Supplemental Dietary Chromium Does Not Influence ACTH, Cortisol, or Immune Responses in Young Calves Inoculated with Bovine Herpesvirus-1

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ABSTRACT: Twelve Holstein bull calves (6 to 8 wk of age) were used to determine the influence of supplemental dietary Cr on ACTH, cortisol, and immune responses of calves experimentally inoculated with bovine herpesvirus-1 (BHV-1). Calves supplemented with Cr received 3 mg Cr/d (Chromium, n = 6) of a high-Cr-yeast product. Following 53 d of treatment, all calves were fitted with jugular catheters, and blood samples were collected every 4 h into tubes containing EDTA. Twenty-four hours later, all calves were inoculated intranasally with BHV-1 (1 × 10⁷ plaque-forming units in each naris). Serial blood collection continued at 4-h intervals for 6 d. Plasma was harvested, immediately frozen in liquid nitrogen, and stored at −20°C. Individual rectal temperatures and urine samples were collected at the same time each day. Rectal temperatures were elevated (P < .05) on d 2, 3, 4, and 5 but were not affected by Cr treatment. Treatment with Cr did not affect secretion of ACTH, cortisol, or plasma tumor necrosis factor-α, although clear circadian variation in ACTH and cortisol occurred. No differences were detected in the concentrations of trace minerals excreted daily in the urine, lymphocyte proliferative response to mitogen stimulation, and neutrophil bactericidal function. The acute phase proteins, ceruloplasmin and fibrinogen, also were not affected by treatment or viral challenge. These data suggest that Cr supplementation using high-Cr yeast (3 mg/d) did not alter stress responses of calves experimentally inoculated with BHV-1.

Key Words: Chromium, Calves, Stress, Trace Elements


Introduction

Chromium is an essential trace nutrient in human nutrition and its effect in glucose tolerance has been reviewed extensively (Anderson, 1988; Mertz, 1993). Recently, the influence of dietary Cr on performance and stress responses of transported calves (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993) and immune responses of lactating dairy cows and newly weaned feedlot calves (Burton et al., 1993, 1994) have been evaluated. In those studies, Cr supplementation caused a decrease in serum cortisol concentrations when fed to stressed feeder calves and altered immune responses of lactating dairy cows and weaned calves. Conversely, in two recent studies, Cr supplementation failed to decrease serum cortisol in stressed feeder calves (Kegley and Spears, 1995) and tended to increase serum cortisol in periparturient dairy cows (Burton et al., 1995b).

The susceptibility of stressed calves to shipping fever is a major economic concern in cattle production systems. The possibility of improving immune competence of stressed calves by nutritional manipulation may be an alternative to medical intervention. In this study, we investigated the influence of supplemental dietary Cr, via a high-Cr-yeast product, on ACTH, cortisol, and immune responses of calves experimentally inoculated with bovine herpesvirus-1 (BHV-1).

Materials and Methods

Animal Care and Use. The procedures used in these experiments were approved by the Kansas State University Institutional Animal Care and Use Committee.
Animals, Diets, and Management. Twelve Holstein bull calves (6 to 8 wk of age) were allotted by weight into one of two dietary treatments (Chromium, n = 6, avg wt = 85 kg or Control, n = 6, avg wt = 83 kg). Calves were fed a mixture of 67% dry ground corn and 33% commercial calf protein supplement, as-fed basis, to achieve an intake of 1.4% of BW (Table 1). A base concentrate mix consisting of 5% dried molasses, 48.8% finely ground corn, and 46.2% soybean meal was fed to each calf at a rate of .11 kg/d. Calves supplemented with Cr received the same base concentrate mix, with high-Cr-yeast (1,000 mg of Cr/kg of Cr-yeast; Alltech, Lexington, KY) replacing finely ground corn at 2.6%. After all of the concentrate was consumed, calves were given ad libitum access to grass hay. This ensured that calves on the Cr treatment consumed 3 mg of supplemental Cr/d. Following 53 d of treatment, all calves were fitted with jugular catheters, and blood samples were collected every 4 h into tubes containing EDTA. Twenty-four hours later, all calves were inoculated intranasally with BHV-1 (1 × 10^7 plaque-forming units in each naris). Serial blood collection continued at 4-h intervals for 6 d. Plasma was harvested immediately from blood samples, rapidly frozen in liquid nitrogen, and stored until analysis for ACTH, cortisol, and tumor necrosis factor-α (TNF-α). Individual rectal temperatures and urine samples were collected at the same time each day. Mineral content of the calf diet and urine trace mineral concentrations were determined using an inductively coupled plasma analyzer (Jobin Yvon 24 Sequential Computer; Peterson Laboratories, Hutchinson, KS) by procedures previously described (Arthington et al., 1996).

