

ORIGINAL ARTICLE

Chromium improves production and alters metabolism of early lactation cows in summer

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Summary

Metabolic demands of early lactation introduce enormous challenges to dairy cows when coincided with environmental stresses. The objective of this study was to determine effects of a chromium (Cr) supplement on feed intake and blood indicators of nutrient metabolism in early lactation cows in summer. Fifteen Holstein cows at 38 ± 6 days in milk were grouped based on parity and randomly assigned to three supplemental doses of 0, 0.05 and 0.10 mg Cr/kg of $BW^{0.75}$. Cows received a basal mixed ration with a forage to concentrate ratio of 57.7:42.3, twice daily at 09:00 and 16:00 h for 9 weeks. The first 2 weeks were for adaptation, and the following 7 weeks were for weekly sampling and data collection. The Cr supplement (a Cr–methionine product with 10% Cr and 90% Met from a compound containing one atom of Cr and three molecules of Met) was mixed with 100 g of ground corn and top dressed with the morning feed. The average ambient temperature–humidity index was 77.7 units during the study. Dry matter intake increased from 21.8 to 24.2 and 23.7 kg/day when 0.05 and 0.10 mg Cr/kg $BW^{0.75}$ was provided respectively. Milk output of energy, fat, protein and total solids increased by providing Cr at 0.05 mg but not 0.10 mg/kg $BW^{0.75}$. Both doses of Cr increased milk protein content, but the higher Cr dose reduced feed efficiency compared with control. While rectal temperature and body condition score were unaffected, cows receiving 0.05 mg and not 0.10 mg Cr/kg $BW^{0.75}$ tended to have greater respiration rate than control cows. Blood insulin and non-esterified fatty acids concentrations and the insulin to glucagon ratio decreased, and serum albumin increased when cows received 0.05 mg of Cr/kg $BW^{0.75}$. Blood glucose, glucagon, insulin-like growth factor-1, total protein, globulins, urea, BHBA, triglycerides, cholesterol, cortisol, progesterone, and high- and very low-density lipoproteins were not affected. Therefore, supplemental Cr supply at 0.05 and 0.10 mg/kg $BW^{0.75}$ improved feed intake, only at 0.05 mg/kg $BW^{0.75}$ increased milk secretion, and mainly at 0.05 mg/kg $BW^{0.75}$ altered peripheral nutrient metabolism in early lactation Holstein cows under high ambient temperatures.

Introduction

An enormous challenge to the dairy industry has been the coincidence of metabolic demands of early

lactation with environmental stresses. Such heavy heat loads from multiple sources raise maintenance energy requirements and affect productivity. Meanwhile, dramatic increases in milk yield over the last

four decades have compromised the ability to cope with high ambient temperatures, thus depressing production (West, 1999). Owing to the negative impact of genetic selection for productivity on stress tolerance and the inadequate effect of modifying housing systems on eliminating heat stress (Beede and Collier, 1986), effective nutritional management remains a promising path to ease metabolic and environmental stresses in associative manners.

