EFFECTS OF CHROMIUM ON PERFORMANCE AND GROWTH OF FEEDLOT STEERS

by

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A DISSERTATION

IN

ANIMAL SCIENCE

Submitted to the Graduate Faculty
of Texas Tech University in
Partial Fulfillment of
the Requirements for
the Degree of

DOCTOR OF PHILOSOPHY

Approved

May, 2000

ACKNOWLEDGEMENTS

The author would like to express his appreciation, gratitude, and respect to Professor C. Reed Richardson. Dr. Richardson was always there to offer the friendship, guidance, and patience needed when things were tough; and that needed push in the right direction or friendly chat when I would begin to slack off. I appreciate everything he did for me during the course of this dissertation.

The author would also like to express his appreciation to Drs. Bob Albin, Mike Galyean, Carlos Villalobos, and Richard Zartman for serving on this committee. In addition, for all of the guidance and direction in project design, research ideas, and interpretation a simple "thank you" is not enough to express the extent to which the author is grateful for their efforts. Furthermore, Dr. Sam Jackson deserves special recognition for not only being a committee member, mentor, and professor, but also for being a friend.

The assistance of Tyler Bramble, Brad Clyburn, Jayden Montgomery, Greg Nunnery, and Ken Wilson (Ken, I did this list in alphabetical order so don't get your

feelings hurt) with sample collection, analysis, critical review, and animal care and feeding is greatly appreciated. In addition to your time, patience, and willingness to help, your friendship is the most important thing that each of you was able to contribute to the author's life. Also, the support and help of the many other friends both within and outside the Animal Science Department will not be forgotten.

The author would like to thank his parents, who made not only this dissertation, but also the entire education of the author possible through their love, support, and sacrifice. One can only hope that all of the sacrifices they made during the last 28 years were worth it, because with out them this dissertation would never have occurred. Thank you for not only being my parents, but for also being my biggest supporters and my closest friends.

Finally, this dissertation is dedicated to my wife, Heather, for all of her love, support, and tremendous sacrifice that she has given to make this dream a reality. Throughout the author's entire graduate career she has been there to listen to each complaint, frustration, accomplishment, and criticism the author has

had. Even when times were tough or when she had tears in her eyes, she always did the best she could to offer support and to help see this dissertation to completion. Finally, I would like to thank Raymond Cade, our son, for being the biggest joy my life has ever known. A smile, a laugh, or a new trick that he learned while this dissertation was in progress made each frustration, each hurdle, and each roadblock a little easier to cross. I knew whenever I finished writing for the day he would always be there to make me realize what true happiness is and what I had to be thankful for.

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CHAPTER I

INTRODUCTION

Mineral nutrition has been studied for over 100 yr by scientists seeking to improve animal performance, profitability, and consumer acceptance through nutritional recommendations. To date, requirements for all macro minerals and eight micro (trace) minerals have been established for beef cattle (NRC, 1996). Most recently, attention has been focused on chromium and determining whether it should be classified as a required nutrient. Because of public demands and consumer pressure to keep animal protein prices low, increased efforts are being made to improve beef production through proper nutrition. It is likely that within the next century, beef cattle requirements for chromium, vanadium, nickel, tin, boron and possibly silicon will be determined and implemented in order to further improve beef production (NRC 1996, 1997).

During the middle of the 20th century, a chromium-containing compound termed glucose tolerance factor (GTF) was discovered. This factor improves insulin binding and increases glucose clearance. Further research with GTF

led to the discovery of many beneficial effects of chromium supplementation. One of the most intriguing aspects associated with chromium is its effect on carcass composition in non-ruminants. The most important effects associated with chromium supplementation in non-ruminants are the increased longissimus muscling and reduced subcutaneous fat. As a result of consumer demands, beef cattle producers have been forced to deliver a high-quality animal protein product, that not only meets their desires for taste, eating quality, and cost, but that also is high in lean tissue, and low in fat. As a result of these demands, many compounds have been evaluated in an effort to improve lean tissue synthesis, while concomitantly decreasing adipose tissue accretion. To date, the majority of these efforts have focused around the use of exogenous growth factors.

Cell culture techniques have been used to determine effects of various compounds on muscle or adipose cells in a laboratory setting. Most recently, these techniques have been used to determine the effects of exogenous hormones on protein deposition and turnover, and to characterize muscle growth in callipyge sheep. These research models allow for a greater understanding of

occurrences at the cellular level than do simple assays such as plasma urea nitrogen, insulin, and blood glucose.

No research has been conducted to determine whether chromium affects beef cattle growth, carcass composition, or performance in a manner that is consistent with non-ruminant animals. Although much of the research in regards to chromium is inconclusive as to its effect on carcass modification, enough convincing evidence is available to promote further research on the ability of chromium to alter growth. The objectives of this study were to determine the effects of chromium on performance and growth of feedlot steers, and to determine the effectiveness of serum from feedlot steers in altering in vitro and in vivo metabolism of glucose and amino acids in muscle cell culture.

CHAPTER II

LITERATURE REVIEW

History

According to NRC (1997), during the last half of the 20th century, chromium (Cr) has become recognized by many nutritionists as an essential nutrient for humans and both laboratory and domestic animals. Curran (1954) discovered that Cr affected cholesterol metabolism and decreased blood glucose levels in rats. Following this discovery, Schwartz and Mertz (1957, 1959) were the first to demonstrate the effectiveness of a component of yeast and pork kidney to influence and improve glucose utilization. This compound was referred to as glucose tolerance factor (GTF), and it was later determined that an active constituent in GTF was Cr (Schwartz and Mertz, 1959). Later, Toepfer et al. (1977) discovered that GTF most likely consists of Cr, cysteine, glutamate, glycine, and nicotinic acid. Today Cr, in the form of GTF, is often prescribed by human health care providers to improve glucose metabolism of patients suffering from diabetes (Mertz, 1993; Kamen, 1994; Olin, 1998). However, to date the only domestic livestock animal to

have a published requirement and approved level of Cr in the diet is swine, at 0.2 ppm (NRC, 1998).

Chromium

Chromium is found in nature and biological systems as primarily, Cr^{+3} (trivalent Cr; NRC, 1997). Other forms in which Cr is found are Cr^{+2} (divalent Cr) and Cr^{+6} (hexavalent Cr); however, both forms are rapidly converted to Cr^{+3} in biological systems. In addition, Cr can also be found in a 0 valence state (Cr^{0}; NRC, 1980). Uses for Cr include, steel and alloy production, paint and dye manufacture, tanning, and electroplating ("chrome"; NRC, 1974). In animal and human nutrition, chromic oxide (Cr_2O_3) has been used extensively as a dietary marker to determine digestibility (Schurch et al., 1950; Woolfolk et al., 1950; Dansky and Hill, 1952; Whitby and Lang, 1960; Owens and Hanson, 1992). One of the main reasons chromic oxide has been so extensively used to estimate diet digestibility is the relatively poor absorption of inorganic Cr (NRC, 1980). According to Anderson (1988), inorganic Cr digestibility is inversely related to dietary intake, and ranges between 0.4 to 3.0%. Therefore, inorganic Cr in the form of

chromic oxide is ideal for estimating digestibility because with the relatively high concentrations fed in marker studies, only minimal amounts would be lost to absorption.

Chromium Sources and Toxicity

According to Bunting (1999), forages and feed grade by-products contain higher levels of Cr than grains and plant protein meals. Animal by-products and brewers yeast are both excellent sources of Cr; however, they only contain between 0.63 to 1.00 mg/kg (Jones and Buckley, 1977). Although Cr levels in corn silage can be as high as 2.03 mg/kg, Cr in ruminant feedstuffs should be considered poorly available (Bunting, 1999). The primary reason for decreased availability of naturally occurring dietary Cr is that the majority of Cr in feedstuffs is a result of contamination from contact with Cr-containing metal surfaces (NRC, 1997; Bunting, 1999). This is possibly the reason why harvested forages, in general, are higher in Cr than other feedstuffs. Therefore, determination of total Cr in any feedstuff is of limited value when determining biological value (McDowell, 1992). With the exception of Cr availability

from brewer's yeast and animal by-products, ruminant feedlot animal diets should be considered deficient in Cr (NRC, 1980), because they are primarily composed of cereal grains and contain little or no brewers yeast and animal protein meals.

Intoxication and animal injury from Cr in natural feedstuffs and by-products is highly unlikely. Chromium is the eighth most abundant metal on earth, and is found in water, soil, and plant and animal matter across the world (McDowell, 1992). Chromium trioxide, chromates, and bichromates are potent protoplasmic poisons because of their protein precipitating and oxidizing properties (NRC, 1980). However, chromic oxide, trivalent Cr salts, and metallic Cr are much less toxic. The LD_{50} of Cr^{+6} for cattle is 700 mg/kg, and 30 to 40 mg/kg for calves. As much as 3,000 mg/kg of trivalent Cr (Cr^{+3}), in the form of chromic oxide, is fed to cattle continuously for weeks during fecal marker trials with no adverse affects. In cattle, signs of Cr toxicity include ulceration of the rumen and abomasum, increased liver and blood concentration of Cr, dermatitis, and lung inflammation (NRC, 1980). In order for any of these signs to be observed, cattle require access to a hexavalent source of

Cr. Given that few cattle if any are grazed in heavy industrial centers, Cr toxicity in cattle is highly unlikely.

Insulin

Numerous facets of the endocrine system can affect protein deposition and muscle growth. Growth hormone (GH), insulin-like growth factor (IGF), insulin, and differentiation inhibitors are the most common factors affecting growth. Insulin is an important hormone in the metabolism and transport of carbohydrate, lipid, and protein in the body (Dickson, 1993). The production of insulin occurs in the beta cells of the Islets of Langerhans in the pancreas (Rhoades and Pflanzer, 1992). Metabolism of glucose, amino acids, and lipids is closely associated because of the action of insulin within the body (Huntington, 1997). However, even with its role in protein deposition, the primary function of insulin is to allow the transport of glucose into muscle and adipose cells across the cell membrane by facilitated diffusion. According to Ganong (1995) insulin is referred to as the "hormone of abundance," meaning that it directs the storage of excess carbohydrate, lipid, and protein.

Insulin promotes anabolic activity, while inhibiting catabolic processes (Lindemann, 1996), thereby leading to an increase in protein synthesis, a decrease in protein degradation, and an increase in lipid accumulation. Insulin secretion is stimulated by an increase in blood glucose, mannose, amino acids (leucine, arginine), fatty acids, certain gastrointestinal hormones (gastrin, secretin), and neural and pharmacological stimuli. Strong inhibitors of insulin secretion include, somatostatin, epinephrine, and norepinephrine (Ganong, 1995). In response to an increase in blood glucose, insulin secretion peaks within 30 min to 1 h, and generally returns to normal within 5 h (Rhoades and Pflanzer, 1992). During the first 30 min, plasma insulin levels increase, in turn increasing tissue uptake of glucose, resulting in decreased plasma glucose levels and a return to basal plasma glucose levels within 3 to 4 h.

