Effect of Chromium Picolinate Supplementation on Early Lactation Performance, Rectal Temperatures, Respiration Rates and Plasma Biochemical Response of Holstein Cows under Heat Stress

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Abstract: Twenty-four multiparous lactating Holstein cows (15-22 days post-partum) were used in this study. Cows were randomly allocated to four treatments based on days in milk, parity and milk yield. Basal diets were supplemented with 0, 3.6, 7.2 and 10.8 milligram chromium/head per day as chromium picolinate in Control (C), Low (L), Moderate (M) and High (H) chromium treatments respectively. The average Temperature-humidity Index (THI) was 79.61 units. The results indicated that: All cows were under heat stress. Significant DMI increase was found among treatments (p<0.001) and DMI increased linearly as the level of Cr supplementation increased (p = 0.004). Milk yield could be increased by adding chromium (p = 0.013), while no significant differences were found between the four treatments in milk fat, protein, lactose, SNF and SCC. And milk yield also increased linearly as the level of Cr supplementation increased (p = 0.003). No significant differences were found between the four treatments in insulin, but there was a trend of decrease with Cr supplementation compared with control (p = 0.079). Concentration of blood glucose and the ratio of blood glucose to insulin were increased by adding chromium (p = 0.019 and p = 0.013, respectively). Adding chromium did not significantly affect rectal temperatures and respiration rates of four treatments (p = 0.310 and p = 0.265 respectively). CK was significantly decreased by adding chromium (p = 0.017), but LDH, AST and ALT were not significantly affected by adding chromium (p = 0.785, p = 0.524 and p = 0.079 respectively). Additionally CK decreased linearly as the level of Cr supplementation increased (p = 0.003). It could be concluded that adding chromium to the diet of dairy cows under heat stress improved milk yield without affecting milk component, chromium supplementation had positive influence on heat-stress cows.

Key words: Chromium picolinate, holstein cow, heat stress, blood parameter

INTRODUCTION
Cows could not endure high temperature, but the southern China especially sichuan province is characterized as humid and hot subtropical climate. So dairy cows are subject to extended periods of high ambient temperature and relative humidity every year from May to September (Du et al., 2007). So one of the greatest challenges to production facing dairy farmers in the southern China is heat stress. Heat stress apparently affects the performance of dairy cows (West, 2003). It has also been reported that heat stress can reduce DMI, milk yield and milk component, increase rectal temperatures, respiration rates and disorder metabolism at temperatures above about 25°C or THI above 72 units (West, 2003; Stanley et al., 1975). Usually, blood biochemical parameters could reflect health status and metabolism of animals, which are used widely in clinical situations. Since heat stress could profoundly affect biochemical parameters (Li et al., 2001). Chromium (Cr) is an essential trace element for the maintenance of normal metabolism of carbohydrates, protein and lipids (Mertz, 1993). Its predominant physiological role is as an integral component of the Glucose Tolerance Factor (GTF) to potentiate the action of insulin (Mertz, 1993). Studies on humans or mice had indicated that various stressors increased urinary excretion of Cr (Borel et al.,1984). During late pregnancy and early lactation, high production dairy cows are subject to great physical and metabolic stressors. Increased metabolism of glucose and mobilization of Cr from body stores during these periods may causes Cr deficiency in cows. Once mobilized, Cr is irreversibly lost in urine (Anderson, 1988). Most cows diets primarily are composed of ingredients from plant origin, which are usually low in Cr (Schroeder, 1971). Therefore, early–lactation and heat-stress dairy cows are more likely to experience Cr deficiency. Supplemental organic Cr had remarkably improved milk yield or immune response of stressed dairy cows (Yu et al., 2006; Bryan et al., 2004; AL-Saiady et al., 2004; Subiyatno et al., 1996). However, evidence for the benefits of supplemental Cr with heat-stress and early-lactation cows is extremely limited. Currently, the NRC (2001)
does not recommend dietary Cr requirement for dairy cows (NRC, 2001). So the objective of this study was conducted to investigate effect of chromium supplementation on early lactation performance, rectal temperatures, respiration rates and blood biochemical response of early lactation Holstein cows under heat stress.