Lymphocyte Blastogenic Response. Purified blood mononuclear cells were collected from 15 mL of heparinized blood on d 4 following BHV-1 challenge. Lymphocyte blastogenic responses to mitogens were evaluated with procedures described previously by Blecha et al. (1984). Briefly, lymphocytes (5 × 10^6 cells/mL) were added in triplicate to wells of a 96-well round-bottom microtiter plate. Each mitogen (100 μL/well) was added to lymphocyte cultures. Cultures were incubated at 37°C in a humidified 95% air, 5% CO_2 atmosphere for 66 h; [3H]thymidine (20 μL; .05 μCi/mL; Amersham Life Science, Arlington Heights, IL) was added to all culture wells for the last 18 h of culture. The same lots of fetal bovine serum, RPMI 1640 culture medium, phytohemagglutinin (PHA, Wellcome Diagnostics, Dartford, U.K.), pokeweed mitogen (PWM, Gibco Laboratories, Grand Island, NY), and concanavalin A (ConA, Pharmacia Laboratories, Piscataway, NJ) were used throughout the experiment to decrease assay variability.

Plasma ACTH, Cortisol, and TNF-α Determination. Conventional RIA was used to determine concentrations of ACTH in bovine plasma (Hoffman et al., 1996). The assay was sensitive to .76 pg/tube. The intraassay CV averaged 7.9% and the interassay CV was 11.8%. Plasma cortisol was measured by RIA identical to the one described for ovine plasma (Minton et al., 1992). For validation in cattle, varying volumes of a pool of bovine plasma were measured in the assay. The regression of volume assayed on concentration of cortisol measured had a slope of -.1 and a 95% confidence interval that included 0. In addition, cortisol added to bovine plasma was quantitatively recovered in the assay. The regression of the concentration of cortisol measured in the assay on concentration added to bovine plasma had a slope of 1.1 and a 95% confidence interval that included 1.0. The assay was sensitive to .7 ng/mL. All samples were evaluated in two assays. The intraassay CV averaged 7.8% and interassay CV was 4.2%. Concentrations of plasma TNF-α were determined by RIA procedures previously validated in cattle (Kenison et al., 1990).

### Table 1. Mineral content of calf diet

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Soybean meal</th>
<th>Grass hay</th>
<th>Corn</th>
<th>Protein supplement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromium, ppm</td>
<td>.49</td>
<td>.27</td>
<td>-</td>
<td>4.89</td>
</tr>
<tr>
<td>Cobalt, ppm</td>
<td>.25</td>
<td>&lt;27</td>
<td>&lt;20</td>
<td>1.88</td>
</tr>
<tr>
<td>Copper, ppm</td>
<td>18.6</td>
<td>3.4</td>
<td>2.01</td>
<td>121</td>
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<tr>
<td>Iron, ppm</td>
<td>149.0</td>
<td>88.8</td>
<td>38.1</td>
<td>1,200.0</td>
</tr>
<tr>
<td>Manganese, ppm</td>
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<td>63.2</td>
<td>9.99</td>
<td>586</td>
</tr>
<tr>
<td>Molybdenum, ppm</td>
<td>3.7</td>
<td>.74</td>
<td>.55</td>
<td>3.84</td>
</tr>
<tr>
<td>Zinc, ppm</td>
<td>70.2</td>
<td>18.7</td>
<td>24.0</td>
<td>499</td>
</tr>
</tbody>
</table>

Calves were fed to achieve an intake of 1.4% of BW (ground corn 2/3 and commercial protein supplement 1/3 of grain ration). Guaranteed analysis of the crude protein content of commercial calf supplement was not less than 32.0% Chromium content of corn was not determined. However, using a value of 12.03 ppm Cr in corn (Chang and Mowat, 1992), the corn/protein supplement provided 9.7 ppm Cr and the base concentrate mix provided 6.1 ppm Cr. The base concentrate mix containing supplemental Cr from high-Cr-yeast provided 31.8 ppm Cr. All calves were given ad libitum access to grass hay.