In addition to the effects of insulin on glucose, its effects on protein and lipid are equally important. Insulin increases the uptake of amino acids by muscle, stimulates protein synthesis, and decreases protein catabolism (Dickson, 1993). However, in the ruminant, effects of insulin on amino acid uptake and protein

synthesis are not always observed to the extent they are in non-ruminants (Lobley, 1994). Therefore, the apparent effect of insulin on ruminant muscle tissue is to regulate protein degradation.

Effects of Chromium on Glucose Metabolism

Glucose metabolism is required for many important processes including, nervous system function, glycolysis, TCA cycle, and utilization and production of reducing equivalents (Huntington, 1997). According to Harmon (1992), insulin production is not affected by diet type (concentrate vs. roughage) in ruminants, but release of insulin is increased by increasing dietary intake. Therefore, the effects of Cr on glucose metabolism, through altered insulin action, should be consistent across the entire feedlot feeding period, with an increase in response seen during high-concentrate feeding when greater quantities of starch and glucose are available to the animal. The normal blood glucose concentration required by ruminants is 30 to 60 mg/dL for most physiological processes (Bergman, 1993).

In ruminants, dietary sources of energy are fermented in the rumen to volatile fatty acids. In

general, less than 25% of carbohydrates consumed pass through the reticulo-rumen intact and are available for further digestion and absorption as glucose in the small intestine (Van Soest, 1994). Less than 10% of the required glucose is absorbed in the ruminant digestive tract, and gluconeogenesis is required to supply more than 90% of the animal's need, with propionate supplying over 50% and amino acids over 15% of the precursors needed for gluconeogenesis (Shirley, 1986). Therefore, glucose regulation in ruminants is much more tightly controlled in terms of blood clearance and liver release because of these lower basal concentrations (Shirley, 1986; Bergman, 1993).

As mentioned previously, Schwartz and Mertz (1957, 1959) were the first to explore the relationship between Cr and glucose metabolism in animals. Today, it is well understood that Cr can alter glucose metabolism by altering the action of insulin following increases in plasma glucose concentrations (Jeejeebhoy et al., 1977; Amoikon et al., 1995; Kegley and Spears, 1995; Yang et al., 1996; Anderson, 1997). Kegley and Spears (1995) reported that plasma glucose clearance of feeder calves was greater for the first 45 min following glucose

infusion when diets were supplemented with Cr. Similar results also have been reported by Amoikon et al. (1995) in experiments with pigs. Bunting et al. (1994) found that plasma glucose clearance was improved in both steers and heifers when Cr picolinate was fed at 0.2 ppm. According to NRC (1997), Cr has a greater effect on glucose clearance in non-ruminants, than in ruminants, although in certain situations stress can cause Cr to be more effective in improving glucose clearance and thus, yield differences between Cr supplemented and non-supplemented diets. This contrast between ruminants and non-ruminants is understandable given the fact that ruminants tend to have lower blood glucose concentrations than non-ruminants, and are considered to be more insulin sensitive than non-ruminants as a result of these lower glucose concentrations.

Glucose Tolerance Factor

Glucose tolerance factor is an organic chromium complex, composed of Cr, cysteine, glutamate, glycine, and nicotinic acid. This complex is responsible for binding insulin to cell membrane receptor sites, thereby potentiating the action of insulin (Kamen, 1994).

Dietary Cr is absorbed in the intestine. Following absorption, the majority of the Cr is transported to the liver where it can be incorporated into GTF (Hossain, 1998). As stated earlier, inorganic Cr is poorly absorbed (< 3%); however, GTF-Cr is absorbed at levels greater than 20% and has a bioavailability of 25 to 30% (Hunt and Stoecker, 1996; NRC, 1997; Hossain, 1998). After improving the binding ability of insulin, and increasing the effectiveness of glucose entry into cells, GTF-Cr is degraded and excreted in the urine (Mordenti et al., 1997). Furthermore, according to Mordenti et al. (1997), GTF-Cr is indispensable for normal sugar, protein, and lipid metabolism. Without an adequate supply of GTF-Cr in the diet, normal body accretion rates of lipid and protein, and metabolism of glucose will be compromised. The result of adequate levels of GTF-Cr can be far reaching, including decreased protein need because of protein sparing, improved growth rate, decreased need for antibiotics, improved lean body composition, improved reproduction, and improved longevity (Mowat, 1996). Most of the early work with GTF-Cr in cattle was centered around the effects of Cr on immune response (Chang and Mowat, 1992; Kegley and Spears, 1995; Kegley et al.,

1996; NRC, 1997). Only recently have the effects of GTF Cr on carcass composition, and lipid and protein metabolism been more thoroughly explored in animals (Page et al., 1993; Gebert and Wenk, 1994; Boleman et al., 1995; Kornegay et al., 1997). By facilitating cellular diffusion and stimulating metabolism of glucose, the sugar will be used more efficiently as a source of energy and improvements in protein synthesis and muscular development can be observed (Mordenti et al., 1997).

Effects of Chromium on Lipid Metabolism

According to NRC (1997), Cr has been suggested to be necessary for normal lipid metabolism and cholesterol regulation in humans. In farm animals, specifically pigs, the effects of Cr on lowering carcass lipid deposition are well noted. Pig adipose tissue uses 25 and 40% of whole body glucose in the insulin stimulated state and basal states, respectively (NRC, 1994). Therefore, any alteration in insulin activity can greatly change the conversion of excess glucose into adipose tissue. Page et al. (1993) reported that 200 ppb of Cr supplementation, in the form of Cr picolinate, decreased back fat thickness in pigs by 13.8 to more than 22% in

three separate experiments. Mordenti (1997) also reported similar effects in rabbits, and Shields (1997) found that Cr can alter lipid content of the carcass and blood in companion animals. Surprisingly, with the consumer movement towards lower fat diets, and the effects of Cr on alteration of carcass lipid content, the only specie with an approved level of Cr in the diet is swine.

Effects of Chromium on Carcass Parameters

Page et al. (1993) conducted some of the earliest research to show that supplemental Cr altered carcass composition in swine. They found that Cr decreased carcass fat deposition at the 10^{th} rib for both lean and obese genotypes tested, and improved longissimus muscle area in genetically fatter genotypes. Lindemann et al. (1995) reported that Cr supplementation neither decreased carcass back fat nor improved longissimus muscle area in the finishing phase; however, in a second trial when pigs were started on Cr at lighter bodyweights and fed for a longer time, Cr improved lean percent and decreased back fat depth. However, Boleman et al. (1995) found that muscling percent and 10^{th} rib back fat were only improved

by Cr supplementation during the finishing phase, and not when Cr was fed during both growing and finishing phases. During this same study (Boleman et al., 1995), Cr also increased Warner Bratzler shear force value when fed during both growing and finishing phases, which is something that was not measured in the previous experiment by Lindemann (1996), and could possibly be detrimental to the use of Cr in swine diets. More recently, Mooney and Cromwell (1997, 1999) conducted experiments to determine the effects of Cr on carcass composition. In the first experiment (Mooney and Cromwell, 1997), they reported that both Cr picolinate and Cr chloride improved longissimus muscle area, and both decreased 10^{th} rib fatness; however, Cr picolinate decreased carcass fatness to a greater extent than Cr chloride. In a second experiment, Mooney and Cromwell, (1999) investigated the effects of Cr on pigs with different lean gain potentials and found that Cr picolinate had no effects on carcass leanness or carcass fatness. These types of differences in responsiveness to Cr supplementation have lead, in part, to the difficulty of approving Cr as a required nutrient (NRC, 1997).

In addition to swine, the effect of Cr on carcass composition also has been studied in several other species. Kitchalong et al. (1995) found that Cr decreased 10^{th} rib fatness in lamb carcasses by 18% and decreased yield grade by over 15%. However, a similar study by Gentry et al. (1999) failed to show any improvement in carcass characteristics for Cr-supplemented lambs. In studies with broilers, Hossain (1998) found results similar to those of Page et al. (1993) and Kitchalong et al. (1995), in pigs and lambs, respectively. Chromium increased breast weight 7%, decreased tissue lipid 54%, and improved yield 8%. In addition, Cr also increased body weight gain of broilers over the first 6-wk period. Hasten et al. (1997a, 1997b), in two separate experiments, found that Cr tended to decrease body fat percentage while not affecting lean muscle mass. The inconsistency of these results have led to the need for further investigation into the effects of Cr in various forms on important growth, carcass, and performance measurements.

Muscle Cell Culture to Investigate Muscle Growth

Initial research with cultured muscle cells revolved around cells harvested from murine and avian sources to study the effects of growth factors on differentiation and cell proliferation (Allen et al., 1979; Florini et al., 1987; Kerth, 1999). It has been suggested that bovine muscle cells might respond differently to hormones and circulating growth factors than non-ruminant cells (Harper et al., 1987). This idea was first attempted in a study by Reecy et al. (1994), in which serum from treated steers was used in muscle cell culture to identify factors involved in regulation of muscle growth. Primary bovine muscle cell culture has been used extensively in the study of ruminant animal growth. In addition, techniques have been recently reviewed and updated in order to make in vitro muscle cell culture a more reliable and accurate means to study bovine animal growth (Woods et al., 1997). The need for such investigative techniques is a result of the fact that muscle accounts for 50 to 75% of a meat animal's live weight (Lawrie, 1991) and having experimental conditions that allow for efficient, low cost, and rapid

determination of effects on muscle growth is needed to reduce the cost of meat production (Woods et al., 1997). Several recent theses and dissertations have used cell culture techniques as a basis for determining the effects of serum, circulating factors, or cellular constituents on muscle cell growth (Thomson, 1996; Kerth, 1999; Morrow, K.J., personal communication, 1999). The use of cell culture is an invaluable tool in the study of the cellular basis for animal responses observed in the scientific study of livestock, and to determine whether certain responses are a result of changes in cellular metabolism.

Conclusions and Objectives

Chromium appears to alter not only growth process, but also metabolism, of glucose, amino acids, and lipid. Unfortunately, these results are not consistently observed, which is most likely a result of differences in stress, previous Cr status, and(or) available Cr in the diet. The most widely accepted mode of action for Cr is its effect on the cellular binding capability of insulin. However, the effects of Cr on ruminant bovine muscle are not well understood as to whether the response observed

in muscle growth is a result of circulating factors, Cr, or increased nutrient supply.

It is unclear whether the improvement in longissimus muscle area in animals supplemented with Cr is a result of improved glucose uptake by the cell, increased protein synthesis, neither, or both. Although most research conducted has yet to investigate these effects, several researchers have indicated that improvements in longissimus muscle area may be a result of changes in serum constituents. Glucose, insulin, and cellular sensitivity to both of these has been well studied; however, no studies have been conducted with bovine serum to determine whether the effects of Cr on muscle growth are reproducible in cell culture.

