute. Immediately after recording pulse rate, rectal temperature (RT) was recorded using mercury in glass clinical thermometer (Qingdao Dacon Trading Co. Ltd., Shandong, China), inserted 7 cm in the rectum for at least 2 min, and the rectal mucosa was in contact with the bulb of the thermometer.

Chemical Analyses and Trace Mineral Estimation

Samples of the feed offered and ort left were collected daily in polyethylene sachets and pooled at weekly intervals for analysis of DM, organic matter, crude protein, ether extract, and total ash [16]. Detergent method was used for estimation of neutral detergent fiber and acid detergent fiber in feed and fodder sample [17].

In the feed offered and ort left, Cr was estimated by using atomic absorption spectrophotometer (Model Z-5000, polarized Zeeman atomic absorption spectrophotometer (AAS), Hitachi High-Technologies Corporation, Tokyo, Japan). For Cr estimation, samples of feeds, fodders, and ort left, during the experimental period, were oven dried (at 80 °C for 16 h) and grounded to pass through a 1-mm sieve. Five grams of grounded samples were placed in glazed ceramic crucibles and ignited in a muffle furnace at 450 °C for 4 h. The ash was

treated with concentrated nitric acid under mild heat to oxidize the trivalent Cr (Cr^{3+}) to hexavalent Cr (Cr^{6+}) for more accurate detection. The acid-extracted cooled samples were filtered through ashless Whatman filter paper no. 1. The crucibles were washed several times with deionized water, and the final volume was made up to 10 ml for the estimation of Cr. A solution of ammonium chloride (20 g/l) was added in the standard and the samples to reduce interferences due to the presence of iron (Fe) in the samples [16].

The feed, ort left, and plasma concentrations of zinc (Zn), copper (Cu), Fe, and manganese (Mn) alongside the plasma concentration of Cr were estimated after digestion in tri-acid mixture ($HNO_3/HClO_4/H_2SO_4$—2.5:1:1 ratio) by using AAS.

Hormone and Energy Metabolite Analyses

Insulin was determined in plasma of calves by "bovine insulin ELISA test kit" (ERK B1009, Endocrine Technologies, New York, USA). The insulin ELISA test kit used was based on the principle of a solid-phase enzyme-linked immunosorbent assay. The assay system utilized anti-insulin antibodies for solid-phase immobilization and mouse monoclonal antibodies in the antibody–enzyme conjugate solution. The test sample was allowed to react simultaneously with the antibodies, resulting in insulin molecules being sandwiched between the solid-phase and enzyme-linked antibodies. After addition of solution of substrate chromogen reagent (TMB), blue color was developed, and the absorbency was measured spectrophotometrically at 450 nm. The intensity of the color formed was proportional to the amount of enzyme present and was directly related to the amount of unlabeled insulin in the sample. Cortisol was determined in plasma of cows by "cortisol EIA kit" (Cayman's Chemical Company, Ann Arbour, Michigan, USA). Cayman's cortisol EIA kit is a competitive assay that can be used for quantification of cortisol in plasma.

The T_3 and T_4 hormone concentrations in plasma were estimated by radioimmunoassay (RIA) using ISOPHARM kits (Bhaba Atomic Research Center, Bombay, India). For T_4 RIA, fixed amounts of ^{125}I-labelled T_4 and anti-T_4 antibody were added to the serum and standards in barbitone buffer. After incubation, the bound and free forms were separated by the addition of poly(ethylene glycol) and centrifuged at 1,000 g for 20 min. The precipitate containing the antibody-bound T_4 was counted in a gamma counter (Iso-Data, 500 series, USA), and the concentrations of T_4 in the unknown samples were derived from a standard curve. Similar method was used to estimate T_3. The results were expressed as nanomole of hormone per liter of plasma. The intra- and inter-assay coefficients of variation were 5.17 and 9.74 % for T_4 and 7.83 and 11.21 % for T_3, respectively. The sensitivity of the assay was 2.5 and 0.15 ng for T_4 and T_3, respectively.