MATERIALS AND METHODS

Animals, diets, treatment and management: The study was conducted with the approval of the Sichuan Agricultural University Institutional Animal Care and Use Committee. The study was carried out at New Hope Group’s Dairy Farm, which was in Hongya county Sichuan China, from July 21 to September 23. Twenty-four multiparous lactating Holstein cows (15-24 days post-partum) were utilized from 3-12 wk of lactation and their parities within the range of second and forth lactation. Cows were divided equally into four treatments based on days in milk, parity and milk yield, with 6 cows per treatment and 6 replicates each. Body Weights (BW) of cows were about 593±25 kg before treatment. Cr was dosed relative to metabolic body size. Treatments were supplementation of 0, 0.03, 0.06 and 0.12 mg of Cr as Cr-Met/kg of BW0.75. So treatments were supplementation of 0, 3.6, 7.2 and 10.8 mg Cr/head per day as chromium picolinate in Control (C), Low (L), Moderate (M) and High (H) Chromium treatments respectively for a period of 9 weeks. The chromium picolinate containing 0.1% Cr was provided by Wuhan New Huayang Corporation. The lactation days in milk were 21.4±2.1, 21.3±2.0, 21.3±2.4 and 21.4±2.3 (Mean±Standard deviation) for cows receiving C, L, M and H, respectively. The parities of dairy cows were 3±0.9, 3±0.9, 3±0.9 and 2.9±1.0 (Mean±Standard deviation) for cows receiving C, L, M and H, respectively. The ingredient composition of the grain supplement fed to all cows was same. Basal diet was formulated to meet nutrient requirements recommended by the NRC (2001). Basal diet consisted of roughage and concentrate. The ingredients and chemical composition of the diets were reported in Table 1. During a 7-day adaptation period, all cows of each treatment were fed the basal diet with Cr gradually reaching corresponding trial level.

All cows were housed under the fan cooling system during the trial period. Animals were kept in a 60 m x 3.6 m x 6 m high tie-stall and stanchion barn with a holding area. Barn was open but covered. The stall was based on size of cows. The length x width x height of stall was 180 cm x 125 cm x 145 cm which did not make cows have a feeling of uncomft. Cows were fed three times daily at 0800, 1400 and 2000 h. First, cows were fed on the mixture of corn silage, wafering Alfalfa hay, wafering oat hay, beet pellet, brewer grain and concentrate, then (about 40 min later) cows were fed on whip grass and alfalfa hay. Cr was added at 0800 and 2000 h by being mixed with 0.5 kg concentrate first, then added to the mixture. Cows had ad libitum access to clean water 24 h a day. Cows were milked three times daily at 0840, 1600 and 2200 h. After milking, cows were allowed to have a rest at shading place on the holding area.

Ambient temperature: Temperature-humidity Index (THI) is a single value representing the combined effects of air temperature and humidity associated with the level of thermal stress. Four thermometers were hung at a height of 150 cm in four different parts of barn. Dry-bulb and wet-bulb temperatures were recorded daily from 0900 to 2100 h at 2-h intervals and THI was calculated according to Maust (Maust et al., 1972).

\[
\text{THI} = 0.72 \left( T_{db} + T_{wb} \right) + 40.6
\]

where \( T_{db} \) was the temperature of dry bulb and \( T_{wb} \) was the temperature of wet bulb.

Rectal temperatures and respiration rates: Rectal temperatures and respiration rates were recorded at 1430 h on two consecutive days every week. Rectal temperatures were determined with a rectal probe thermometer (Model CV 2006, shanghai Medical Instruments Co. Ltd., Shanghai, China). Respiration rates were determined at this time by counting flank movements.