The first objective of this research is to determine the effects of Cr on beef steer performance, growth, and carcass characteristics. Because the effects observed in carcass composition have not been fully explained, the second objective is to determine whether serum from Cr-supplemented steers affects glucose uptake and protein synthesis is a muscle cell culture system.

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CHAPTER III

EFFECTS OF ORGANIC CHROMIUM ON GROWTH, EFFICIENCY, AND CARCASS CHARACTERISITICS OF FEEDLOT STEERS

Abstract

The objective of this research was to determine the effects of supplemental chromium (Cr) during growing and finishing periods on steer feedlot performance and carcass characteristics. English and Continental crossbred steers (n = 105, BW = 283 ± 22 kg) were allotted randomly to three dietary treatments: (1) control (CON, basal diet); (2) basal diet with 0.2 ppm of supplemental Cr; and (3) basal diet with 0.4 ppm supplemental Cr. Chromium was supplied as high-Cr yeast in a ground grain sorghum premix added to the diet at either 0.25% or 0.50% in replacement of steam-flaked grain sorghum. All steers were fed a growing diet for 35 d, followed by a finishing diet for 161 d. Neither control nor treated steers experienced any significant health problems throughout the trial. Dry matter intake tended (P = .22) to be lower by steers fed CON and 0.4 ppm Cr diets than by steers fed the 0.2 ppm Cr diet (8.22 and 7.80 vs. 8.96 kg/d). Daily gain was greater (P <

.05) by steers fed CON and 0.2 ppm Cr than by steers fed 0.4 ppm Cr diet (1.11 and 1.16 vs. 0.96 kg/d). Gain-to-feed (GF) ratio was greater ($P < .05$) for steers fed CON and 0.2 ppm Cr than for those fed 0.4 ppm Cr diet (135 and 129 versus 118 g gain/kg feed). Serum cortisol was unaffected by Cr feeding throughout the trial, except on d 196 when 0.2 ppm Cr steers had elevated ($P < .05$) serum cortisol levels compared with CON and 0.4 ppm fed steers. Steers fed the 0.2 ppm Cr diet had heavier ($P < .05$) carcass weights than steers fed 0.4 ppm Cr, with CON steers being intermediate (315, 276, and 302 kg, respectively). Longissimus muscle area was greatest ($P < .05$) with the 0.4 ppm Cr diet and least with the CON diet, with 0.2 ppm Cr diet being intermediate (81.6, 77.9, and 81.4 cm^2, respectively). Marbling score was decreased ($P < .05$) by 0.4 ppm Cr diet compared with the CON and 0.2 ppm Cr diets (395 vs. 472 and 446). Dressing percent was increased ($P < .05$) by the CON and 0.2 ppm Cr diets compared with the 0.4 ppm Cr diet (63.37 and 63.65 vs. 62.16). Final yield grade was increased ($P < .05$) by the CON and 0.2 ppm Cr diets and decreased by the 0.4 ppm Cr diet (2.53 and 2.32 vs. 1.56). Feeding Cr failed to improve growth performance and gain efficiency; however,

Cr improved many economically important carcass characteristics, which is important when cattle are sold on a system, that emphasizes hot carcass weight, longissimus muscle area, and final yield grade.

Introduction

Rate of growth and feed conversion by feedlot cattle are influenced by feed intake and energetic efficiency (NRC, 1996). Chromium (Cr) is an important trace element in the regulation of blood glucose and immune response in humans and laboratory animal species (NRC, 1997). Chromium is known to be a structural component of a glucose tolerance factor, which potentiates the action of insulin and is an essential trace mineral for normal metabolism of carbohydrates and lipids (NRC, 1989; Mertz, 1993). Recently, Cr has been shown to improve reproduction, growth, and carcass characteristics of pigs fed organic sources of chromium (Boleman et al., 1995; Lindemann et al., 1995; Kornegay et al., 1997; Mooney and Cromwell, 1997). In cattle, supplemental Cr has enhanced cell-mediated immunity, decreased blood cortisol concentrations and increased antibody titers (Change and Mowat, 1992; Kegley et al., 1996; Mallard and Borgs,

1997). Moonsie-Shageer and Mowat (1993) demonstrated the efficacy of supplemental Cr for decreasing morbidity in stressed feeder calves. Effects of Cr on performance and growth variables have been less consistent than the effects of Cr on immune factors in beef cattle.

The effects of Cr as a potential carcass modifier have been well documented with swine (Page et al., 1993; Gebert and Wenk, 1994; Boleman et al., 1995). Kornegay et al. (1997) found that Cr increased longissimus muscle area, and when pigs were fed Cr at lighter weights for a longer period of time, the increase in longissimus muscle area was greater than for shorter feeding periods. Similar results were reported by Mooney and Cromwell (1997), who in addition to increased longissimus muscle area, noted that Cr improved carcass protein deposition rate and decreased percentage of carcass fat. Hasten et al. (1997a) reported decreased fat accretion in rats when supplemental Cr was fed. In a subsequent study, Hasten et al. (1997b) supported their previous findings, again noting a decreased percentage of carcass fat in rats supplemented with Cr.

The objectives of the present study were to determine the effects of two levels of supplemental

organic Cr (Cr yeast) on feed intake, growth rate, feed conversion, blood cortisol, and carcass characteristics of feedlot steers.

Materials and Methods

The 196-d experiment was conducted at the Texas Tech University Burnett Center, a research feedlot on the southern High Plains of Texas, during winter, 1997 and spring, 1998. One hundred five crossbred steers (283 ± 22 kg) of primarily Angus, Hereford, Charolais and Simmental breeding were housed outdoors in pens (2.9 m x 5.6 m) equipped with a partially slotted concrete floor and an automated feed delivery system. Feed for each pen was individually mixed and delivered at approximately 0900 h each morning. Before feeding, feed remaining in bunks was estimated visually, which was used to adjust the daily delivery of feed. Treatments consisted of a basal diet (CON), basal diet plus 0.2 ppm Cr from Bio-Chrome (0.2 ppm Cr), and basal diet plus 0.4 ppm Cr from Bio-Chrome (0.4 ppm Cr).

Diets fed daily to the steers were typical feedlot diets consisting of a steam-flaked grain sorghum and cottonseed hull base (Table 3.1). On arrival at the

feedlot, steers were immediately processed, including clostridial vaccination (Ultrabac/Somubac™, SmithKline Beecham,), deworming (Ivomec™, Meriel), prophylactic medication (Micotil™, Elanco Animal Health), implanting (Synovex-S™, Fort Dodge Animal Health), an individual ear tag, and an individual BW measurement. After processing, steers were fed a 50% concentrate/50% roughage receiving diet containing chlortetracycline and sulfamethiazine (AS 700, Roche Animal Health). The following day, animals were sorted by BW and assigned randomly by BW to five pens per treatment with seven steers per pen, for a total of 35 steers per treatment. The Cr yeast treatments were started immediately after sorting and fed during the time steers were adapted to the high-concentrate diet (35 d), and continued for the remainder of the study.

The 0.2 ppm Cr treatment group received a Cr premix as .25% of the diet that consisted of ground grain sorghum and high-Cr yeast that contained 80 ppm of Cr. The 0.4 ppm Cr treatment group received the same premix at 0.50% of the diet. The Cr premix in each treatment group replaced steam-flaked grain sorghum in the basal diet to ensure that diets were iso-nitrogenous and iso-caloric for each treatment group.

Individual steer BW were recorded in a hydraulic squeeze chute fitted with load cells at 28-d intervals during the experiment. Average daily gain, GF, and DMI were calculated for each 28-d period. Blood samples were collected from each steer before feeding in the morning on d 0, 14, 28, 56, and 196 via jugular venipuncture for determination of cortisol. Immediately after collection, these samples were placed on ice until they were transported back to the laboratory, where blood was centrifuged at 3,000 x *g*. Serum was separated and stored at-20°C until cortisol analysis was conducted using a cortisol specific radioimmunoassay kit (Coat-A-Count, Diagnostic Products Corporation).

At the conclusion of the study, steers were transported to a commercial packing plant where liver abscess scores, final yield grade, marbling score, dressing percent, longissimus muscle area, and USDA quality grade were measured by trained personnel from the Texas Tech University Meats Laboratory. A trained person familiar with differing backfat measurements also visually estimated backfat thickness to the nearest 0.05 cm.

This completely random design was analyzed using the GLM procedure of SAS (1995). Pen was the experimental unit, and means were separated using Fischer's Protected Least Significant Difference Test.

Results and Discussion

Initial weights of steers did not differ among treatments. No differences were detected for ADG, DMI or GF during the first 56-d of the study (Table 3.2). These data are consistent with previous studies conducted by Chang et al. (1992) and Kegley et al. (1996) who also found that ADG, DMI and FG were not affected by Cr supplementation. However, steers fed the 0.4 ppm Cr treatment tended ($P = .17$) to have a lower DMI during the first 56 d period.

The effects of Cr on performance during the finishing phase are shown in Figures 3.1, 3.2, and 3.3. During the final 140 d of the study (Table 3.2), no differences were detected among treatments for DMI; however, steers receiving 0.4 ppm Cr had lower ($P < .05$) ADG and decreased ($P < .05$) GF than CON steers and those fed 0.2 ppm of Cr. These results are consistent with those of Chang and Mowat (1992), who found that

supplemental Cr at 0.2 ppm did not improve performance compared to control cattle. In contrast, Moonsie-Shageer and Mowat (1993) found that Cr improved DMI and ADG when fed at 0.2 ppm to feedlot steers over a 56-d trial period. Average daily gain and DMI increased for all treatments during the final 140-d when compared with performance during the initial 56-d of the study. Overall, performance by cattle receiving 0.2 ppm Cr did not differ ($P < .05$) from control steers (Table 3.2); however, cattle on the 0.4 ppm Cr treatment had a lighter ($P < .05$) final live weight, as well as lower ($P < .05$) ADG and lower ($P < .05$) GF than cattle on the control and 0.2 ppm Cr treatments.

Carcass characteristics of steers following the 196-d feeding period are presented in Figures 3.4, 3.5, 3.6, 3.7, and 3.8. The 0.2 ppm Cr treatment increased ($P < .05$) hot carcass weight compared with the 0.4 ppm Cr treatment, with control steers being intermediate (Figure 3.4). Dressing percent, marbling score, and final yield grade were decreased ($P < .05$) compared with control and 0.2 ppm Cr treatments when the 0.4 ppm Cr treatment was fed. Longissimus muscle area was increased ($P < .08$) with Cr supplementation (Figure 3.6). Kornegay et al.