Plasma glucose was quantified by glucose oxidase method with the use of an enzymatic colorimetric assay [18]. Assay is based on the principle that glucose oxidase oxidizes glucose to gluconic acid and hydrogen peroxide. The modified copper soap solvent extraction method was adopted for the estimation of plasma NEFA [19].

Statistical Analysis

Generated data were analyzed using the general linear model procedure of Statistical Package for the Social Sciences (SPSS for Windows, V19.0; SPSS Inc., Chicago, IL, USA). The statistical model was used to estimate sampling day effect, treatment groups (control, 0.5, 1.0, and 1.5 ppm Cr), and their interaction as follows:

$$Y_{ijk} = \mu + T_i + D_j + (T \times D)_{ij} + e_{ijk}$$

Where

Y_{ijk}=Dependent variable
μ=Overall mean of the population
T_i=Mean effect of Cr level
D_j=Mean effect of day of sampling (j=0, 15, 30, 45, 60, 75, 90, 105, and 120 days of dietary treatment) with day as a repeated factor
$(T \times D)_{ij}$=Effect of the interaction between effect of Cr supplementation and day of sampling
e_{ijk}=Unexplained residual error assumed to be independent and normally distributed

The model included Cr group, period, and their interaction as fixed effects, and calves within group as the random effect. The pairwise comparison of means was carried out using Tukey's honestly significant difference test. Differences among treatments were considered to be significant when $P<0.05$.

Results

Climatic and Physiological Variables

In the present experiment, THI decreased with the decrease in environmental temperature and found minimum during the month of January. In contrary to THI, RH was found maximum during the month of January (Fig. 1). The overall THI and RH (in percent) during the 120 days of experimental period were 58.1±1.4 and 52.0±2.8, respectively (Table 1). THI values below 72 are used to indicate no stress on animals [20, 21]. Therefore, the animal in the present trial experienced no stress. In the present experiment, dietary Cr supplementation did not affect RT, PR, and RR in buffalo calves ($P<0.05$) exposed to low ambient temperature (Table 2).

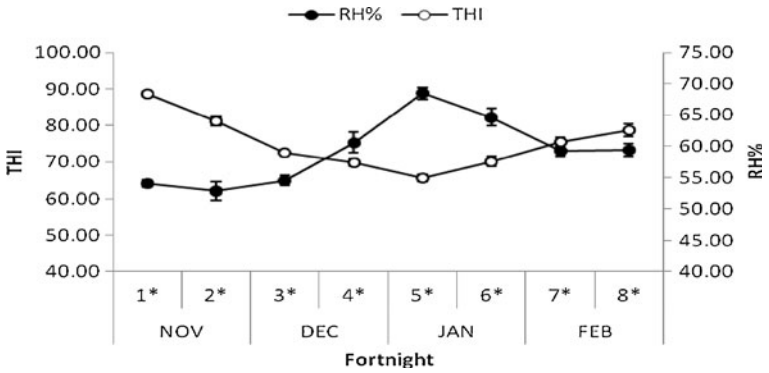

Fig. 1 Temperature humidity index and relative humidity changes during experimental period * Fortnight. *THI* temperature humidity index, *RH* relative humidity

Chemical Composition, Nutrient Intake, and Growth Performance

Ingredients and chemical composition of basal diet fed to the experimental calves are presented in Tables 2 and 3. Nutrient requirement of buffalo calves were met by feeding concentrate mixture and berseem fodder. Nutrient composition in all four groups was similar. The Cr content in the basal diet averaged 0.20 mg/kg DM of Cr; however, in other three groups, additional 0.5, 1.0, and 1.5 mg/kg DM of Cr was supplemented. Statistical analysis revealed no significant ($P>0.05$) effects of dietary Cr supplementation on DMI, body mass gain, and average daily gain (ADG) over a period of 120 days during winter season (Table 4). Similarly, supplementation of different Cr levels did not have any effect ($P>0.05$) on average daily feed intake (ADFI) and feed conversion efficiency (FCE) of the buffalo calves.

