Growth performance, feeding behavior, health status, and blood metabolites of environmentally heat-loaded Holstein dairy calves fed diets supplemented with chromium

S. Kargar,^{*1} F. Mousavi,[†] S. Karimi-Dehkordi,[†] and M. H. Ghaffari^{‡2}

^{*}Department of Animal Science, School of Agriculture, Shiraz University, Shiraz 71441–65186, Iran

[†]Department of Animal Science, College of Agriculture, Shahrood University, Shahrood 34141–88186, Iran

[‡]Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton T6G 2P5, Canada

ABSTRACT

We investigated the effect of Cr supplementation on growth performance, feeding behavior, health status, and blood metabolites of summer-exposed dairy calves during the pre- and postweaning periods. A total of 24 newborn Holstein female calves (1 d of age; 42.1 ± 0.89 kg of body weight) were assigned randomly to a control group (no Cr supplement; Cr–) or a Cr group (Cr+) receiving 0.05 mg of Cr/kg of body weight^{0.75}. During the study period, the average maximum temperature-humidity index was 81.3 units, indicating a high environmental heat load. Chromium was provided in colostrum and milk during the preweaning period, and in the starter feed during the postweaning period. Calves had free access to fresh water and starter feed throughout the experiment and were weaned on d 63. Starter feed intake and total dry matter intake were greater in Cr+ calves. Despite decreased meal frequency in Cr+ calves, during the preweaning period the starter feed intake increased due to increases in meal duration and meal size and a tendency for greater meal interval. Increased postweaning starter intake in Cr+ calves was attributed to a tendency for an increase in meal duration. Chromium-supplemented calves had greater overall weight gain, but their feed efficiency and rectal temperature were not affected by Cr treatment. Chromium supplementation tended to increase the heart girth during the preweaning and overall periods and increased the hip width during the postweaning and overall periods. Respiration rates were lower in Cr+ calves than Cr– calves during the preweaning and overall periods. During the preweaning period, the time spent in eating and ruminating per 12-h period was not affected by Cr treatment. During the postweaning

period, the rumination time (min per 12-h period) was unaffected but total eating time (min per 12-h period) increased in Cr+ calves. Times spent resting, drinking, standing, lying, and in nonnutritive oral behaviors were not affected by treatments during the pre- and postweaning periods. Chromium supplementation had no effects on serum concentrations of cortisol, triiodothyronine, and thyroxine measured at weaning or the end of the trial. The Cr+ calves tended to have higher serum concentrations of glucose and a higher ratio of insulin to glucose measured at the end of the trial. In conclusion, Cr supplementation of both liquid and solid feeds improved growth performance in summer-exposed calves as a result of reduced respiration rate and increased feed intake; however, no beneficial effects were found on feed efficiency and insulin metabolism.

Key words: dairy calf, chromium, summer

INTRODUCTION

During summer months, calves exhibit poor growth performance (West, 2003), low immunity (Tao and Dahl, 2013), increased respiratory rate (Spain and Spiers, 1996), and greater susceptibility to diseases due to reduced feed intake (West, 2003). The consequences of thermal stress are costly due to economic losses associated with reduced weight gain, and increased mortality and morbidity (Roland et al., 2016). Chromium supplementation may be a viable approach to improve calf performance and welfare during summer as it may alleviate the negative effects of environmental stress (Pechova and Pavlata, 2007) and improve the physiological responses in dairy calves exposed to thermal stress (Yari et al., 2010). Chromium as a part of chromodulin (also called low-molecular-weight chromium-binding substance) plays an important role in the activation of insulin receptor and allows glucose to enter the cells by recruiting the GLUT 4 transporters from the interior of cells to the cell surface (Vincent, 2015). In buffalo calves, dietary Cr supplementation (0.5 to 1.5 mg/kg of DM) during the summer months

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¹Corresponding author: skargar@shirazu.ac.ir or skargar@wisc.edu

²Current address: Institute of Animal Science, Physiology & Hygiene Unit, University of Bonn, 53115 Bonn, Germany.

improved their heat tolerance and immune status, and the effectiveness of insulin with no effects on feed intake, growth performance, or triiodothyronine (T_3) and thyroxine (T_4) concentrations (Kumar et al., 2015).

Beneficial effects of Cr supplementation on production responses in dairy cows during normal (Sadri et al., 2009) and heat stress conditions (Soltan, 2010) were also reported. However, the data on the effect of Cr supplementation on intake and growth performance in calves are not consistent (Yari et al., 2010; Ghorbani et al., 2012; Kumar et al., 2015). Such inconsistencies may be explained by (1) the chemical form of Cr used [inorganic ($CrCl_3$) vs. organic (Cr-yeast or chelated-Cr; Kegley and Spears, 1995)], (2) environmental conditions (normal vs. heat stress condition), (3) method of offering Cr (in the starter or liquid feed), or (4) Cr dosage.

There is a paucity of data describing the effect of Cr supplementation on the starter feed intake, growth performance, feeding behavior, health status, and blood metabolites in dairy calves under summer conditions. Furthermore, the mechanism by which feed intake may be affected by Cr supplementation is not completely clear. Investigation of feeding behavior in Cr-supplemented calves is also required to clarify the mechanism through which Cr affects the feed intake. The objective of this study was to determine the effect of Cr supplementation on feed intake, meal pattern, health status, blood metabolite, and growth performance in environmentally heat-loaded newborn Holstein calves during the pre- and postweaning periods. It was hypothesized that supplemental Cr would enhance growth performance in dairy calves during summer, possibly through increased starter feed intake.