(1997) and Mooney and Cromwell (1997) both reported a similar effect on longissimus muscle area in swine supplemented with 0.2 ppm Cr. In contrast, Kitchalong et al. (1995) found that 0.2 ppm Cr decreased final yield grade, while having no effect on longissimus muscle area in lambs. No effects of treatment on serum cortisol concentrations (Table 3.3) were noted on d 0, 14, 28, and 56; however, on d 196 steers on the 0.2 ppm Cr treatment had increased ($P < .05$) serum cortisol compared with the other two treatments. Similar results were reported by Arthington et al. (1997), who found that Cr did not influence cortisol production in calves.

Implications

Feedlot cattle supplemented with Cr yeast had a significantly larger longissimus muscle area than those fed a control diet. Hot carcass weight also was increased by the 0.2 ppm supplementation of Cr. Average daily gain and DMI were decreased, and GF was decreased, in cattle supplemented with 0.4 ppm of Cr. These data indicate that under our conditions, high-Cr yeast supplemented at 0.2 ppm improved two economically important carcass traits: hot carcass weight and

longissimus muscle area. In addition, when Cr was supplemented at 0.4 ppm, longissimus muscle area was increased to an even greater extent than with 0.2 ppm Cr.

Table 3.1. Composition and analysis of finishing diets (DM)

	TREATMENTS		
Ingredients	**Control**	**0.2 ppm Cr**	**0.4 ppm Cr**
Grain sorghum, Steam flaked	77.22	76.92	76.61
Cottonseed hulls	9.50	9.50	9.50
Cottonseed meal	2.52	2.52	2.52
Molasses, cane	3.88	3.88	3.88
Fat, yellow grease	3.10	3.10	3.10
Urea	0.63	0.63	0.63
Limestone, ground	1.04	1.04	1.04
Salt	0.12	0.12	0.12
Potassium chloride	0.39	0.39	0.39
Mineral premix[a]	0.23	0.23	0.23
Vitamin A premix[b]	0.30	0.30	0.30
Vitamin E premix[c]	0.16	0.16	0.16
Drug premix[d]	0.91	0.91	0.91
Chromium premix[e]	--	0.30	0.61
Total	100.00	100.00	100.00

	TREATMENTS		
Item	**Control**	**0.2 ppm Cr**	**0.4 ppm Cr**
Crude protein[f], %	13.59	13.53	13.68
NEm, Mcal/kg[g]	2.02	2.02	2.02
NEg, Mcal/kg[g]	1.36	1.36	1.36

[a]Trace mineral premix contained (ppm) I, 559; Mn, 3,815; Zn, 3,815; Cu, 375; Co, 23; Fe, 1,840.
[b]Vitamin A premix provided 300,000 IU of Vitamin A acetate/ kg.
[c]Vitamin E premix provided 1,765 IU of α-tocopherol/ kg.
[d]Rumensin/Tylan premix provided 360 mg/ steer daily of Monensin and 66.64 mg/ steer daily of Tylosin
[e]Chromium premix contained 80 ppm of Cr.
[f]Kjeldahl N analysis method.
[g]Calculated energy value.

Table 3.2. Effect of Cr supplementation on average daily gain, dry matter intake, and gain efficiency during the diet adaptive period (Day 0 to 56)[1] and during the finishing period (Day 57 to 196)

	TREATMENT			
ITEM	Control	0.2 ppm Cr	0.4 ppm Cr	SE
(DAY 0 to 56)				
Initial wt., kg	291.69	287.24	280.32	6.20
Dry matter intake, kg/d	8.26	8.30	7.66	0.93
Average daily gain, kg/d	0.74	0.74	0.75	0.12
Gain:feed, g/kg	94.43	93.98	98.33	3.23
(Day 56 to 196)				
Initial wt., kg	333.13[a]	328.72[a]	322.56[b]	2.18
Dry matter intake, kg/d	8.40	9.37	8.04	1.34
Average daily gain, kg/d	1.24[a]	1.31[a]	0.99[b]	0.18
Gain:feed, g/kg	147.93[a]	139.47[a]	123.61[b]	4.55

[1]No differences were found between treatments.

[a,b]Means in the same row with different superscripts differ (P<.05).

Table 3.3. Effect of Cr supplementation on serum cortisol concentrations (ug/dL) of growing and finishing beef steers

	TREATMENT			
Day	Control	0.2ppm Cr	0.4 ppm Cr	SE
0	3.54	4.24	3.44	0.35
14	3.88	3.98	3.21	0.45
28	5.35	5.80	4.67	0.53
56	5.06	5.48	4.67	0.56
196	5.19[a]	6.91[b]	4.83 [a]	0.47

[a,b]Means in the same row with different superscripts differ (P<.05).

Table 3.4. Effect of Cr supplementation on quality grade of steers during a 196-day feeding period

	TREATMENT		
ITEM	Control	0.2ppm Cr	0.4 ppm Cr
Choice, %	32.2	14.7	2.9
Select, %	58.9	64.7	40.0
Standard, %	5.9	20.6	57.1

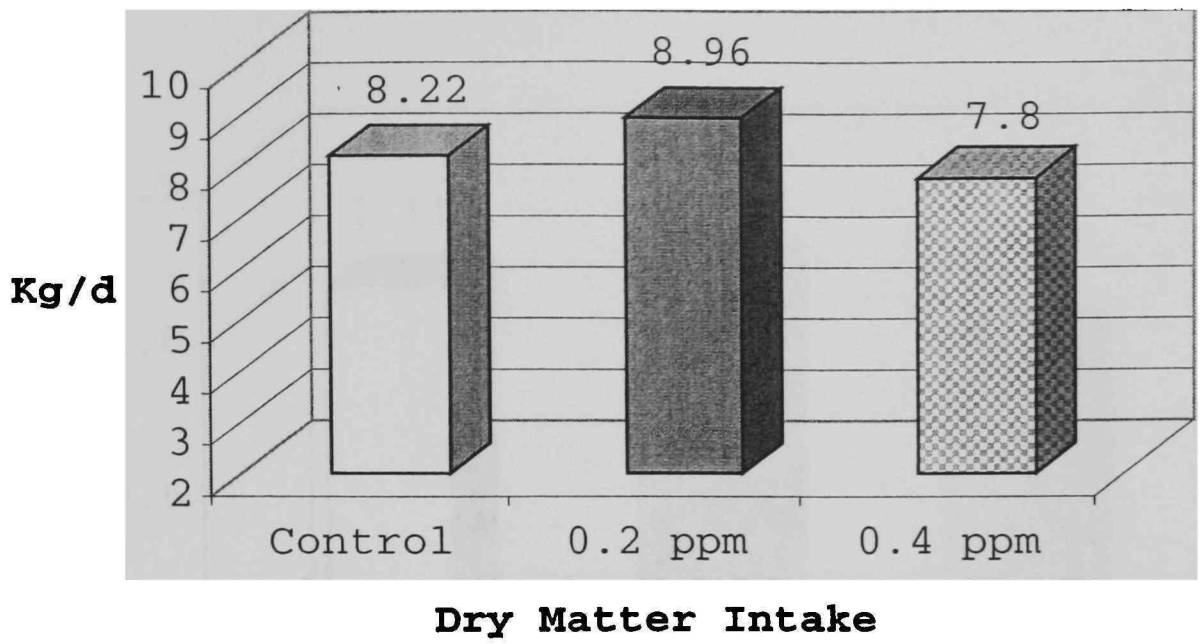

Figure 3.1. Effect of Cr supplementation on dry matter intake during a 196-d feeding period (SEM = 1.21).

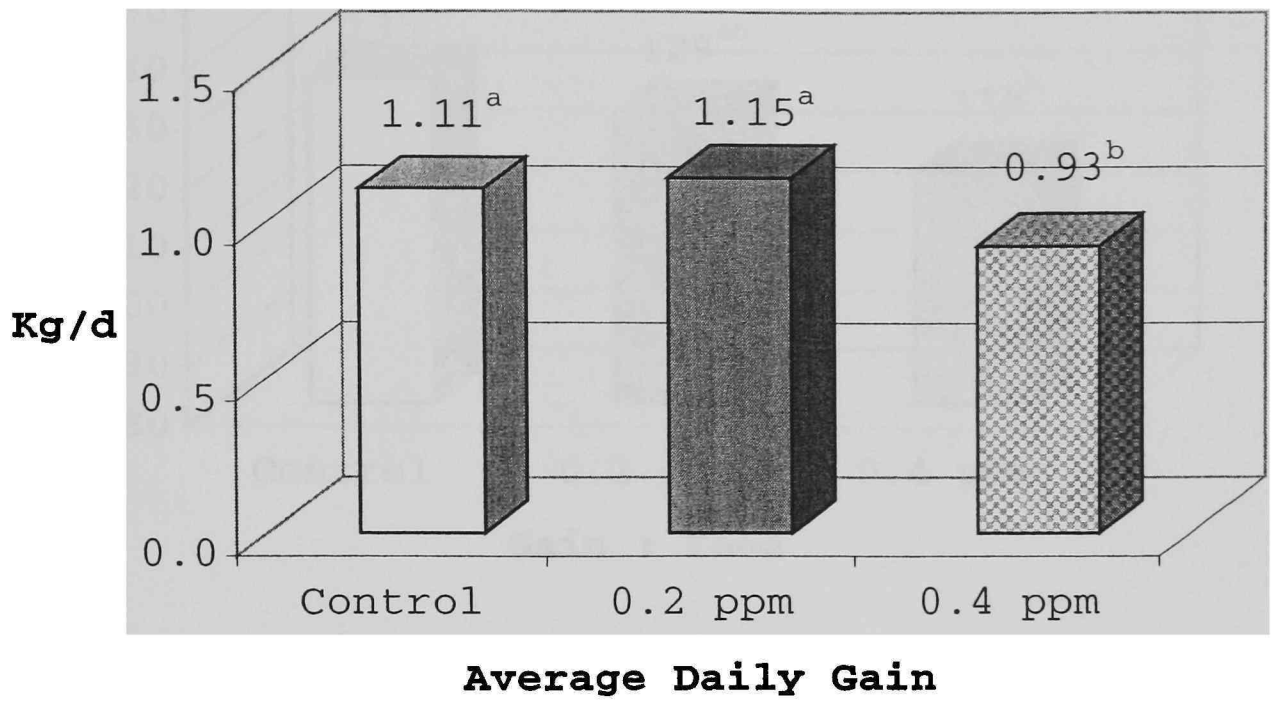

Figure 3.2. Effect of Cr supplementation on average daily gain during a 196-d feeding period (SEM = 0.03). [a,b]Means with different superscripts differ (P<.05).