Sampling collection and analytical procedure: The amount of feed offered and refused was recorded daily.
for each treatment of cows was recorded and dry matter was calculated. Production efficiency was calculated as the ratio of the average milk yield to the average DMI. Samples of feed offered to each treatment were collected biweekly and composite at the end of the trial, dried in a forced-air drying oven (Model DHG-9023A, Shanghai Medical Instruments Co. Ltd., Shanghai, China) at 65°C and then grindend (Grinder Model FW100, Tianjin Taisite Instruments Corp. Ltd., Tianjin, China) for further chemical analysis. Basal diet contents were analyzed according to AOAC for dry matter, CP, calcium, total phosphorus and trace elements (AOAC, 1990). CP was analyzed by the methods of Kjeldahl determination. Dietary calcium and phosphorous were determined by potassium permanganate titration and colorimetric method respectively. The trace elements were determined by atomic absorption (Model PU 9100, Philips Scientific, Cambridge). Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) were assayed using method of Van Soest et al. (1991). The Net Energy for Lactation (NEl) concentration of the diet was calculated according Weiss (1998). Measures of energy utilization were calculated with co-efficient from NRC (2001).

Daily milk yield from all cows of each treatment were recorded on three consecutive days every week. Fresh milk samples were collected biweekly. Milk samples were collected in a plastic bottle containing 2-brom-2-nitro-1, 3-propanediol (a preservative) on 7, 21, 42 and 56 trial day. Milk samples were sent to New Hope Group’s milk analysis laboratory (Hongya county, Sichuan China) for determination of concentrations of fat, protein, lactose, SNF and SCC with lactostar 3560 (Funke Gerber, Berlin, Germany).

Five cows were chosen from each treatment randomly. Cows were bled from the same five cows per each treatment at 0700h on 1, 28 and 56 trial day. Blood samples were collected from jugular vein-puncture into tubes containing anticoagulant and tubes were kept on ice for 30 min, then samples were centrifuged at 3000 x g (centrifuge Model 0406-1, shanghai Medical Instruments Co. Ltd., Shanghai, China) for 10 min. Plasma was obtained, then kept at-20°C until further laboratory analyses at Animal Nutrition laboratory (Ya’an, Sichuan, China).

The insulin was analyzed using established radioimmunoassay procedures with standard assay kit (Nanjing Jiancheng Biotechnology Co., Ltd, Nanjing, China). Radioimmunoassay kit was used to determine insulin (Kit No. DF-00064) concentrations in the plasma in Sichuan Agriculture University 101 laboratory (Ya’an, Sichuan, China). The known sensitivities of the insulin assays was 0.28 (µIU/ml). The intra- and interassay coefficients of variations were 5.4 and 7.5% for insulin, respectively.

Samples were analyzed for enzyme activities of Creatine Kinase (CK), glutamic-oxal (o) acetic transaminase (AST), Glutamate-pyruvate Transaminase (ALT) and Lactate Dehydrogenase (LDH). The activities were determined with an automatic biochemical analyzer (CL-7200, Shimadzu Instruments Manufacturing. Co., Ltd., Suzhou, China) using standard assay kits (Nanjing Jiancheng Biotechnology Co., Ltd, Jiangsu, China) and results were expressed as international units per liter as 25°C. Enzymatic assay was conducted to determine concentration of plasma glucose by using standard assay kit (Maker Biotechnology Co., Ltd, Chengdu, China) using the same instrument. All plasma samples were assayed in duplicate and measurements resulting in errors greater than 7% were reanalyzed.

**Statistical analysis:** Treatment was the experimental unit for DMI, milk yield, milk composition and biochemical parameters. Statistical analysis was performed using SPSS12.0 (SPSS Inc., Chicago, IL). Data was analyzed by one-way ANOVA. LSD multiple comparisons were used to test the differences between treatments, which were denoted by different letter superscripts. Polynomial contrasts were used to test linear and quadratic effects of Cr supplementation. Statistical significance was accepted at p<0.05 and P between 0.1 and 0.05 were interpreted as indicating a trend towards significance.