MATERIALS AND METHODS

This study was conducted from July 5 to October 12, 2015, at the FKA Agri-Animal Production Co., Isfahan, Iran. Animal experimentation followed the protocol #19356 of the Iranian Council of Animal Care (1995). Air temperature and relative humidity (**RH**) in the stall areas were recorded daily by Hobo Pro Series Temp probes (Onset Computer Corporation, Pocasset, MA). Temperature-humidity index (**THI**) was calculated using the equation: $THI = 0.8 \times \text{maximum } T + (\text{minimum } RH/100) \times (\text{maximum } T - 14.4) + 46.4$, in which T is the air temperature ($^{\circ}C$) and RH denotes the relative humidity (%; NRC, 2001).

Calves, Treatments, and Management

Twenty-four newborn Holstein female calves (1 d old; 42.1 ± 0.89 kg of BW $\pm$ SE) were separated from their

dams immediately after birth, weighed, and randomly transferred to individual pens (2.9 m $\times$ 1.1 m $\times$ 1.8 m; length $\times$ width $\times$ height). Fresh sawdust bedding was added every 3 d, but manure was removed daily as needed to keep the pens visibly clean and dry. Each calf was fed 6 L of colostrum over 2 d starting from 2 to 8 h after birth. From d 2 onward, the calves were individually fed pasteurized waste milk, containing $3.34 \pm 0.12\%$ fat, $2.86 \pm 0.09\%$ CP, and $4.87 \pm 0.07\%$ lactose, in steel buckets in 2 meals of equal volume (daily at 0800 and 1600 h). Milk was pasteurized by heating for 30 min at $62.5^{\circ}C$ (Pazoki et al., 2017). Representative samples of milk ($n = 36$) were taken from both the morning and evening meals twice per week, preserved with potassium dichromate, stored at $4^{\circ}C$, and then analyzed for concentrations of fat, protein, and lactose by MilkoScan (134 BN Foss Electric, Hillerød, Denmark; AOAC International, 1990). The calves were offered milk 3 L/d from 3 to 15 d of age, 4 L/d from 16 to 20 d of age, 5 L/d from 21 to 25 d of age, 6 L/d from 26 to 60 d of age, and 3 L/d from 61 to 63 d of age. The calves were weaned on d 63 and the study was terminated on d 91. Calves were assigned randomly to 2 treatments ($n = 12$): a control group (**Cr-**) and a group receiving supplemental Cr (**Cr+**). The rate of chromium supplementation was chosen according to Vincent (2014), who suggested doses between 0.08 to 1 mg of Cr/kg of BW for a positive effects of Cr on metabolism. Chromium was added to the liquid feed (300 mL of colostrum or milk) and fed to calves in bottles up to wk 9 to supply 0.05 mg of Cr/kg of $BW^{0.75}$; the calves received the remaining colostrum/milk according to the feeding schedule described above. The chromium supplement [MicroPlex 1000, supplied 1,000 mg of Cr/kg as Cr-Met (90% Met and 10% Cr, wt/wt); Zinpro Animal Nutrition Inc., Eden Prairie, MN] was provided by Sana Dam Co. (Tehran, Iran). After weaning, the same dosage of Cr was added in the starter feed. To ensure that each calf consumed the calculated amount of Cr, the Cr-Met was mixed with 50 g of daily starter feed allowance and top-dressed. Chromium supplementation was adjusted based on weekly BW throughout the entire experimental period (Yari et al., 2010; Ghorbani et al., 2012). Adjustment of the level of Cr fed each week is not how Cr would be expected to be supplemented in real world feeding situations; therefore, feeding rates of Cr supplements should be based on the manufacturer's recommendations.

Calves were provided ad libitum access to fresh water and starter feed (mashed form) formulated according to the Cornell Net Carbohydrate and Protein System, version 5.1 (CNCPS), allowing at least 10% refusals (i.e., the portion of the starter not consumed over a 24-h period). Grains (corn and barley), used in the

starter feed, were ground using a hammer mill with a 2-mm screen size (model 5543 GEN, Isfahan Dasht, Isfahan, Iran). Second-cut alfalfa hay at 50% flowering was chopped (particle size distribution: $5.0 \pm 0.3\%$ greater than 18 mm, $28.9 \pm 1.4\%$ between 8 and 18 mm, $41.8 \pm 0.9\%$ between 1.18 and 8 mm, and $24.3 \pm 1.3\%$ less than 1.18 mm, and geometric mean particle 4.05 ± 0.14 mm) with a theoretical length of 30 mm, using a harvesting machine with screen size regulator (Golchin Trasher Hay Co., Isfahan, Iran). Calves received a starter feed containing 8% alfalfa hay as TMR with concentrate throughout the study. The ingredients and nutrient composition of the basal diet are shown in Table 1. Starter feed refusal was removed at 0800 h. Feed or refusal were mixed thoroughly and ground to pass a 1-mm screen in a Wiley mill (Ogawa Seiki Co., Ltd., Tokyo, Japan) before samples were analyzed for DM (AOAC International, 1990; method 934.01) and CP (AOAC International, 1990; method 988.05) contents. Neutral detergent fiber was analyzed without

sodium sulfite but with α -amylase included (Van Soest et al., 1991).