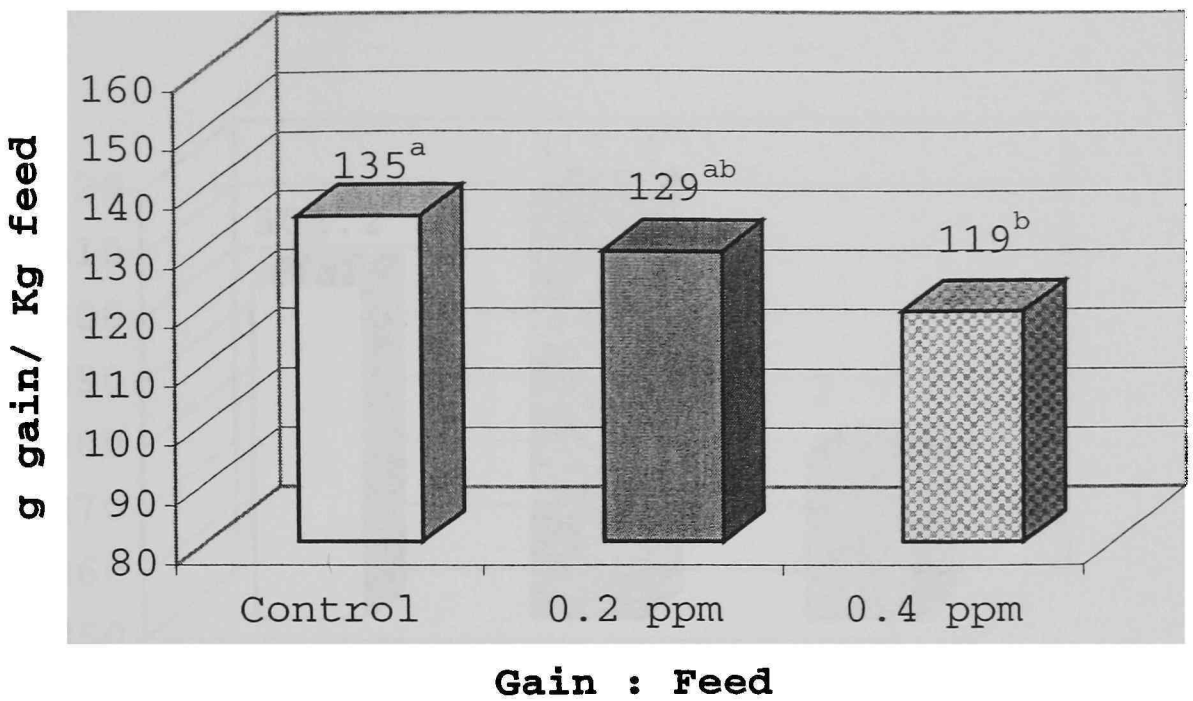

Figure 3.3. Effect of Cr supplementation on gain to feed during a 196-d feeding period (SEM = 8.33). [a,b]Means with different superscripts differ (P<.05).

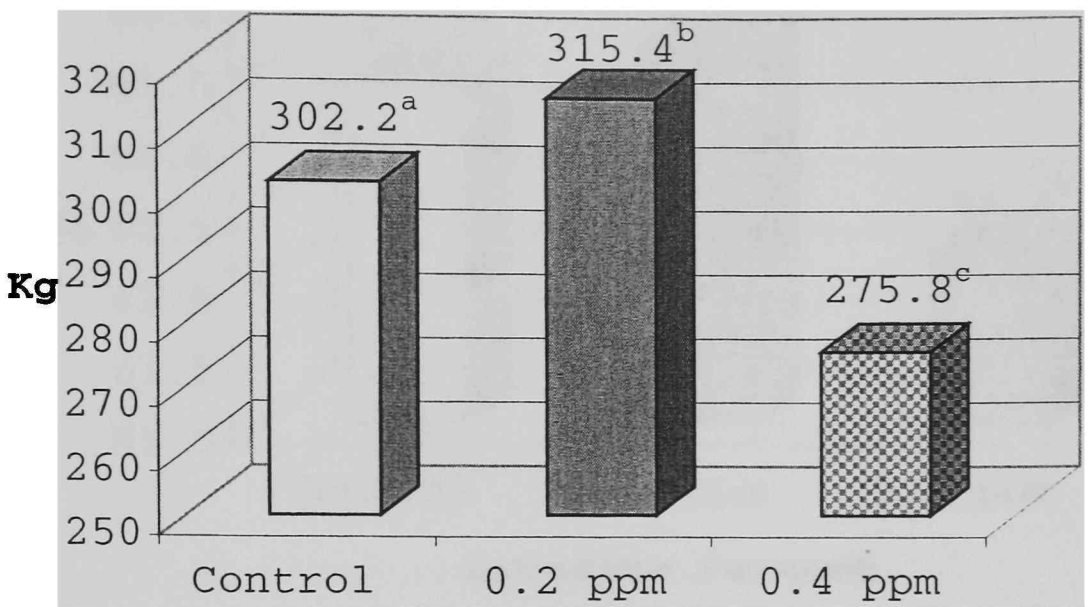

Figure 3.4 Effect of Cr supplementation on hot carcass weight during a 196-d feeding period (SEM = 4.1).
[a,b,c]Means with different superscripts differ (P<.05).

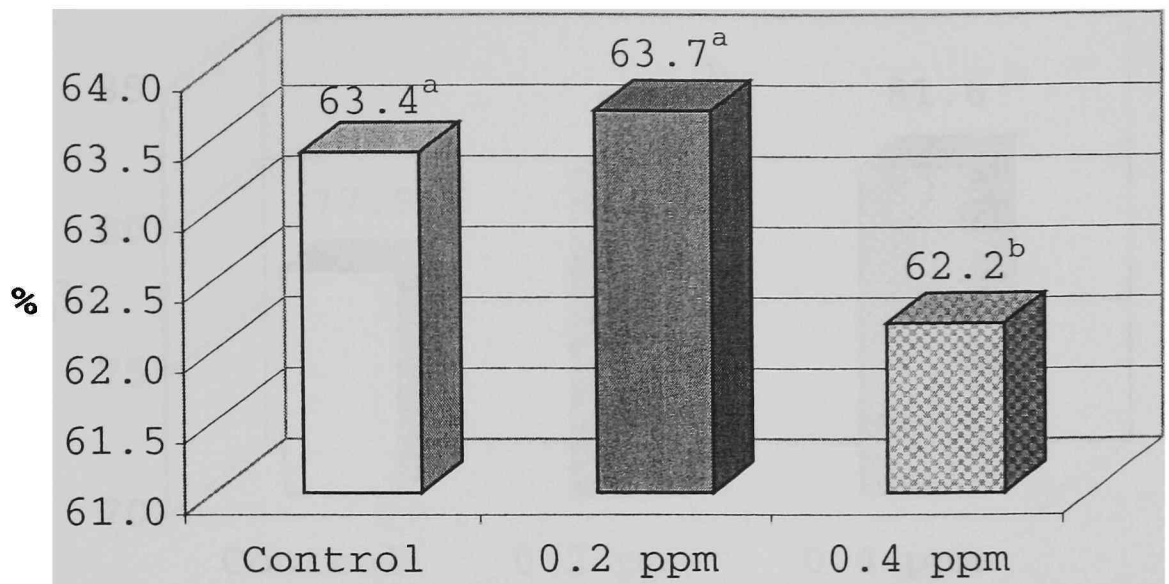

Figure 3.5. Effect of Cr supplementation on dressing percentage during a 196-d feeding period (SEM = 0.14). [a,b]Means with different superscripts differ (P<.05).

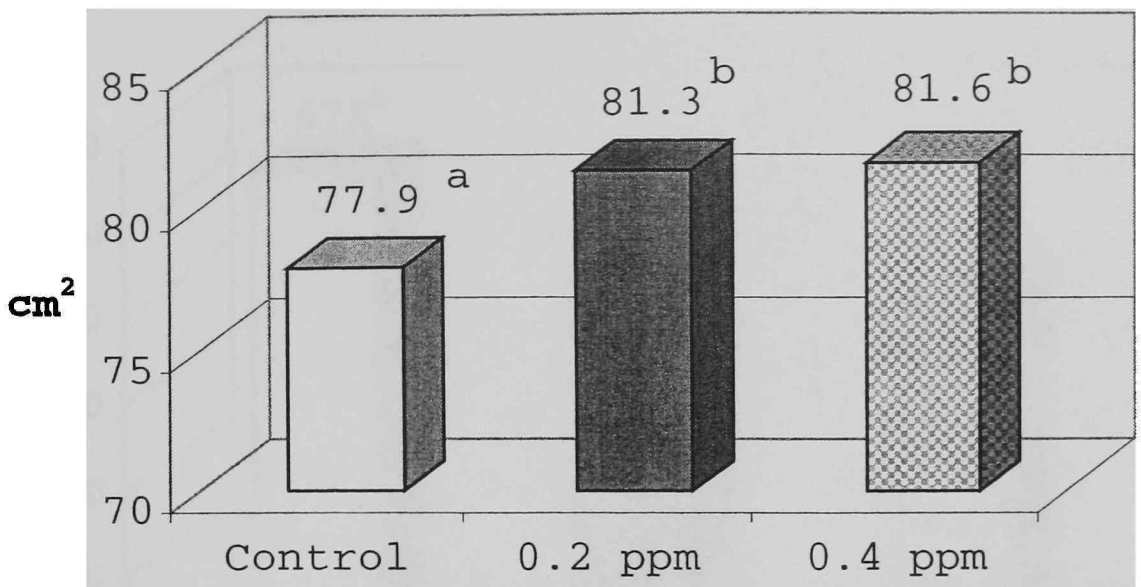

Figure 3.6. Effect of Cr supplementation on longissimus muscle area during a 196-d feeding period (SEM = 0.09). [a,b]Means with different superscripts differ (P<.05).