### Neutrophil Isolation and Determination of Bactericidal Activity. Jugular blood (30 mL) was collected in heparinized tubes on d 0 and 4 relative to BHV-1 challenge. Neutrophils were isolated, and the percentage of opsonized Staphylococcus aureus killed was determined by methods previously described (Arthington et al., 1995).
Plasma Ceruloplasmin and Fibrinogen Determination. Ceruloplasmin and fibrinogen concentrations were determined in blood samples collected at 8-h intervals for 6 d following BHV-1 inoculation. Plasma ceruloplasmin concentrations were measured with calorimetric procedures described by Demetriou et al. (1974). Assay variation was controlled by high and low standard pools established in our laboratory. All results were expressed as milligrams per deciliter (mg/dL) using a conversion equation described by King (1965). Plasma fibrinogen concentration was determined using a commercial fibrinogen determination kit (Sigma procedure No. 880, Sigma Diagnostics, St. Louis, MO). Results are expressed as milligrams/deciliter determined from a standard curve generated from a human fibrinogen reference (Sigma Diagnostics).

Whole Blood Constituents and Cell Differentials. Jugular blood (5 mL) was collected into tubes containing EDTA on d 0 and 4 relative to BHV-1 challenge. Analyses of whole blood, including total leukocytes and erythrocytes, hemoglobin, and hematocrit, were made with an electronic cell counter (Danam Particle Cell Counter, Dallas, TX). Differential leukocyte counts were determined by counting 100 cells from Wright's-stained (Camco Quick Stain, American Scientific Products, McGaw Park, IL) blood films. Monocytes were not differentiated from lymphocytes. Cell numbers were calculated by multiplying the percentage of cell type by total leukocyte counts.

Statistical Analyses. Analysis of variance was performed with the GLM procedures of SAS (1985). For analyses involving multiple measurements over time, a split-plot design was used with the effect of pen in the whole plot and time and time × treatment effects in the subplot. When time × treatment interactions were significant (P < .05), treatment means within times were compared by least significant differences. For some variables (rectal temperature; plasma ACTH, cortisol, and TNF-α; and urinary Cu and Zn) in which neither a treatment nor treatment × time interaction was noted, data from both treatments were pooled and time means presented to more clearly illustrate changes following BHV-1 challenge.

Results and Discussion

Calf Weight and Rectal Temperature Change. During the Cr feeding period, calf ADG did not differ (.65 and .64 kg/d for Control and Chromium calves, respectively). There were no treatment or treatment × time interactions noted for rectal temperature (Figure 1). However, BHV-1 challenge induced significant increases in rectal temperature on d 2, 3, 4, and 5 (Figure 1, inset). On d 3 after BHV-1 challenge, rectal temperatures of Control calves were .6°C higher than those of Cr-supplemented calves; however, this difference was not significant (time × treatment interaction; P = .63). Moonsie-Shageer and Mowat (1993) reported a decrease in rectal temperatures of stressed feeder calves supplemented with a high-Cr yeast source and entering the feedlot. In comparing these studies, the type of stress producing the observed changes in rectal temperatures must be considered. The stress produced from viral infection (BHV-1 challenge) may have had considerably different immunological implications than the stress incurred by shipping.

