ABSTRACT

STAHLHUT, HEATHER SUZANNE. The Effect of Supplemental Chromium and Copper Status on Glucose Metabolism, Performance, and Reproduction of Beef Cattle. (Under the direction of Jerry W. Spears).

A trial was conducted to determine the effect of supplemental chromium and copper status on glucose metabolism, performance, and reproduction of beef cattle. Pregnant Angus (n=83) and Simmental (n=69) cows were blocked by age and breed and randomly assigned to one of two free-choice mineral supplements. Supplements consisted of: 1) control (no supplemental Cr) and 2) 40 mg Cr /kg of mineral (from Cr picolinate). Mineral supplements were formulated to contain all minerals typically supplemented to cattle diets with the exception of Cu. The study began approximately 75 d prepartum, at which point half of the cows in each treatment received a 25 g Cu oxide needle bolus. Over the course the study, cows supplemented with chromium had lower plasma glucose and nonesterified fatty acid concentrations than control cows. Angus had higher plasma glucose concentrations than Simmental cows for the duration of the study.

Glucose challenges were conducted pre- and postpartum to determine the effect of chromium supplementation on glucose metabolism in late gestation and early lactation in beef cows. Following a glucose challenge prepartum, plasma glucose and serum insulin concentrations were lower in animals receiving supplemental chromium. In cows receiving a copper bolus, chromium supplementation resulted in higher plasma glucose concentrations following a glucose challenge postpartum; however, no difference in plasma glucose concentrations was observed between treatments in animals that did not receive a copper bolus. Serum insulin was lower at 10 and 20 minutes following glucose infusion in the

postpartum challenge in cows receiving supplemental chromium. Area under the curve and glucose clearance rates were not affected by treatment in either the pre- or postpartum glucose challenges.

Breed differences were observed in basal plasma glucose as well as plasma glucose, NEFA, and serum insulin following the glucose challenges. Angus had higher plasma glucose concentrations following the postpartum glucose challenge. Serum insulin was higher and plasma NEFA concentrations were lower in Angus cows following glucose infusion during prepartum and postpartum glucose challenges when compared to Simmental cows.

Cows receiving supplemental chromium lost less weight throughout the course of the study than control animals, and tended to have higher pregnancy rates than control cows. Over the course of the study as well as the period postpartum, young cows (2 or 3 years of age) receiving supplemental chromium lost less weight than controls. Control cows in replicates 2 (4 and 5 years of age) and 3 had higher plasma NEFA concentrations when compared to cows receiving chromium in the same replicates; indicating greater mobilization of fat stores. Simmental cows lost more weight than Angus over the course of the study and during the postpartum period. Chromium supplementation lowered plasma NEFA concentrations in cows that did not receive a copper bolus, and tended to lower NEFA concentrations in cows supplemented with copper.

Chromium supplementation did not affect calf performance, morbidity, and mortality. Simmental calves, male calves, and calves born to older cows (replicates 1 and 2) had higher birth and weaning weights than Angus calves, heifer calves, or calves born to young cows.

In conclusion, chromium and copper status altered glucose metabolism in reproducing beef cows. Supplemental chromium decreased the amount of weight lost by cows postpartum, and may increase pregnancy rates in beef cows.

THE EFFECT OF SUPPLEMENTAL CHROMIUM AND COPPER STATUS ON GLUCOSE METABOLISM, PERFORMANCE, AND REPRODUCTION OF BEEF CATTLE.

by

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DEDICATION

The author wishes to dedicate this work in loving memory of her grandfather, Mr. Wallace M. Cherry; on whose farm she spent the happiest summers of her youth. Through him, she was first exposed to agriculture: An experience that, by his patience and attention, was fostered into a love and deep respect for animals and earth.

BIOGRAPHY

Heather Suzanne Stahlhut was born in Union City, Georgia on December 2, 1980 to Suzanne and David M. Stahlhut. She was raised in Peachtree City, Georgia, along with her older brother, Michael. The author graduated from Counterpane Montessori School in May of 1999. In August 1999, she enrolled in Colorado State University in Fort Collins, Colorado. She received a Bachelor of Science degree with a major in Animal Science from Colorado State University in December of 2002. In January of 2003, she entered North Carolina State University's Animal Science program in pursuit of a Master of Science degree.

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Introduction

Chromium is a transitional element with an atomic number of 24 and an atomic weight of 51.9996. Chromium was first shown to be essential by Schwarz and Mertz (1957) when they isolated "glucose tolerance factor" (GTF) from swine kidney. This organometallic molecule, composed of nicotinic acid, glutamic acid, glysine, cysteine, and trivalent chromium, was shown to reverse impaired glucose tolerance in rats. Chromium has been found to play essential roles in the activity of certain enzymes, the metabolism of proteins and nucleic acids, and may impact immune function (Beitz et al., 1997); however, only its function as related to glucose metabolism is sufficiently understood.

The most striking evidence for chromium's essential role in glucose metabolism was observed in a woman receiving total parenteral nutrition. After three years of receiving total parenteral nutrition, the woman experienced a 15% weight loss, neuropathy, glucose intolerance, and a need for the administration of exogenous insulin, despite the fact that serum insulin levels were normal (Jeejeebhoy et al., 1977). Caloric intake was increased to approximately 1000 kcal/day more than what she had initially received in attempts to maintain the patient's weight. Neuropathy and glucose intolerance, however, persisted until 250 μg of chromium (as $CrCl_3$) per day was added to the infusate for a period of two weeks (Jeejeebhoy et al., 1977). Treatment with supplemental chromium reversed impaired glucose tolerance, neuropathy, removed the need for exogenous insulin, and resulted in a return to normal body weight even when caloric intake was reduced to previous levels (Jeejeebhoy et al., 1977).

Since there is no adequate measure of chromium status, establishing dietary requirements for livestock and humans is difficult. While the recommended intake for

chromium is 50 – 200 μg per day (National Research Council, 1989) in humans; currently, there is no established chromium requirement for ruminants. Improvement in impaired glucose tolerance after chromium supplementation is the most sufficient means to determine deficiency.

Chromium Metabolism

Chromium is abundant in water, soil, and living matter, but it is poorly absorbed and has often been used as a marker for passage of nutrients through the GI tract, as it is almost totally excreted in the feces (Underwood and Suttle, 1999). Chromium occurs in -2 to +6 oxidation states; however, 0, +2, +3, and +6 oxidation states are most common (McDowell, 1992). Chromium is most stable, and abundant, in its trivalent (Cr^{+3}) form (Beitz et al., 1997). Underwood and Suttle (1999) suggest that the form of chromium in the diet may be more important than the amount present in terms of absorption. While hexavalent (Cr^{+6}) forms of chromium are absorbed three to five times better than Cr^{+3} when administered directly to the intestine (Anderson, 1987), Cr^{+6} is reduced to Cr^{+3} in the acidic environment of the stomach (Mertz, 1984).

Chen et al. (1973) observed increased absorption of ^{51}Cr in fasted versus non-fasted rats, suggesting that the inorganic chromium was complexing with feedstuffs within the digestive tract of non-fasted animals. When oxalate and phytate were added to the diet, chromium absorption increased and decreased respectively, lending greater credence to contents of the digestive tract impacting absorption. The authors postulated that oxalate formed ligands with chromium to prevent olation and the subsequent precipitation of chromium that renders it unavailable (Chen et al., 1973). Complexing chromium to proteins, free amino acids, and low-molecular weight ligands, to form synthetic organic forms of

chromium such as chromium picolinate (CrPic) and chromium nicotinate (CrNic), has resulted in greater absorption efficiency (Beitz et al., 1997).

The primary site of chromium absorption appears to be the jejunum of the small intestine, as shown in an *in vitro* study using $^{51}CrCl_3$ in everted gut sacs of Sprague-Dawley rats (Chen et al., 1973). In humans consuming a self-selected diet, chromium absorption was reported to be approximately 0.4% in non-supplemented and chromium supplemented (200 μg $CrCl_3$) subjects (Anderson et al., 1983). Anderson and Kozlovsky (1985) determined that chromium absorption was inversely related to average dietary intake of chromium in humans consuming self-selected diets.

Mertz and Roginski (1971) reported that facilitated diffusion was the predominant means of chromium absorption using everted guts sacs of rats treated with 0 to 10 μg of chromium. Active transport was ruled out as an important means of absorption when the addition of glucose or acetate, both energy-supplying substrates, did not affect the rate of chromium transport (Mertz and Roginski, 1971). However, when inorganic, trivalent chromium was added to cultures of vascularly perfused rat small intestine, absorption was determined to be a non-saturable process; ruling out carrier-mediated transport (Dowling et al., 1989). The authors postulate that inorganic, trivalent chromium may be absorbed via aqueous channels in plasma membranes or paracellulary through tight junctions (Dowling et al., 1989). This does not eliminate the possibility that chromium may be absorbed by facilitated diffusion under certain circumstances.

Once absorbed, chromium is transported to the body tissues by transferrin. Hopkins and Schwarz (1964) report that transferrin is only 30% saturated with iron under normal conditions, but with saturation with iron *in vitro*, chromium competes with iron for these

binding sites. More recent *in vitro* studies indicate that transferrin has two binding sites (A and B), one of which has greater affinity for iron, depending upon pH (Borel and Anderson, 1984). However, as concentrations of iron increase, competition between iron and chromium for the binding sites increases as well.

While an average 0.5% of dietary trivalent chromium is absorbed, there is currently no reliable means of measuring whole-body chromium status (Beitz et al., 1997). Anderson (1987) reports typical concentrations of circulating chromium to be 0.01 – 0.3 μg/L; however, plasma is cleared of chromium relatively quickly when compared to chromium stored in tissue (Borel and Anderson, 1984). Due to this disparity between whole-body and circulating chromium, plasma concentrations are not an effective measure of chromium status (Beitz et al., 1997).

Excretion of Chromium

The majority of absorbed chromium is excreted via the urine, however small amounts may be lost in bile, perspiration, and hair. Mertz (1984) suggests that comparison of daily urinary Cr loss with daily dietary intake may act as a reasonable measure of absorption efficiency. Beitz et al., (1997) concluded that it was not an effective measure of dietary chromium status after several studies showed oral loading of glucose (Borel and Anderson, 1984), stress, and exercise in humans increased urinary excretion of chromium.

Dietary chromium absorption, at average daily intakes, is inversely related to dietary intake and urinary excretion of chromium (Anderson and Kozlovsky, 1985). Anderson et al. (1982) observed a four-fold increase in urinary chromium concentrations when free-living individuals were supplemented with 200 μg of $CrCl_3$. While 80% of control subjects had increased excretion of chromium following a glucose challenge, the authors concluded that

this response was not related to the chromium status of the individuals (Anderson et al., 1982). Similarly, Anderson et al. (1983) observed increased chromium excreted in urine in free-living humans with daily supplementation of 200 μg of $CrCl_3$. Chromium absorption in these individuals remained at approximately 0.4% regardless of chromium supplementation; meaning that in those individuals receiving supplemental chromium, urinary excretion increased fivefold (Anderson et al., 1983). In humans receiving low-chromium diets, daily supplementation of 200 μg of $CrCl_3$ increased urinary chromium excretion (Anderson et al., 1991). Urinary chromium losses increased following a glucose challenge in all subjects regardless of treatment.

Biologically active compounds

Chromium was found to be an active component of glucose tolerance factor, an organometallic molecule which potentiates the action of insulin (Mertz, 1969). Toepfer et al. (1977) postulated that glucose tolerance factor is composed of trivalent chromium, nicotinic acid, glutamic acid, glycine, and cysteine; however, its exact structure has not been determined.

The addition of chromium tripicolinate to cultures of rat skeletal muscle resulted in an increase in glucose uptake by the tissue; however, glucose oxidation was not observed when chromium tripicolinate was added to isolated rat adipocytes (Evans and Pouchnik, 1993). Conversely, Evans and Pouchnik (1993) observed that the addition of chromium dinicotinate resulted in significant glucose oxidation in rat adipocytes, but had no effect on glucose uptake by skeletal muscle. These results indicate that these two compounds differ not only in chemical composition, but also in biological activity.

An oligopeptide known as low-molecular-weight, chromium-binding substance has been identified in bovine colostrum (Yamamoto et al., 1988) as well as bovine (Davis and Vincent, 1997) and porcine (Sumrall and Vincent, 1997) liver. This compound is composed of chromium, aspartic acid, glutamic acid, glycine, and cysteine. Addition of low-molecular-weight, chromium-binding substance from colostrum to cultures of rat adipocytes had no effect on glucose oxidation; however, when exogenous insulin was added to the medium as well, the rate of glucose oxidation increased significantly (Yamamoto et al., 1988). When measuring glucose incorporation into lipids, Yamamoto et al. (1988) observed that low-molecular-weight, chromium-binding substance from colostrum with and without the addition of exogenous insulin, resulted in increased glucose incorporation rates.

Davis and Vincent (1997) observed that the addition of bovine liver low-molecular-weight, chromium-binding substance (LMWCr), or chromodulin, to the membranes of rat adipocytes resulted in increases in membrane kinase activity only in the presence of insulin; however, LMWCr had no effect on kinase activity when insulin could not bind to its receptor. These results indicate that LMWCr's site of action is the insulin receptor itself. Titration of apoLMWCr with different levels of chromic ions was shown to influence its ability to activate kinase activity. The addition of four chromic ions per oligopeptide resulted in maximal activity; however, addition of six chromic ions per oligopeptide resulted in inhibition of kinase activation (Davis and Vincent, 1997).

Sun et al. (2000) observed that in the presence of apoLMWCr, loading of transferrin with chromium is substantially depressed, indicating that, even at near neutral pH, LMWCr competes with transferrin for chromium. During a 25,000 minute incubation with

apotransferrin, LMWCr did not release any of its chromium to apotransferrin in contrast to significant chromium migration observed from transferrin (Sun et al., 2000).

It is postulated that chromium acts as a part of an autoamplification system for the insulin signaling pathway (Vincent, 2000). It is proposed that apochromodulin is stored in the cells of insulin-sensitive tissues, such as muscle and adipose. Increases in blood insulin concentrations cause insulin to bind to its receptor on these cells; resulting in a conformational change and subsequent activation of tyrosine kinase; signaling the movement of chromium from the blood into the cells (Vincent, 2004). Vincent (2000) reports apochromodulin to possess a large chromic ion binding constant, enabling this molecule to bind chromium in response to a sudden flux into the cell. Holochromodulin may then bind to its receptor, assisting in maintaining the receptor's active conformation, and amplifying insulin signaling (Vincent, 2004). As insulin levels decrease, holochromodulin is released from the receptor and excreted from the cell to ultimately be voided in the urine (Vincent, 2004).

Sumrall and Vincent (1997) have explored a potential link between LMWCr and GTF as first isolated from porcine kidney by Schwartz and Mertz (1959). LMWCr isolated from porcine kidney was found to be extremely susceptible to hydrolysis; resulting in the formation of smaller molecular weight species of chromium containing compounds (Sumrall and Vincent, 1997). The authors suggest that techniques used in previous studies to isolate and purify GTF from crude extracts would destroy LMWCr; potentially resulting in GTF or a GTF-like species (Sumrall and Vincent, 1997).

Effect of Chromium on Carbohydrate Metabolism

Since there is no adequate measure of chromium status, establishing dietary requirements for livestock and humans is difficult. Currently, there is no established chromium requirement for ruminants. Improvement in impaired glucose tolerance after chromium supplementation is the most sufficient means to determine deficiency. Chromium deficiency is marked primarily by disturbances in glucose, lipid, and protein metabolism, decreased insulin sensitivity of peripheral tissues, as well as impaired growth and longevity in experimental animals (Underwood, 1977).

Mertz et al. (1964) observed increased glucose removal rates following a glucose challenge in chromium deficient rats supplemented with 5 ppm $CrCl_3$. Following a hypoglycemic dose of insulin, plasma glucose concentrations were shown to decrease half as much, and remain lower for a shorter amount of time in rats fed a diet low in protein and chromium, when compared to rats receiving similar levels of protein but 2 ppm $CrCl_3$ (Roginski and Mertz, 1968). Higher glycogen reserves in animals receiving supplemental chromium indicates greater tissue response to insulin in these animals, resulting in the greater hypoglycemic response (Roginski and Mertz, 1968). Ranhorta and Gelroth (1986) observed lower peak glucose concentrations in rats supplemented with 45 μg Cr/100g diet as high-chromium yeast when compared to control animals; however supplementation of $CrCl_3$ did not result in a significant decrease in peak glucose concentrations.

Hopkins et al. (1968) observed hypoglycemia and impaired glucose tolerance in malnourished Jordanian and Nigerian infants. However, the infants from Jordan came from two different regions, and while all were malnourished, only those from the Jordanian hill area exhibited severe glucose intolerance. The authors attributed this difference to lower

levels of chromium present in the drinking water in the Jordanian hill area.

Supplementation of 250 μg $CrCl_3$ resulted in improved glucose clearance rates and overall glucose tolerance in infants exhibiting impaired glucose tolerance (Hopkins et al., 1968). However, supplementation of 250 μg $CrCl_3$ in malnourished Egyptian children did not affect glucose tolerance (Carter et al., 1968). Carter et al. (1968) determined that while the diets of children in that area were protein deficient, the diets were high in chromium; suggesting that the children were adequate in chromium and that an effect on glucose tolerance would not be expected when chromium status is normal.