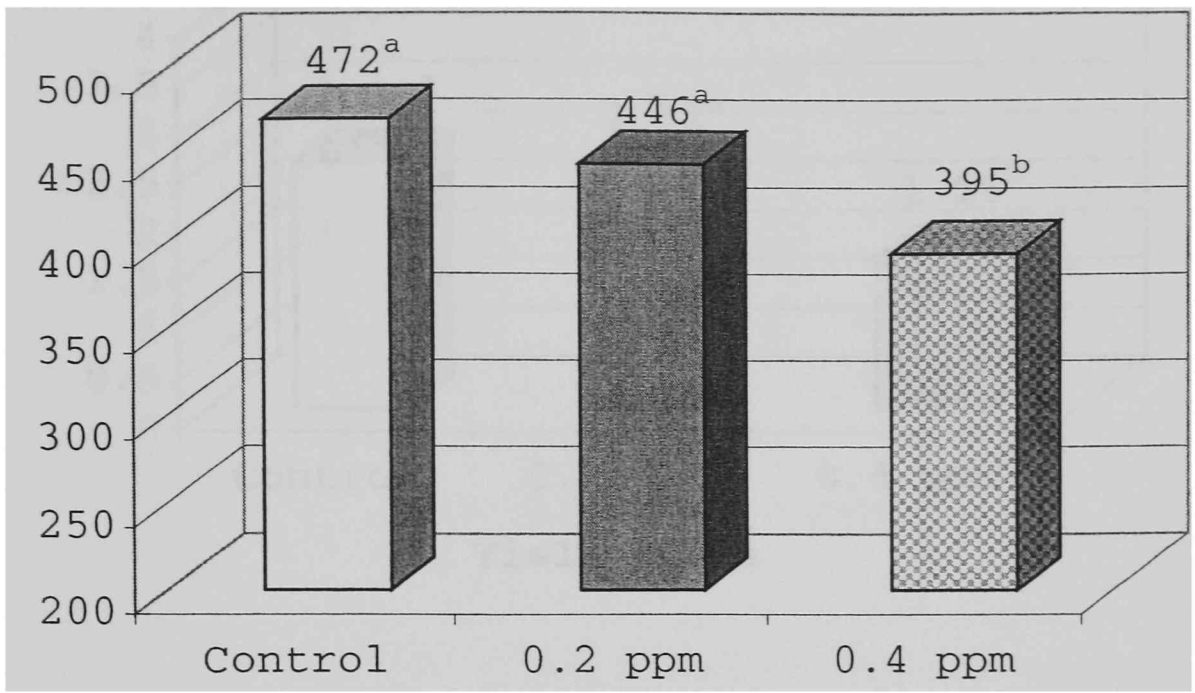

Figure 3.7. Effect of Cr supplementation on marbling score during a 196-d feeding period (SEM = 10.69).
[1]Marbling scores: 300=traces, 400=slight, 500=small.
[a,b]Means with different superscripts differ (P<.05).

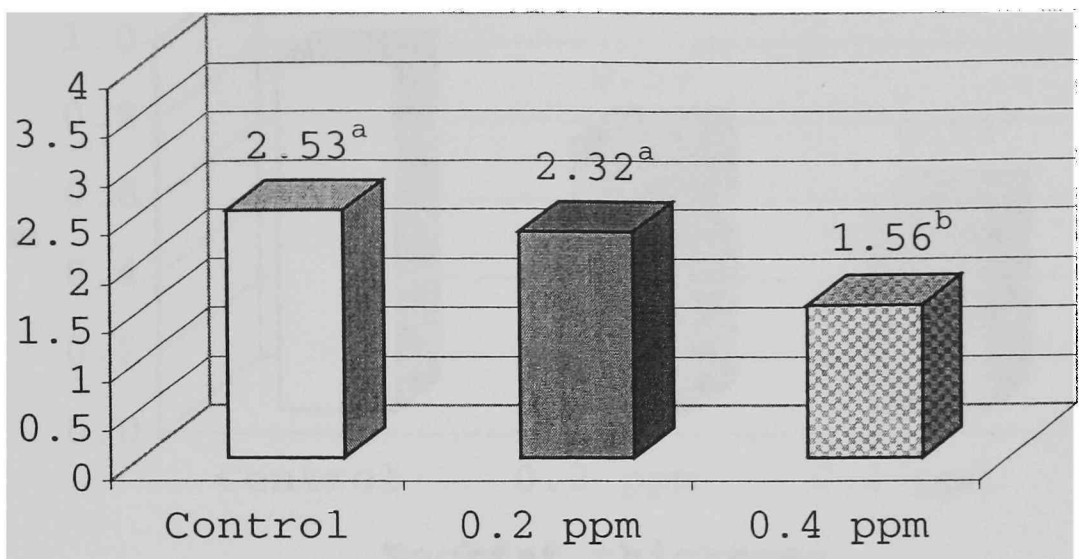

Figure 3.8. Effect of Cr supplementation on final yield grade during a 196-d feeding period (SEM = 0.13).
[a,b]Means with different superscripts differ (P<.05).

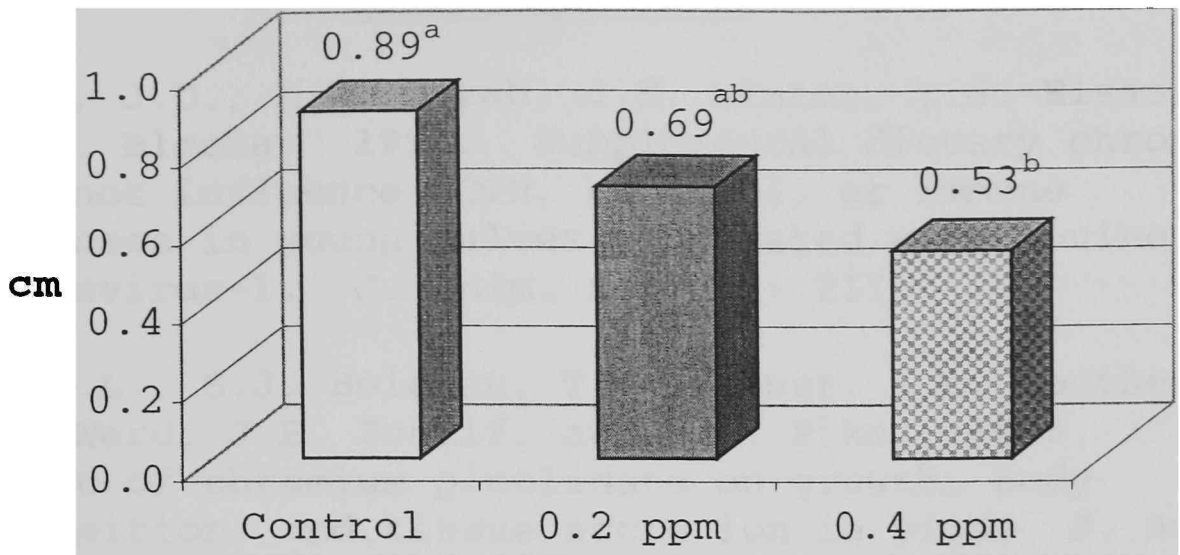

Figure 3.9. Effect of Cr supplementation on backfat thickness during a 196-d feeding period (SEM = 0.16). [a,b]Means with different superscripts differ (P<.05).

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CHAPTER IV
EFFECTS OF LEVEL OF ORGANIC CHROMIUM ON IN VITRO DRY MATTER DISAPPEARANCE OF STARCH AND CELLULOSE

Abstract

Minerals are known to affect digestibility of forages and diets, both in vitro and in vivo. The effects of organic Cr on digestibility were investigated using in vitro digestibility techniques to determine whether Cr supplementation at levels currently fed in research trials affected digestibility of starch or cellulose. Previous research with Cr has indicated that diet digestibility is not decreased when Cr is supplemented at levels of 0 to 0.4 ppm in swine diets. The effects of four different levels of Cr (0 ppm, 0.2 ppm, 0.4 ppm, and 0.8 ppm) were determined by in vitro analysis for DM disappearance (DMD). A commercially available form of high-Cr yeast was combined with either corn starch or solka floc to simulate the effects of Cr on high-concentrate or high-roughage diets. Substrate and ruminal fluid mixtures were incubated for 12, 24, and 48 h for corn starch DMD and 24, 48, and 96 h for solka floc DMD. No consistent differences were found for any level of Cr with either corn starch or solka floc during

the final two incubation periods. However, during the initial incubation period, 12 and 24 h for corn starch and solka floc, respectively, substrates without Cr had lower ($P < .05$) DMD. As expected, corn starch had greater ($P < .05$) levels of DMD at 24 and 48 h incubation times than solka floc. These data indicate that Cr supplementation might not have any deleterious effects on diet digestibility with either high-starch or high-roughage diets.

Introduction

Minerals, both trace and macro, have been shown to affect diet digestibility in both in vitro and in vivo studies (Minson, 1990; McDowell, 1992; Petit and Tremblay, 1995). According to Van Soest (1994), rare earth minerals are capable of suppressing diet digestibility even at relatively low levels. Silica has also been shown to decrease cellulose digestibility, regardless of whether it is associated with the plant cell wall fraction (Smith et al., 1971). McDowell (1992) reported that vanadium, when fed at levels as low as 0.5 ppm, decreased in vitro DMD of a cellulose diet. Minson (1990) and McDowell (1992) have both reported in vitro

and in vivo digestibility increases in response to additions of S, Zn, and Mg to fermentation systems deficient in one or more of these minerals. However, Mg tended to decrease digestibility of in vitro mixtures when inclusions exceeded 40 ppm. Recently, Salyer and Galyean (1999) found that level and source of mineral is important in determining the effect of a specific mineral on DMD; however, it was concluded that neither source nor level of Cu or Zn dramatically affected DMD even when included at three times the NRC (1996) recommendation.

The effects of Cr have been well documented in regards to its effect on carcass composition, glucose tolerance, and immune response (Chang and Mowat, 1992; Boleman et al., 1995; Kegley et al., 1996; NRC, 1997). However, the effects of organic Cr on diet digestibility are not well understood. Only two studies (Gebert and Wenk, 1994; Kornegay et al., 1997) have addressed the effects of organic Cr on diet digestibility with swine diets, and no information is available on the effects of Cr on ruminal digestion. Therefore, the objective of the present study was to determine whether organic Cr supplied in an in vitro digestibility system, decreased

or increased digestibility of a corn starch or solka floc substrates.

Materials and Methods

Substrates for the in vitro culture system were made by combining Cr in the form of high Cr yeast with either solka floc or corn starch (Agro®, Englewood Cliffs, NJ). Initial mixtures were calculated to contain 1.6 ppm of Cr in either solka floc or corn starch. Enough of each substrate and Cr yeast were combined in separate 1-L canning jars to bring the final concentration of Cr to 1.6 ppm. The jars were then mixed by hand, tumbling for 3 min. This initial mixture was used to make all test mixtures for the in vitro trial. The in vitro trial consisted of four levels of Cr in each substrate (0 ppm, 0.2 ppm, 0.4 ppm, and 0.8 ppm). The final concentrations of Cr were made by diluting the initial mixture with enough substrate to make the desired concentration. The initial mixture was the only mixture used to make the resulting Cr substrates.

For example, 500 g of initial (1.6 ppm of Cr) mixture were combined with 500 g of Cr-free mixture to make the 0.8 ppm Cr mixture. .5 (1.6 ppm) = 0.8 ppm

Substrates were weighed into 100-mL in vitro culture tubes and combined with ruminal fluid buffered with McDougall's artificial saliva containing urea. Ruminal fluid was obtained from a steer fitted with a rumen cannula, fed a predominately forage diet for the solka floc trial or a concentrate diet for the corn starch trial. Fresh ruminal fluid was collected at the Texas Tech University Farm in New Deal, TX and immediately transported back to the nutrition lab. In the laboratory, ruminal fluid was strained through four layers of cheesecloth and combined with McDougall's artificial saliva in a mixture of three parts ruminal fluid to seven parts of artificial saliva. In vitro tubes were then filled with 50 mL of ruminal fluid:artificial saliva mixture under 95% CO_2 and placed into a 37°C water bath. Following incubation, in vitro cultures were removed from the water bath and mixed with 2-mL of 0.2 N HCl solution to inhibit further digestion and placed into a -5°C freezer until filtering. Thawed in vitro samples were filtered using Gouch crucibles (Coors™, Golden, CO) filled with sufficient fiberglass angel hair (Fisher, Houston, TX) to prohibit loss of

substrate during filtering. A water vacuum was applied to the crucible during the filtering process. Crucibles containing the filtered substrate were immediately placed into a 55°C oven for 48 h and then weighed to determine DMD. The procedures used for the in vitro portion are the Moore modification of the Tilley and Terry procedure as described by Summers and Sherrod (1974) and Harris (1970).