Glucose, NEFAs, and Hormone

Effect of Cr supplementation on energy metabolites, i.e., glucose and NEFAs, in buffalo calves is listed in Table 5. Supplemental Cr did not have any impact ($P>0.05$) on blood glucose and NEFAs concentration, and the range of glucose (54–56 mg/dl) and NEFAs (130–135 μmol/l) concentration was found within the normal range [22] (Table 5). Effect of Cr supplementation on insulin, cortisol, and T_3 and T_4 hormones in growing buffalo calves were estimated fortnightly (Table 5). Overall mean values of insulin and cortisol hormones were significantly ($P<0.05$) decreased as the levels of

Table 1 Mean ± SEM of environmental variables during winter seasons

Temperature			
Maximum and minimum	Max[a]	Min[b]	Mean
	26.4±0.8	5.7±1.2	15.9±0.9
Dry bulb	Morning	Evening	Mean
	6.3±1.2	24.0±0.6	15.1±0.9
Wet bulb	Morning	Evening	Mean
	4.0±1.6	14.3±1.2	9.2±1.4
RH[c] (%)	Morning	Evening	Mean
	74.0±2.3	34.1±3.4	52.0±2.8
THI[d]	Morning	Evening	Mean
	48.0±2.0	68.1±1.2	58.1±1.4

[a] *Max* maximum, [b] *Min* minimum, [c] *RH* relative humidity, and [d] *THI* temperature humidity index

Table 2 Ingredient and nutrient composition of experimental diet

Ingredients	Parts
Concentrate mixture	
Maize grain (yellow)	33.0
Groundnut cake (oiled)	21.0
Mustard oil cake (oiled)	12.0
Wheat bran	20.0
Deoiled rice bran	11.0
Mineral mixture	2.0
Common salt	1.0
Nutrient compositions of basal diet	Content (% DM basis)
Berseem fodder	19.8
Wheat straw	20.2
Ground yellow maize	23.0
Groundnut cake	15.7
Mustard cake	5.2
Wheat bran	6.4
Rice bran	8.3
Mineral mixture with vitamins premix[a]	1.0
Salt	0.3
$CrCl_3.6H_2O$ [b]	Variable [c]

[a] Premix composition per kilogram: vitamin A 500,000 IU, vitamin D_3 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, and BHT antioxidant 3,000 mg

[b] $CrCl_3.6H_2O$ (minimum assay 97 %; Hi-Media Laboratories Ltd., Mumbai, India)

[c] 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

Table 3 Chemical compositions of basal diet

Nutrients	Content (DM basis)
Dry matter, %	76.4
Organic matter, %	90.2
Crude proteins, %	17.8
Crude fiber, %	29.1
Total ash, %	9.8
Neutral detergent fiber, %	39.4
Acid detergent fiber, %	26.3
Chromium, mg/kg	0.20
Copper, mg/kg	22.81
Zinc, mg/kg	46.61
Iron, mg/kg	298.12
Manganese, mg/kg	60.82

supplemental Cr increased and were found lowest in 1.0 and 1.5 mg/kg DM of Cr supplemented buffalo calves. However, supplementation of Cr did not alter ($P>0.05$) the circulating plasma T_3 and T_4 level among all dietary treatments.

Plasma Trace Mineral Levels

In present study, plasma Cr level was found significantly higher ($P<0.01$) in 1.0 and 1.5 mg/kg DM of Cr fed buffalo calves (Table 5). However, plasma Cr level in both control and 0.5 mg/kg DM of Cr was found similar ($P>0.05$). Supplementation of Cr did not affect ($P<0.05$) plasma mineral levels of Cu, Zn, Fe, and Mn.

Discussion

Variation in ambient temperature and RH during winter season did not exert any adverse affect on the physiological variables, feed intake, and growth performance. Similar respiration rate, pulse rate, and rectal temperatures indicate that buffalo calves were not experiencing stressful conditions. Therefore, dietary Cr supplementation did not have beneficial effects on physiological variables. Similarly, Holstein cows receiving 0.00, 0.05, and 0.10 mg Cr/kg $BW^{0.75}$ has similar rectal temperature among all treatments [23]. However, increased respiration rate and decreased rectal temperature in Cr supplemented Holstein female calves [24].