**RESULTS**

Ambient temperature and thi: Average of maximum and minimum ambient air temperature (by dry-bulb’s reading) during the experimental period was 29.8± 2.6°C and 25.0±1.7°C, respectively. The calculated THI averaged 79.61 units.

Milk yield and composition: Supplementation of chromium picolinate to the diet of cows under heat stress increased milk yield significantly (p = 0.013 (Table 2), but did not show any significant effect on milk composition (Table 2). Results indicated that adding Cr significantly improved (p<0.001) feed intake as compared to the control (Table 2) no differences in production efficiency were found. Furthermore, DMI and milk yield increased both in a linear manner (p = 0.004 and p = 0.003, respectively, Table 2) as the level of Cr supplementation increased.

Rectal temperature, respiration rates and plasma biochemical response: The effects of supplemental Cr on rectal temperatures, respiration rates and plasma biochemical response were shown in Table 3. Adding Cr had no significant effect on rectal temperatures, respiration rates (p = 0.310 and p = 0.265, respectively, Table 3). Plasma glucose concentration and molar ratio
Table 2: Effect of Cr supplementation on lactation performance and energy efficiency of Holstein cows

<table>
<thead>
<tr>
<th>Response variables</th>
<th>Least square mean</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily dry matter intake (kg)</td>
<td>Control</td>
<td>Low</td>
</tr>
<tr>
<td>Daily milk yield (kg/d)</td>
<td>24.34</td>
<td>25.29</td>
</tr>
<tr>
<td>Fat (g/l)</td>
<td>3.22</td>
<td>3.29</td>
</tr>
<tr>
<td>Protein (g/l)</td>
<td>2.92</td>
<td>2.89</td>
</tr>
<tr>
<td>Lactose (g/l)</td>
<td>4.81</td>
<td>4.83</td>
</tr>
<tr>
<td>Production efficiency (kg milk/kg feed)</td>
<td>1.38</td>
<td>1.40</td>
</tr>
</tbody>
</table>

Notes: Different letters within row mean significant difference (p<0.05). *P values between treatments

Table 3: Effect of Cr supplementation on rectal temperatures, respiration rates and plasma biochemical response

<table>
<thead>
<tr>
<th>Response variables</th>
<th>Least square mean</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectal temperatures</td>
<td>Control</td>
<td>Low</td>
</tr>
<tr>
<td>Respiration rates</td>
<td>64.8</td>
<td>63.91</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>0.301</td>
<td>0.310</td>
</tr>
<tr>
<td>Insulin (µIU/ml)</td>
<td>14.47</td>
<td>13.92</td>
</tr>
<tr>
<td>Glucose/Insulin</td>
<td>0.207</td>
<td>0.231</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>107.94</td>
<td>106.33</td>
</tr>
<tr>
<td>CK (IU/L)</td>
<td>98.39</td>
<td>95.99</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>607.75</td>
<td>602.75</td>
</tr>
</tbody>
</table>

Notes: Different letters within row mean significant difference (p<0.05). *P values between treatments