This completely randomized experiment was analyzed by analyses of variance using GLM procedure of SAS (1995). Tube was the experimental unit, and means were separated using Fischer's Protected Least Significant Difference Test.

Results and Discussion

Dry matter disappearance increased ($P < .05$) as length of incubation increased for both corn starch and solka floc treatments. As expected, corn starch had greater ($P < .05$) DMD than solka floc. Results for corn starch and solka floc DMD are presented in Figures 4.1 and 4.2, respectively. Chromium improved ($P < .05$) 12 h corn starch DMD (Figure 4.1) by an average of 8%. In addition, Cr improved 24 h solka floc DMD (Figure 4.2) by

an average of more than 100%. These results are supported by Gebert and Wenk (1994), who found that Cr supplementation improved DM digestibility of swine diets. Kornegay et al. (1997) found that corn-soybean meal swine diets supplemented with 0.2 ppm Cr had more than 2% greater DM digestibility. It was not anticipated that Cr would have such a dramatic effect on initial DMD of solka floc in the present study. Effects of Cr on ruminant diet digestibility have not been reported previously. These results indicate that supplemental organic Cr improves initial diet DMD of both starch and cellulose diets. Increased digestibility of high-cellulose diets, may be one reason why Cr has been indicated to improve immune status of newly received feeder cattle. Improvements in health might not only be a function of altered immune function, but possibly may be a result of altered nutritional status, specifically energy.

Implications

Organic Cr, as high-Cr yeast, was effective in improving initial in vitro DMD. These results indicate that Cr might improve feed utilization during early digestion periods. The potential of improving initial

digestion is important during physical or disease challenges when animals might be more likely to consume less feed, and therefore have lower total nutrient intakes. Further research is needed to determine whether Cr affects mixed diets or other in vitro systems, and to determine whether these effects can be carried over to in vivo systems.

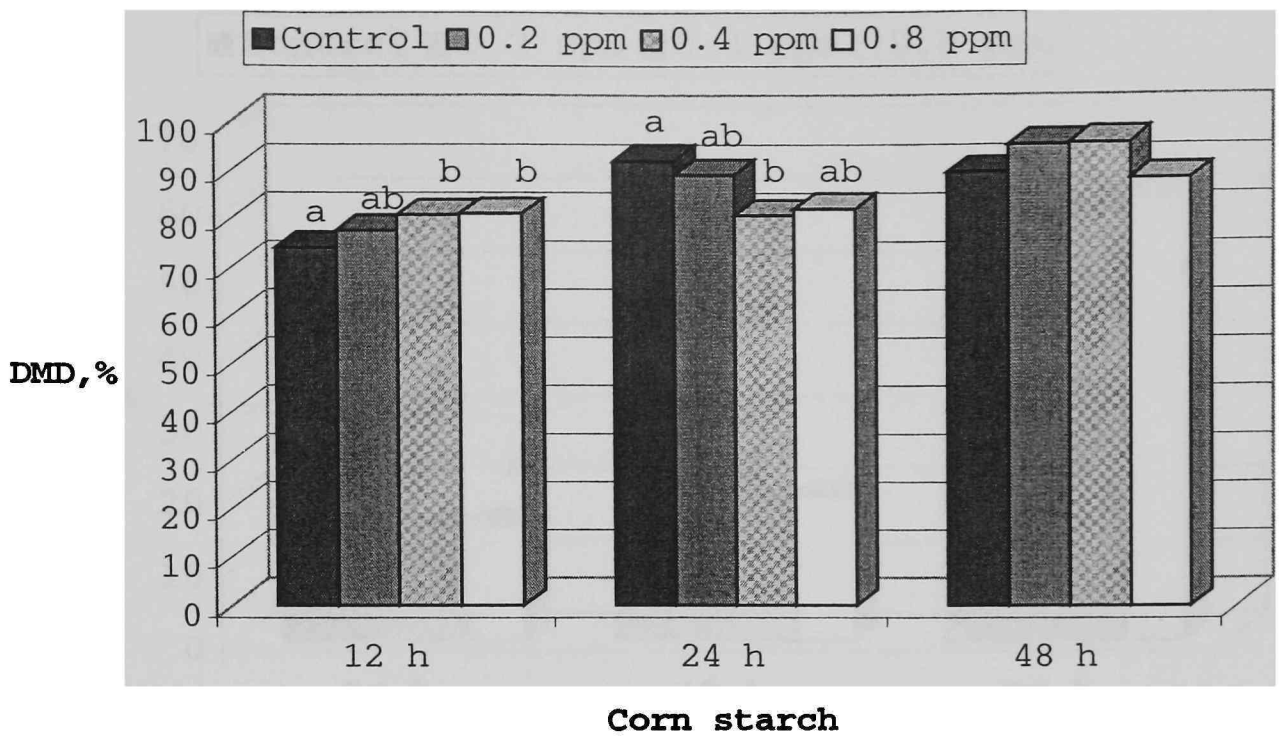

Figure 4.1. Effects of organic Cr on dry matter disappearance of corn starch (SEM: 12 h = 1.60, 24 h = 2.40, 48 h = 3.67). [a,b]Means with different superscripts differ ($P < .05$).

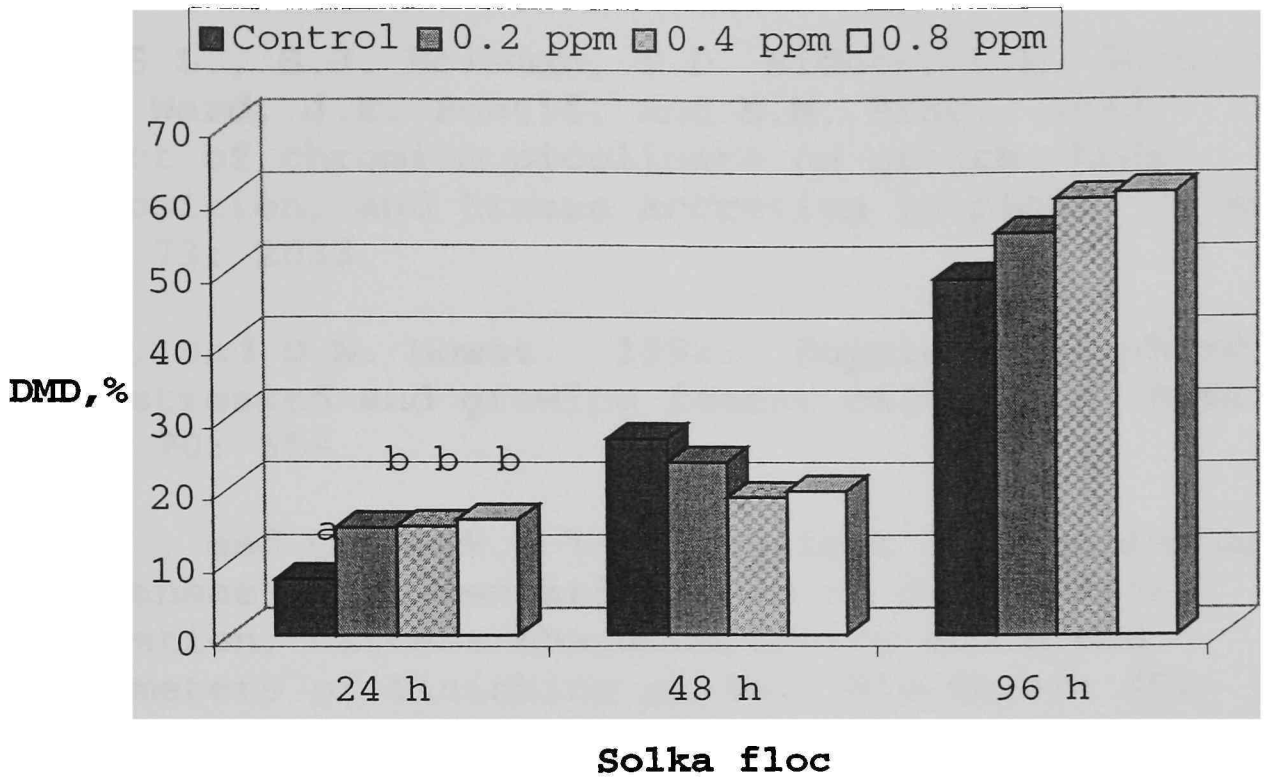

Figure 4.2. Effects of organic Cr on dry matter disappearance of solka floc (SEM: 24 h = 2.40, 48 h = 3.67, 96 h = 3.35). [a,b]Means with different superscripts differ ($P < .05$).

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CHAPTER V

LABORATORY EVALUATION OF ORGANIC CHROMIUM: EFFECTS SERUM FROM STEERS ON MUSCLE CELL PROTEIN SYNTHESIS AND GLUCOSE, UPTAKE IN A CELL CULTURE SYSTEM, AND IN VIVO GLUCOSE UTILIZATION BY LAMBS

Abstract

The objective of this study was to evaluate the effects of organic chromium (Cr) on glucose metabolism and protein synthesis in ruminants. In vitro cell culture glucose uptake and protein synthesis were measured using serum from feedlot steers fed organic chromium diets, and glucose clearance was studied utilizing sheep as a model. One hundred five crossbred steers (283 kg ± 22 kg) were fed typical finishing diets for 196 d and serum samples were collected via jugular venipuncture (Chapter III) for in vitro glucose uptake and protein synthesis determination. Cultures of primary fetal bovine muscle cells were grown in RPMI media and 10% fetal bovine serum until 100% confluent. Protein synthesis and glucose uptake were measured as a percentage of increase in disintegrations per minute (dpm) for serum-treated cells over non-serum treated

cells, utilizing [^{14}C] labeled amino acid mixture and [^{3}H] labeled glucose. Sixteen crossbred lambs were used to determine glucose clearance following a 21-d feeding trial. Fasted sheep were challenged with 500 mg of glucose/kg of BW introduced via i.v. venipuncture, followed by collection of blood samples at -10, 0, 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, and 150 min. Treatments investigated were: control (0 ppm supplemental Cr), 0.2 ppm, and 0.4 ppm of supplemental Cr from high-Cr yeast. Harvested serum from 0.2 ppm Cr and 0.4 ppm Cr-treated steers increased ($P < .01$) protein synthesis in primary bovine muscle cells. Glucose uptake by muscle cells also was increased ($P = .088$) by 0.2 ppm Cr and 0.4 ppm Cr serum. No differences ($P > .05$) were noted for ADG by lambs during the 21-d feeding period. Glucose clearance lambs was improved ($P < .05$) by addition of Cr to the diet. These results are interpreted to suggest that carcass modifications in ruminants fed Cr containing diets are a result of alterations in amino acid uptake and glucose metabolism within muscle cells through indirect effects of factors found serum.