Anderson et al. (1983) observed no effect of chromium supplementation (200 μg $CrCl_3$) on fasting insulin and glucose concentrations in free-living individuals exhibiting little difference between fasting glucose concentrations and concentrations 90 minutes after a glucose challenge. In those individuals that had higher fasting glucose than 90 minutes after the challenge, glucose concentrations were decreased by chromium supplementation. Chromium supplementation increased glucose concentrations in those individuals with lower fasting concentrations of glucose when compared to concentrations 90 minutes following glucose administration. In a later study, Anderson et al. (1991) observed improved glucose tolerance, insulin and glucagon activity in mildly hyperglycemic individuals fed a low-chromium diet supplemented with 200 μg $CrCl_3$. Results of these studies indicate that chromium supplementation may be beneficial to both hyperglycemic and hypoglycemic individuals.

Steele et al. (1977) observed no effect on peak glucose concentrations, half life, or glucose clearance in swine supplemented with 100 mg of glucose tolerance factor. There was no effect of GTF observed on peak insulin, suggesting that swine tissue is very sensitive

to insulin. However, glucose tolerance factor did augment the hypoglycemic action of insulin following an intravenous insulin challenge (Steele et al., 1977). Similarly, Matthews et al. (2001) observed increased glucose clearance rates and decreased glucose half-life in growing-finishing swine supplemented with chromium (200 ppm chromium picolinate or chromium propionate) following an insulin challenge; indicating improved insulin sensitivity.

Volatile fatty acids produced by microbes present in the rumen serve as the primary energy substrates for ruminant animals. Due to the fact that ruminants must synthesize their own glucose, their response to insulin is different than that observed in non-ruminants. Growing steers tended to have lower plasma glucose concentrations when supplemented with chromium (0.2 ppm high chromium yeast); however, chromium supplementation did not affect insulin (Chang and Mowat, 1991). Chang et al. (1995) observed lower plasma glucose concentrations in growing calves supplemented with high-chromium yeast (0.75 mg Cr/kg DM) on d-57, as well as d-55 for those supplemented with $CrCl_3$, but no effect in those supplemented with $CrCl_3$ plus niacin (0.75 mg Cr/kg DM plus 100 mg niacin/kg DM). Mowat et al. (1993) also observed decreased plasma glucose in growing calves supplemented with soybean meal and chelated chromium (1 mg Cr/kg DM). However, in a second trial, chromium supplementation (0.5 mg Cr/kg DM as high chromium yeast or chelated chromium) had no effect on plasma glucose concentrations in stressed feeder calves (Mowat et al., 1993).

Following a glucose challenge, glucose clearance rates increased, while area under the curve and half life were decreased with chromium supplementation (370 μg chromium picolinate/kg DM) in steers fed a corn-cottonseed hull diet (Bunting et al., 1994). Insulin

concentrations and area under the curve were not affected by chromium supplementation following a glucose challenge. Tissue sensitivity to insulin was enhanced by chromium picolinate.

Conversely, Kegley et al. (1997a) observed no effect of chromium supplementation (0.4 mg Cr/kg of DM as $CrCl_3$ or Cr-nicotinic acid complex) on glucose clearance rate and half life following a glucose challenge in calves fed a milk replacer diet. Calves receiving $CrCl_3$ had lower peak insulin than those animals receiving the control diet or Cr-nicotinic acid complex (Cr-NAC) following a glucose challenge. However, before infusion, control calves had higher insulin than those calves supplemented with $CrCl_3$. Following an infusion of insulin, calves supplemented with $CrCl_3$ had greater decreases in plasma glucose concentrations than controls from 15 to 180 minutes. Supplementation of Cr-NAC resulted in lower plasma glucose concentrations from 45 to 180 minutes relative to controls, and from 90 to 180 minutes as compared to calves supplemented with $CrCl_3$. Serum insulin concentrations were not affected by supplementation; however, decreases in plasma glucose in response to treatment indicate increased tissue sensitivity to insulin.

Plasma glucose and serum insulin concentrations were higher in stressed calves fed a corn silage diet supplemented with Cr-NAC (0.4 mg Cr/kg DM) when compared to controls as well as those supplemented with high chromium yeast or $CrCl_3$ following a glucose challenge (Kegley and Spears, 1995). Plasma glucose concentrations were not affected by supplementation of 0.4 mg Cr-NAC/kg DM in steers stressed by shipping (Kegley et al., 1997b).

Hayirli et al. (2001) observed increased serum insulin in prepartum multiparous dairy cows fed a total mixed ration supplemented with chromium methionine (0.03, 0.06, and 0.12

mg Cr/kg $BW^{0.75}$). Chromium supplementation was not shown to have an effect on plasma glucose, serum glucagon, or the molar ratio of insulin to glucose prepartum. Postpartum concentrations of serum insulin and the molar ratio of insulin to glucose decreased in a quadratic manner with increasing level of supplemental chromium. Other blood metabolites such as glucose and serum glucagon were not affected by chromium supplementation postpartum. Glucose challenges were conducted both pre- and postpartum to determine chromium's effect on glucose metabolism in cows in late gestation as well as early lactation. Plasma glucose concentrations and area under the curve (AUC) were not affected by chromium supplementation in either the prepartum or postpartum glucose challenges. However, postpartum peak glucose and clearance rates (CR) decreased, while half life and the time to reach basal concentrations increased quadratically with increasing chromium supplementation (Hayirli et al., 2001). Basal insulin was higher in cows receiving chromium prepartum. Control animals had higher peak serum insulin and greater AUC during the postpartum challenge than those animals receiving chromium. AUC decreased linearly with increasing Cr-Met following glucose infusion prepartum.

Subiyatno et al. (1996) observed differences in glucose metabolism between primiparous and multiparous dairy cows supplemented with 0.05 ppm chromium in late gestation and early lactation. Serum insulin and the insulin to glucose ratio decreased in response to chromium supplementation in primiparous animals following a prepartum glucose challenge; however, the ratio of insulin to glucose was increased postpartum. Chromium supplementation decreased peak insulin and AUC in primiparous cows in the first 30 min following glucose infusion during the prepartum challenge. Multiparous animals had lower basal insulin than primiparous cows; however, concentrations were lower for all cows

postpartum than prepartum. Plasma chromium concentrations were lower in chromium supplemented animals, though during the glucose challenges, plasma chromium remained fairly constant with chromium supplementation, but increased for primiparous control animals.

Chromium and Lipid Metabolism

High blood cholesterol and triglycerides are recognized as risk factors in the occurrence of atherosclerosis. In rats fed a chromium deficient diet, marginal chromium deficiency resulted in slight elevation in blood cholesterol levels; however, supplementation of high-chromium yeast (45 μg Cr/100 g diet) resulted in a significant decrease in serum cholesterol (Ranhotra and Gelroth, 1986). Supplementation of $CrCl_3$ had no effect on serum cholesterol. Serum triglycerides were lowered by both high-chromium yeast and $CrCl_3$; however, the effect was more pronounced in those animals receiving high-chromium yeast. In rabbits fed a high cholesterol diet, supplementation of chromium (20 μg potassium chromate/day) resulted in the regression of atherosclerotic plaques as well as cholesterol content of the aortic tissue when compared to non-chromium supplemented controls (Abraham et al., 1980).

Riales and Albrink (1981) observed that adult men supplemented with 100 μg $CrCl_3$ for 12 weeks had progressively increasing high-density lipoprotein cholesterol (HDL-C) and decreasing serum triglyceride concentrations when compared to those individuals not receiving supplemental chromium. A slight decrease in weight was also observed in those men receiving supplemental chromium; however, serum total cholesterol and the ratios of total cholesterol or low-density lipoprotein cholesterol (LDL-C) to HDL-C were not significantly affected by treatment (Riales and Albrink, 1981). The authors suggested that

low HDL-C and high triglycerides are a result of insulin resistance (Riales and Albrink, 1981).

Page et al. (1993) observed lower serum cholesterol and back fat thickness over the tenth rib in growing-finishing swine supplemented with chromium picolinate when compared to controls; however, serum triglycerides were not affected by supplementation. In another study, chromium picolinate did not influence average backfat or 10^{th} rib backfat in growing-finishing swine; however, percent of fat tissue in the carcass and percent lipid in the muscle were decreased by supplementation (Mooney and Cromwell, 1995). Similarly, Matthews et al. (2001) observed no effect of chromium supplementation (200 ppm Cr as chromium picolinate or chromium propionate) on backfat thickness in growing-finishing swine. Supplementation of chromium picolinate resulted in lower plasma nonesterified fatty acid concentrations when compared to swine supplemented with chromium propionate and controls; however, serum cholesterol, HDL cholesterol, and HDL cholesterol to total cholesterol ratio were not affected by chromium, regardless of source (Matthews et al., 2001).

Wether lambs supplemented with 250 μg chromium picolinate/kg DM had lower plasma NEFA concentrations than non-supplemented animals; however, no effect was observed on serum cholesterol (Kitchalong et al., 1995). Kitchalong et al. (1995) observed lower lean carcass yield grades, decreased fat over the 10^{th} rib, and a trend for decreased pelvic fat in wethers supplemented with chromium picolinate. Chromium supplementation (0.2 ppm high chromium yeast) did not affect cholesterol or free fatty acids in growing steers (Chang and Mowat, 1992). Similarly, Bunting et al. (1994) did not observe an effect of

chromium picolinate (370 μg Cr/kg DM) on concentrations of plasma NEFA in steers fed a corn-cottonseed hull diet; however, a decrease in serum cholesterol was observed.

Hayirli et al. (2001) observed lower plasma NEFA concentrations in prepartum multiparous dairy cows fed a total mixed ration supplemented with chromium methionine (0.03, 0.06, and 0.12 mg Cr/kg $BW^{0.75}$) when compared with control animals. Plasma NEFA concentrations were not affected by chromium supplementation in postpartum cows; however, basal NEFA concentrations decreased linearly with increasing Cr-Met following glucose infusion prepartum. No effect was observed on plasma NEFA concentrations in dairy cows supplemented with 0.5 mg chelated chromium from 6 weeks prepartum to 16 weeks postpartum, regardless of parity (Yang et al., 1996). Subiyatno et al. (1996) observed a decrease in serum triglycerides in primiparous dairy cows supplemented with 0.05 ppm chromium in late gestation; however, serum triglycerides increased during early lactation.

Chromium and Protein Metabolism

In rats fed a low-protein diet, chromium supplementation resulted in increased transport of the amino acid analog, α-aminoisobutyric acid, into tissues (Roginski and Mertz, 1969). Roginski and Mertz (1969) also observed that incorporation of glycine, serine, and methionine into protein was depressed in chromium deficient animals. Similarly, chromium supplementation was shown to increase protein concentrations in the heart and liver of rats fed a low-protein diet (Mertz and Roginski, 1969). These increases in protein accumulation in the organs of these animals resulted in greater overall weight gain when compared to rats not receiving supplemental chromium.

Lean body mass in humans participating in an aerobics class increased significantly with supplementation with chromium picolinate; however, lean body mass of individuals

supplemented with chromium nicotinate was not affected (Evans and Pouchnik, 1993). In swine, supplementation of chromium picolinate increased longissimus muscle area and percentage of muscling in the carcass (Page et al., 1993). Mooney and Cromwell (1995) observed increases in accretion rate of muscle with supplementation of chromium picolinate in growing-finishing pigs; however, supplementation did not affect longissimus muscle area. Conversely, Matthews et al. (2001) found no effect of chromium picolinate supplementation on longissimus muscle area or percent muscling of carcass in growing-finishing barrows. Chang et al. (1992) observed no effect of high-chromium yeast on carcass loin eye areas of growing-finishing cattle.

Chromium and Immune Response

Reported signs and symptoms of chromium deficiency in ruminants include increased prevalence of morbidity and decreased humoral immune response (Anderson, 1994). Kelley (1988) postulated that increases in plasma cortisol concentrations are responsible for decreases in T-cell mediated immune response induced by stress. The stress associated with weaning, marketing, transport, and adaptation to feedlots increases the susceptibility of animals to Bovine Respiratory Disease Complex (BRDC) which may result in increased mortality and morbidity as well as poor performance and losses due to shrinkage (Wright et al., 1993). High chromium yeast, supplemented (0.4 ppm) to stressed calves during the first 28 d post market and transport, had no effect on morbidity (Chang and Mowat, 1992). However, Chang and Mowat (1992) observed decreases in serum cortisol during a 70 day growing period in steers supplemented with chromium (0.2 ppm high chromium yeast). Mathison and Engstrom (1995) supplemented chromium as an amino acid chelate (0.5 mg

Cr/kg DM) to stressed calves, but observed no effect on morbidity in the growing or finishing phase of the trial.

Supplementation of high-chromium yeast (0, 0.2, 0.5, and 1 ppm Cr) was observed to decrease morbidity and rectal temperatures in stressed steer calves fed a corn silage diet (Moonsie-Shageer and Mowat, 1993). On day 28 of the study serum cortisol was shown to decrease linearly with increasing level of chromium supplementation; however, no effect was observed on day 7, 14, or 21.

Kegley and Spears (1995) observed no effect of chromium supplementation (0.4 mg Cr/kg of DM as high-Cr yeast, Cr-NAC, or $CrCl_3$) on serum cortisol in stressed feeder calves. In steers, no effect of chromium supplementation was seen upon serum cortisol prior to shipping (Kegley et al., 1997b). However, after a 2 hour transport, serum cortisol was higher in shipped steers than those steers that had not been shipped. No difference between treatments was seen in serum cortisol 7 days after shipping.

Similarly, Chang et al. (1995) observed little affect of chromium supplementation (0.75 mg Cr/kg DM as high-chromium yeast, $CrCl_3$, or $CrCl_3$ plus 100 mg niacin/kg DM) on serum cortisol in stressed feeder calves. Supplementation of high-chromium yeast resulted in decreased serum cortisol concentrations on d 57 of the study, but the inorganic form, with or without supplemental zinc, had no effect on any sampling day (Chang et al., 1995). The authors suggested that the apparent ineffectiveness of the inorganic chromium was a result of low availability for absorption (Chang et al., 1995). Supplementation of chromium, regardless of source, did not affect overall morbidity in this study.

Chelated chromium (1 mg Cr/kg DM) decreased serum cortisol in steers during a 56 d growing period; however, in a second trial, chromium supplementation (0.5 mg Cr/kg DM as

high-chromium yeast or chelated chromium) did not affect serum cortisol concentrations (Mowat et al., 1993). Both sources of chromium decreased morbidity in the steers during the second trial period, though a greater decrease was observed in animals receiving chelated chromium. Mowat et al. (1993) postulated that this large decrease in morbidity was a result of early chromium supplementation relative to the onset of animal illness.

Cr-NAC supplementation (0.4ppm) to calves fed milk replacer resulted in lower body temperatures following a disease challenge with an intranasal dose of infectious bovine rhinotracheitis (Kegley et al., 1996). White cell counts were also higher in calves receiving Cr-NAC supplementation five days after disease challenge than in controls, but at 12 days post challenge, no difference remained. Both Cr-NAC and $CrCl_3$ lowered serum cortisol concentrations in the calves 5 days after the challenge. Prior to the challenge, differences in cortisol concentrations between the treatments were not detectable. Ratio of neutrophils to lymphocytes, a stress indicator in calves, was not affected by Cr supplementation.

Cell-mediated Immune Function

Cell-mediated immune function was shown to be enhanced in Holstein bull calves fed a milk replacer diet supplemented with 0.4 ppm of Cr-NAC or $CrCl_3$ (Kegley et al., 1996). Skinfold thickness was greater in calves supplemented with Cr-NAC at 6, 12, 24, and 48 h after injection of phytohemagglutinin (PHA) than controls, while $CrCl_3$ supplemented animals only showed greater response at 24 and 48 h (Kegley et al., 1996). Increased skin fold thickness following an intradermal injection of PHA was also observed in Angus cross steers fed a corn silage diet provided with 0.4 mg of high-chromium yeast/kg of DM, when compared to those steers receiving $CrCl_3$, CrNAC, or the control diet (Kegley and Spears, 1995). In contrast, cell-mediated immune function was shown to be depressed in stressed

steers supplemented with chromium (0.4 mg Cr/kg of DM as Cr-NAC), as measured by decreased response to dinitrochlorobenzene (DNCB) 24 and 48 h after application (Kegley et al., 1997b). Conversely, supplementation of high-chromium yeast (0, 0.2, 0.5, or 1 ppm Cr) to stressed steer calves was not shown to affect contact sensitivity to DNCB (Moonsie-Shageer and Mowat, 1993).

Responses of lymphocytes from calves to phytohemagglutinin or pokeweed mitogen (PWM) were not affected by chromium supplementation (0.4 ppm of Cr-NAC or $CrCl_3$) in vitro (Kegley et al., 1996). Blastogenic response of unstimulated steer lymphocytes or those cultured with 10 and 5 μg/mL of PWM, or 6.25 μg/mL PHA, were not affected by chromium supplementation (0.4 mg Cr/kg of DM of high-chromium yeast, Cr-NAC, or $CrCl_3$). However, greater blastogenic response to 12.5 μg PHA/mL was observed in peripheral lymphocytes from steers supplemented with Cr-NAC than with lymphocytes from controls, or $CrCl_3$ (Kegley and Spears, 1995). Following incubation of peripheral blood mononuclear cells (PBMC) from periparturient dairy cows in concanavalin A, blastogenic activity was increased in cells from cows supplemented chromium (0.5 ppm amino acid chromium chelate) from 6 weeks prepartum through 16 weeks postpartum, when compared to controls (Burton et al.., 1993).