Data Collection and Sampling

Sub-samples of the starter feed ($n = 13$; one sample per week) were prepared (Nasrollahi et al., 2012) and analyzed for Cr contents (flame photometer; GBC Integra EXL ICP, Melbourne, Australia). Glassware were acid-washed to avoid sample contamination; a blank was run with the samples to test for possible contamination. The amount of starter feed intake was calculated based on the weight of feed offered and refusal collected daily. The calves were weighed at birth and once weekly thereafter using an electronic scale, which was calibrated by the manufacturer's agent before initiation of the study and every month thereafter. Average daily weight gain (kg/d) and gain-to-feed ratio [kg of BW gain/total DMI (DMI = milk DM + starter feed DM)] were calculated. In addition, the barrel, heart girth, and hip width measurements were recorded weekly (Pazoki et al., 2017). Respiration rate (**RR**) was measured at 1400 h daily by visual observation at 3 separate minutes (Kargar et al., 2015). Immediately after recording RR, rectal temperature (**RT**) was measured using a thermometer (Qingdao Dacon Trading Co. Ltd., Shandong, China) placed in the rectum for 1 min.

Behavioral data [eating, ruminating, resting, drinking, standing, lying, and nonnutritive oral behaviors (**NNOB**)] were monitored visually by trained personnel, unaware of the treatment, during a 12-h period for 3 consecutive days before weaning (d 58 to 60 of the trial) and after weaning (d 86 to 88 of the trial). A total of 4 individuals in 2-person teams rotated throughout the 12-h period; 2 individuals observed the calves simultaneously for 2 h with a 2-h rest period before recording again. All activities were noted every 5 min and each activity was assumed to persist for the entire 5-min interval between observations (Kargar et al., 2010, 2013, and 2014). A period of eating was defined as at least 1 observation of eating activity occurring after at least 5 min without eating. Meal frequency was defined as the number of bouts during a 12-h period. The meal duration (min/meal) was computed as the time from the beginning of the 1st feeding event until an interval between events, and averaged for each calf. Intervals (min) between feeding events were computed from the end of a feeding event to the beginning of the next, and averaged for each calf. The meal size (g of starter DM/meal) was the total amount of starter DM ingested during each meal. The same procedure was used to calculate the rumination pattern.

Table 1. Ingredients and chemical composition of the experimental starter diet on a DM basis

Item	Diet
Ingredient composition, % of DM	
Alfalfa hay	8.0
Corn grain, ground	43.9
Barley grain, ground	9.2
Soybean meal	29.4
Extruded soybean	4.7
Fat supplement ¹	0.5
Calcium carbonate	1.7
Sodium bicarbonate	1.1
Monocalcium phosphate	0.5
Vitamin and mineral mixture ²	0.5
Salt	0.5
Chemical composition, % of DM unless otherwise indicated	
DM, %	89.2
CP	21.6
NFC ³	53.1
NDF	14.7
ADF	6.3
Ether extract (EE)	4.3
Ash	4.3
Calcium ⁴	0.98
Phosphorus ⁴	0.52
Chromium, mg/kg of DM	0.95
ME, ⁴ Mcal/kg of DM	3.10
NE _M , ⁴ Mcal/kg of DM	2.32
NE _G , ⁴ Mcal/kg of DM	1.76

¹Palmac 80–16 (IOI Oleochemical Industries Sdn Bhd, Prai, Malaysia). Product contained 2% C12:0, 5% C14:0, 80% C16:0, 2% C18:0, 9% C18:1, and 3% C18:2.

²Contained per kilogram of supplement: 975,000 IU of vitamin A, 750,000 IU of vitamin D, 1,800 IU of vitamin E, 143 g of Zn, 76 g of Mn, 48.6 g of Cu, 19.5 g of Se, 18.4 g of Fe, 8 g of Ca, and 1.3 g of Co.

³NFC = $100 - (\text{CP} + \text{NDF} + \text{EE} + \text{ash})$ (NRC, 2001).

⁴Calculated from NRC (2001).

Blood samples were collected 4 h after the morning meal into vacuum tubes on d 63 (weaning day) and 91 of life, placed on ice, and centrifuged at $3,000 \times g$ for 20 min at 4°C . Serum (1.5 mL) was transferred into 2-mL cryotubes and stored at -20°C . Serum concentration of glucose was determined spectrophotometrically (UNICO, 2100; Zistchemi, Tehran, Iran) using commercially available kit [Pars Azmoon Co., Tehran, Iran; catalog number: glucose (1–500–017)]. Serum concentrations of insulin, T_3 , T_4 , and cortisol were determined using ELISA (Bio-Tek Instruments Inc., Winooski, VT) commercial kits (Diaplus Inc., North York, ON, Canada).