The trend of performance expressed as DMI, ADG, and FCE in winter-exposed buffalo calves was similar. The information on this aspect about how the supplementation of Cr affects the performance in buffalo calves during winter season is lacking. However, findings of present trial were in accordance with results in other species reported by many researchers. Supplementation of 0.4 mg of Cr/kg DM in the form of $CrCl_3$, high-Cr yeast, or Cr nicotinic acid complex has similar results as found in present feeding trial [25]. Similarly, 0.2 and 1.0 mg of chelated Cr/kg DM supplementation did not have any effect on the rate and efficiency of body weight gain in steers [26]. In calves, feeding of 100, 200, or 400 μg Cr from high-Cr yeast/kg DM did not have beneficial effect on ADG and gain efficiency [27]. In lambs, supplementation of Cr as Cr picolinate (Cr Pic) at the levels ranging from 0.5 to 1.5 ppm did not have any effect on growth performance [28, 29]. Similarly, no effect of feeding 400 or 800 μg Cr/kg of DM as Cr-L-methionine was observed on ADG or ADFI [30]. In Suffolk lambs, supplementation Cr Pic also did not have any

Table 4 Effect of Cr supplementation on growth performance, nutrient intake, and physiological variables

Parameters	Supplemental Cr (mg/kg of DMI)				SEM[a]	Effects, P value		
	0	0.5	1.0	1.5		Group (G)	Period (P)	GxP
Initial body mass, kg	170.46	171.27	169.65	177.08	17.18	0.555	0.999	0.998
Final body mass, kg	225.17	227.35	224.38	229.01	16.54	0.687	0.666	1.000
Gain, kg	54.71	56.08	54.73	51.93	0.35	0.369	0.686	1.000
ADG[b], kg/day	0.455	0.467	0.456	0.432	0.29	0.078	0.566	0.999
ADFI[c], kg/day	5.47	5.51	5.43	5.55	0.04	0.258	0.666	1.000
DMI, kg/100 kgBW	2.54	2.50	2.59	2.53	0.6	0.666	0.699	1.000
FCR[d]	12.02	11.80	11.91	12.85	0.001	0.365	0.666	0.998
Respiration rate, min^{-1}	16.23	15.82	16.76	15.75	0.61	0.090	0.087	0.998
Pulse rate, min^{-1}	57.10	59.01	56.87	59.05	0.65	0.258	0.358	1.000
Rectal temperature, °C	38.17	38.13	38.17	38.02	0.19	0.356	0.666	0.668

[a] *SEM* standard error of the mean, [b] *ADG* average daily gain, [c] *ADFI* average daily feed intake, [d] *FCR* feed conversion rate

The experiment lasted for 120 days ($n=6$ per treatment group). The buffalo calves were fed with a basal diet devoid of supplemental Cr (control) or basal diet supplemented with 0.5, 1.0, and 1.5 mg Cr/kg DM

Table 5 Effect of Cr supplementation on energy metabolites, endocrine variables, and plasma Cr concentration

Parameters	Supplemental Cr (mg/kg of DMI, ppm)				SEM	Effects, *p* value		
	0	0.5	1.0	1.5		Group (G)	Period (P)	G x P
NEFAs, μmol/l	134.67	133.27	131.83	130.58	11.42	0.303	<0.001	0.379
Glucose, mg/dl	54.96	55.39	55.95	54.62	3.34	0.120	0.221	0.370
Insulin, ng/ml	1.63	1.61	1.58	1.48	0.05	<0.001	<0.001	<0.001
Glucose/insulin	33.71	34.40	35.41	36.90	2.10	0.003	0.910	0.961
Cortisol, μg/dl	3.92	3.84	3.66	3.55	0.18	<0.001	<0.001	0.019
Thyroxine (T_4), nmol/l	68.97	71.48	70.93	73.48	6.66	0.001	0.534	0.686
Triiodothyronine (T_3), nmol/l	1.86	1.86	1.79	1.93	0.30	0.014	0.133	0.826
T_4:T_3	37.08	38.43	39.63	38.07	1.54	0.326	0.446	0.995
Plasma Cr, ppb	0.21	0.29	0.40	0.43	0.09	0.001	<0.001	1.000
Plasma Cu, ppm	0.68	0.67	0.69	0.70	0.05	0.015	0.463	0.824
Plasma Zn , ppm	1.28	1.26	1.31	1.30	0.09	0.345	0.564	1.000
Plasma Fe, ppm	1.84	1.88	1.85	1.89	0.04	0.394	0.637	0.865
Plasma Mn, ppm	1.32	1.30	1.33	1.31	0.07	0.441	0.544	1.000