Humoral Immune Response

The effect of chromium supplementation on humoral immune response in ruminants has yielded variable results. Total antibody, immunoglobulin G, and IgM titers to porcine red blood cells were not affected in calves when chromium (0.4 ppm $CrCl_3$ or Cr-NAC) was added to a milk replacer diet (Kegley et al., 1996). Antibody titers to *Pasteurella hemolytica* were not found to be affected by chromium in the calves after administration 5 d following a

disease challenge with infectious bovine rhinotracheitis virus (IBRV). Kegley and Spears (1995) observed greater serum IgG concentrations in steers supplemented with Cr-NAC (0.4 mg Cr/kg DM) when compared to controls and those receiving high-Cr yeast, and $CrCl_3$. Chromium did not affect total serum IgM or antibody titers to *P. hemolytica* vaccination.

In a study to determine the effect of chromium supplementation and stress on immune response, serum IgM concentrations were not affected by supplementation (0.4 mg Cr/kg of DM as Cr-NAC) in non-stressed animals, while IgG concentrations decreased (Kegley et al.., 1997). Stressors included shipping, food and water deprivation, and a disease challenge. Chromium did not affect serum total IgM concentration or antibody responses to IBRV and porcine red blood cells (PRBC) post shipping; however, total IgG concentrations were decreased by treatment. High chromium yeast (0.2 ppm) added to a SBM supplement increased IgM and total immunoglobulins, and tended to increase IgG1 and IgG2 in growing steers (Chang and Mowat, 1991).

Moonsie-Shageer and Mowat (1993) observed increased primary antibody response to human erythrocytes with supplementation of high-chromium yeast on day 14 of the study, however, no effect was observed on day 21. IgM and IgG2 were not affected by high-chromium yeast.

Burton et al. (1993) observed increased primary and secondary antibody response to ovalbumin in dairy cows supplemented with chromium (0.5 ppm amino acid chromium chelate) to ovalbumin when compared to controls. However, supplemental chromium had no effect on antibody response when these animals were injected with human erythrocytes.

Chromium and Production

Potential benefits of supplementing chromium to livestock have been shown including improved performance in growing and finishing swine and ruminants. Page et al. (1993) observed increased average daily gains and dressing percentages in growing-finishing swine supplemented with chromium picolinate. Improved growth rates and total gain of lean tissue were reported in growing-finishing swine supplemented with chromium picolinate; however, no effect of supplementation was observed on feed efficiency (Mooney and Cromwell, 1995). Conversely, Matthews et al. (2001) reported decreases in daily gain and average daily feed intakes of growing-finishing swine supplemented with 200 ppm chromium picolinate or chromium propionate during the growing phase. Chromium supplementation did not affect average daily gain, average daily intake, gain to feed ratio, or carcass characteristics during the finishing phase of the trial (Matthews et al., 2001).

Kegley et al. (1997b) found that average daily gains, dry matter intake, and gain to feed ratios were not affected by chromium supplementation (0.4 mg Cr-nicotinic acid complex/kg DM) in steers prior to or following shipping and disease challenge. However, Cr-nicotinic acid complex (Cr-NAC) supplementation did increase average daily gains over the course of the entire 80 day study. Supplementation of 0.4 ppm high chromium yeast increased average daily gains (ADG) and feed efficiency in stressed feeder calves during first 28 d following market and transport (Chang and Mowat, 1991). However, when calves received both a long-acting oxytetracycline injection 48 h after arrival to the feedlot, and supplemental chromium, no effect on performance was observed. During the 70 d growing phase of the trial, chromium supplementation (0.2 ppm high chromium yeast) had no effect on ADG, DMI, or feed efficiency.

Chang et al. (1995) observed source differences in the effect of chromium supplementation on performance in stressed feeder calves. When 0.75 mg Cr/ kg DM was added to the corn silage diet as high chromium yeast, no effect on performance was observed in the steers. However, when $CrCl_3$ (0.75 mg Cr/kg DM) and niacin (100 mg niacin/kg DM) were added to the diet, steers had increased weight gains and feed efficiencies during the initial 28-d stress period (Chang et al., 1995). No effect was observed during the 28-d growing phase.

Similarly, Wright et al. (1994) observed positive effects of chromium supplementation and vaccination on performance in stressed feeder calves. While treatment had no effect on feed efficiency, dry matter intake was higher in those animals receiving 0.14 mg amino acid chelated chromium/kg DM plus an intramuscular vaccination cocktail (4 ml i.m. vaccine with IBR, Parainfluenza-3 (PI3), Bovine viral diarrhea (BVD), and Bovine Respiratory Syncytial Virus (BRSV), as well as a 2 ml i.m. vaccine against *Pasteurella haemolytica*) and those animals just receiving the vaccines when compared to controls. Average daily gains were also higher in those steers receiving chromium plus the vaccinations when compared to controls, but they did not differ from those animals receiving only chromium or only the vaccines.

In a study conducted by Kegley and Spears (1995), chromium supplementation (0.4 mg Cr/kg DM of $CrCl_3$, high chromium yeast, or Cr-NAC) did not affect average daily gains, feed intake, or gain to feed ratios in steers fed a corn silage diet. Chromium supplementation (0.5 mg/kg DM as amino acid chelate) failed to have an effect on carcass characteristics (grade or liver abscesses), when added to a corn silage diet fed to finishing steers (Mathison and Engstrom, 1995). Mowat et al. (1993) observed no effect on average daily gains, dry

matter intake, or feed efficiency in steers fed a corn silage diet supplemented with soybean meal and 1 mg/kg of a chromium chelate.

Moonsie-Shageer and Mowat (1993) observed increased average daily gains and dry matter intake in steer calves supplemented different levels of high-chromium yeast (0, 0.2, 0.5, and 1 ppm Cr) to a corn silage diet. At 0.2 and 1 ppm of supplemental chromium, average daily gains were increased by 27 percent. Dry matter intake was increased at supplementation levels of 0.2 and 0.5 ppm over the course of the 30 day trial, but feed efficiency was not affected.

Kegley et al. (1997a) observed higher average daily gains and feed to gain ratios between d 28 and 42 in calves fed milk replacer supplemented (0.4mg Cr/kg of DM) with Cr-NAC, when compared to controls and those receiving $CrCl_3$. However, overall performance was not affected. Growth performance in Holstein calves fed a corn-cottonseed hull diet was not affected by chromium picolinate supplementation (370 μg Cr/kg diet), though animals exhibited little indication of physiological stress (Bunting et al., 1994). It is possible that the lack of performance response to chromium supplementation was due to Cr status of the animal, the form of chromium that was used, or stress factors affecting the animals.

In studies conducted in dairy cows, chromium supplementation has been shown to increase milk yields, dry matter intake (DMI), and decreased placental retention, and udder edema in older cows. Hayirli et al. (2001), found that supplementing chromium (0, 0.03, 0.06, and 0.12 mg of Cr-Met/kg of $BW^{0.75}$ as a gel bolus) to multiparous Holsteins from 21 days prior to 28 days post calving resulted in quadratic increases in milk yield, fat, and lactose. Dry matter intake was increased by increasing Cr-Met supplementation in prepartum

and postpartum periods. Supplementation did not affect body weights, but body condition score increased with increasing levels of Cr-Met postpartum (Hayirli et al., 2001).

Increased milk yields were also observed in primiparous Holsteins supplemented 0.5 mg/kg DM of chelated chromium from 6 weeks prepartum through 16 weeks postpartum; however there was no effect on milk yield observed in multiparous animals (Yang et al., 1996). Weight loss occurred in chromium supplemented primiparous cows and weight gain in control animals, however there was no effect seen in multiparous animals regardless of treatment, or in BCS or energy balance in any of the animals. Reproductive performance was not adversely affected in primiparous cows despite increased milk yield, and may have decreased the number of primiparous cows that did not conceive. Days to first estrus, teat and udder edema tended to be decreased, in multiparous and parity 3 or more cows respectively, with supplementation of chromium. Conversely, Subiyatno et al. (1996) observed no effect of chromium supplementation (0.5 ppm) on milk yield in either primiparous or multiparous cows when they were supplemented from late gestation through early lactation. Dry matter intake, body condition score, and body weight loss were also unaffected. In a second experiment conducted only in multiparous cows, Subiyanto et al. (1996) did observe an increase in daily milk production in weeks 2 and 6 with supplementation.

Supplementation of organic chromium (3.5 mg Cr/h/d) during the last 9 weeks of pregnancy decreased the incidence of placental retention in multiparous Holsteins with a high herd history of this reproductive problem (Villalobos-F et al., 1997). Calf birth weight was not affected by chromium supplementation. Villalobos-F et al. (1997) suggest a link between placental retention and high serum cortisol levels induced by the stress associated with

parturition. Chromium may have influenced serum cortisol in those animals receiving supplementation resulting in a decrease in placental retention.

Copper in Ruminants

The essentiality of copper for cattle was first established when a copper deficiency was observed in grazing Jersey cattle in Florida (Becker et al., 1965). This wasting disease, termed "salt sick", was found to be caused by deficiencies in cobalt, iron, and copper. Estimates of the copper requirements of sheep, beef, and dairy cattle have been established by the National Research Council to be 7-11 mg/kg, 10 mg/kg, and 10 mg/kg, respectively (NRC, 1985, 1996, 1989). In 1933, scientists in Northern Europe discovered that a wasting disease in sheep and cattle with diarrhea, loss of appetite, and anemia was caused by Cu deficiency (McDowell, 1992). Animals suffering from the disease were cured by copper therapy. In Western Australia, copper supplementation prevented the occurrence of enzootic neonatal ataxia of sheep caused by copper deficiency (Bennetts and Chapman, 1937). "Falling-disease", a seasonally linked disorder, resulting in sudden death in cattle was associated with severe copper deficiency in parts of south-western Australia (Bennetts and Hall, 1939).

Chapman and Bell (1963) found that copper source has a profound impact on absorption in steers with cupric carbonate being the most readily available source of inorganic copper. Despite its relatively low absorption rate, cupric oxide needles are often administered to cattle. Due to their particle size, cupric oxide needles move slowly through the gastro-intestinal tract and act as a method of slow-release copper supplementation (Chapman and Bell, 1963).

Ruminants are more likely to suffer from Cu deficiency than monogastrics, under grazing conditions due to low absorption (1-3% of copper intake) of copper (McDowell, 1992). The ability of the rumen to degrade organic and inorganic sources of sulfur to sulfide makes ruminants more susceptible to Cu deficiency than non-ruminants (Underwood and Suttle, 1999). Sulfides created by rumen protozoa bind to copper in the rumen causing it to precipitate out as copper sulfide, making it unavailable to the animal. Thiomolybdates, formed when molybdenum and sulfur are rich in the diet, also bind copper into insoluble complexes, preventing absorption.

Copper deficiency may manifest itself in a variety of visual and physiological abnormalities. An animal's species, age, sex, environment, and duration of deficiency all affect how the copper deficiency will express itself. Anemia, bone deformities, neonatal ataxia, depigmentation of hair or wool, defective keritinization of wool, fibrosis of the myocardium, and scouring are all manifestations of copper deficiency. Copper deficiency primarily manifests itself as achromotrichia in many species. This condition, attributed to a lack of tyrosinase, results in the inability for tyrosine to be converted to melanin (McDowell, 1992).

Howell and Davidson (1959) reported the cause of neonatal ataxia to be decreased cytochrome oxidase activity leading to degeneration of nervous tissue in the brain and spinal chord. The sudden cardiac failure typified by "falling disease", is due to atrophy of the myocardium with extensive fibrosis. Underwood (1966) states that while the degree of fibrosis is not well linked to animal death, most deaths occurred in the spring while animals are in a state of moderate to severe anemia. Decreased lysyl oxidase in the heart is thought to be the cause of myocardial fibrosis (McDowell, 1992). The Cu-containing enzyme, lysyl

oxidase, is essential in the formation of cross-links in collagen and elastin which lend structural integrity and elasticity to proteins. This cross-linking between collagen fibers is achieved only after lysyl oxidase adds a hydroxyl group to the lysine residues present in collagen (McDowell, 1992).

Anemia in sheep and cattle is often associated with ataxia, poor keritinization of wool, and 'falling disease,' but it can be very mild and does not occur in all animals exhibiting other symptoms. Copper is needed in trace amounts to catalyze the reaction which allows the body to use iron for hemoglobin formation. Iron must be converted from the ferric to the ferrous form to be incorporated in hemoglobin or myoglobin, or be mobilized from stored ferritin (McDowell, 1992). Ferrous iron must be converted back to the ferric form by ceruloplasmin before it can be stored as ferritin or incorporated into transferrin for transport (Curzon, 1960).

In copper deficient animals, lipid metabolism is often altered, resulting in elevated levels of triglyceride, phospholipids, and cholesterol in the serum (McDowell, 1992). Alterations in lipid metabolism have also been observed following supplementation of copper at levels at or above NRC requirements. Serum cholesterol, longissimuss muscle cholesterol, and backfat thickness were decreased in steers supplemented with 10 or 20 mg of copper sulfate/kg diet dry matter relative to controls (Engle and Spears, 2000). Similarly, Engle et al. (2000) observed decreased backfat and longissimuss muscle cholesterol in steers supplemented 10 or 40 mg of copper sulfate. The authors suggest that supplemental copper alters the metabolism of adipose tissue as well as its response to hormones responsible for lipolysis (Engle et al., 2000).

Effect of Breed on Copper Status

Copper status is known to vary between species, but there is evidence that different breeds within a species differ in their copper requirements as well. This was first observed in work with different breeds of sheep. Wiener et al. (1969) observed that plasma concentrations of copper were lower in Scottish Blackface than Welsh Mountain sheep when animals were kept in the same environment. In later work, three sheep breeds (Scottish Blackface, Welsh Mountain, and North Ronaldsay) were used to determine differences in copper metabolism in a repletion study (Wiener et al., 1978). Lambs of the three breeds were fed the depletion diet until plasma copper concentrations reached approximately 0.3 mg Cu/liter. When copper was added to the diet, North Ronaldsay lambs had a much higher rate of repletion relative to the other two breeds. Welsh Mountain lambs demonstrated intermediate repletion rates, while Scottish Blackface had the slowest. The same trend in repletion rate among the lamb breeds was observed after two subsequent depletions.

Smart and Christensen (1985) observed lower concentrations of plasma copper in Simmental-sired heifers when compared to Hereford and Angus-sired heifers in a study to determine the effects of copper intake, sire breed and age on copper status. Ward et al. (1995) observed similar results in Angus, Charolais, and Simmental heifers and calves fed either a control diet (4.5 ppm Cu), or one supplemented with copper sulfate (14.5 ppm Cu). Heifers receiving supplemental copper did not differ significantly in plasma copper concentrations over the course of the study; however, differences were observed in those animals not receiving supplementation. Plasma copper concentrations and ceruloplasmin activity were higher in Angus heifers than in Charolais and Simmentals when animals did not receive supplementation (Ward et al., 1995). However, plasma copper concentrations of

Charolais and Simmentals did not differ at any sampling day during the study. Among calves born to copper supplemented heifers, Angus calves had higher plasma copper than Simmental calves. Ward et al. (1995) suggested that the higher plasma copper concentrations observed in Angus calves were resultant of copper supplementation allowing greater Cu storage in the fetuses of Angus than those of the other two breeds. Among calves born to heifers not receiving supplemental copper, Angus had higher plasma copper and ceruloplasmin activity than Simmental calves and tended to have higher plasma copper than Charolais on d 196. Even though Angus had higher plasma copper, all calves were copper deficient based on plasma copper concentrations; Simmentals being severely deficient (Ward et al., 1995).

To further investigate the large difference in plasma copper concentrations between Angus and Simmental animals, steers were used in a metabolism study to examine copper absorption and retention between the two breeds (Ward et al., 1995). Angus steers had higher plasma copper concentrations than Simmental at weaning, as well as the beginning and end of the metabolism crate phase. Simmental had lower apparent absorption of copper than Angus steers did, indicating that absorption was lower in Simmental steers or endogenous losses were higher (Ward et al., 1995). The authors observed no difference in urinary excretion between the two breeds and suggested that the gastrointestinal tract of Angus is more permeable to Cu than that of Simmental, thus resulting in higher copper retention and copper status in Angus animals (Ward et al., 1995).

Similarly, Mullis et al. (2003a) found Simmental steers had lower plasma copper concentrations than Angus throughout the duration of a study to determine the effect of copper and zinc source on mineral status of steers fed a diet high in iron. Copper source had

no effect on serum copper concentrations, ceruloplasmin activity, or liver copper concentrations. Simmental had lower ceruloplasmin activity and liver copper concentrations than Angus throughout the duration of the study.