Statistical Analyses

Data were checked for normality using the UNIVARIATE procedure (SAS 9.4, SAS Institute Inc., Cary, NC). The data that were not normally distributed were transformed logarithmically. Starter feed intake, total DMI, ADG, feed efficiency, skeletal growth, RR, and RT values were analyzed for 3 discrete periods as preweaning (d 1 to 63), postweaning (d 64 to 91), and overall (d 1 to 91) periods. Data on starter feed intake, total DMI, ADG, feed efficiency, skeletal growth, RR, and RT were analyzed using the MIXED MODEL procedure (SAS 9.4) as

$$Y_{ijkl} = \mu + \text{Calf}_i + \text{Treatment}_j + \text{Time}_k + (\text{Treatment} \times \text{Time})_{jk} + \beta(X_i - \bar{X}) + e_{ijkl},$$

where Y_{ijkl} is the dependent variable; μ is the average experimental value; Calf_i is the random effect of calf; Treatment_j is the fixed effect of Cr i ($i = 0$ and $0.05 \text{ mg/kg of BW}^{0.75}$); Time_j is the fixed effect of day or week j ($j = \text{number of day or week}$); $(\text{Treatment} \times \text{Time})_{ij}$ represents the effect of the interaction between treatment and time; $\beta(X_i - \bar{X})$ designates the covariate variable, where the β is the regression coefficient relating covariate factor to the variable measured, X_i is the covariate factor for the i th factor, and $\bar{X}$ is the overall mean of covariate factor; and e_{ijkl} is the error term.

Time (day or week) was modeled as a repeated measurement by using an autoregressive covariance structure (type 1), which was determined by the smallest Akaike's information criterion. Initial BW was considered as a covariate for BW (d 63 and 91) and skeletal growth analyses. Least squares means for treatment effects were separated using a Tukey adjustment when the overall F -test was $P < 0.05$. Trends were declared at $0.05 \leq P \leq 0.10$.

Data on BW, feeding and chewing behavior, and blood variables were subjected to ANOVA according to the following model:

$$Y_{ijk} = \mu + \text{Calf}_i + \text{Treatment}_j + e_{ijk},$$

where Y_{ijk} is the dependent variable; μ is the average experimental value; Calf_i is the random effect of calf; Treatment_j is the fixed effect of Cr j ($j = 0$ and $0.05 \text{ mg/kg of BW}^{0.75}$); and e_{ijk} is the error term.

RESULTS

The average maximum THI, RH, and maximum temperature were 81.3 , 11.5% , and 36.9°C , respectively, during the experiment (Figure 1).

Feed Intake and Performance

Results of feed intake, BW, ADG, and FE are shown in Table 2. Calves supplemented with Cr had greater starter feed intake and total DMI during the preweaning ($P = 0.001$), postweaning ($P = 0.04$), and overall ($P = 0.003$) periods than Cr– calves. Irrespective of the treatment, total DMI increased with increasing calf age (week effect: $P < 0.001$). Calves supplemented with Cr had a higher total DMI at weaning (d 63) as compared with Cr– calves (treatment $\times$ week effect: $P = 0.01$; Figure 2). Initial BW (41.3 vs. 43.0 kg for Cr– vs. Cr+), and weaning (76.4 vs. 80.8 kg) and final (106.8 vs. 114.4 kg) weights were not affected by Cr treatment. Average daily gain was not affected by treatments during the pre- (0.56 vs. 0.60 kg/d ; $P = 0.11$) or postweaning (1.10 vs. 1.20 kg/d ; $P = 0.12$) periods; however, Cr+ calves had a greater ($P = 0.02$) ADG during the whole experiment. No differences in feed efficiency were detected between treatments during the preweaning, postweaning, and overall periods ($P > 0.05$). However, calves supplemented with Cr tended to have higher ADG (treatment $\times$ week effect: $P = 0.07$; Figure 3) and a greater feed efficiency (treatment $\times$ week effect: $P = 0.03$; Figure 4) during the first week of study.

Skeletal Growth and Health Status

Skeletal growth, RR, and RT data are presented in Table 3. Chromium supplementation tended to increase the heart girth during the preweaning ($P = 0.05$) and overall periods ($P = 0.10$), with no difference between treatments during the postweaning period. No differences were observed in body barrel between treatments

Table 2. Intake, BW, ADG, and feed efficiency of summer-exposed calves supplemented with (Cr+) or without (Cr-) chromium

Item	Treatment		SEM	P-value ¹		
	Cr–	Cr+		T	W	T × W
Starter intake, kg of DM/d						
Prewaning (d 1 to 63)	0.39 ^b	0.50 ^a	0.03	0.001	<0.001	0.004
Postweaning (d 64 to 91)	2.07 ^b	2.31 ^a	0.08	0.04	<0.001	0.77
Overall (d 1 to 91)	0.91 ^b	1.06 ^a	0.04	0.003	<0.001	0.01
Total DMI, ² kg/d						
Prewaning (d 1 to 63)	1.04 ^b	1.15 ^a	0.03	0.001	<0.001	0.004
Postweaning (d 64 to 91)	2.07 ^b	2.31 ^a	0.08	0.04	<0.001	0.77
Overall (d 1 to 91)	1.35 ^b	1.51 ^a	0.04	0.002	<0.001	0.01
BW, kg						
Initial (d 1)	41.25	43.00	0.89	0.17	—	—
At weaning (d 63)	76.38	80.79	1.85	0.20	—	—
At end (d 91)	106.83	114.39	2.45	0.14	—	—
ADG, kg/d						
Prewaning (d 1 to 63)	0.56	0.60	0.03	0.11	<0.001	0.06
Postweaning (d 64 to 91)	1.10	1.20	0.05	0.12	<0.001	0.20
Overall (d 1 to 91)	0.72 ^b	0.79 ^a	0.02	0.02	<0.001	0.07
Feed efficiency ³						
Prewaning (d 1 to 63)	0.50	0.51	0.02	0.80	<0.001	0.02
Postweaning (d 64 to 91)	0.54	0.52	0.02	0.47	<0.001	0.15
Overall (d 1 to 91)	0.51	0.51	0.02	0.93	<0.001	0.03