Chromium (Cr) is an essential micronutrient that can reduce risks of neuropathy, infertility, immune malfunction, arteriosclerosis, hypercholesterolemia and hyperlipidemia, extracellular hyperpressure and cell metabolic irregularities and death (Burton, 1995; National Research Council, 1997; Pechova and Pavlata, 2007). It can also improve metabolic rate by improving thyroid hormones inter-conversions (Burton, 1995), increasing DMI, and reducing blood NEFA (Yang *et al.*, 1996; Hayirli *et al.*, 2001). Chromium has been gaining worldwide interest in attenuating different stresses in high-producing livestock (Mowat, 1997), as it is additionally associated with glucose tolerance factor (Pechova and Pavlata, 2007). Increased plasma insulin has increased Cr supply to peripheral cells in humans and rats (Morris *et al.*, 1993). Chromium is carried in blood by beta-globulins and is transferred into tissues by 'transferrin' whose cell receptors are responsive to insulin (Kandror, 1999). At cellular levels, Cr plus apo-chromodulin give rise to chromodulin that will presumably enhance insulin signalling (Pechova and Pavlata, 2007) by activating insulin receptors tyrosine kinase and membranes phosphotyrosine phosphatase (Davis and Vincent, 1997). Stress increases glucose and insulin turnover and elevates blood cortisol and other glucoregulatory hormones. Cortisol is anti-insulin and prevents glucose use by muscle and fat cells to prioritize it for brain and liver in non-ruminants and the mammary gland in the lactating cow (Mowat, 1997). The essentiality of Cr for efficient insulin function suggests that Cr can improve liver metabolism and health during high metabolic demands of early and peak lactation coupled with external heat overload (McNamara and Valdez, 2005). Earlier studies on Cr have mostly used calves, heifers, sheep and transition cows (Jackson *et al.*, 1993; Kitchalong *et al.*, 1995; Kegley *et al.*, 1997, 2000; Hayirli *et al.*, 2001; Pechova *et al.*, 2002; Sumner *et al.*, 2007), but while critical in many regions, little information exists on the effect of Cr in dairy cows undergoing metabolic pressures of early lactation while exposed to high ambient temperature. We hypothesize that increased Cr supply

to early lactation cows attenuates metabolic and environmental pressures in associative manners, thereby improving DMI and milk yield more noticeably. Our objective was to determine effects of increased dietary Cr supply on DMI, blood metabolites and hormones, and milk production of early lactation cows in summer.

Materials and methods

Experimental design and cow management

Twelve high-producing multiparous and three primiparous Holstein cows were grouped based on parity and randomly assigned to three treatments within parities. As a result, each treatment group had four multiparous cows and one primiparous cow. Treatments were diets supplemented with either 0, 0.05 or 0.10 mg supplemental Cr/kg of metabolic body weight ($BW^{0.75}$). The cows were in 38 ± 6 (mean \pm SE) days in milk and had 620 ± 20 kg BW at the commencement of the study. The study lasted for 9 weeks with the first 2 weeks for adaptation to the experimental conditions followed by 7 weeks of sampling and data collection. Experimental cows, started the study at the same time, were housed in free individual boxes (4×4 m), and were fed to permit approximately 7% daily orts (i.e., the portion of the TMR not consumed by individual cows over the last 24 h), and had unlimited access to fresh water. The boxes were equipped with individual feed troughs and waterers, and were bedded with clean wood shavings that were replaced twice daily to maintain acceptable hygienic conditions. The total mixed ration (TMR) shown in Table 1 was formulated with the Cornell Net Carbohydrate and Protein System program and was fed to all cows individually at 09:00 and 16:00 h. Chromium was provided as a Cr–methionine product (10% Cr and 90% Met from a compound containing one atom of Cr and three molecules of Met; MicroPlex, Zinpro, Eden Prairie, MN, USA), which was mixed and top dressed with 100 g of ground corn on the morning feed. The methionine was rather a carrier than a major supplement, as it was provided in only minor amount. Body condition was scored weekly using a five-score system with the score of one being an emaciated cow and the score of five being an extremely obese cow. Cows were weighed at the beginning and the end of the study, and were cared for according to the guidelines of the Iranian Council of Animal Care (1995). Cows were allowed daily to walk outside for approximately 30 min just before the noon milking. This experiment was

Table 1 Dietary ingredients and nutrient composition (DM basis) of basal diet

Ingredient %	
Alfalfa hay	22.30
Corn silage	20.00
Coarsely ground barley	11.80
Coarsely ground corn	15.15
Soybean meal	17.20
Whole cottonseeds	5.30
Cottonseed meal	3.50
Fat supplement	2.45
Calcium carbonate	0.76
Sodium bicarbonate	0.76
Mineral and vitamin premix	0.76
Chemical analysis	
NE _L ^a , Mcal/kg	1.65
DM, %	57.5
CP, %	18.2
NDF, %	37.1
ADF, %	24.2
Non-fibre carbohydrates, %	36.0
Ether extract, %	5.0
Ca, %	0.77
P, %	0.40

^aCalculated using the following formula: $2.323 - (0.0216 \times \text{NDF}\%)$.

conducted at the Dairy Research Facilities of Lavark Research Station (Isfahan University of Technology, Isfahan, Iran) from June to September of 2007.