Mullis et al. (2003b) used Simmental and Angus heifers to further explore the differences in copper requirements between the two breeds. Heifers were fed a corn silage-based diet with 0, 7, or 14 mg of Cu/kg of DM supplemented as copper sulfate for 160 days. During the growing phase of the trial, copper supplementation increased plasma copper and ceruloplasmin activity in Simmental heifers. However, copper supplementation did not affect ceruloplasmin in Angus heifers and only served to increase plasma copper concentrations on day 93 and 121 (Mullis et al., 2003b). Copper supplementation increased liver copper concentrations, but no difference between breeds was observed. Breed differences were observed between unsupplemented animals as well as those heifers receiving 14 mg of supplemental copper. In control heifers, Simmental had lower plasma copper concentrations and ceruloplasmin activity than Angus, but in those heifers fed supplemental copper at 14 mg/kg Simmental heifers had higher plasma copper concentrations than Angus on day 65 and 121 (Mullis et al., 2003). No breed difference in plasma copper levels were observed in those heifers receiving 7 mg/kg, and no difference in ceruloplasmin activity was observed with either 7 mg/kg or 14 mg/kg of supplemental copper. The second trial began as heifers reached the last trimester of pregnancy to determine the effect of breed on copper requirements in late gestation and early lactation. Simmental heifers on the control diet had lower plasma copper concentrations than Angus, but no breed difference was observed in those animals receiving 7 mg/kg, and Simmental were shown to have only slightly lower concentrations than Angus on three of the sampling

dates (Mullis et al., 2003b). Ceruloplasmin was shown to be lower in Simmental heifers receiving the control diet and 7 mg/kg copper when compared to the Angus heifers on the same treatments. However, at 14 mg/kg of supplementation, Simmental heifers had lower ceruloplasmin activity than Angus on only one sampling day. Mullis et al. (2003b) found that Angus had higher final liver copper concentrations that Simmental heifers, and that liver copper in both breeds was greatly increased by copper supplementation. In the calves of control heifers, Simmental had lower plasma copper levels than Angus. Simmental calves receiving 7 mg/kg had lower plasma copper concentrations than Angus on day 73 and 101 after calving; however, plasma copper concentrations of those calves fed 14 mg/kg did not differ between breeds.

The difference in copper status between these two breeds may be explained in terms of biliary copper excretion. Gooneratne et al. (1994) found that biliary copper concentrations and copper excretion were approximately two times higher in Simmental cattle than in Angus. The propensity of Simmental to excrete copper may explain why deficiency occurs more frequently in Simmental cattle, resulting in higher copper requirements to meet the animal's needs.

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RUNNING HEAD: EFFECTS OF CHROMIUM ON METABOLISM

Effect of Chromium Supplementation and Copper Status on Glucose and Lipid Metabolism in Reproducing Beef Cows[1]

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ABSTRACT: Pregnant Angus (n=83) and Simmental (n=69) cows were blocked by age and breed and randomly assigned to one of two free-choice mineral supplements to determine the effect of dietary chromium (Cr) and copper (Cu) status on glucose metabolism in beef cows. Supplements consisted of: 1) control (no supplemental Cr) and 2) 40 mg Cr /kg of mineral (from Cr picolinate). Mineral supplements were formulated to contain all minerals typically supplemented to cattle diets with the exception of Cu. The study began approximately 75 d prepartum, at which point half of the cows in each treatment received a 25 g Cu oxide needle bolus. Blood was collected from 35 cows on d 28, 58, 97 (approximately 20 d postpartum), 155, 210, and 279 for plasma glucose and non-esterified fatty acid (NEFA) determination. Chromium supplementation reduced ($P < 0.05$) plasma glucose concentrations. Plasma glucose concentrations were also affected by breed x Cu bolus ($P < 0.05$). In non-Cu supplemented cows, plasma glucose levels were higher ($P < 0.05$) in Angus than in Simmental. In animals receiving a Cu bolus, plasma glucose levels were similar in both breeds. Plasma NEFA concentrations were affected by time ($P < 0.01$), Cr x Cu bolus ($P < 0.05$), Cr x time ($P < 0.01$), and Cr x replicate ($P < 0.01$). On d 97 and 155, plasma NEFA concentrations were lower ($P < 0.01$) in cows receiving Cr relative to controls. Chromium supplementation reduced ($P < 0.01$) plasma NEFA concentrations in 2 and 3 (rep 3) and 4 and 5 year-old cows (rep 2) but not in older cows (rep 1), relative to control animals in those replicates. Chromium supplemented animals had lower ($P < 0.05$) plasma NEFA concentrations than controls in animals that did not receive Cu bolus. No significant difference in plasma NEFA concentrations were observed between treatments in animals that received a Cu bolus. At approximately 1 mo prepartum and 1 mo postpartum, 12 cows were cannulated and glucose tolerance tests (GTT) were conducted. Cows used in

GTT received 0.15 g/kg body weight of a 50% dextrose solution. Chromium supplemented animals had lower plasma glucose ($P < 0.01$), serum insulin ($P < 0.05$), and NEFA ($P < 0.01$) concentrations following the GTT conducted prepartum than control cows. Clearance rates for glucose were not affected by treatment. In postpartum GTT, plasma glucose was affected by an interaction between Cr-supplementation and Cu status. Chromium-supplemented animals that received a Cu bolus had higher ($P < 0.001$) plasma glucose after glucose administration than cows not supplemented with Cu. No significant difference in plasma glucose was observed between control cows regardless of Cu status. Chromium supplemented animals had lower ($P < 0.05$) serum insulin concentrations 10 to 45 minutes after glucose administration than controls. Plasma NEFA concentrations were affected by a Cr x Cu bolus x time interaction ($P < 0.01$) following the glucose challenge. Results of this study indicate that plasma glucose is lower in cows receiving supplemental Cr and that an interaction between Cr and Cu status may alter glucose metabolism.

Key Words: Chromium, Cattle, Copper, Glucose Tolerance

1. Introduction

Chromium potentiates the action of insulin (Davis and Vincent, 1997; Matthews et al., 2001). Altered glucose metabolism has been reported following chromium supplementation in rats (Schwartz and Mertz, 1959), humans (Anderson et al., 1991), swine (Matthews et al., 2001), and ruminants (Bunting et al., 1994; Chang et al., 1995; Kegley et al., 1997; Hayirli et al., 2001).

Previous chromium research in ruminants has been conducted in pre-ruminant calves (Kegley et al., 1997), growing and finishing steers (Bunting et al., 1994; Chang et al., 1995) and dairy cattle (Subiyatno et al., 1996; Hayirli et al., 2001); however, no research has been

done to determine the effect of dietary chromium on glucose metabolism in reproducing beef cows. Volatile fatty acids produced by microbes present in the rumen serve as the primary energy substrates for ruminant animals. Due to the fact that ruminants must synthesize their own glucose, their response to insulin is different than that observed in non-ruminants (Fahey and Berger, 1988).

Currently, there is no established chromium requirement for ruminants. Improvement in glucose tolerance after chromium supplementation is the most sufficient means to determine if chromium is limiting insulin responsiveness. Delayed insulin response and general glucose intolerance has also been shown in copper-deficient rats (Choudry et al., 1981; Hassel et al., 1983); suggesting that copper may play a role in glucose metabolism as well. Consequently, the objective of this study was to determine the effect of supplemental chromium and copper status on glucose metabolism and blood metabolites in reproducing beef cows.

2. Materials and Methods

2.1. Protocol

Care, handling, and sampling of animals in this study were approved by the North Carolina State University Animal Care and Use Committee. One-hundred and fifty-two pregnant Angus (n=83) and Simmental (n=69) cows were blocked by breed and age and randomly assigned to one of two free-choice mineral supplements to determine the effect of dietary chromium and copper status on glucose metabolism in beef cows. Supplements consisted of: 1) control (no supplemental chromium) and 2) 40 mg chromium/kg of mineral (from chromium picolinate). Mineral supplements were formulated to contain all minerals typically supplemented to cattle diets with the exception of copper (Table 1). Cows were

divided into three replicates per treatment, according to their age with Angus and Simmental equally represented in each treatment. Replicate one contained cows 6 years of age or older (584.4 ± 6.5 kg). The second replicate contained cows of 4 to 5 years of age (542.2 ± 7.9 kg). Replicate three was composed of 2 and 3 year old females (468.9 ± 6.9 kg). The study began approximately 75 d prepartum, at which point half of the cows in each treatment received a 25 g copper oxide needle bolus (Copasure®).

Cattle were rotated among tall fescue pastures at 14-day intervals to correct for any pasture differences during the grazing season. During the winter cattle were fed grass hay free choice. In addition to hay, cows in all replicates were fed 5.5 kg (DM) of corn silage and 0.9 kg of corn gluten feed following calving; however, on a $DM \cdot kg^{-1}$ of body weight basis, heifers (replicate 3) received more energy and protein than older cows. Hay and silage samples were taken during the non-grazing months at 28-day intervals for copper analysis.

Calves were born between d 43 (October 16) and 104 (December 15). Calves born to copper supplemented dams were given a 12.5 g copper oxide needle bolus (Copasure®) on d 196 (March 17). Cows were synchronized using an injection of gonadotropin releasing hormone (GnRH, Fertagyl, 100 μg) followed seven days later by an injection of prostaglandin F2α (PGF, Lutalyse, 25 mg). Cows were observed for estrus twice daily for 72 hours following the Lutalyse injection and were inseminated using the AM-PM protocol if detected in estrus. All cows not observed in estrus by 72 hours were injected with another dose of GnRH and artificially inseminated on d 155 (February 5). Cows were then exposed to Angus sires for 28 days, beginning on d 159 (February 9). Cows were palpated to determine pregnancy and calves were weaned on d 284 (June 14). One cow died on d 5 of the study from bovine leukemia. Two cows died on d 70 and d 217 of the study from cancer.

Four cows were removed from the study on d 111; two of which had calves born dead, and two that were found to be open.

Cows were weighed at 28-d intervals throughout the 284-d trial. Thirty-five cows were bled via jugular venipuncture on d 28, 58, 97, 155, 210, and 279 for the determination of plasma glucose and non-esterified fatty acid (NEFA) concentrations. Samples for plasma glucose and NEFA were collected in tubes containing potassium oxalate and sodium fluoride (Vacutainer 6383, Becton Dickinson, Franklin Lakes, NJ).

Glucose challenges were conducted on d 65 (n=11) and d 126 (n=12). One cow calved before the prepartum glucose challenge could be conducted. Two cows per replicate per treatment were used in the glucose challenge; however, only 3 year old cows were chosen to represent replicate three. Cows were cannulated in the jugular vein approximately 24-hours prior to the glucose challenge to minimize the effects of stress. Cows were moved into barns approximately 3 days prior to each challenge and fed hay (ad libitum) and corn silage top dressed with 0.11 kg of their treatment mineral supplement. Following the regular morning feeding, 0.15 g/kg of body weight of a 50% dextrose solution was administered via the jugular catheter over a period of 2 minutes. Blood samples were taken at -5, 0, 5, 10, 20, 30, 45, 60, 90, and 120 minutes relative to infusion. Blood samples for serum insulin determination were put into glass tubes and kept on ice until centrifuged. Blood for plasma glucose and NEFA concentration determination were put in tubes containing potassium oxalate and sodium fluoride (Vacutainer 6383) and kept on ice until centrifuged.

2.2. Analytical procedures

Blood was transported on ice to the laboratory and centrifuged at 2500 x *g* for 20 min. Plasma and serum were removed and frozen at -20°C until analysis for glucose, insulin, and

NEFA concentrations. Plasma glucose was determined by electrochemical sensor coupled to a membrane-immobilized glucose oxidase enzyme (Industrial Analyzer, Model 27, Yellow Springs Instrument, Yellow Springs, OH). Serum insulin was determined by radioimmunoassay using antibody coated tubes (ImmuChem^{TM} Insulin Coated Tube, ICN Pharmaceuticals, Inc., Costa Mesa, CA). Plasma NEFA concentrations were determined by an enzymatic, colorimetric method (WAKO Pure Chemical Industries, Ltd., Richmond, VA).

2.3. Data analysis

Data were analyzed by repeated measures using the Proc Mixed procedure of SAS (1988). Individual animal was used as the experimental unit for blood data. The model for blood data included treatment, breed, bolus, time, replicate, treatment x breed, treatment x bolus, treatment x time, treatment x replicate, bolus x breed, breed x time, and bolus x time. The model for the prepartum glucose challenge included treatment, time, breed, treatment x time, breed x time, and the breed x treatment interaction. Bolus was not included in the model for the prepartum glucose challenge due to one cow calving prior to the glucose challenge and a lack of degrees of freedom. In the postpartum glucose challenge the model included treatment, time, breed, bolus, treatment x time, breed x time, treatment x breed, treatment x bolus, and the bolus x time interaction. Interactions that were not significant ($P < 0.05$) for parameters of interest were deleted from the models.

3. Results

3.1. Mineral consumption and analysis

Average mineral disappearance during the course of the experiment was 0.084 ± 0.008 and 0.087 ± 0.007 $kg \cdot cow^{-1} \cdot d^{-1}$ for the control and chromium treatments, respectively.

Based on mineral intake, chromium consumption in the chromium picolinate treatment averaged 3.5 $mg \cdot cow^{-1} \cdot d^{-1}$.

3.2. Basal levels of blood metabolites

Plasma glucose concentrations were affected by time ($P < 0.01$), treatment ($P < 0.01$), breed ($P < 0.01$), and breed x Cu bolus ($P < 0.02$). Plasma glucose was not affected by replicate or a replicate x treatment interaction. Plasma glucose concentrations increased with time until d 155 in all treatment groups (Figure 1). Cows receiving supplemental chromium had lower ($P < 0.01$) plasma glucose concentrations than control animals. Plasma glucose was not affected by a breed x time interaction (Figure 2). Angus cows had higher ($P < 0.01$) plasma glucose than Simmental in cows that did not receive supplemental copper. In those animals that received a copper bolus, plasma glucose concentrations did not differ between breeds (Table 2).

Plasma NEFA concentrations were affected by treatment x time ($P < 0.01$), treatment x copper bolus ($P < 0.04$; Table 3), and treatment x replicate x time ($P < 0.01$). Chromium supplemented cows had lower plasma NEFA concentrations on d 97 ($P < 0.01$) and d 155 ($P < 0.01$). In cows that did not receive a copper bolus, supplemental chromium reduced ($P < 0.01$) plasma NEFA concentrations. In cows receiving copper, supplemental chromium only tended ($P < 0.16$) to reduce plasma NEFA concentrations when compared to control animals. Chromium supplemented cows in replicates 2 and 3 had lower ($P < 0.01$) NEFA concentrations than controls in those same replicates. In cows 6 years of age or older (replicate 1), chromium did not affect plasma NEFA.

3.3. Glucose tolerance tests

In the glucose challenge conducted prepartum, plasma glucose concentrations were affected by time ($P < 0.01$) and treatment ($P < 0.01$; Figure 3). Cows receiving supplemental chromium had lower ($P < 0.01$) plasma glucose concentrations following the glucose challenge than controls. However, plasma glucose was not affected by a treatment x time interaction and glucose clearance rates were not affected by treatment (Table 4).

Serum insulin following the prepartum glucose challenge was affected by time ($P < 0.01$), treatment ($P < 0.05$), breed ($P < 0.01$), and a breed x time interaction ($P < 0.03$). Cows receiving supplemental chromium had lower ($P < 0.05$) serum insulin following the glucose challenge when compared to controls (Figure 4). Simmental cows had lower serum insulin than Angus at 10 ($P < 0.01$), 20 ($P < 0.01$), and 30 ($P < 0.05$) minutes after glucose challenge (Figure 5).

Plasma NEFA concentrations were affected by time ($P < 0.01$), treatment ($P < 0.01$), and a breed x treatment interaction ($P < 0.01$). Angus receiving supplemental chromium had lower ($P < 0.01$) plasma NEFA concentrations than Angus controls (174.69 ± 13.84 vs. 260.85 ± 11.30 $mEq \cdot ml^{-1}$). No effect of treatment on plasma NEFA concentrations was observed in Simmental cows.

In the glucose challenge conducted postpartum, plasma glucose concentrations were affected by time ($P < 0.01$), treatment ($P < 0.01$), and a treatment x bolus interaction ($P < 0.01$; Figure 7). In cows receiving a copper bolus, chromium supplementation increased ($P < 0.01$) plasma glucose concentrations following the postpartum glucose challenge; however, chromium did not affect plasma glucose concentrations in non-copper supplemented cows. Glucose clearance rate was not affected by treatment (Table 4).

Serum insulin was affected by time ($P < 0.01$), treatment ($P < 0.01$), breed ($P < 0.01$), breed x treatment ($P < 0.01$), breed x time ($P < 0.01$), and treatment x time ($P < 0.03$) following the glucose challenge postpartum. Chromium supplementation resulted in lower serum insulin at 10 ($P < 0.01$) and 20 ($P < 0.05$) minutes following glucose infusion when compared to controls (Figure 8). Supplementation of a copper bolus had no effect on serum insulin regardless of treatment or breed. Supplemental chromium reduced ($P < 0.01$) serum insulin following glucose infusion in Angus but did not significantly affect serum insulin in Simmental cows (Figure 9). Angus had higher ($P < 0.01$) serum insulin, regardless of treatment, than Simmentals at 10 and 20 minutes following the glucose challenge, and tended ($P < 0.16$) to have higher serum insulin at 30 minutes post infusion (Figure 10).