^{a,b}Means within a row with different superscripts are significantly different ($P < 0.05$).
¹T = treatment effect; W = week effect; and T × W = treatment by week interaction.
²Total DMI = milk DM + starter feed DM.
³Feed efficiency was calculated by dividing ADG by average total daily DMI (milk DM + starter feed DM).

Figure 1. Temporal patterns of minimum (Min), mean, and maximum (Max) temperature-humidity index (THI) over the experimental period. The average maximum THI, relative humidity, and maximum temperature were 81.3, 11.5%, and 36.9°C, respectively.

Figure 2. Daily total DMI ($\pm$ SE; kg/d) of calves supplemented with (Cr+) or without (Cr-) chromium, over 13 wk of age. SEM = 0.04. Effects in the model: treatment (T): $P = 0.002$; week (W): $P < 0.001$; T $\times$ W: $P = 0.01$. For each time point, * denotes significant difference at $P < 0.05$.

Figure 3. Average daily gain ($\pm$ SE; kg/d) of calves supplemented with (Cr+) or without (Cr-) chromium, over 13 wk of age. SEM = 0.02. Effects in the model: treatment (T): $P = 0.02$; week (W): $P < 0.001$; T $\times$ W: $P = 0.07$. For each time point, † denotes a tendency to differ at $0.05 \leq P \leq 0.10$.

Table 3. Skeletal growth, respiration rate, and rectal temperature of summer-exposed calves supplemented with (Cr+) or without (Cr-) chromium

Item	Treatment		SEM	P-value ¹		
	Cr-	Cr+		T	W	T × W
Heart girth, cm						
Prewaning (d 1 to 63)	86.5 ^b	87.5 ^a	0.35	0.05	<0.001	0.56
Postweaning (d 64 to 91)	103.1	103.7	0.54	0.44	<0.001	0.08
Overall (d 1 to 91)	91.7	92.5	0.33	0.10	<0.001	0.27
Body barrel, cm						
Prewaning (d 1 to 63)	93.8	94.0	0.44	0.78	<0.001	0.94
Postweaning (d 64 to 91)	119.4	120.9	0.82	0.22	<0.001	0.02
Overall (d 1 to 91)	101.7	102.2	0.39	0.38	<0.001	0.32
Hip width, cm						
Prewaning (d 1 to 63)	24.1	24.3	0.10	0.12	<0.001	0.29
Postweaning (d 64 to 91)	30.3 ^b	30.8 ^a	0.15	0.02	<0.001	0.53
Overall (d 1 to 91)	26.0 ^b	26.3 ^a	0.08	0.01	<0.001	0.37
Respiration rate, breaths/min						
Prewaning (d 1 to 63)	41.9 ^a	39.5 ^b	0.80	0.03	0.01	0.57
Postweaning (d 64 to 91)	40.2	39.2	0.73	0.35	0.13	0.45
Overall (d 1 to 91)	41.4 ^a	39.4 ^b	0.60	0.02	0.01	0.51
Rectal temperature, °C						
Prewaning (d 1 to 63)	39.08	39.07	0.02	0.86	0.04	0.35
Postweaning (d 64 to 91)	39.09	39.02	0.04	0.24	0.71	0.22
Overall (d 1 to 91)	39.08	39.06	0.02	0.43	0.13	0.16

^{a,b}Means within a row with different superscripts are significantly different ($P < 0.05$).

¹T = treatment effect; W = week effect; and T × W = treatment by week interaction.

($P > 0.05$). The hip width was greater for Cr+ calves than Cr- calves during the postweaning and overall periods but not during the preweaning period. Rectal

temperature was not affected by treatments ($P > 0.05$), but RR was significantly lower in Cr+ calves during the preweaning ($P = 0.03$) and overall ($P = 0.03$) periods.

Figure 4. Feed efficiency ($\pm$ SE; kg/d) of calves supplemented with (Cr+) or without (Cr-) chromium, over 13 wk of age. SEM = 0.02. Effects in the model: treatment (T): $P = 0.93$; week (W): $P < 0.001$; T × W: $P = 0.03$. For each time point, * denotes significant difference at $P < 0.05$.

Chewing Behavior and Meal and Rumination Patterns

Table 4 presents the total time devoted to each behavior in a 12-h observation period. During the preweaning period, the times spent in eating and ruminating were not affected by treatments ($P > 0.05$). Times devoted to resting, drinking, standing, lying, and in NNOB were not affected by treatments. During the postweaning period, eating time, expressed as minutes per 12 h, increased (128.3 vs. 105.8 min; $P = 0.03$) in Cr+ calves. During the postweaning period, rumination time (min per 12-h period) as well as the times devoted to resting, drinking, standing, lying, and NNOB were not different ($P > 0.05$) between treatments.