Plasma ACTH, Cortisol, and TNF-α Concentrations. Neither a treatment nor a treatment × time interaction was noted for plasma ACTH (74.6 ± 4.5 and 74.8 ± 4.8 pg/mL), cortisol (9.4 ± 1.2 and 8.7 ± 1.3 ng/mL), or TNF-α (167 ± 7 and 157 ± 9 pg/mL) concentrations for Control and Chromium, respectively. These findings suggest that neither Cr supplementation nor the BHV-1 challenge affected the pituitary-adrenal axis or activation of peripheral macrophages to secrete TNF-α. Plasma ACTH, cortisol, and TNF-α were measured because current dogma holds that inflammation, whether from bacterial, viral, or parasitic infection, tissue damage, or tumor growth, produces an acute rise in plasma proinflammatory cytokines and activation of the hypothalamic-pituitary-adrenal axis (Fey and Gauldie, 1990; Myers and Murtaugh, 1995). Furthermore, dietary Cr has been suggested to reduce circulating cortisol in stressed feeder cattle, albeit delayed relative to the onset of stress. Chang and Mowat (1992) and Moonsie-Shageer and Mowat (1993) reported significant decreases in serum cortisol in stressed feeder calves receiving supplemental Cr. However, these decreases were detected at later times following the induction of stress compared to the current study. Moonsie-Shageer and Mowat (1993) did not detect a decrease in serum cortisol concentrations until d 28 following transportation stress (differences on d 7, 14, and 21 were not significant), whereas Chang and Mowat (1992) detected a difference in sample means collected on d 60 and 69 of the growing period. In contrast, Kegley and Spears (1995) did not detect a treatment effect on serum cortisol concentrations at d 7 and 35 of Cr supplementation, and Burton et al. (1995b) found that serum cortisol tended to be increased in periparturient dairy cows supplemented with Cr. Similarly, our data do not support a reduction in ACTH or cortisol in virus-infected calves in response to Cr. We did not specifically design this study to evaluate circadian variation in adrenal cortical function, which is present in cattle (Thun et al., 1981), although our data clearly reveal a close concordance in the circadian rhythmicity in plasma ACTH and cortisol (Figure 2).

Further, six distinct peaks in both hormones seem to occur in six 24-h periods (Figure 2), suggesting that viral challenge and fever did not disrupt this rhythmicity.

Clearly, BHV-1 induced an inflammatory response as evidenced by the rise in rectal temperature 2 to 5 d
Figure 1. Mean rectal temperatures of calves following intranasal BHV-1 inoculation. Calves were fed Control or Cr-supplemented diets for 53 d and then challenged with BHV-1. Chromium treatment did not affect rectal temperature response to BHV-1 challenge (pooled SEM = .17 and .25 for Control and Chromium, respectively). However, rectal temperatures were increased ($P < .05$) on d 2, 3, 4, and 5 (Figure inset; superscript denotes increased temperatures relative to d 0).

Figure 2. Mean plasma cortisol and ACTH concentrations of calves fed Control or Cr-supplemented diets for 53 d and then challenged with BHV-1. No significant time $\times$ treatment interactions occurred; therefore, treatment means within times were pooled (pooled SEM = 5.5 ng/mL and 20.5 pg/mL for cortisol and ACTH, respectively).

after BHV-1 infection (Figure 1, inset). Despite this, pulmonary macrophage production of proinflammatory cytokines seemed not to be involved in BHV-1-induced fever, as evidenced by failure of plasma TNF-$\alpha$ to change. The lack of change in TNF-$\alpha$ suggests that BHV-1-induced fever occurs via different mechanisms than endotoxin-induced fever and may account for the lack of effect of BHV-1 on plasma ACTH and cortisol. Our data showing that Cr supplementation did not alter concentrations of
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Figure 3. Urinary Zn concentrations of calves fed Control or Cr-supplemented diets for 53 d and then challenged with BHV-1. Control and Chromium did not differ \((P > .2)\). Chromium treatment did not affect urinary Zn concentrations following BHV-1 challenge. However, urinary Zn was increased \((P < .01)\) on d 0 and 1 (Figure inset; superscript denotes increased temperatures relative to d \(-1\)).

plasma TNF-\(\alpha\) are in agreement with those of Burton et al. (1995b).