An effect of a bolus x treatment x time interaction ($P < 0.01$) was observed on plasma NEFA concentrations following a postpartum glucose challenge (Figure 11). Chromium supplementation resulted in lower ($P < 0.01$) plasma NEFA concentrations relative to controls in non-copper supplemented animals. Though p-values differed at different times, the treatment effect observed in non-copper supplemented animals was significant ($P < 0.01$) for the entire sampling period. No effect was observed between treatments in animals that received a copper bolus. Plasma NEFA concentrations were lower ($P < 0.01$) in Angus following the glucose challenge postpartum when compared to Simmental cows (253.83 ± 19.39 vs. 335.27 ± 19.39 $mEq \cdot ml^{-1}$).

4. Discussion

4.1. Basal levels of blood metabolites

The present study indicates that supplementation of chromium lowers plasma glucose concentrations in reproducing beef cattle. Previous studies (Chang et al., 1995; Mowat et al.,

1993) demonstrated that chromium supplementation lowered plasma glucose concentrations in growing and finishing steers. However, other trials (Mowat et al., 1993; Kegley et al., 1997) have found no effect of chromium supplementation on plasma glucose concentrations in cattle.

Plasma nonesterified fatty acid concentrations were lower in cows receiving supplemental chromium on d 97 and 155; differences occurring shortly after calving. Greater weight loss observed in control cows (Stahlhut, 2004) was accompanied by higher plasma NEFA concentrations in control cows in replicates 2 and 3 relative to cows receiving supplemental chromium in the same replicates; indicative of fat mobilization from body stores to meet the demands of lactation. Hayirli et al. (2001) reported that chromium supplementation did not affect body weights in dairy cows, but body condition score increased with increasing levels of chromium methionine postpartum. Matthews et al. (2001) reported that swine supplemented with chromium picolinate had lower plasma NEFA concentrations than controls. Similarly, studies conducted in sheep (Kitchalong et al., 1995) and dairy cattle (Hayirli et al., 2001) demonstrated that chromium supplementation lowered plasma NEFA concentrations. Conversely, supplemental chromium had no effect on plasma NEFA concentrations in growing steers (Chang and Mowat, 1992; Bunting et al., 1994). Yang et al. (1996) also observed no effect on plasma NEFA concentrations in dairy cattle supplemented with chelated chromium. In the present study, responses in plasma NEFA concentrations to chromium were affected by copper status. Chromium supplementation lowered plasma NEFA concentrations in non-copper supplemented cows, but only tended to lower NEFA concentrations in those cows that received a copper bolus. Supplemental copper alters the metabolism of adipose tissue as well as its response to hormones

responsible for lipolysis (Engle et al., 2000b); however, in several studies, supplemental copper did not alter plasma NEFA concentrations in growing and finishing beef steers (Engle et al., 2000a; Engle et al., 2000b).

4.2. Glucose tolerance tests

Following a glucose challenge prepartum, plasma glucose concentrations were lower in animals receiving supplemental chromium. Conversely, Kegley and Spears (1995) reported no effect of supplementation with chromium nicotinic acid complex on plasma glucose concentrations following a glucose challenge in calves. In cows receiving a copper bolus, chromium supplementation resulted in higher plasma glucose concentrations following a glucose challenge postpartum. However, no difference in plasma glucose concentrations was observed between treatments in animals that did not receive a copper bolus. Glucose clearance rates were not affected by treatment in either the prepartum or postpartum glucose challenges. Hayirli et al. (2001) reported no effect of supplemental chromium, from chromium methionine, on plasma glucose concentrations or area under the curve in dairy cows following either prepartum or postpartum glucose challenges.

Serum insulin was lower in cows receiving supplemental chromium following a glucose challenge prepartum, and was lower at 10 and 20 minutes following glucose infusion in the postpartum challenge. Subiyanto et al. (1996) observed that chromium supplementation decreased serum insulin in primiparous dairy cows following a glucose challenge prepartum. Conversely, Hayirli et al. (2001) reported higher serum insulin in dairy cows supplemented with chromium methionine following a prepartum glucose challenge; however, following the postpartum challenge, serum insulin was lower in cows receiving chromium. Decreased levels of plasma glucose and serum insulin following the prepartum

glucose challenge in chromium supplemented cows suggest that less insulin was required to clear glucose from the blood; indicating greater tissue sensitivity to insulin. Bunting et al. (1994) observed increased tissue sensitivity to insulin in growing steers fed a corn-cottonseed hull diet supplemented with chromium picolinate.

4.3. Breed differences

Breed differences were observed in basal plasma glucose as well as plasma glucose, NEFA, and serum insulin following the glucose challenges. Angus had higher plasma glucose concentrations than Simmental cows for the duration of the study, as well as during the postpartum glucose challenge. Serum insulin was higher and plasma NEFA concentrations were lower in Angus cows following glucose infusion during prepartum and postpartum glucose challenges when compared to Simmental cows. Jones et al. (1991) reported that Angus heifers had higher serum insulin concentrations than Simmental heifers prior to puberty; however, glucose concentrations were not affected by breed. Higher milk production in Simmental (Marston et al., 1992) may explain lower plasma glucose as the glucose is utilized in the synthesis of lactose. Mobilization of fat stores to meet the energy demands of lactation may also explain the higher concentration of plasma NEFA in Simmental cows. Differences in copper requirement (Smart and Christensen, 1985; Ward et al., 1995; Mullis et al., 2003), average daily gain (Crouse et al., 1985; Gregory et al., 1994), fatty acid composition of carcass (Laborde et al., 2001), and milk production (Marston et al., 1992) have been reported between Angus and Simmental cattle; indicating a potential for differences in carbohydrate and lipid metabolism between the two breeds.

5. Conclusions

Supplementation with chromium resulted in lower plasma glucose concentrations in beef cows throughout the course of the study. Plasma NEFA concentrations were reduced by chromium in early lactation but not prepartum or in late lactation. Chromium altered glucose metabolism following glucose challenges in reproducing beef cattle; however, an unanticipated interaction between chromium supplementation and copper status was observed postpartum. Further investigation is needed to understand the interaction between chromium supplementation and copper status, as well as the apparent differences in carbohydrate and lipid metabolism between Simmental and Angus cows.

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Table 1. Composition of free-choice mineral supplement

Ingredient	%
Mono-Dicalcium Phosphate (21% P)	38.0
Calcium carbonate	13.5
Magnesium oxide (58% Mg)	18.5
Salt	16.0
Cane molasses	5.0
Rice meal by-product	5.5
Zinc sulfate	0.694
Manganese sulfate	0.846
EDDI	0.004
Cobalt carbonate	0.008
Sodium selenite premix (0.2% Se)	1.5
Mineral oil	0.5
Vitamin A	50,000 IU[a]
Vitamin D	6,818 IU[a]
Vitamin E	57 IU[a]

[a] IU are on a per kg basis.

Table 2. Effect of dietary chromium and copper supplementation on plasma NEFA concentrations in beef cows

	Treatment			
	- Chromium	+ Chromium	SE	P-value
Plasma NEFA, mEq/dL [a,b]	420.6	313.4	17.3	0.01
- Cu	452.4	289.5	25.6	0.01
+ Cu	388.8	337.3	24.4	0.16
Time, day				
d 28	297.0	220.3	42.0	0.20
d 58	451.4	377.3	42.0	0.21
d 97	568.9	389.3	42.6	0.01
d 155	668.7	373.1	42.0	0.01
d 210	248.8	272.9	42.7	0.69
d 279	289.0	247.6	42.7	0.49

[a] Treatment x copper bolus ($P < 0.05$).
[b] Treatment x time ($P < 0.01$).

Table 3. Effect of breed and copper supplementation on plasma glucose concentrations in beef cows

	Breed			
	Angus	Simmental	SE	P <
Plasma glucose, mg/dL [a]	55.6	53.7	0.004	0.01
- Cu	56.3	52.9	0.006	0.01
+ Cu	54.9	54.4	0.006	0.58

[a] Breed x copper bolus ($P < 0.05$).

Table 4. Effect of dietary chromium on glucose clearance rates following glucose challenges

	Treatment			
	- Chromium	+ Chromium	SE	P-value
Glucose clearance rate, %/min				
Prepartum	1.41	1.54	0.17	0.61
Postpartum	2.18	1.67	0.29	0.25

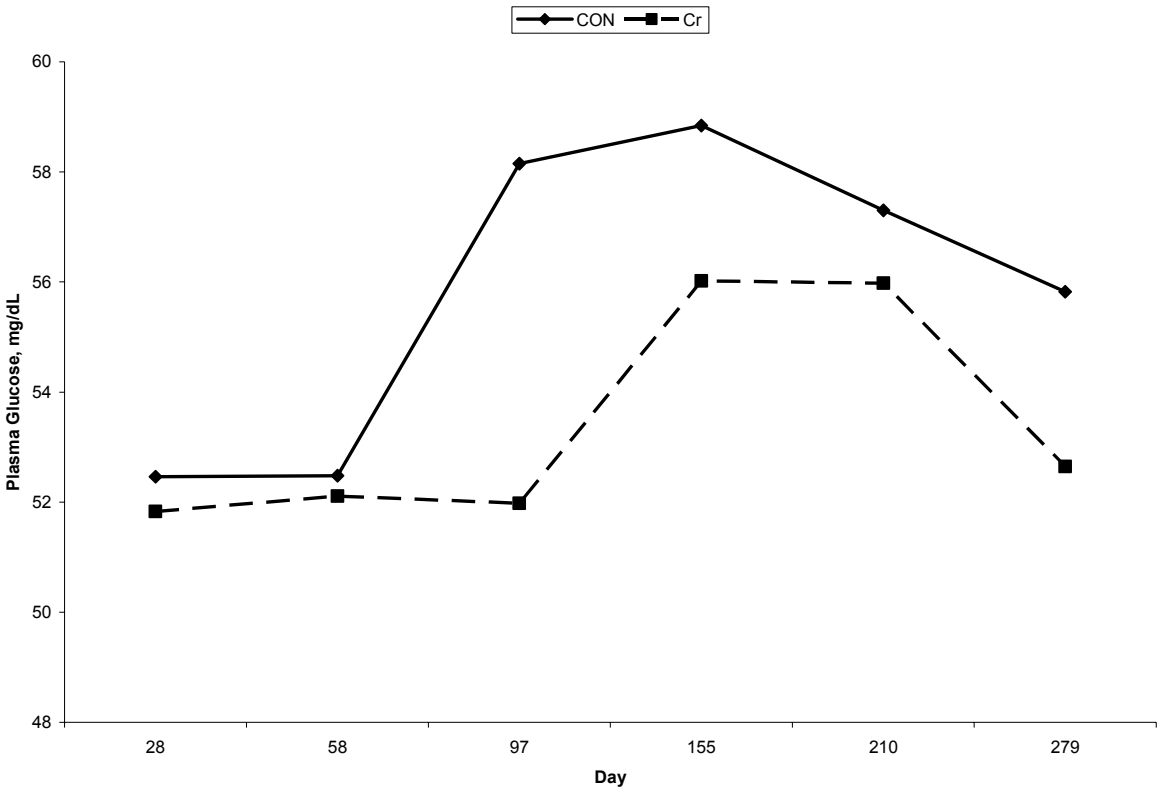

Figure 1. Effect of chromium on plasma glucose concentrations in reproducing beef cows. Pooled SEM = 0.42. Treatment (P < .01). Time (P < .01)

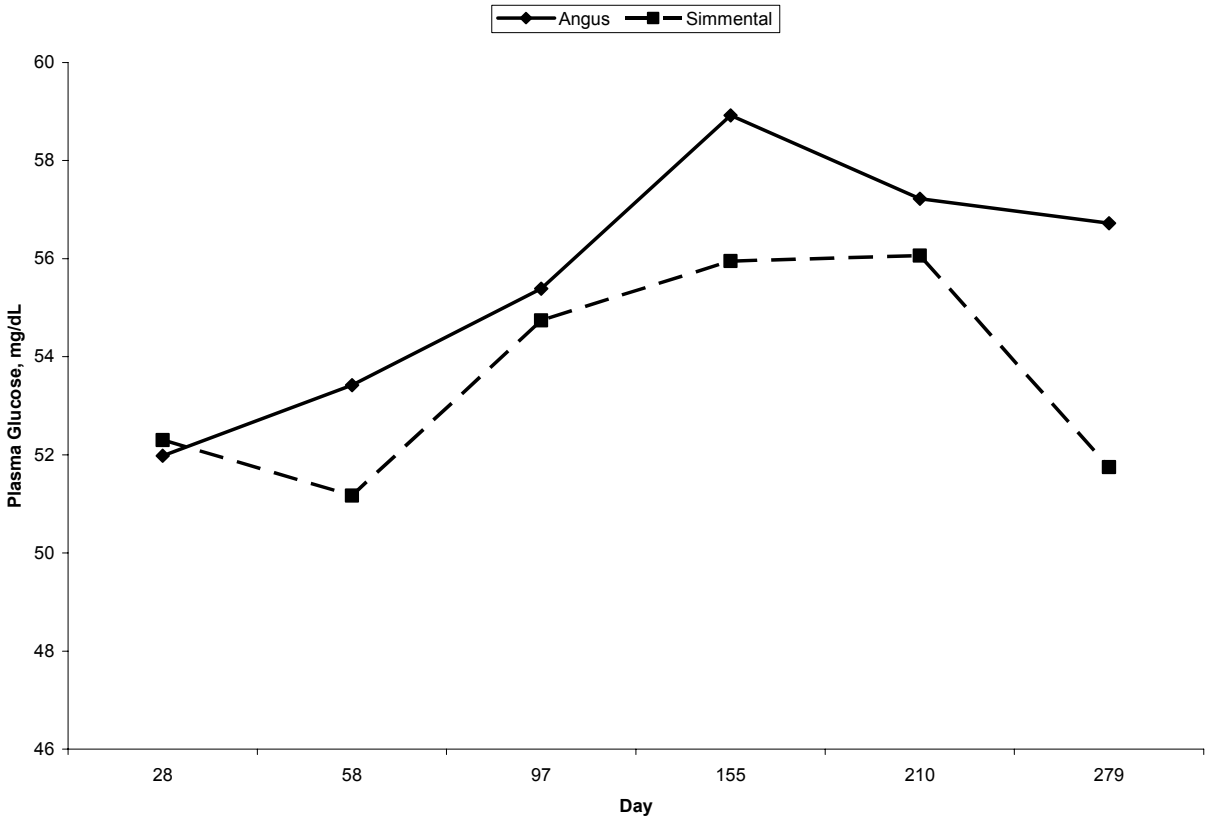

Figure 2. Effect of breed on plasma glucose concentrations in Angus and Simmental cows. Pooled SEM = 0.40. Breed (P < .01). Time (P < .01).

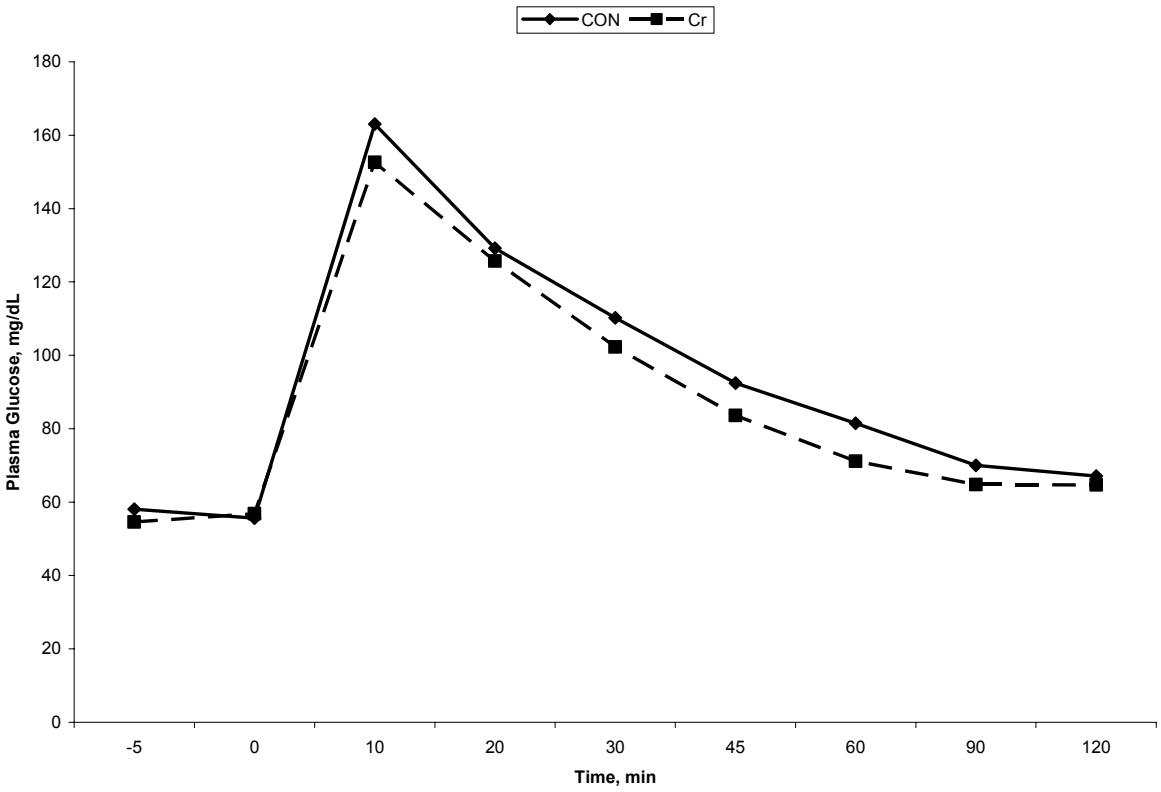

Figure 3. Effect of chromium on plasma glucose during prepartum glucose challenge. Pooled SEM = 1.12. Treatment (P < .01). Time (P < .01).