The data on meal and rumination patterns are presented in Table 5. During the preweaning period, meal frequency decreased (5.2 vs. 6.4 bouts/12 h; $P = 0.04$) but meal duration (14.5 vs. 11.7 min/meal; $P = 0.04$), meal size (99.8 vs. 72.3 g of starter DM; $P = 0.04$), and inter-meal interval (124.0 vs. 100.8 min; $P = 0.08$) increased in Cr+ calves. Chromium supplementation decreased the number of rumination bouts (6.0 vs. 7.5 per 12 h; $P = 0.03$) during the preweaning period. However, rumination duration (min/meal, 19.3 vs. 16.3; $P = 0.06$) and interval between bouts (100.7 vs. 79.7 min, $P = 0.04$) increased in Cr+ calves compared with Cr– calves. During the postweaning period, meal patterns were not affected by treatments except for meal length, which tended to be greater for Cr+ calves than for Cr– calves (15.7 vs. 13.7 min; $P = 0.08$). Furthermore,

rumination patterns were not different between treatments.

Table 6 presents the overall means of blood glucose and hormone concentrations at weaning and at the end of the trial. At weaning, concentrations of blood glucose, insulin, T₃, T₄, and cortisol, and insulin to glucose and T₃ to T₄ ratios were not affected by treatments. Blood glucose concentration measured at the end of the trial tended to be lower ($P = 0.09$) in Cr+ calves. Serum concentration of insulin ($P = 0.01$), and insulin to glucose ratio ($P = 0.02$) increased in Cr+ calves compared with Cr– calves. However, concentrations of cortisol and thyroid hormones, and T₃ to T₄ ratio were not affected by Cr supplementation.

DISCUSSION

During the experiment, the average maximum THI, RH, and maximum temperature were 81.3, 11.5%, and 36.9°C, respectively, indicating a high environmental heat load (Yari et al., 2010; Beiranvand et al., 2016). We have ignored the potential effect of methionine as a part of Cr-Met supplement in the current study and discussed the results by focusing only on the possible effect of Cr. Chromium concentration in the starter ingredients varies widely and has not always reported due to the difficulty in analysis of Cr. The starter ingredients in the current study contained 0.95 mg Cr/kg of DM, which is in the range (approximately 1 to 3 mg of Cr/kg of DM) reported by Lindemann (2007). However, the bio-availability of Cr in the starter feed

Table 4. Chewing behaviors and times devoted to drinking, standing, lying, and nonnutritive oral behaviors of summer-exposed calves supplemented with (Cr+) or without (Cr–) chromium

Item	Treatment		SEM	P-value ¹
	Cr–	Cr+		T
Preweaning period (d 58 to 60)				
Eating time, min/12 h	73.3	72.9	5.78	0.95
Ruminating time, min/12 h	121.3	111.3	7.77	0.37
Resting time, min	444.2	464.2	13.27	0.19
Drinking time, min	12.5	14.6	1.89	0.44
Nonnutritive oral behaviors, ² min	68.8	57.1	7.55	0.28
Standing time, min	267.9	257.1	12.83	0.55
Lying time, min	452.1	462.9	12.83	0.55
Postweaning period (d 86 to 88)				
Eating time, min/12 h	105.8 ^b	128.3 ^a	6.92	0.03
Ruminating time, min/12 h	149.2	145.8	10.80	0.82
Resting time, min	384.6	371.7	14.82	0.49
Drinking time, min	20.4	23.8	3.15	0.46
Nonnutritive oral behaviors, ² min	60.0	50.4	4.40	0.13
Standing time, min	287.5	312.1	12.46	0.17
Lying time, min	432.5	407.9	12.46	0.17

^{a,b}Means within a row with different superscripts are significantly different ($P < 0.05$).

¹T = treatment effect.

²Nonnutritive oral behaviors refer to licking any surface, tongue rolling.

Table 5. Meal and rumination patterns of summer-exposed calves supplemented with (Cr+) or without (Cr–) chromium

Item	Treatment		SEM	<i>P</i> -value ¹
	Cr–	Cr+		T
Prewaning period (d 58 to 60)				
Meal frequency, bouts/12 h	6.4 ^a	5.2 ^b	0.41	0.04
Meal duration, min/meal	11.7 ^b	14.5 ^a	0.93	0.04
Meal interval, min	100.8	124.0	9.30	0.08
Meal size, g of starter DM	72.3 ^b	99.8 ^a	9.25	0.04
Rumination frequency, bouts/12 h	7.5 ^a	6.0 ^b	0.46	0.03
Rumination bout length, min/bout	16.3	19.3	1.09	0.06
Rumination bout interval, min	79.7 ^b	100.7 ^a	7.95	0.04
Postweaning period (d 86 to 88)				
Meal frequency, bouts/12 h	7.8	8.3	0.40	0.47
Meal duration, min/meal	13.7	15.7	0.77	0.08
Meal interval, min	78.6	71.1	5.62	0.28
Meal size, g of starter DM	171.6	170.2	13.62	0.94
Rumination frequency, bouts/12 h	7.5	6.4	0.44	0.19
Rumination bout length, min/bout	20.9	22.6	1.14	0.31
Rumination bout interval, min	75.1	89.9	7.75	0.21

^{a,b}Means within a row with different superscripts are significantly different ($P < 0.05$).