Lymphocyte Blastogenesis and Neutrophil Bactericidal Activity. Neither Cr treatment nor BHV-1 challenge seemed to affect proliferative responses of mitogen-stimulated lymphocytes (data not shown). Burton et al. (1993) reported that supplemental Cr, via a Cr amino acid chelate, prevented a decrease in ConA-induced mononuclear cell proliferation in prepartum dairy cows. Although cortisol levels were not reported, it was reasonable to hypothesize that this effect may have been due to a decrease in serum cortisol concentrations in the Cr-supplemented cows. Increases in serum cortisol, as a result of stress, have been associated with decreases in lymphocyte responsiveness to mitogen stimulation (Blecha and Minocha, 1983; Coppinger et al., 1991; Minton et al., 1992). Shipping stress also has been shown to decrease mitogen-induced lymphocyte responsiveness in calves (Blecha et al., 1984). Kegley and Spears (1995) reported an increase in PHA-induced lymphocyte blastogenic responses of calves supplemented with Cr-nicotinic acid vs calves receiving Cr-chloride, but not in calves receiving the control diet. Serum from Cr-supplemented cows increased ConA-induced blastogenic responses of donor cow lymphocytes even though cortisol tended to be increased by Cr supplementation (Burton et al., 1995b). These comparisons suggest that the effects of dietary Cr on stress responses are dependent on several variables, including the type and duration of stress challenge under consideration.

Neutrophil bactericidal abilities also were unaffected by treatment and BHV-1 challenge (data not shown). Although we are unaware of other reports investigating the effect of supplemental Cr on neutrophil function, cortisol-treated calves have suppressed neutrophil function (Murata and Hirose, 1991). Therefore, investigation of neutrophil function coupled with plasma cortisol concentration merited attention in the current study.

Plasma Ceruloplasmin and Fibrinogen Concentrations. Concentrations of plasma ceruloplasmin and fibrinogen were not affected \((P > .10)\) either by Cr treatment or BHV-1 inoculation (pooled mean ± SE concentrations = 8.9 ± .5 and 9.6 ± .6 mg/dL ceruloplasmin and 233.6 ± 18.5 and 241.0 ± 20.2 mg/dL fibrinogen for Control and Chromium, respectively). Ceruloplasmin and fibrinogen are two of several plasma acute-phase proteins that often are increased in response to inflammatory signals (Conner et al., 1988; Baumann and Gauldie, 1994). Previously, we have reported increases in both proteins following BHV-1 inoculation in growing beef heifers (Arthington et al., 1996). The absence of a similar response in the current study is unclear. During both instances, increased rectal temperatures along with typical clinical symptoms of respiratory distress were observed. The acute-phase response in younger calves, such as were used in the current study, may be
different in terms of the quantity and pattern of acute-phase protein release. Total plasma protein concentrations are low in calves and gradually increase as animals age (Schalm, 1986).

Urinary Copper and Zinc Excretion. Concentrations of Cu and Zn excreted in urine were not affected by either treatment or BHV-1 challenge (Cu data not shown; Zn shown in Figure 3); however, urinary Zn excretion was increased on d 0 and 1 after BHV-1 challenge (Figure 3, inset). This may have been a response to the stress experienced through the implantation of the jugular catheters. Orr et al. (1990) reported an increase in both urinary Cu and Zn excretion following inoculation with infectious bovine rhinotracheitis. This response was maximal on d 12 after viral challenge. Urine samples in the current study were collected only through d 6 following BHV-1 challenge.

Whole Blood Constituents and Cell Differentials. Chromium-supplemented calves tended to have a greater (P = .10) increase in neutrophil number following BHV-1 challenge (Table 2). Whole blood measures of hemoglobin and hematocrit were not affected by Cr treatment or BHV-1 challenge. Increases in blood cortisol have been shown to cause a decrease in the expression of adhesion molecules important for the migration of neutrophils across the vascular endothelium (Burton et al., 1995a). However, because cortisol was not influenced by Cr supplementation, it is unlikely that neutrophil trafficking was influenced by blood cortisol following BHV-1 challenge in this study.

Implications

In this study, chromium supplementation, via a high-chromium-yeast product, failed to affect various stress measures in young Holstein calves experimentally inoculated with bovine herpesvirus-1. Other researchers have reported decreases in serum cortisol as well as improved immune responses in chromium-supplemented, stressed cattle. Nevertheless, the body of evidence suggesting a stress-modulating function for dietary chromium is still inconclusive. Currently, supplemental dietary chromium is not available for incorporation into diets for livestock; however, further possibilities for decreasing livestock stress, perhaps through dietary manipulations, are exciting and deserve attention.

Literature Cited


Burton, J. L., B. A. Mallard, and D. N. Mowat. 1993. Effects of supplemental chromium on immune responses of peripar-