The experiment lasted for 120 days ($n = 6$ per treatment group). The buffalo calves were fed with a basal diet devoid of supplemental Cr (control) and the basal diet supplemented with 0.5, 1.0, and 1.5 mg Cr/kg DM

effect on DMI and ADG [31, 32]. Similar to calves and lambs, supplementation of organic Cr did not have effect on performance of pigs [33–35]. In contrary to present finding, supplementation of 1.0 ppm Cr during the stress period had beneficial effect on weight gain and feed efficiency [36, 37]. This apparent discrepancy in different studies might be attributed to the chemical form of the Cr used (i.e., inorganic CrCl3 or organic Cr), or the Cr level was assumed to be not high enough to influence physiological variables, nutrient intake, and growth performance.

The plasma glucose and NEFAs concentrations were not affected by Cr supplementation and were found within the normal level [22]. Similar to present findings, feeding of 250 ppb of Cr as Cr Pic in Suffolk lambs did not have any effect on plasma glucose concentration [31]. In similar study, supplementation of Cr Pic did not have any effect on glucose clearance rate and half-life during the intravenous glucose tolerance tests. Similar to present findings, 400 ppb Cr Pic fed lambs showed no effect on glucose clearance rate [32]. The 0.03 mg of supplemental Cr/kg of $BW^{0.75}$ did not affect blood glucose in Holstein female calves [24]. In another study, supplementation of Cr nicotinic acid complex did not alter plasma glucose concentrations following a glucose challenge in calves [25]. Conversely, supplementation of 0.2 mg Cr/kg of DM basis in steers did not have any effect on glucose concentration [38, 39]. In contrast to present findings, other trials have found lowered plasma glucose concentration in growing and finishing steers [40–43]. Similar to current study, no difference were found in NEFAs concentrations in Holstein calves supplemented with Cr-L-methionine [30] and Cr-tripicolinate [44]. The sheep that consumed diets containing either 0 or 1 mg of Cr/kg from a high-Cr yeast and were exposed from to a cold environment (0 to 4 °C) did not have any effect on plasma NEFAs concentrations [45]. Similarly, no effect of supplemental Cr on plasma NEFAs concentrations was found in growing steers [44, 46] and dairy cattle [47]. In contrast to present findings, other authors reported decreased NEFAs concentrations [32, 39, 48, 49]. Plasma NEFAs concentrations are used as a measure of energy balance. When dietary energy intake is insufficient to meet the animal's metabolic needs, then stored fat is mobilized from adipose tissues in the form of NEFAs. No change in plasma NEFAs concentrations in the present experiment showed that experimental animals were not in negative energy balance.

Supplemental Cr reduced plasma insulin and cortisol levels in winter-exposed buffalo calves. However, plasma insulin and cortisol levels were in normal physiological range in all the groups throughout the 120-days trial period [50]. Similar to present finding, Cr supplemented cows had lower serum insulin concentrations [49, 51]. Reduced plasma insulin concentrations were also observed in broiler birds supplemented with 1,600 and 3,200 ppb chromium picolinate [52]. In contrast to current study, dietary supplementation of 1 mg of Cr/kg from high Cr yeast did not have any impact on plasma insulin concentrations in sheep exposed to cold environment (0 to 4 °C) [45]. However, higher circulatory insulin concentration was observed in black Bengal goats supplemented with 0.5 mg Cr as chromic chloride [53]. Decreased levels of

plasma glucose and serum insulin following the Cr supplementation suggested that less insulin was required to clear glucose from the blood, indicating greater tissue sensitivity to insulin. This is consistent with the role of Cr in potentiating the action of insulin [54]. Cr, as the active component of the glucose tolerance factor, is involved in stimulation and regulation of the action of insulin, that is, chromium's physiological role to empower insulin action [55]. Cr can stimulate the biological activity of insulin by increasing the insulin-sensitive cell receptors or binding activity [56].