Climatic data

Maximum air temperature (*T*) and minimum relative humidity (RH) data were obtained from daily reports of Najaf Abad Meteorological Network Station (Najaf Abad, Isfahan, Iran) to calculate the T–H index or THI according to the following formula: $[\text{THI} = 0.8 \times (\text{maximum } T) + (\text{minimum RH}/100) \times (\text{maximum } T - 14.4) + 46.4]$; Garcia-Ispierto *et al.*, 2006). The average maximum temperature, minimum relative humidity, and the average maximum THI during the study were 35.3 °C, 14.76% and 77.7% units respectively. These data indicate medium degrees of heat stress. Physiological responses to heat stress such as depressed DMI and milk yield and increased maintenance energy expenditure begin to occur at a THI of approximately 72 (Johnson, 1987).

Feed sampling and processing

Feed samples were collected weekly, and orts for individual cows were weighed daily and sampled weekly. Grab faecal samples were taken weekly at

11:00 h and frozen at –20 °C. The frozen faecal samples were thawed at room temperature and dried at 60 °C for 72 h. Ground samples were analysed for CP, NDF, ADF, ether extract, Ca and P (AOAC, 2002). The Cr content of the control TMR (i.e. without Cr supplement) was 1.79 mg/kg DM determined by atomic absorption in three replicates. The acid detergent insoluble ash (AIA) was used as an internal marker and measured in feed and faeces to calculate apparent total tract nutrient digestibility coefficients (Van Keulen and Young, 1977; Nikkhah *et al.*, 2004).

Milk sampling and processing

Cows were milked three times daily at 04:00, 12:00 and 20:00 h in the milking parlour. Milk yield was recorded at each milking, and representative milk samples were collected weekly for three consecutive milkings. Before milking, cows were monitored for udder inflammation, skin colour change and milk clots in the nipples to ensure no occurrence of mastitis. The amount of milk produced for each cow was read from graduated jars (Agri & SD, Frankfurt, Germany). Milk samples were preserved with potassium dichromate and kept at 4 °C until shortly analysed for fat, protein and lactose (Milk-O-Scan 134 BN Foss Electric, Hillerød, Denmark).

Blood sampling and analysis, rectal temperature, and respiration rate

Blood samples were taken from individual cows starting 2-weeks after introduction of experimental diets. Blood was collected weekly at 11:30 h via coccygeal vein for 7 weeks. Blood was placed on ice immediately post-collection and centrifuged at 2000 *g* for 15 min. The serum was preserved at –20 °C until analysed for metabolites and hormones. The concentrations of insulin, glucagons, progesterone, IGF-1 and cortisol were measured using radio immunoassays (RIA) with commercial kits (Pars Azmoon Kits; Pars Azmoon, Tehran, Iran) and an automatic gamma-counter (Biosource, Milan, Italy). The serum concentrations of NEFA, BHBA were measured using commercial kits (Pars Azmoon Kits, Pars Azmoon, Tehran, Iran) with a Technician-RA 1000 Auto-analyzer (DRG, Marburg, Germany). Serum total proteins, albumin, triglycerides, HDL, VLDL, urea nitrogen, cholesterol and glucose were determined using Pars Azmoon Kits (Pars Azmoon, Tehran, Iran). Rectal temperature and respiration rate were measured 4 days a week during the seven

sampling weeks. Respiration rate was counted at 15:00 h for three separate min and an average was calculated.