¹T = treatment effect.

ingredients is lower than that in Cr-Met (Lindemann, 2007). Accordingly, we attempted to supplement an additional 2 mg of Cr/d to determine its effect on growth performance, feeding behavior, and well-being of dairy calves.

In the current study, Cr supplementation increased starter feed intake and total DMI in summer-exposed calves, which indicates that Cr may have been deficient in calves. It has been reported that Cr requirement of calves is increased under a variety of stress conditions (Chang and Mowat, 1992; Pechova and Pavlata,

2007). A major break in the heat load occurred at 49 d, resulting in a drop of 8 to 10 units in the maximum THI (Figure 1), which may have exacerbated the feed intake difference between the 2 treatments (Figure 2), and perhaps generated the treatment by week interaction on the feed intake (Table 2). Regardless of the environmental heat load, the birth and weaning processes can be stressful for the calf (Jonasen and Krohn, 1991; Murray and Leslie, 2013). Stressful situations may increase the urinary excretion and thus Cr requirements (Chang and Mowat, 1992; Pechova and Pavlata,

Table 6. Blood serum metabolite and hormones of summer-exposed calves supplemented with (Cr+) or without (Cr–) chromium

Item	Treatment		SEM	<i>P</i> -value ¹
	Cr–	Cr+		T
At weaning (d 63)				
Glucose, mg/dL	97.40	93.80	2.00	0.23
Insulin, μ IU/mL	13.82	20.20	5.90	0.46
Insulin:glucose	0.14	0.21	0.06	0.44
Triiodothyronine (T ₃), ng/dL	176	148	13.6	0.18
Thyroxine (T ₄), μ g/dL	6.42	5.22	0.55	0.16
T ₃ :T ₄	28.34	29.08	3.33	0.87
Cortisol, μ g/dL	1.78	1.12	0.31	0.18
At end (d 91)				
Glucose, mg/dL	97.20	93.00	2.43	0.09
Insulin, μ IU/mL	10.04 ^b	31.94 ^a	4.87	0.01
Insulin:glucose	0.11 ^b	0.34 ^a	0.05	0.02
T ₃ , ng/dL	202	268	28.0	0.13
T ₄ , μ g/dL	7.84	8.38	0.98	0.70
T ₃ :T ₄	26.86	31.90	2.63	0.21
Cortisol, μ g/dL	1.16	1.38	0.98	0.54

^{a,b}Means within a row with different superscripts are significantly different ($P < 0.05$).

¹T = treatment effect.

2007; Yari et al., 2010). In studies performed under normal conditions, Cr supplementation did not affect the starter feed intake or total DMI (Bunting et al., 2000; Ghorbani et al., 2012). It seems that calves in the present study may have experienced some type of stress at birth or weaning. According to Yari et al. (2010), Cr supplementation (either 0.02 or 0.04 mg/kg of BW^{0.75}) to the starter diets had no effects on preweaning total DMI, but decreased total DMI in postweaning calves in summer. Part of the reason for this discrepancy may be explained by differences in the level of supplementation and method of offering Cr (milk only or colostrum plus milk).

Unlike previous research (Yari et al., 2010; Kumar et al., 2015) in which supplemented and nonsupplemented calves had similar weight gain, Cr+ calves in the current study had greater overall ADG, structural growth (hip width and heart girth), and numerically greater BW measured at weaning (+4.4 kg) and at the end of the trial (+7.6 kg) than Cr− calves. However, Cr acted fast to improve ADG during the first week but its effect disappeared after 2 wk of supplementation. One possible cause of this improved ADG could be the greater starter feed intake relative to Cr− calves (Ghorbani et al., 2012). Similarly, Ghorbani et al. (2012) reported a positive effect of adding Cr to colostrum and milk on weight gain in dairy calves under normal conditions. Part of the discrepancy between our results and others might be due to the source of Cr (Kegley and Spears, 1995).

The RT was normal and similar between treatments, indicating a reasonable calf capability to overcome the potential stresses in the present trial. The respiratory rates of 39 to 42 breaths per minute in summer-exposed calves were similar to those observed in dairy calves during the summer (40–43 per minute; Yari et al., 2010), but was greater than the respiratory rates under normal conditions (31–34 per minute; Ghorbani et al., 2012). Our results are similar to those of Yari et al. (2010), who showed that RR at wk 5 was decreased in Cr-supplemented individuals. The decrease in RR in Cr+ calves may indicate that they suffered less heat load accompanied by greater well-being. The conserved energy may have been directed at increasing the final BW (which was numerically greater in Cr+ calves than in Cr− calves) or used to mitigate the higher metabolic heat production due to the increased DMI.

To our knowledge, the effect of Cr supplementation on meal patterns in calves has not been reported previously. Analysis of meal patterns in young calves may be helpful in clarifying the questions about the short-term control of feed intake (Allen et al., 2009; Kargar et al., 2010, 2013). In the current study, meal and rumination patterns were greatly influenced by Cr

supplementation. The meal frequency (5–6 meals in 12 h) was similar to that observed in dairy calves eating solid feed (Miller-Cushon et al., 2015), but was lower than that in dairy cows (Kargar et al., 2010, 2013, 2014). Meal size (27.6%) and meal duration (19.3%) increased, but the meal frequency (23.1%) decreased in Cr+ calves compared with Cr− calves. Despite lowered meal frequency, Cr+ calves had similar time to eating intervals (min/12 h).