DISCUSSION

Heat stress is commonly assessed by the Temperature-humidity Index (THI), the sum of dry and wet bulb temperatures. Elevated ambient temperature during hot summer months imposes an additional heat load. It is well documented that in the south of China dairy cows suffered from heat stress even with the use of artificial cooling (Du et al., 2007). The meteorological results indicated a heat stress on trial cows during the entire trial period, because it exceeded the upper critical limit (72 THI units) for dairy cattle (Johnson, 1987). As THI exceeded the upper critical limit, four treatments cows showed similar types of responses to heat stress (e.g., elevated rectal temperatures and respiration rates), but there were slight differences in the degree of response. Control treatment cows had greater elevation in rectal temperatures and respiration rates. There was increasing evidence that adding Cr may have multiple nutritional benefits for domestic livestock. In this experiment, there was a trend of linear increase with Cr supplementation in DMI and milk yield as the level of Cr supplementation increased. It may be related to possible elimination of Cr deficiency. Cr requirements in human and farm animals are well known to increase during stress, the heat stress and early lactation could excrete Cr irreversibly through urine (Borel et al., 1984). In general, the present results agreed with previous studies which reported that heat stress decreased feed intake and adding Cr to the diet relieved this effect (AL-Saiady et al., 2004; Hayirli et al., 2001). However, feed intake was not affected by supplemental Cr in the study of Yang et al. (1996). It might be that cows were not under heat stress, Cr deficiency was not as serious as early-lactation cows under heat stress. Cows fed the diet with Cr had the highest peak milk production over the control. It was not yet clear how supplemental Cr increased milk yield in early lactation. The increased milk yield can may be explained by higher DMI and a reduction in the rate of mobilization of fatty acids from adipose tissue (McNamara and Valdez, 2005). Cr deficiency can cause insulin resistance. The
ratio of glucose to insulin can be considered as a crude index of tissue sensitivity to insulin (Evock-Clover et al., 1993). Cows receiving Cr had higher molar ratio of glucose to insulin and lower insulin than cows receiving no Cr. So insulin sensitivity was increased. If Cr increased insulin sensitivity, lipogenesis should be stimulated and lipolysis inhibited. A reduction in the rate of mobilization of fatty acids from adipose tissue may allow a greater increase in feed intake, stabilize hepatic fat metabolism and reduce hepatic ketogenesis, all working to increase milk production (Blum et al., 1983; Kronfeld, 1976). Cows receiving Cr had higher blood glucose than cows receiving no Cr, which might be attributed to higher DMI and gluconeogenesis. Cr supplementation normalized blood glucose by decreasing glucose in hyperglycemic subjects, having no effect on normal subjects and increasing that of hypoglycemic subjects (Subiyatno et al., 1996; Yang et al., 1996). Glucose uptake by bovine mammary gland is the major determinant of rate of milk secretion (Kronfeld, 1976). Glucose is required by the gland not only for synthesis of lactose and the glycerol moiety of milk lipids but also for provision of reducing equivalents in the form of NADPH for lipogenesis (Subiyatno et al., 1996). So higher blood glucose could enhance milk yield partially. The amount of any single enzyme in blood indirectly reflected its concentration in the cell, extent of cell injury, normal cell death or apoptosis and its degradation in plasma (Kaneko et al., 1997). In general, enzymes were surrounded by cellular membrane, so they could not easily get across cellular membrane into blood in normal condition. But extreme condition, such as heat stress could change the cellular membrane permeability, so enzyme activity increased when animals were under heat stress (Li et al., 2001). ALT and AST are two of the most reliable markers of hepatocellular injury or necrosis. ALT primarily exists in liver, but AST exists in various tissues like heart, liver, kidney and so on. During the trial, adding Cr tended to decrease ALT, while did not affect AST significantly, which indicated that Cr could protect the liver of cows under heat stress. CK is sensitive indicator of heat stress, which is an organ-specific enzyme in muscle (Li et al., 2004). Adding Cr decreased plasma CK activity, which indicated adding Cr could be beneficial to muscle. It might be explained that sensitivity of insulin improved in muscle (Besong et al., 1996). Usually, aerobic metabolism is unable to supply energy needs and the animal must call upon anaerobic pathways with resultant production and accumulation of lactic acid, LDH increased accordingly. But LDH did not show any significant differences with Cr supplementation. Maybe LDH is less sensitive to Cr than CK. These datas from DMI and milk yield suggested that there might have been slight Cr toxicity at our highest dose as lactation progressed, although the toxic level of Cr for ruminants was unknown. Maybe, Release of Cr from Cr picolinate for use in cells requires reduction of the chromic center, a process that can lead potentially to the production of harmful hydroxyl radicals (John, 2000).

**Conclusion:** The results of present study indicated that Cr supplementation of dairy cows at the early and peak of lactation under heat stress increased milk yield but not changing its composition. The increase in milk yield could be attributed to increase of DMI and improvement of the physiological status of the animals. Moderate level of Cr was recommended.

**REFERENCES**