Statistical analysis

Data were analysed with mixed procedures of SAS (2003) for repeated weekly measures. The final model included fixed effects of treatment, weeks and their interaction plus the random effect of cow within treatment. The best-fit covariance structure used for the analysis of blood IGF-1, NEFA and cortisol was autoregressive (1), and for other measures was autoregressive heterogeneous (1). The method of estimating least square means was maximum likelihood and that of calculating denominator degrees of freedom was Kenward–Roger. The CONTRAST statement of the SAS (2003) was utilized to obtain linear and quadratic responses, and the PDIF option was used to separate means. Significance was declared at $p \leq 0.05$ and tendencies were stated at $0.05 < p \leq 0.10$.

Results

Dry matter intake (DMI) increased quadratically by Cr–Met ($p < 0.01$, Table 2), and it was greater with both the moderate and the higher Cr dose compared

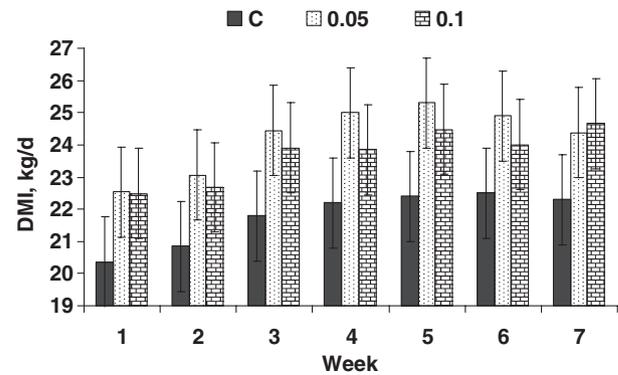


Fig. 1 Dry matter intake (DMI) patterns during the study in control cows (C) and Cr supplemented cows at 0.05 and 0.10 mg Cr/kg BW^{0.75}. The study lasted for 9 weeks with the first 2 weeks for adapting cows to the experimental conditions. Cows entered the study at 38 ± 6 days in milk.

with control. Figure 1 illustrates weekly patterns in DMI during the study. Milk protein percentage increased ($p < 0.01$) at both Cr doses while milk protein yield increased ($p < 0.05$) with the moderate Cr dose (Table 2). Milk fat percentage tended to increase ($p < 0.10$) and milk fat yield and lactose percentage increased ($p < 0.05$) when Cr was supplied at 0.05 mg/kg BW^{0.75}. Similarly, Cr at the moderate but not at the higher dose improved milk

Table 2 Effects of Cr–Met supplementation on productivity of early lactation heat-stressed dairy cows

Item	Treatment (Trt or mg Cr/kg BW ^{0.75})			SEM	p-value				
	0	0.05	0.10		Trt	Week	Trt × Week	Linear	Quadratic
Raw milk yield, kg/day	38.0	39.6	37.8	0.95	0.34	0.75	0.99	0.84	0.58
FCM yield†, kg/day	35.0***	37.8*	34.1**	1.1	0.04	0.02	0.97	0.57	0.46
ECM yield‡, kg/day	36.7**	39.8*	36.1**	1.1	0.05	0.06	0.74	0.73	0.39
Milk fat, %	3.46***	3.69*	3.36**	0.12	0.13	0.0003	0.75	0.55	0.65
Fat yield, kg/day	1.32**	1.46*	1.27**	0.05	0.03	0.002	0.91	0.51	0.47
Milk protein, %	2.73**	2.85*	2.82**	0.02	0.003	0.07	0.70	0.01	0.001
Protein yield, kg/day	1.04**	1.13*	1.06***	0.03	0.04	0.73	0.97	0.63	0.10
Milk lactose, %	5.67**	5.79*	5.59**	0.04	0.0002	0.75	0.99	0.08	0.69
Lactose yield, kg/day	2.16***	2.29*	2.11**	0.05	0.04	0.82	0.99	0.51	0.51
Milk total solids, %	12.05**	12.53*	11.98**	0.11	0.001	0.0007	0.89	0.62	0.15
Total solids yield, kg/day	4.59**	4.96*	4.51**	0.12	0.01	0.28	0.99	0.64	0.29
Dry matter intake, kg/day	21.8**	24.2*	23.7*	0.53	0.004	0.33	1.00	0.01	0.001
FCM/DMI	1.62*	1.59*	1.45**	0.05	0.03	0.0003	0.94	0.02	0.10
DM digestibility, %	65.2	63.1	65.0	1.5	0.51	<0.0001	0.05	0.93	0.52
Body weight change, kg/7 weeks	20.6*	−9.0**	8.2***	10.5	0.14	–	–	0.38	0.10
Body condition score	3.03	3.01	2.97	0.04	0.58	0.0004	0.37	0.32	0.46
Rectal temperature, °C	39.0	39.4	39.1	0.19	0.23	0.84	0.51	0.55	0.19
Respiration rate, per min	55.4***	58.6*	55.1**	1.2	0.10	0.008	0.99	0.85	0.36