Feed intake is a function of meal size and meal frequency, determined by satiety and hunger, respectively (Allen et al., 2009). In fact, the temporal patterns of fuel absorption, mobilization, and metabolism can affect the feed intake in ruminants by altering the meal size and frequency (Allen et al., 2009). Of fuels metabolized by the ruminant liver, propionate is probably a primary satiety signal because its flux into the liver increases greatly during meals. Propionate is used for gluconeogenesis or oxidized in the liver and stimulates oxidation of acetyl CoA. Accordingly, meals can be terminated by signals carried from the liver to the brain via afferents in the vagus nerve, and hepatic oxidation of fuels (including propionate) and generation of ATP (Allen et al., 2009) affect these signals. In the current study, an increase in starter feed intake (by larger meal size and longer meal length) and a decrease in RR may indicate that Cr+ calves can cope better with environmental heat load and cause a reduction in meal frequency as calves become metabolically capable of consuming more starter feed per meal. We supposed that this increase in starter feed intake may have increased rumen propionate formation (although we did not measure it) and flux into the liver and thereby terminated the meals and reduced meal frequency. However, further studies are warranted to confirm this supposition.

The environmental heat load may have also decreased the rumination activity (Soriani et al., 2013). In heat-loaded animals, the fractional digesta passage rate in the gastrointestinal tract is slower, reflecting reduced rumen motility and thereby decreasing the feed intake (Silanikove, 1992). The lack of treatment effect on rumination time may be an indication of no severe environmental heat load imposed on the calves in the present study. The mechanism(s) by which Cr affected the rumination patterns remains unclear, but might involve the insulin-potentiating pathway. Evidence indicates that insulin has a stimulatory effect on rumination by increasing glucose entry in the cells and thereby reticulo-rumen motility (Bareille and Faverdin, 1996). Bareille and Faverdin (1996) reported increased rumination time when glucose was infused intravenously (2.75 mmol/kg) to dairy cows. However, supplemental Cr did not affect blood insulin concentration at weaning, and rumination time during the

preweaning period in the current study, despite the fact that rumination patterns changed (decreased rumination frequency and increased rumination duration and interval). Therefore, the relationship between blood insulin concentration and rumination patterns needs to be investigated further.

Chromium supplementation had small effects on blood glucose and insulin in calves under normal or heat-stress conditions (Yari et al., 2010; Ghorbani et al., 2012). Kumar et al. (2015) reported reduced blood insulin concentration in heat-stressed buffalo calves when Cr was supplemented at 1 and 1.5 mg/kg of DMI but not at 0.5 mg/kg of DMI. Consistent with our results, Bunting et al. (2000) reported that blood concentrations of glucose and insulin decreased and increased, respectively, when dairy calves were supplemented with 0.5 mg of Cr/kg of DMI. Chromium supplementation increased insulin concentration and insulin to glucose ratio (an indirect indicator of insulin sensitivity), suggesting that more insulin was needed to remove glucose from the blood (less tissue sensitivity to insulin) and that resistance to insulin increased over that time period (Bunting et al., 2000).

Calves in the present study did not show any change in blood concentrations of T_3 and T_4 . Similar findings were observed in buffalo calves that received 0.5, 1, and 1.5 mg of Cr/kg of DMI (Kumar et al., 2015). However, Cr supplementation (0.03 mg of Cr/kg of $BW^{0.75}$) increased blood T_4 concentration and reduced T_3 to T_4 ratio in dairy calves (Ghorbani et al., 2012). In contrast, increasing Cr doses (from 0 to 0.02 and 0.04 mg Cr/kg of $BW^{0.75}$) resulted in quadratic reduction in serum T_4 , whereas blood T_3 decreased only with the higher Cr dose supplementation in dairy calves (Yari et al., 2010).

Our data did not support a reduction in serum cortisol concentrations in response to Cr supplementation. Some previous studies reported that Cr supplementation decreased sensitivity to stress by diminishing blood cortisol concentrations in birth-stressed dairy calves (Ghorbani et al., 2012), in feeder calves under transportation stress (Moonsie-Shageer and Mowat, 1993), and in goats under a short-term (72 h) feed restriction (Haldar et al., 2006). Nevertheless, supplemental Cr did not change (Arthington et al., 1997) or increased (Yari et al., 2010) serum concentration of cortisol in calves inoculated with bovine herpesvirus-1 or experiencing heat stress conditions, respectively. Such inconsistencies may be explained by such factors as species, age, the level, type, and duration of stress, and also type and duration of Cr supplementation. However, the relationship between Cr and blood cortisol concentration needs to be clarified further.

CONCLUSIONS

Chromium supplementation to environmentally heat-loaded dairy calves increased the feed intake via larger meal size and longer meal duration despite reducing the meal frequency. Chromium-supplemented calves performed similarly in terms of BW and feed efficiency compared with Cr– calves. Blood glucose concentration was not different between treatment groups, but blood insulin concentration and insulin to glucose ratio were higher in Cr+ calves at d 91 of age. In general, Cr supplementation may be beneficial in heat stress abatement by reducing RR and increasing feed intake in dairy calves; but its effect on growth performance may be marginal.

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