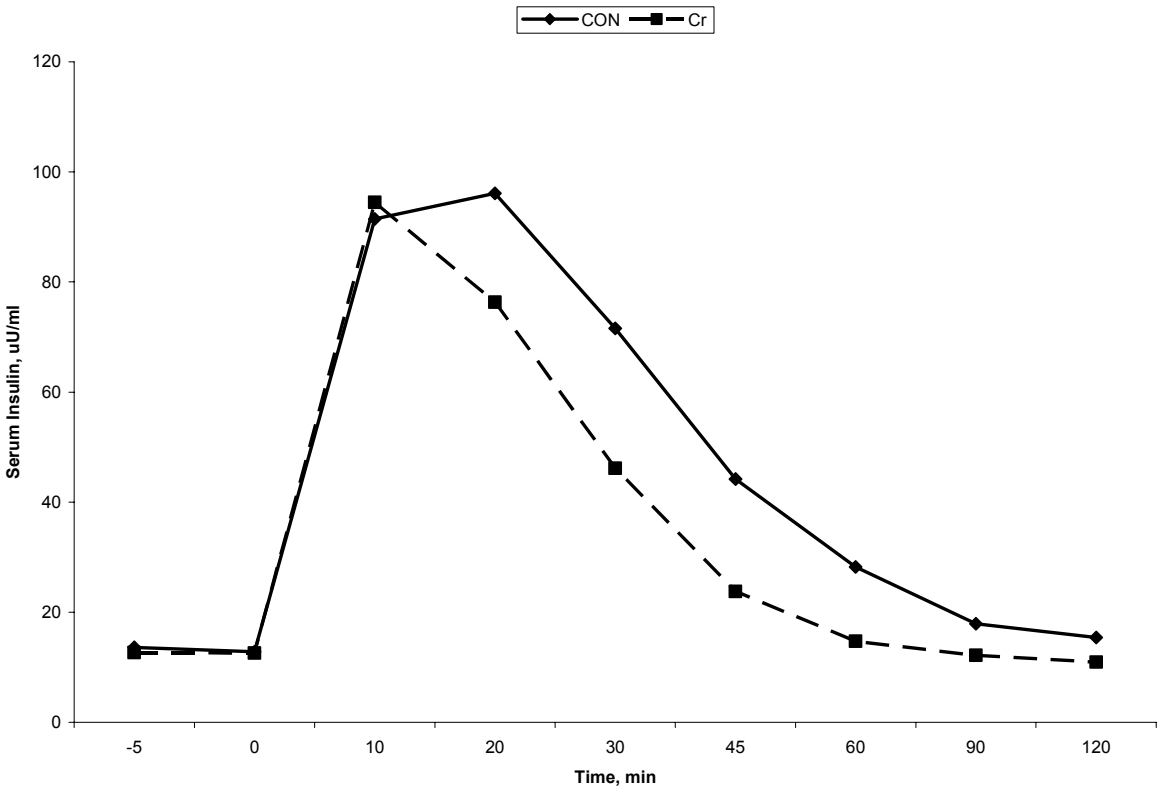

Figure 4. Effect of chromium on serum insulin concentrations during a glucose challenge prepartum. Pooled SEM = 3.35. Treatment (P < .01). Time (P < .01).

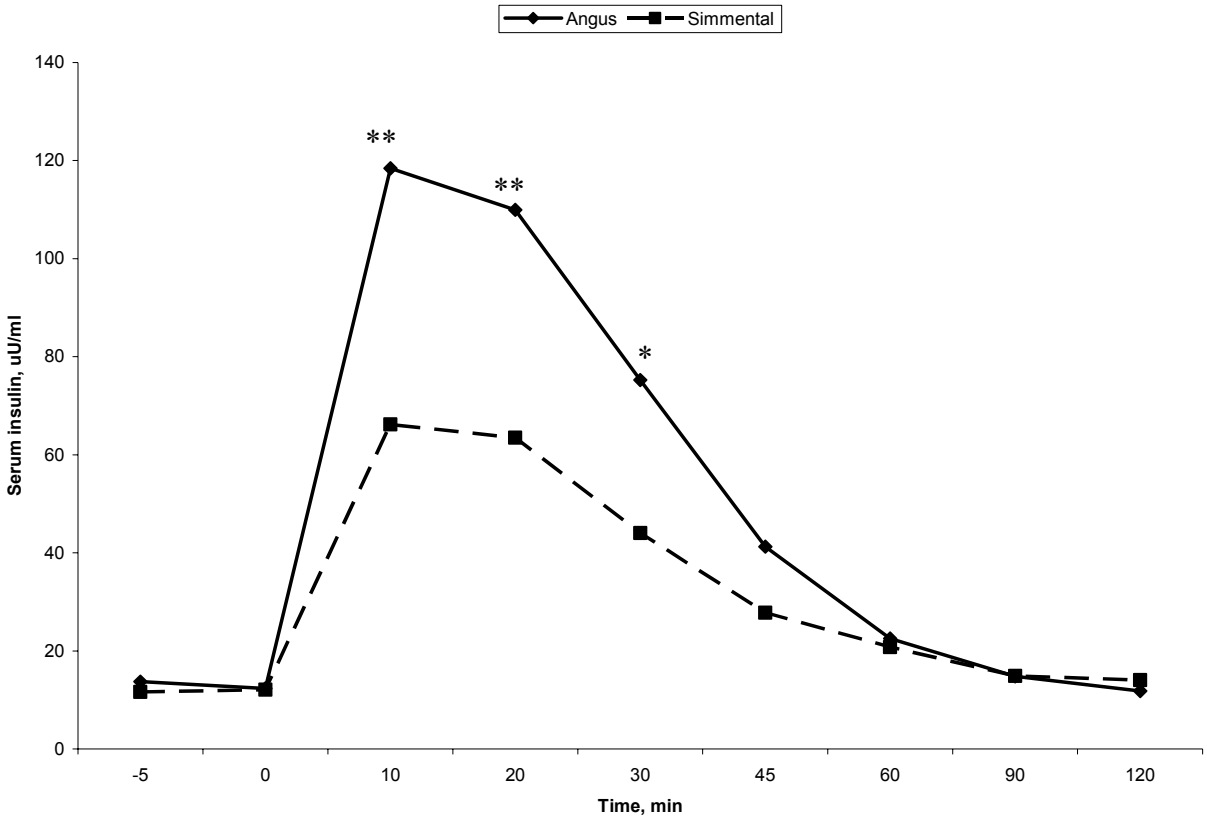

Figure 5. Effect of breed on serum insulin concentrations following a glucose challenge prepartum. Pooled SEM = min -5 = 10.0; min 0 = 10.0; min 10 = 10.5 (Angus), 9.55 (Simmental); min 20 = 10.5 (Angus), 9.55 (Simmental); min 30 = 10.5 (Angus), 9.55 (Simmental); min 45 = 10.0; min 60 =10.0; min 90 = 10.0; min 120 = 10.0. Breed x time interaction (P < .03). * P < .05 ** P < .01

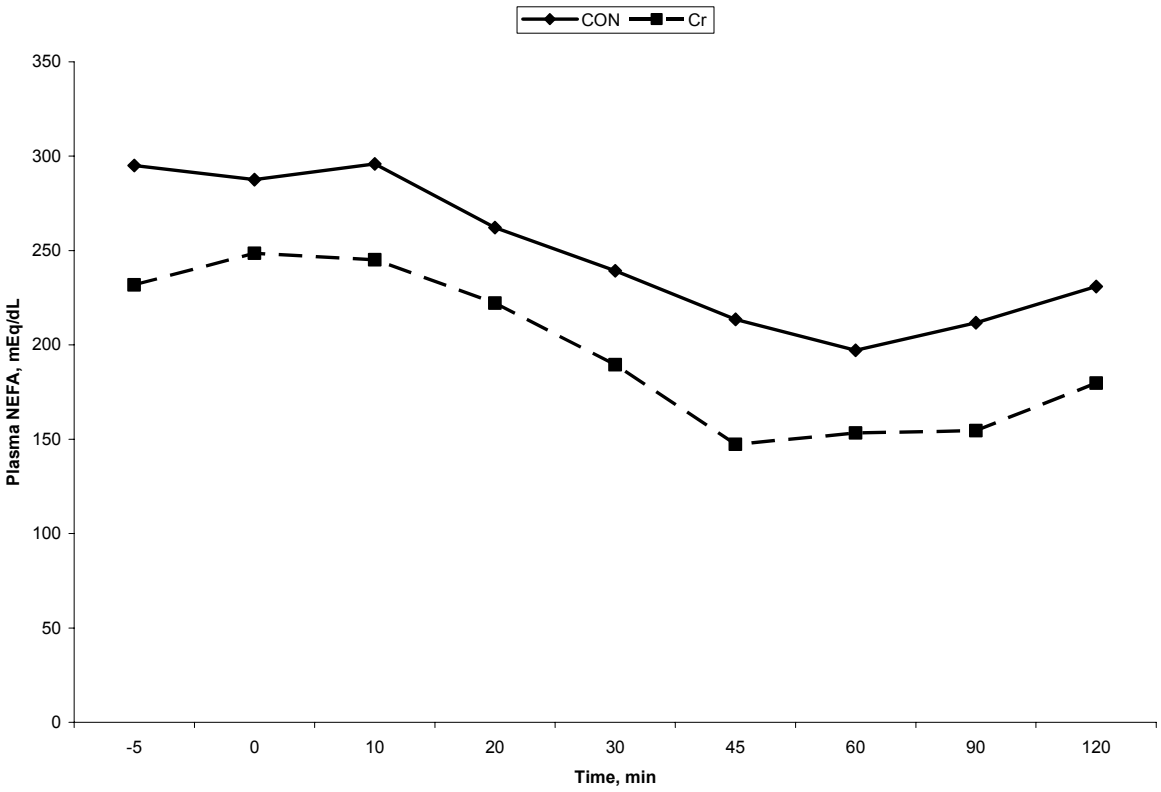

Figure 6. Effect of chromium on plasma NEFA concentrations during a glucose challenge prepartum. Pooled SEM = 8.46. Treatment (P < .01). Time (P < .01).

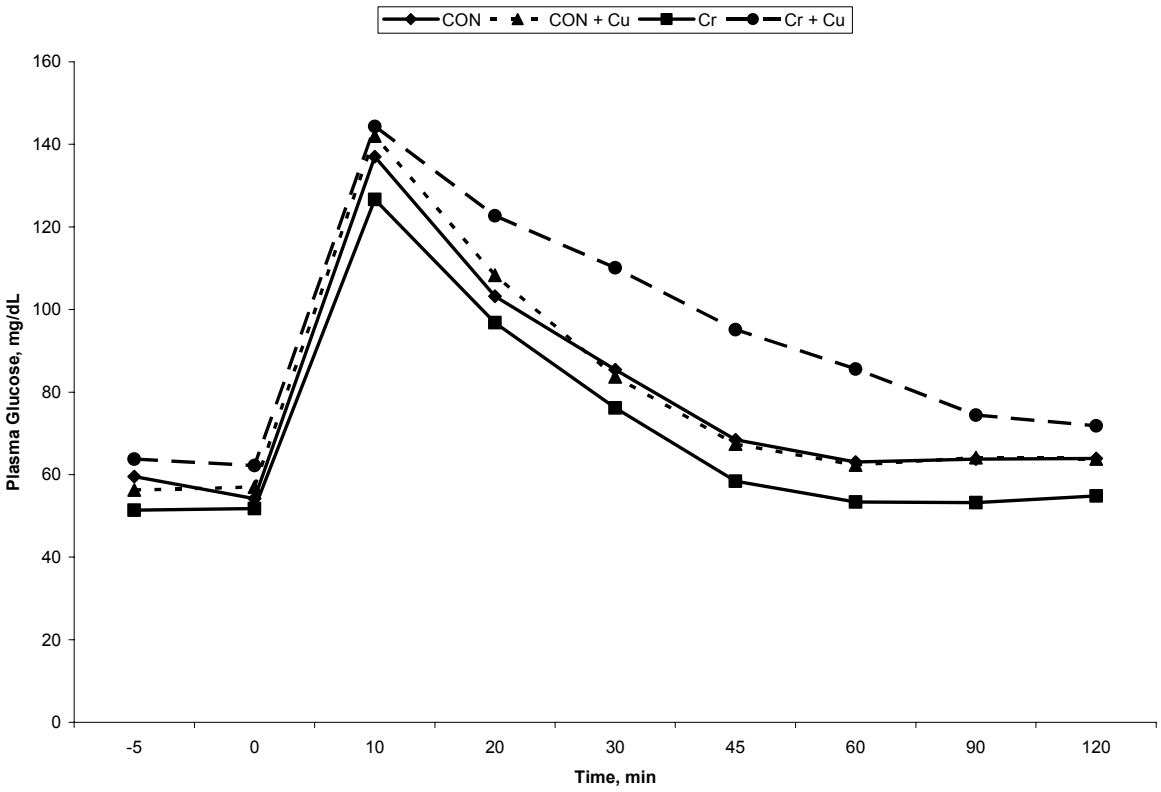

Figure 7. Effect of chromium and copper on plasma glucose concentrations during a glucose challenge postpartum. Pooled SEM = 2.15. Treatment x Cu bolus interaction (P < .01). Treatment (P < .01). Time (P < .01).

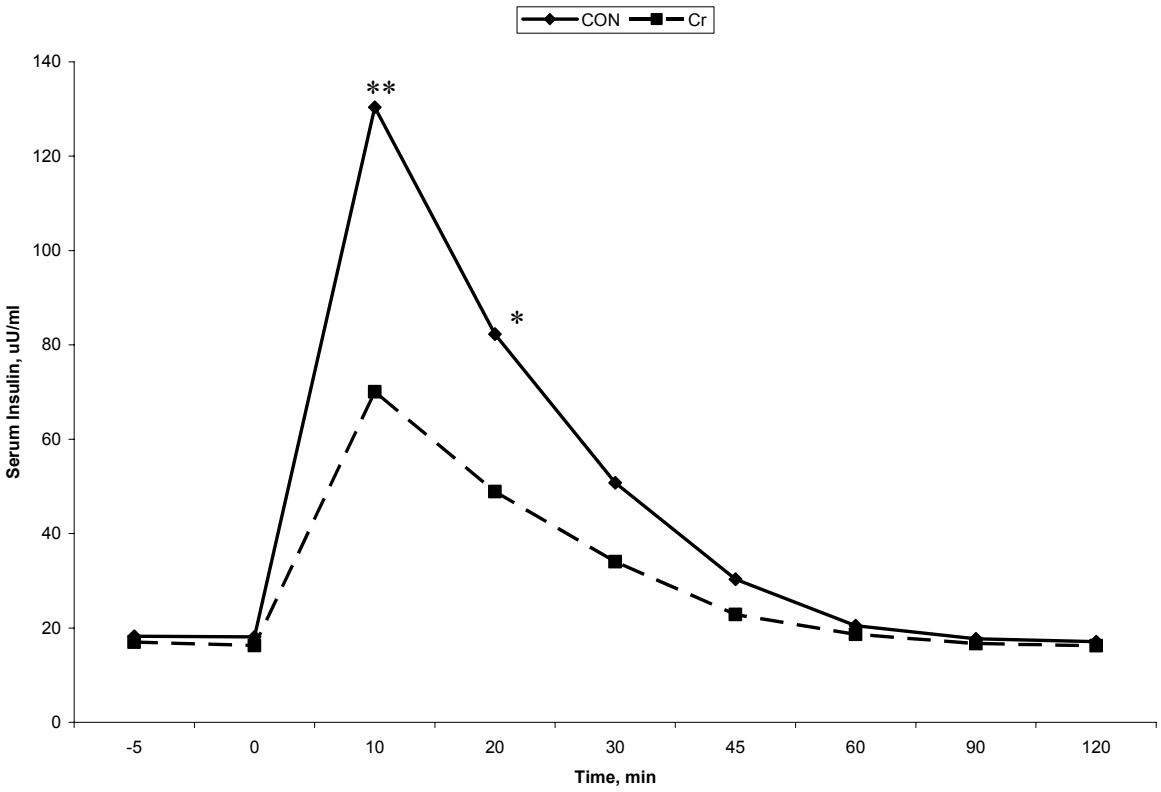

Figure 8. Effect of chromium on serum insulin concentrations during a glucose challenge postpartum. Pooled SEM = 9.47. Treatment x time interaction (P < .03). * P < .05 ** P < .01