***Means with different superscripts within each row differ at $p \leq 0.05$.

†4% Fat-corrected milk yield = $(0.399 \times \text{kg daily milk yield}) + (15.02 \times \text{kg daily milk fat yield})$.

‡Energy-corrected milk yield = $(\text{kg of milk} \times 0.3246) + (\text{kg of milk fat} \times 12.96) + (\text{kg of milk protein} \times 7.04)$.

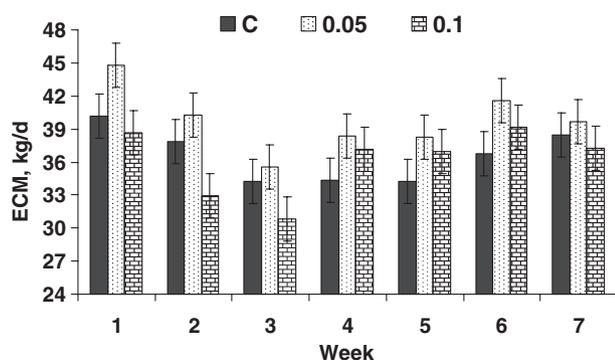


Fig. 2 Energy corrected milk yield (ECM) patterns during the study in control cows (C) and Cr supplemented cows at 0.05 and 0.10 mg Cr/kg BW^{0.75}. The study lasted for 9 weeks with the first 2 weeks for adapting cows to the experimental conditions. Cows entered the study at 38 ± 6 days in milk. Weeks 2, 0.05 vs. 0.10, $p = 0.10$.

total solids content and yield ($p < 0.01$, Table 2). Treatments did not interact with time on DMI and milk production. Weekly patterns in energy corrected milk yield (ECM) are shown in Fig. 2. Raw milk yield and apparent total tract DM digestibility over the experimental period were not affected by treatments. Cows receiving control TMR and the moderate dose of Cr had superior feed efficiency than cows receiving the higher dose of Cr.

Respiration rate tended to increase ($p = 0.07$) when cows received 0.05 mg but not 0.10 mg of Cr/kg of BW^{0.75} (Table 2). Rectal temperature was similar among groups ($p = 0.23$). While BCS changes were similar ($p = 0.58$), cows on the moderate dose of Cr had a modest BW loss of approximately 9 kg during the study, or 124 g/day, whereas cows on no and high doses of supplemental Cr gained respectively approximately 20 and 8 kg BW during the trial (Table 2). Statistically, cows on the moderate Cr dose were different in BW changes from control cows ($p = 0.05$).

Serum concentrations of glucose, glucagon, IGF-1, BHBA, urea, globulin, total proteins, cholesterol, triglycerides and lipoproteins were not affected by treatments (Table 3). Serum insulin and NEFA decreased ($p < 0.05$) when 0.05 mg but not 0.10 mg of supplemental Cr/kg of BW^{0.75} was provided (Table 3). As a result, blood ratios of insulin to glucagon decreased by Cr. Chromium at 0.05 mg but not at 0.10 mg per kg of BW^{0.75} increased serum albumin (Table 3).