The results of present study indicated that the feeding of inorganic Cr in buffalo calves during winter season reduces plasma cortisol concentration. Reduced circulating cortisol concentration was also observed in multiparous Holstein dairy cows fed on 8 mg of Cr-Met per head per day [57]. Similarly, reduced plasma cortisol concentration was also observed in Holstein female calves supplemented with 0.03 mg of supplemental Cr/kg of $BW^{0.75}$ [24]. To strengthen the result of present findings, supplementation of 400 μg Cr Pic/kg diet under low ambient temperature (6.9 °C) had shown decreased plasma corticosteroid concentration in hens [58, 59]. Circulating cortisol concentrations decreased in diabetic rats treated with a combination of the biotin and Cr Pic supplements [60]. A number of authors also reported decreased sensitivity to stress in 0.5 ppm chelated Cr supplemented animals through reduced concentration of cortisol in the blood [61–63]. Reduced plasma cortisol concentrations were also found in another rat model supplemented with 80 μg Cr Pic per kilogram body mass [64]. Five grams per day Cr-Met supplementation tended to decrease cortisol concentration in multiparous Holstein cows [65]. In contrast to current study, dietary supplementation of 1 mg of Cr/kg from high Cr yeast did not have any impact on plasma cortisol concentration in sheep exposed to cold environment (0 to 4 °C) [45]. Other authors also did not found any effect on plasma cortisol concentration in calves [25, 66] and lambs [31, 32]. Dietary Cr supplementation reduces stress in livestock by lowering circulatory cortisol and potentiating the action of insulin. The reduction of cortisol level induced by the supplemental Cr might contribute to better glucose utilization, since cortisol promotes hyperglycemia by stimulating gluconeogenesis and reducing glucose utilization.

Growing buffalo calves in the present study did not exhibit any change in plasma T_3 and T_4 concentrations. Similar to present findings, feeding of 0.25 or 0.35 mg Cr-yeast in male Rambouillet lambs also did not affected plasma T_4 and T_3 levels [67]. Similar findings were reported in Suffolk lambs supplemented with 250 ppb of Cr as Cr Pic [31]. However, supplementation of 0.03 mg of supplemental Cr/kg of $BW^{0.75}$ did not affect plasma T_3 concentration, but increased plasma T_4 level was found in Holstein female calves [24]. Nevertheless, increasing Cr doses caused quadratic declines in serum T_4, whereas blood T_3 declined only with the higher Cr dose supplementation in Holstein calves [68].

It is evident from the changes in plasma Cr concentration that there was a significant effect of dietary treatments on plasma Cr concentration. Supplementation of Cr resulted in dose-dependent linear increase in tissue Cr concentration [69]. However, blood Cr concentration might reflect to a certain extent 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 [70]. Cr supplementation protected stress-induced losses of several trace elements in mice [71]. However, increased intake of trace minerals were observed in $CrCl_3$ supplemented heifers [72]. Increased urinary excretion of Zn and Cu has been reported in Cr supplemented animals [73].

Conclusion

The results of present findings indicated that dietary inorganic Cr supplementation does not have any impact on physiological variables and growth performance of winter-exposed buffalo calves. However, dietary Cr supplementation reduced circulating concentration of insulin and glucose in 1.0 and 1.5 mg of Cr/kg DM indicates increased insulin potency and better glucose utilization. Furthermore, reduced cortisol concentration in 1.0 and 1.5 mg of Cr/kg DM fed groups shows increased insulin or anabolic activity. Results also suggested that supplemental Cr also improve blood plasma Cr level without affecting plasma concentration of other trace minerals.

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