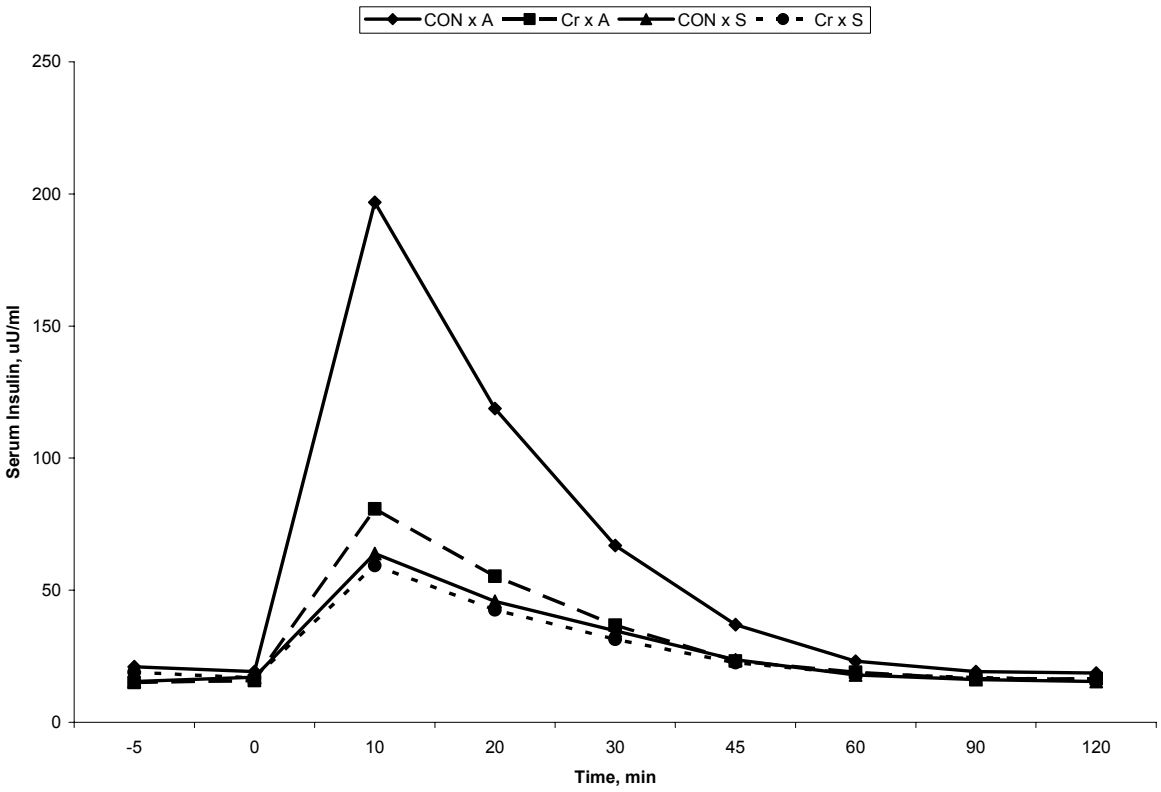

Figure 9. Effect of breed and chromium on serum insulin concentrations in Angus and Simmental cows during a glucose challenge postpartum. Pooled SEM = 4.46. Treatment x breed interaction (P < .01).

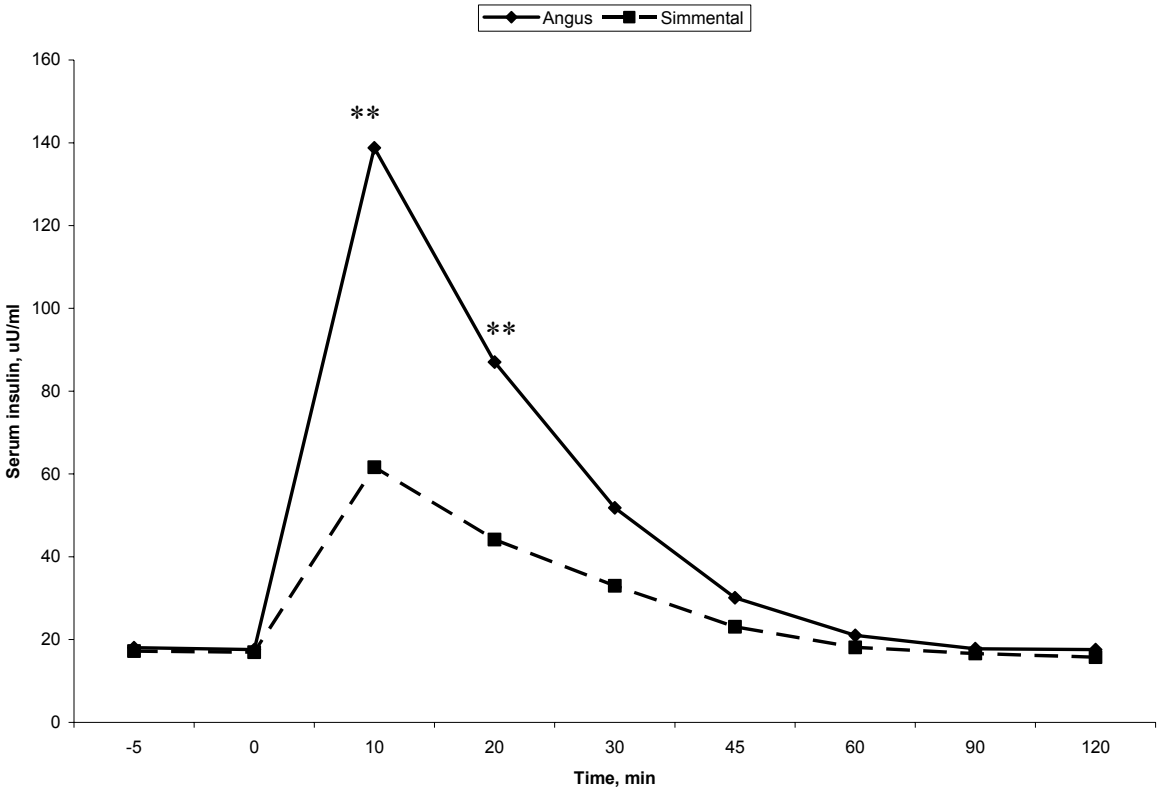

Figure 10. Effect of breed on serum insulin concentrations following a glucose challenge postpartum. Pooled SEM = 9.47. Breed x time interaction (P < .01). ** P < .01

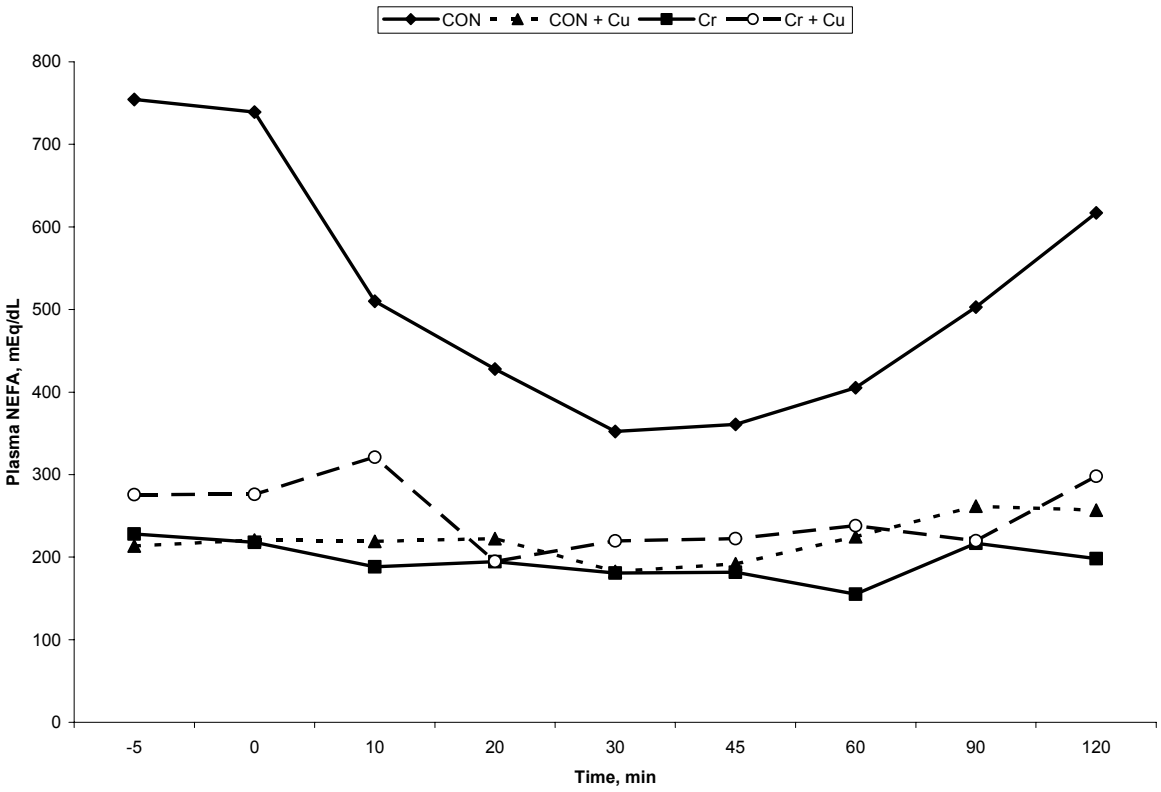

Figure 11. Effect of chromium and copper bolus on plasma NEFA concentrations in postpartum glucose challenge. Pooled SEM = 41.8. Treatment x bolus x time interaction (P < .01).

RUNNING HEAD: EFFECTS OF CHROMIUM ON PERFORMANCE AND REPRODUCTION

Effect of Chromium Supplementation and Copper Status on Performance and Reproduction of Beef Cows[1]

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ABSTRACT: Pregnant Angus (n=83) and Simmental (n=69) cows were blocked by age and breed and randomly assigned to one of two free-choice mineral supplements to determine the effect of dietary chromium (Chromium) and copper (Cu) status on glucose metabolism in beef cows. Supplements consisted of: 1) control (no supplemental Chromium) and 2) 40 mg Chromium /kg of mineral (from Chromium picolinate). Mineral supplements were formulated to contain all minerals typically supplemented to cattle diets with the exception of Cu. The study began approximately 75 d prepartum, at which point half of the cows in each treatment received a 25 g Cu oxide needle bolus. Blood was collected from 35 cows on d 0, 28, 58, 97 (approximately 20 d postpartum), 155, 210, and 279, and from 35 calves on d 196 and 279 for plasma copper determination. Liver biopsies were taken on d 0 and 279 to determine initial and final liver copper concentrations in cows. Plasma copper concentrations were affected by copper bolus x time ($P < 0.05$), breed x time ($P < 0.01$), and breed x bolus ($P < 0.01$) interactions in cows, and by a treatment x time interaction ($P < 0.05$) in calves. Liver copper concentrations were affected by breed x time ($P < 0.01$) and copper bolus x time ($P < 0.05$) in cows. Cows receiving a copper bolus had higher ($P < 0.05$) plasma copper on d 0 and 97, and higher ($P < 0.05$) liver copper on d 279 relative to those animals that did not get a bolus. Simmental cows had lower ($P < 0.01$) plasma copper from on d 28 and at subsequent sampling days, and lower ($P < 0.01$) liver copper on d 0 and 279 than Angus. Simmental cows that received a copper bolus had higher ($P < 0.01$) plasma copper concentrations than Simmental that did not receive supplemental copper. Supplemental chromium resulted in higher ($P < 0.05$) plasma copper concentrations in calves on d 279 when compared to controls. Overall weight loss, and weight loss postpartum, in cows was affected by breed ($P < 0.01$ and $P < 0.05$) and treatment x rep ($P < 0.01$). Overall

and postpartum weight loss was lower in Angus ($P < 0.05$). Chromium supplemented cows in replicates 2 (4 and 5 year-old; $P < 0.05$) and 3 (2 and 3 year-old; $P < 0.01$) experienced less weight loss postpartum than control cows in the same replicates. Overall weight loss was less ($P < 0.01$) in chromium supplemented young cows (rep 3) relative to controls. Cows supplemented with chromium tended ($P < 0.06$) to have higher pregnancy rates than controls. Calf birth weights and weaning weights were not affected by chromium or copper bolus.

Keywords: Chromium, Cattle, Reproduction, Performance

1. Introduction

Reproductive and growth performance, are key factors that effect the economics of the livestock industry. Improving production parameters such as conception rates, weaning weight, and animal health by nutritional means would increase profitability as well as enhance animal well-being. Supplemental chromium has been shown to improve performance in stressed calves (Chang and Mowat, 1992; Chang et al., 1995; Moonsie-Shageer and Mowat, 1993), enhanced immune response in stressed feeder calves (Moonsie-Shageer and Mowat, 1993; Kegley et al., 1997), increased milk yield, and decreased placental retention in dairy cattle (Hayirli et al., 2001; Yang et al., 1996; Villalobos-F et al., 1997).

No research has been done to determine the effect of supplemental chromium on performance and reproduction of beef cows. The objective of this study was to determine the effect of supplemental chromium picolinate and copper status on performance and reproduction of beef cows.

2. Materials and Methods

2.1. Protocol

Care, handling, and sampling of animals in this study were approved by the North Carolina State University Animal Care and Use Committee. One-hundred and fifty-two pregnant Angus (n=83) and Simmental (n=69) cows were blocked by breed and age and randomly assigned to one of two free-choice mineral supplements to determine the effect of dietary chromium (Chromium) and copper (Cu) status on glucose metabolism in beef cows. Supplements consisted of: 1) control (no supplemental Chromium) and 2) 40 mg Chromium /kg of mineral (from Chromium picolinate). Mineral supplements were formulated to contain all minerals typically supplemented to cattle diets with the exception of Cu (Table 1). Cows were divided into three replicates per treatment, according to their age with Angus and Simmental equally represented in each treatment. Replicate one contained cows 6 years of age or older (584.4 ± 6.5 kg). The second replicate contained cows of 4 to 5 years of age (542.2 ± 7.9 kg). Replicate three was composed of 2 and 3 year old females (468.9 ± 6.9 kg). The study began approximately 75 d prepartum, at which point half of the cows in each treatment received a 25 g Cu oxide needle bolus (Copasure®).

During the grazing season, cattle were rotated among tall fescue pastures at 14-day intervals to correct for any pasture differences. During the winter cattle were fed grass hay free choice. Additionally, cows in all replicates were fed 5.5 kg (DM) of corn silage and 0.9 kg of corn gluten feed postpartum; however, on a $DM \cdot kg^{-1}$ of body weight, heifers (replicate 3) received more energy and protein than older cows. Hay and silage samples were taken during the non-grazing months at 28-day intervals for copper analysis.

Calves were born between d 43 (October 16) and 104 (December 15). On d 196 (March 17), calves born to copper supplemented dams were given a 12.5 g copper oxide needle bolus (Copasure®). Cows were synchronized using an injection of gonadotropin releasing hormone (GnRH, Fertagyl, 100 μg) followed seven days later by an injection of prostaglandin F2α (PGF, Lutalyse, 25 mg). Cows were observed for estrus twice daily for 72 hours following the Lutalyse injection and were inseminated using the AM-PM protocol if detected in estrus. All cows not observed in estrus by 72 hours were injected with another dose of GnRH and artificially inseminated on d 155 (February 5). Beginning on d 159 (February 9), cows were exposed to Angus sires for 28 days. Rectal palpation was used to determine pregnancy, and open cows were removed from the study when calves were weaned on d 284 (June 14). One cow died on d 5 of the study from bovine leukemia. Two cows died on d 70 and d 217 of the study from cancer. Four cows were removed on d 111 of the study; two of which were found to be open, and two of which gave birth to stillborn calves.

Cows were weighed at 28-d intervals throughout the 284-d trial. Thirty-five cows were bled via jugular venipuncture on d 0, 28, 58, 97, 155, 210, and 279 for the determination of plasma copper concentrations. Liver biopsies were taken from these same animals on d 0 and 279 to determine initial and final liver copper concentrations (Engle and Spears, 2000). Calves (n = 35) were bled via jugular venipuncture on d 196 and 279 for plasma copper determination. Samples for plasma copper were collected in tubes containing sodium heparin (Vacutainer 9735, Becton Dickinson, Franklin Lakes, NJ) and kept on ice until centrifuged.

2.2. Analytical procedures

Blood was transported on ice to the laboratory and centrifuged at 2500 x *g* for 20 min. Plasma was removed and frozen at -20°C until it was analyzed for copper concentrations. For determination of plasma copper, plasma was diluted 1:3 with 5% nitric acid and then centrifuged for 20 minutes at 2500 RPM at room temperature. Feed and liver samples were wet ashed using mineral grade nitric acid and hydrogen peroxide in a microwave digester (Model MDS-81D, CEM, Matthews, NC, USA) as described by Gengelbach et al. (1994) in preparation for copper analysis. Atomic absorption spectroscopy was used to determine copper concentrations of plasma, feed, and liver (Model AA-6701F, Shimadzu Scientific Instruments, Japan).

2.3. Data analysis

Data were analyzed by repeated measures using the Proc Mixed procedure of SAS (1988). Individual animal was used as the experimental unit. The model for plasma copper and liver data included treatment, breed, bolus, time, treatment x breed, treatment x bolus, treatment x time, bolus x breed, breed x time, and the bolus x time interaction. Cow weight loss was analyzed using a model that included treatment, bolus, breed, rep, rep x treatment, rep x bolus, breed x treatment, and breed x bolus interactions. The model for calf birth and weaning weights included treatment, bolus, breed, sex, and rep. Pregnancy rate, and calf morbidity and mortality were analyzed using the Proc Logistic procedure of SAS. The model for cow performance data included treatment, breed, bolus, rep, and time. Proc GLM was used to check for interactions between factors for reproductive performance data; however, no significant interactions were observed. Interactions that were not significant ($P < 0.05$) for parameters of interest were deleted from the models.