Discussion

The improved DMI agrees with other studies in fresh and early lactation cows under normal environments (Hayirli et al., 2001; McNamara and Valdez, 2005),

Table 3 Effects of Cr–Met supplementation on blood metabolites and hormones of early lactation heat-stressed dairy cows

Item	Treatment (Trt or mg Cr/kg BW ^{0.75})			SEM	p-value				
	0	0.05	0.10		Trt	Week	Trt × Week	Linear	Quadratic
Glucose, mg/dl	68.1	66.5	67.1	0.8	0.37	0.20	0.14	0.38	0.19
Insulin, μ U/ml	8.9 ^a	8.2 ^b	8.3 ^{ab}	0.3	0.13	0.96	0.32	0.14	0.05
Glucagon, pg/ml	74.9	78.5	80.6	2.9	0.37	0.73	0.47	0.16	0.19
Insulin:glucose	0.131	0.123	0.126	0.004	0.41	0.99	0.30	0.39	0.22
Insulin:glucagon	0.123 ^a	0.108 ^b	0.109 ^b	0.005	0.08	0.87	0.34	0.06	0.03
IGF-1, ng/ml	248.5	245.3	246.6	6.8	0.95	0.12	0.29	0.84	0.76
Total proteins, g/dl	7.35	7.40	7.38	0.06	0.87	0.45	0.33	0.78	0.64
Albumin, g/dl	3.77 ^b	3.91 ^a	3.88 ^{ab}	0.05	0.07	0.26	0.02	0.07	0.02
Globulin, g/dl	3.58	3.48	3.49	0.06	0.39	0.89	0.26	0.24	0.17
Albumin:globulin	1.06 ^b	1.14 ^a	1.13 ^a	0.03	0.08	0.71	0.04	0.06	0.03
Urea nitrogen, mg/dl	9.1	9.2	9.0	0.4	0.93	0.97	0.95	0.79	0.93
NEFA, μ Eq/l	158.9 ^a	144.6 ^b	157.3 ^{ab}	4.7	0.07	0.11	0.13	0.82	0.17
BHBA, mmol/l	4.2	4.2	4.4	0.15	0.73	0.51	0.19	0.49	0.68
Triglycerides, mg/dl	19.3	18.3	19.9	0.74	0.31	0.14	0.06	0.61	0.78
Cholesterol, mg/dl	130.4	133.1	131.2	1.3	0.30	0.23	0.25	0.47	0.19
HDL, mg/dl	25.5	27.9	26.6	0.9	0.21	0.93	0.27	0.44	0.14
VLDL, mg/dl	4.0	3.83	3.72	0.14	0.41	0.40	0.24	0.18	0.21
Cortisol, μ g/dl	14.6	14.5	14.7	0.4	0.95	0.38	0.99	0.83	0.96
Progesterone, ng/dl	27.6	25.5	26.1	1.2	0.47	<0.0001	0.55	0.41	0.24

HDL, high-density lipoproteins; VLDL, very low-density lipoproteins.

Within rows, means with different superscripts differ at $p < 0.05$.

thus uniquely extending the positive impact of Cr on DMI to early lactation cows under high ambient temperatures. Improved insulin action facilitates glucose entry into the cell and can suppress lipolysis. Decreased fat mobilization will decrease reliance on body reserves and reduce peripheral NEFA (Yang *et al.*, 1996; Kegley *et al.*, 2000). Decreased NEFA can in turn increase DMI via reversing the lipostatic mechanism, as high circulating NEFA concentrations depress feed intake (Forbes, 1995; McNamara and Valdez, 2005). Decreased blood insulin and NEFA levels and the insulin to glucagon ratio with unchanged glucose levels in the present study suggest an improvement in insulin action to sustain the increased milk production (Hayirli *et al.*, 2001). This cascade may have derived a rise in DMI (National Research Council, 2001). The Met content of the Cr–Met supplement might have had only some minor contribution to increased mammary Met supply given its trivial amount. Increased milk fat with the lower Cr dose was consistent with the increased milk total solids yield at the same Cr dose. These results agree with earlier data on Cr in dairy cattle under normal conditions (Jackson *et al.*, 1993; Subiyatno *et al.*, 1996; Pechova *et al.*, 2002). Given the similar ‘insulin:glucagon’ and ‘albumin:globulin’ between the moderate and the higher Cr doses, the lack of Cr benefits on milk energy output and blood NEFA with the higher dose, compared with control, might suggest an alleviated Cr mediation of improved insulin sensitivity. Toxic levels of Cr for lactating cows have not conclusively been reported, but our data show no metabolic and productive effects of Cr at 0.10 g/kg BW^{0.75} despite the improved DMI. Non-linear production responses to increased Cr supply were observed in periparturient cows with 0.03, 0.06 and 0.12 mg supplemental Cr/kg BW^{0.75} (Hayirli *et al.*, 2001). It is a scientific postulation that Cr oversupply might interfere with parallel reductions in lipolysis and increases in hepatic gluconeogenesis, and the mammary supply of substrates. Nevertheless, because of the absence of such direct evidence thus far, the sample size of this study could be a reason for observing no metabolic effects at the highest Cr dose, and hence, future larger studies are warranted for delineation.

The increased feed efficiency with the moderate than the highest Cr dose can be explained by the reasonably high DMI and yet the lower FCM yield of the cows on the higher Cr dose. Increased DMI and reduced blood insulin with the moderate dose of Cr indicate greater mammary nutrient flow to sustain the increased milk secretion (Faverdin, 1986).

The data suggest that biosynthesis in the mammary gland was prioritized over anabolism and oxidation in peripheral adipose and muscle tissues (Subiyatno *et al.*, 1996). Because of the increased milk contents of fat, protein and lactose, ECM was improved by Cr. McNamara and Valdez (2005) provided cows 10 mg/day Cr from 21 days pre-partum to 35 post-partum and observed an increase in DMI and milk yield which was associated with reduced lipolysis, suggesting an increased glucose uptake by adipose tissue that would allow DMI to rise (National Research Council, 2001). Hence, the literature provides compelling support to the unique data obtained in the present study with peak cows under summer conditions.

The tendency for increased respiration rate was consistent with production data and suggests an improvement in hepatic nutrient flow and mammary substrate uptake under such metabolic and environmental pressures. Similar rectal temperatures indicate that cows were not experiencing extremely stressful conditions. The BW data suggest a mild anabolic status of control cows vs. a mild catabolic status of the cows on moderate Cr. Dietary energy intake does not usually meet energy requirements of early lactation cows, which can be exacerbated by depressed DMI in summer. Our data suggest that cows on the moderate Cr dose were able to maintain and even increase DMI, and effectively sustain the increased milk secretion. Increased DMI may have boosted heat tolerance at times of enormous metabolic demands (Al-Saiadi *et al.*, 2004). Treatment similarities in blood glucose, glucagon, IGF-1, BHBA, urea, globulin, total proteins, cholesterol, triglycerides and lipoproteins were consistent with the improved DMI and suggest that increased Cr supply maintained blood levels of milk precursors and major indicators of hepatic N metabolism.

The insulin to glucagon ratio data concur with the results of Subiyatno *et al.* (1996) testing propionate tolerance in primiparous cows fed supplemental Cr. In light of the increased DMI and milk solids yield; the similar blood glucose and BHBA, and reduced insulin and NEFA suggest that Cr may have enabled the liver and internal adipose tissue to more effectively orchestrate nutrient partitioning toward the mammary gland (Drackley *et al.*, 2005). Similarly, others have reported a decrease in blood insulin by Cr under normal conditions (Hayirli *et al.*, 2001; Wang *et al.*, 2007). Decreased blood insulin may mean a decreased demand for insulin by peripheral adipose tissue (Faverdin, 1986). Reduced insulin

requirements could partly be due to an increase in mammary metabolite uptake at the expense of their peripheral oxidation and accretion as well as an improvement in insulin action. The latter agrees with the enhancing effect of Cr on insulin efficiency as evident by the reduced blood insulin (Table 3) (McNamara and Valdez, 1995; Vincent, 2000). Chromium is known to improve insulin communication with cell membrane receptors (Pechova and Pavlata, 2007).