3. Results

3.1. Mineral consumption and analysis

During the course of the experiment, average mineral disappearance was 0.084 ± 0.008 and 0.087 ± 0.007 $kg \cdot cow^{-1} \cdot d^{-1}$ for the control and chromium treatments, respectively. Based on mineral intake, chromium consumption in the chromium picolinate treatment averaged 3.5 $mg \cdot cow^{-1} \cdot d^{-1}$. Copper contents of supplemental hay and silage were found to be 9.85 ± 0.24 and 7.29 ± 0.42 $mg \cdot kg^{-1}$, respectively.

3.2. Copper status

Liver and plasma concentrations are indicative of copper status in cattle. Liver copper concentrations in cows were affected by breed ($P < 0.01$), time ($P < 0.05$), copper bolus x time ($P < 0.01$), and breed x time ($P < 0.01$) interactions, and tended ($P < 0.07$) to be affected by supplementation with a copper bolus. Simmental cows had lower initial ($P < 0.01$) and final ($P < 0.01$) liver copper concentrations than Angus (Table 2). In cows that did not receive a copper bolus, liver copper concentrations were lower ($P < 0.05$) on d 279 than on d 0 (Table 3). Liver copper did not decrease between d 0 and 279 in cows receiving a copper bolus. Liver copper tended ($P < 0.06$) to be higher in cows that received a copper bolus on d 279 relative to those that did not receive supplemental copper. Chromium supplementation had no effect on liver copper concentrations.

Plasma copper concentrations were affected by breed ($P < 0.01$), time ($P < 0.01$), breed x time ($P < 0.01$), copper bolus x time ($P < 0.05$), and a breed x bolus ($P < 0.01$) interaction in cows.

Supplementation of a copper bolus increased plasma copper concentrations in cows on d 97 ($P < 0.01$) relative to cows that did not receive a bolus (Table 4). Simmental cows

had lower ($P < 0.01$) plasma copper concentrations than Angus from d 28 through d 279. Plasma copper concentrations were lower ($P < 0.01$) in Simmental that did not receive a copper bolus than in Simmental cows that had received supplemental copper; however, no differences were observed between Angus cows regardless of copper supplementation (Table 5). Angus had higher ($P < 0.01$) plasma copper concentrations than Simmental cows whether or not they had received a copper bolus.

Plasma copper concentrations in calves were affected by treatment ($P < 0.01$), time ($P < 0.01$), and a treatment x time interaction (Table 6). Chromium supplementation increased ($P < 0.01$) plasma copper concentrations in calves on d 279, but not on d 196. Copper supplementation in calves did not affect plasma copper concentrations.

3.3. Cow performance

Cows supplemented with chromium tended ($P < 0.06$) to have a higher rate of pregnancy than control animals (Table 7). Breed, copper status, and age of dam did not affect pregnancy rates.

Overall cow weight loss was affected by treatment ($P < 0.01$), breed ($P < 0.01$), rep ($P < 0.01$), and rep x treatment ($P < 0.01$; Table 8). Two and three year-old cows (rep 3) receiving supplemental chromium lost less ($P < 0.01$) weight when compared to controls in rep 3. Chromium did not affect weight change in older cows. Simmental cows lost more weight overall ($P < 0.01$) than Angus (Table 9).

3.4. Calf performance

Chromium supplementation and copper status did not affect birth and weaning weights of calves (Table 10). Birth and weaning weights of calves were affected by rep ($P < 0.01$), breed ($P < 0.01$), and sex ($P < 0.01$). Calves born to young females (rep 3) had lower

($P < 0.01$) birth and weaning weights than calves born to older cows. Simmental x Angus calves had higher ($P < 0.01$) birth and weaning weights than Angus calves, regardless of age of dam. Bull calves were heavier ($P < 0.01$) at birth and weaning than heifers. Calf morbidity and mortality were not affected by treatment.

4. Discussion

4.1. Copper status

Ruminants are more likely to suffer from Cu deficiency than monogastrics, due to low absorption (1-3% of copper intake) of copper (McDowell, 1992). Based on liver and plasma copper concentrations (Underwood and Suttle, 1999), cows in the non-copper-supplemented group were not deficient in copper in the present study. This can explain the lack of effect of supplemental copper on calf weaning weights. Chromium supplementation had no effect on liver or plasma copper concentrations in cows; however, chromium supplementation increased plasma copper concentrations in calves on d 279.

Liver copper concentrations in cows that received a copper bolus tended to be higher on d 279 relative to those cows that did not receive supplemental copper. Supplementation with a copper bolus increased plasma copper concentrations in cows on d 97 relative to cows that did not receive a bolus. Similarly, Ahola et al. (2004) observed increased plasma and liver copper concentrations in reproducing beef cows supplemented with copper, zinc, and manganese when compared to controls.

Simmental cows had lower liver copper concentrations than Angus at the beginning and end of the study. Mullis et al. (2003a) reported that Simmental steers had lower liver copper concentrations than Angus throughout the duration of a study to determine the effect of copper and zinc source on mineral status of steers fed a diet high in iron.

Similarly, Mullis et al. (2003b) found that Angus heifers had higher liver copper concentrations than Simmental heifers at the end of a study to determine differences in copper status between the two breeds.

Simmental cows had lower plasma copper concentrations than Angus from d 28 through d 279. This is in agreement with previous studies reporting that Angus have higher plasma copper concentrations than Simmental (Smart and Christensen, 1985; Ward et al., 1995; Mullis et al., 2003a; Mullis et al., 2003b). Plasma copper concentrations were lower in Simmental that did not receive a copper bolus than in Simmental that had received supplemental copper; however, no differences were observed between Angus cows regardless of copper supplementation.

The difference in copper status between these two breeds may be explained in terms of biliary copper excretion. Gooneratne et al. (1994) found that biliary copper concentrations and copper excretion were approximately two times higher in Simmental cattle than in Angus. The propensity of Simmentals to excrete copper may explain why deficiency occurs more frequently in Simmental cattle, resulting in higher copper requirements to meet the animal's needs.

4.2. Cow performance

Cows supplemented with chromium tended to have higher pregnancy rates than control animals. Yang et al. (1996) reported that reproductive performance was not adversely affected in primiparous cows supplemented with chromium, despite increased milk yield, and supplementation with chromium may have decreased the number of primiparous cows that did not conceive. Previous studies (Richards et al., 1986; Houghton et al., 1990) indicate that weight loss postpartum and cow body condition loss impact conception rates in reproducing

beef cows. Young cows (rep 3) receiving supplemental chromium lost less weight during the study than control animals. It is possible that decreased weight loss in chromium supplemented cows may have resulted in greater fertility.

Hayirli et al. (2001) reported that chromium supplementation did not affect body weights in dairy cows, but body condition score increased with increasing levels of Chromium-Met postpartum. Control cows in replicates 2 and 3 had higher plasma NEFA concentrations when compared to cows receiving chromium in the same replicates (Stahlhut, 2004); suggesting greater mobilization of fat stores. Conversely, Yang et al. (1996) reported weight loss in chromium supplemented primiparous dairy cows and weight gain in control animals. Simmental cows lost more weight than Angus during the study. Heavier calf birth weights and higher milk production in Simmental cows (Marston et al., 1992) may explain the greater weight loss observed in Simmentals when compared to Angus cows.

4.3. Calf performance

Calf performance was not affected by treatment or copper status in the present study. Birth and weaning weights were higher for calves born to older cows (replicates 1 and 2) than to 2 and 3 year-old females. Similarly, Kress et al. (1990) reported that younger cows weaned lighter calves than older dams. In agreement with previous research (Woldehawariat et al., 1977; Kress et al., 1990), bull calves had heavier birth and weaning weights than heifers in the present study. Simmental x Angus calves had heavier birth and weaning weights than Angus calves in agreement with findings by Jenkins and Ferrell (1994). Higher milk production in Simmental cows (Marston et al., 1992) and greater average daily gains in Simmental calves (Crouse et al., 1985; Gregory et al., 1994) may attribute to the difference in weaning weights observed between Angus and Simmental-cross calves.

Previous research in growing and finishing cattle indicates that chromium supplementation enhances immune function (Moonsie-Shageer and Mowat, 1993; Kegley and Spears, 1995; Kegley et al., 1996). Morbidity and mortality among calves were not affected by treatment. Similarly, no effect on morbidity was observed in stressed feeder calves supplemented with chromium (Chang and Mowat, 1992; Chang et al., 1995; Mathison and Engstrom, 1995). Conversely, Mowat et al. (1993) observed lower occurrence of morbidity in stressed steers supplemented with chromium. Supplementation of high-chromium yeast was observed to decrease morbidity and rectal temperatures in stressed steer calves fed a corn silage diet (Moonsie-Shageer and Mowat, 1993).

5. Conclusions

Supplementation of a copper bolus resulted in higher copper status in cows and calves, but had no effect upon reproduction or calf weaning weights. Chromium supplementation resulted in decreased weight loss in reproducing cattle, especially in young cows. Cows receiving supplemental chromium tended to have higher pregnancy rates when compared to controls. The results of this study indicate that supplementation of chromium may improve fertility and decrease weight loss in reproducing beef cows.

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Table 1. Composition of free-choice mineral supplement

Ingredient	%
Mono-Dicalcium Phosphate (21% P)	38.0
Calcium carbonate	13.5
Magnesium oxide (58% Mg)	18.5
Salt	16.0
Cane molasses	5.0
Rice meal by-product	5.5
Zinc sulfate	0.694
Manganese sulfate	0.846
EDDI	0.004
Cobalt carbonate	0.008
Sodium selenite premix (0.2% Se)	1.5
Mineral oil	0.5
Vitamin A	50,000 IU [a]
Vitamin D	6818 IU [a]
Vitamin E	57 IU [a]

[a] IU are on a per kg basis.

Table 2. Effect of breed on liver copper in beef cows

	Breed			
	Angus	Simmental	SE	P <
Liver Cu, ppm [a]	137.1	49.0	8.3	0.01
Time, day				
d 0	169.7	44.9	11.8	0.01
d 279	104.5	53.1	11.6	0.01

[a] Breed x time (P < 0.01).

Table 3. Effect of copper supplementation on liver copper in beef cows

	Bolus			
	- Cu	+ Cu	SE	P-value
Liver Cu, ppm [a]	89.7	96.4	8.3	0.57
Time, day				
d 0	116.3	98.3	11.8	0.29
d 279	63.2	94.4	11.6	0.06

[a] Copper bolus x time ($P < 0.05$).

Table 4. Effect of bolus x time and breed x time interactions on plasma copper in reproducing beef cows

	Bolus				Breed			
	- Cu	+ Cu	SE	P <	Angus	Simmental	SE	P <
Plasma Cu, ppm[a,b]	0.94	0.97	0.01	0.07	1.04	0.87	0.01	0.01
Time, day								
d 0	0.98	0.89	0.03	0.05	0.94	0.92	0.03	0.66
d 28	0.91	0.98	0.03	0.16	1.05	0.83	0.03	0.01
d 58	0.85	0.89	0.03	0.39	0.97	0.77	0.03	0.01
d 97	0.84	0.97	0.03	0.01	1.01	0.80	0.03	0.01
d 155	1.10	1.11	0.03	0.75	1.16	1.05	0.03	0.01
d 210	1.03	1.04	0.03	0.74	1.17	0.91	0.03	0.01
d 279	0.89	0.93	0.03	0.29	0.99	0.83	0.03	0.01

[a] Copper bolus x time ($P < 0.05$).
[b] Breed x time ($P < 0.01$).

Table 5. Effect of breed and bolus on plasma copper concentrations in reproducing beef cows

	Bolus			
	-Cu	+Cu	SE	P <
Plasma Cu, ppm [a]				
Breed				
Angus	1.05[b]	1.03[b]	0.017	0.45
Simmental	0.83[b]	0.91[b]	0.018	0.01

[a] Breed x bolus (P < 0.01).

[b] Values in same column with same subscripts are different (P < 0.01).

Table 6. Effect of chromium supplementation on plasma copper in beef calves

	Treatment			
	- Chromium	+ Chromium	SE	P <
Plasma Cu, mg/dL [a]	0.77	0.85	0.018	0.01
Time, day				
d 196	0.74	0.75	0.026	0.53
d 279	0.81	0.95	0.026	0.01

[a] Treatment x time (P < 0.05).

Table 7. Effect of chromium supplementation on cow pregnancy rates and calf morbidity and mortality

	Treatment			
	CON	Cr	SE	P <
Cows				
Pregnancy Rate, %	81.0	89.2	4.24	0.06
Calves				
Morbidity, %	5.28	1.17	2.10	0.19
Mortality, % [a]	2.45	3.63	2.05	0.45

[a] Includes calve that were born dead.

Table 8. Effect of chromium supplementation and age of dam on weight change in reproducing beef cows

	Treatment			
	CON	Cr	SE	P <
Weight change, kg[a]				
Overall (Sept – June)	-70.2	-56.7	2.83	0.01
Rep 1	-66.9	-73.1	4.46	0.33
Rep 2	-69.3	-60.0	5.40	0.23
Rep 3	-74.4	-37.1	4.78	0.01

[a] Treatment x rep ($P < 0.001$).

Table 9. Effect of breed on weight change in reproducing beef cows

	Breed			
	Angus	Simmental	SE	P <
Weight change, kg				
Sept – June	-56.1	-70.8	2.82	0.01
Jan – June	-51.5	-60.4	2.72	0.02

Table 10. Effect of treatment, breed, and copper status on calf birth and weaning weights

	Calf performance Parameters					
	Birth wt, kg	SE	P <	Weaning wt, kg	SE	P <
Treatment						
CON	39.2	0.51	0.27	215.2	2.28	0.56
Chromium	40.0	0.51	0.27	217.1	2.28	0.56
Breed						
Angus	37.8	0.49	0.01	203.8	2.22	0.01
Simmental x Angus	41.4	0.53	0.01	228.4	2.36	0.01
Copper status						
-Cu	39.4	0.52	0.61	218.0	2.29	0.26
+Cu	39.8	0.51	0.61	214.3	2.28	0.26

SUMMARY

A study was conducted to determine the effect of chromium supplementation and copper status on glucose metabolism, performance, and reproduction of beef cattle. Simmental and Angus cows were kept on tall fescue pastures and had access to a free-choice mineral supplement containing all minerals typically supplemented to cattle with the exception of copper. One half of the cows in each treatment received a 25 g copper oxide needle bolus (Copasure®) at the beginning of the study. Supplementation with chromium picolinate (40 mg Cr · kg^{-1}) to a free-choice mineral supplement affected several aspects of glucose metabolism in Simmental and Angus cows. Over the course of a 279 d study, cows supplemented with chromium had lower plasma glucose and nonesterified fatty acid concentrations than control cows. Angus had higher plasma glucose concentrations than Simmental cows for the duration of the study.

Glucose challenges were conducted pre- and postpartum to determine the effect of chromium supplementation on glucose metabolism in late gestation and early lactation in beef cows. Following a glucose challenge prepartum, plasma glucose and serum insulin concentrations were lower in animals receiving supplemental chromium. In cows receiving a copper bolus, chromium supplementation resulted in higher plasma glucose concentrations following a glucose challenge postpartum. However, no difference in plasma glucose concentrations was observed between treatments in animals that did not receive a copper bolus. Serum insulin was lower at 10 and 20 minutes following glucose infusion in the postpartum challenge in cows receiving supplemental chromium. Area under the curve and glucose clearance rates were not affected by treatment in either the pre- or postpartum glucose challenges.

Breed differences were observed in basal plasma glucose as well as plasma glucose, NEFA, and serum insulin following the glucose challenges. Angus had higher plasma glucose concentrations following the postpartum glucose challenge. Serum insulin was higher and plasma NEFA concentrations were lower in Angus cows following glucose infusion during prepartum and postpartum glucose challenges when compared to Simmental cows.

Cows receiving supplemental chromium lost less weight throughout the course of the study than control animals, and tended to have higher pregnancy rates than control cows. Over the course of the study as well as the period postpartum, young cows (age 2 or 3 years) receiving supplemental chromium lost less weight than control heifers. Control cows in replicates 2 (4 or 5 year-olds) and 3 (2 or 3 year-olds) had higher plasma NEFA concentrations when compared to cows receiving chromium in the same replicates; indicating greater mobilization of fat stores. Simmental cows lost more weight than Angus over the course of the study and during the postpartum period. Chromium supplementation lowered plasma NEFA concentrations in cows that did not receive a copper bolus, and tended to lower NEFA concentrations in cows supplemented with copper.

Chromium supplementation did not affect calf performance, morbidity, and mortality. Simmental calves, bull calves, and calves born to older cows (replicates 1 and 2) had higher birth and weaning weights than Angus calves, heifer calves, or calves born to young cows.

In conclusion, chromium and copper status altered glucose metabolism in reproducing beef cows. Supplemental chromium decreased the amount of weight lost by cows postpartum, and may increase pregnancy rates in beef cows.