The small effect of Cr on blood proteins was in agreement with other reports from dairy calves (Bunting *et al.*, 2000) and lambs (Kitchalong *et al.* 1995). Pechova *et al.* (2002) reported an increase in serum albumin by supplemental Cr in periparturient cows. Albumin is synthesized by the liver and is essential for circulating metabolite transportation (National Research Council, 2001). Given the BW loss and unchanged blood globulins and total proteins, the time-related rise in serum albumin by the moderate Cr dose suggests an improved hepatic amino acid balance and albumin synthesis to maintain the augmented hepatic and mammary nutrient turnover. During stress (e.g. early lactation and/or under high ambient temperatures), the immune system is challenged and the need to synthesize essential proteins such as albumin and globulins increases (West, 1999; National Research Council, 2001). Thus, the Cr may have contributed to sustaining adequate nutrient supply and boosting immunity while maintaining milk production peak, when metabolic pressures coincided with high ambient temperatures (Al-Saiadi *et al.*, 2004). Logically, thus, the increased albumin to globulin ratio agrees with other reports (Al-Saiadi *et al.*, 2004). The lack of Cr effects on blood urea agrees with other reports (Kitchalong *et al.*, 1995; Bunting *et al.*, 2000) and suggests no or little compromise of the liver function in ammonia detoxification.

Reduced NEFA alongside the decreased insulin concentrations and enhanced milk secretion suggest that the Cr improved nutrient availability at times of high metabolic demands. The declining effect of Cr on serum NEFA concurs with other reports in dairy cows (Subiyatno *et al.*, 1996), lambs (Kitchalong *et al.* 1995) and pregnant beef cows (Stahlhut *et al.*, 2006). The comparable blood BHBA and its relatively low concentrations suggest that negative energy balance was not severe but rather moderate. This concurs with the modest BW loss. Chromium improves insulin efficiency, and reduces lipolysis (McNamara and Valdez, 2005), circulating NEFA

and hepatic ketogenesis (Hayirli *et al.*, 2001), and may increase peripheral BHBA use (Mertz, 1993; Kegley *et al.*, 2000).

The similar serum cortisol concentrations were consistent with other reports under normal environments (Arthington *et al.*, 1997; Kegley *et al.*, 1997). Cortisol antagonizes insulin and prevents glucose entry into peripheral cells to spare it for use by tissues of greater demand (National Research Council, 1997). Changes in circulating cortisol can usually be a useful indicator of short-term stresses, but seemingly not as much reliable to indicate chronic stresses. Chromium may decrease cortisol to progesterone ratio likely by reducing the activity of 11- β -hydroxylase, responsible for progesterone conversion to cortisol (Mertz, 1993; Arthington *et al.*, 1997). The absence of treatment effects on serum cortisol and 'cortisol:progesterone' suggests that cows were not exposed to severe changes in the degree of stress. However, the sum of internal metabolic and external pressures was adequate for the Cr effects to be detected on DMI, serum insulin and NEFA, and milk production.

In conclusion, dietary use of a chromium–methionine (Cr–Met) supplement for approximately 2 months in peak and early lactation cows under natural high ambient temperatures to provide 0.05 mg Cr/kg of BW^{0.75} improved DMI, and milk fat, protein and total solids yields. Increased Cr supply, when the metabolic demands of early lactation were superimposed on high ambient temperatures, increased respiration rate and serum albumin coupled with a mild weight loss, whereas it reduced serum NEFA and insulin. Supplemental Cr at 0.10 mg/kg BW^{0.75}, however, did not have such metabolic and production effects. These data suggest reduced requirements for insulin or a rise in insulin efficiency to prioritize milk secretion over peripheral nutrient oxidation or deposition. Therefore, findings indicate beneficial effects of increased dietary Cr supply on metabolism and production of early lactation Holstein cows under high ambient temperatures.

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