Introduction

Effects of Cr on protein deposition have been studied extensively. However, previous research using Cr with regard to improvements in carcass muscling has dealt almost exclusively with non-ruminants (Page et al., 1993; Hasten et al., 1997a, 1997b; Hossain, 1998; Mooney and Cromwell, 1999). However, in one study conducted with lambs (Kitchalong et al., 1995), Cr did not affect longissimus muscle area in lambs fed a 0.2 ppm Cr diet. In addition to the effect of Cr on carcass muscling in swine and poultry, the effect of Cr on glucose clearance also has been broadly investigated in both ruminants and non-ruminants (Bunting et al., 1994; Amoikon et al., 1995; Kegley and Spears, 1995; Kitchalong et al., 1995). At present, results are inconclusive as to what role, if any, Cr plays in glucose clearance by ruminants.

The objective of this study was to characterize muscle growth by measuring protein synthesis and glucose uptake in bovine myoblasts treated with serum from Cr-fed steers, and to determine whether glucose clearance was altered in lambs fed diets containing Cr.

Materials and Methods

The cell culture portion of this experiment was performed at the Texas Tech University Health Sciences Center in the laboratory of Dr. John Morrow. Primary bovine myoblast cultures were prepared using procedures described by Hembree et al. (1991) as adapted by Kerth (1999), and by the procedure of Woods et al. (1997). A cell line previously established by Kerth (1999) was used for all cell culture experiments. Amino acid uptake was determined using procedures outline by Reecy et al. (1994). Media were removed from a culture flask containing primary bovine myoblasts. Cells were immediately covered with a solution containing 0.25% trypsin (wt/vol) and 1 mM ethylenediamine tetraacetic acid (EDTA), and left at room temperature for approximately 8 min. After cells were found to be released from the bottom of the flask, the media was poured into a 15-mL centrifuge tube and centrifuged at 1,000 X *g* for 5 min. The supernatant fluid was decanted, and 8 mL of RPMI 1629 medium with 10% fetal bovine serum (vol/vol, Atlanta Biologicals, Norcross, GA) was added. The tube was immediately vortexed to resuspend cells, a 1-mL portion of the media and cells was placed on a

hemocytometer to determine the number of cells per milliliter. Sufficient media were transferred to each well of a 24-well culture plate (Corning®, Fisher, Houston, TX) so that approximately 5,000 cells were introduced to each well. Cells were allowed to grow to 100% confluency (72 h), at 37°C in 5% CO_2:95% air with 100% humidity in a CO_2 cell incubator (Queue Systems Model 2210, Parkersburg, VA). When confluency was reached, media were aspirated and replaced with 1 mL of skeletal muscle basal media (SkBM; Atlanta Biologicals, Norcross, GA) containing the treatment serum (i.e., control, 0.2 ppm Cr, or 0.4 ppm Cr serum from feedlot steers in Chapter III) and allowed to incubate for 24 h. Pooled serum from each pen served as the treatment and was added to the SkBM media at 5% (vol/vol). The following day, 1 μCi of ^{14}C-labelled amino acid mixture (New England Nuclear, Boston, MA, 10% labeled mixture) was added to each well and allowed to incubate for 2 h at 37°C. Labeling media were removed, and well plates were rinsed with fresh media three times to remove any residual ^{14}C. Immediately following the final rinse, 0.5 mL of 1 M NaOH was added to each well, and the plate was returned to the incubator for 2 h. After 2 h, without removing the NaOH,

0.5 mL of cold 20% (wt/vol) TCA solution were added, and plates were placed in a 4°C refrigerator for 12 h. Plates were then removed from the refrigerator, and cells were scraped from the well using a pipette tip, and filtered onto a glass fiber filter in a vacuum manifold. Wells were washed with a minimum of 5 mL of 5% (wt/vol) TCA. Glass fiber filters (Fisher, Houston, TX) were dried under a heating lamp for 20 to 30 min until all moisture appeared to be removed. Filters were then transferred into 7 mL scintillation vials and covered with 5 mL of scintillation counting cocktail (ICN Pharmaceuticals Ecolite (+), Costa Mesa, CA). Disintegrations per minute were counted in a liquid scintillation counter (Beckman LS 6500, Fullerton, CA). All assays were performed in triplicate and expressed as a percentage of increase over the SkBM control (without serum).

Glucose uptake by primary bovine myoblasts was determined using procedures similar to those described above. However, instead of 1 μCi of ^{14}C-labelled amino acid mixture, 1 μCi of ^{3}H-labeled glucose was used. Also, the labeling incubation period was changed to 15 min, and culture plates were placed on a shaker in a 37°C

incubator during the labeling process. Additional procedures used for glucose uptake were outlined by Morris et al. (1995) in Cr work using mouse myotubes.

To determine glucose clearance rates in vivo, crossbred lambs (n = 16, BW = 41.3 kg) were used. Lambs were housed outdoors in 7 X 10 m pens at the Texas Tech University Sheep Center, located at New Deal, TX. All pens were equipped with fence line feed bunk and an automatic water supply. Feed was delivered once per d at approximately 0900 h, all sheep were fed at three percent of BW. Diets consisted of corn-cottonseed hull base, and were the same diet as those being fed at the Sheep Center at the time of this study (Table 5.1). Dietary treatments consisted of control (basal), 0.2 ppm Cr (basal diet plus 0.2 ppm Cr), 0.4 ppm Cr (basal diet plus 0.4 ppm Cr), and 0.8 ppm Cr (basal diet plus 0.8 ppm Cr). Chromium was added to each diet by combining 100 kg of basal diet with 2 kg of Cr premix in a ground, grain sorghum base. All diets received 2 kg of ground grain sorghum to ensure that diets remained iso-nitrogenous and iso-caloric. On d 21, i.v. glucose challenge tests were conducted using procedures described by Kitchalong et al. (1995), Kegley and Spears (1995), and Gentry et al.

(1999). Blood glucose was determined on serum using a glucose specific kit (Sigma Diagnostics®, kit No. 510). Initial blood glucose values for -10 and 0 min blood samples were averaged and used as a baseline to determine response to the glucose challenge for each individual animal.

Statistical analyses for both the cell culture experiments and blood glucose challenge test were analyzed as a completely random design by analyses of variance using GLM procedure of SAS (1995). For the cell culture portion of this experiment, each series of three wells utilizing pooled serum from a single pen were considered to be an experimental unit. During the sheep trial, each animal was considered an experimental unit. For both experiments means were separated using Fisher's Protected Least Significant Difference Test.

Results and Discussion

Cell culture. Protein synthesis as indicated by amino acid uptake was increased ($P < .05$) by serum from Cr fed steers (Figure 5.1). These findings are supported by previous research with pigs (Page et al., 1993; Mooney and Cromwell, 1999), broilers (Hossain, 1998). In the

aforementioned study (Chapter III) Cr supplementation was shown to increase longissimus area of cattle. In the present study, chromium improved amino acid uptake in cell culture by 73% when serum from 0.2 ppm Cr fed steers was used, and 71% when serum from 0.4 ppm Cr fed steers was used. These data indicate that Cr alters amino acid uptake by bovine muscle cells because of factors found in serum.

Glucose uptake by bovine myoblasts in cell culture also was increased (P = .09) by serum from Cr-fed steers (Figure 5.2). These data are in agreement with Morris et al. (1995) who reported that Cr was capable of increasing glucose uptake in primary myoblasts. In work with murine myotubes, Morris et al. (1995) found that Cr increased glucose uptake by as much as 90% over control cells. In the present study, however, the effects of Cr were not as dramatic, Cr only improved glucose uptake by 30% for 0.2 ppm Cr serum treated cells and by over 22% when 0.4 ppm Cr treated serum was used. These data indicate that Cr alters energetic efficiency in bovine muscle cells because of factors found in serum.

Glucose clearance. In the 21-d feeding trial, no effects on ADG were noted from Cr (Figure 5.3); however,

lambs were limit fed during the entire trial. Therefore differences in ADG across treatments were not expected as a result of the lowered daily gains, because of limit feeding. These results agree with previous research by Kitchalong et al. (1995) and Gentry et al. (1999), in which no evidence that Cr alters ADG of lambs was reported. Lambs fed 0.4 ppm Cr and 0.8 ppm Cr diets had lower ($P < .05$) peak serum glucose levels. Throughout the entire glucose challenge, the 0.8 ppm Cr diet had the lowest numerical glucose levels, whereas the control diet had the highest numerical glucose levels (Figure 5.4). Glucose clearance rate was increased ($P < .05$) by the addition of 0.2 ppm Cr and 0.8 ppm Cr to the diets of feedlot sheep. This result is consistent with previous reports with sheep (Kitchalong et al., 1995; Gentry et al., 1999) and cattle (Kegley and Spears, 1995). These data indicate that 0.8 ppm Cr and 0.4 ppm Cr diets lowered ($P < .05$) the initial peak in blood glucose noted at 10 min, and that Cr tends ($P < .11$) to increase glucose clearance throughout a 150 min challenge test.

Implications

Serum from steers receiving Cr had large effects on primary muscle cells in vitro. The data presented here support many previous observations made in vivo with both pigs and cattle. Chromium significantly increased both amino acid and glucose uptake by primary fetal bovine muscle cells. In addition to alterations at the cellular level, Cr seemed to alter peak blood glucose in sheep following a glucose challenge.

Table 5.1. Composition of lamb diets (AF)

	TREATMENTS			
Ingredients	**Control**	**0.2 ppm Cr**	**0.4 ppm Cr**	**0.8 ppm Cr**
Corn, cracked	60.75	60.75	60.75	60.75
Cottonseed hulls	15.00	15.00	15.00	15.00
Soybean meal	13.75	13.75	13.75	13.75
Molasses, cane	6.25	6.25	6.25	6.25
Limestone, ground	1.50	1.50	1.50	1.50
Ammonium chloride	0.50	0.50	0.50	0.50
Mineral premix	0.13	0.13	0.13	0.13
Vitamin premix	0.12	0.12	0.12	0.12
Grain sorghum, grd	2.00	--	--	--
0.2 ppm Cr premix	--	2.00	--	--
0.4 ppm Cr premix[e]	--	--	2.00	--
0.8 ppm Cr premix	--	--	--	2.00
Total	100.00	100.00	100.00	100.00

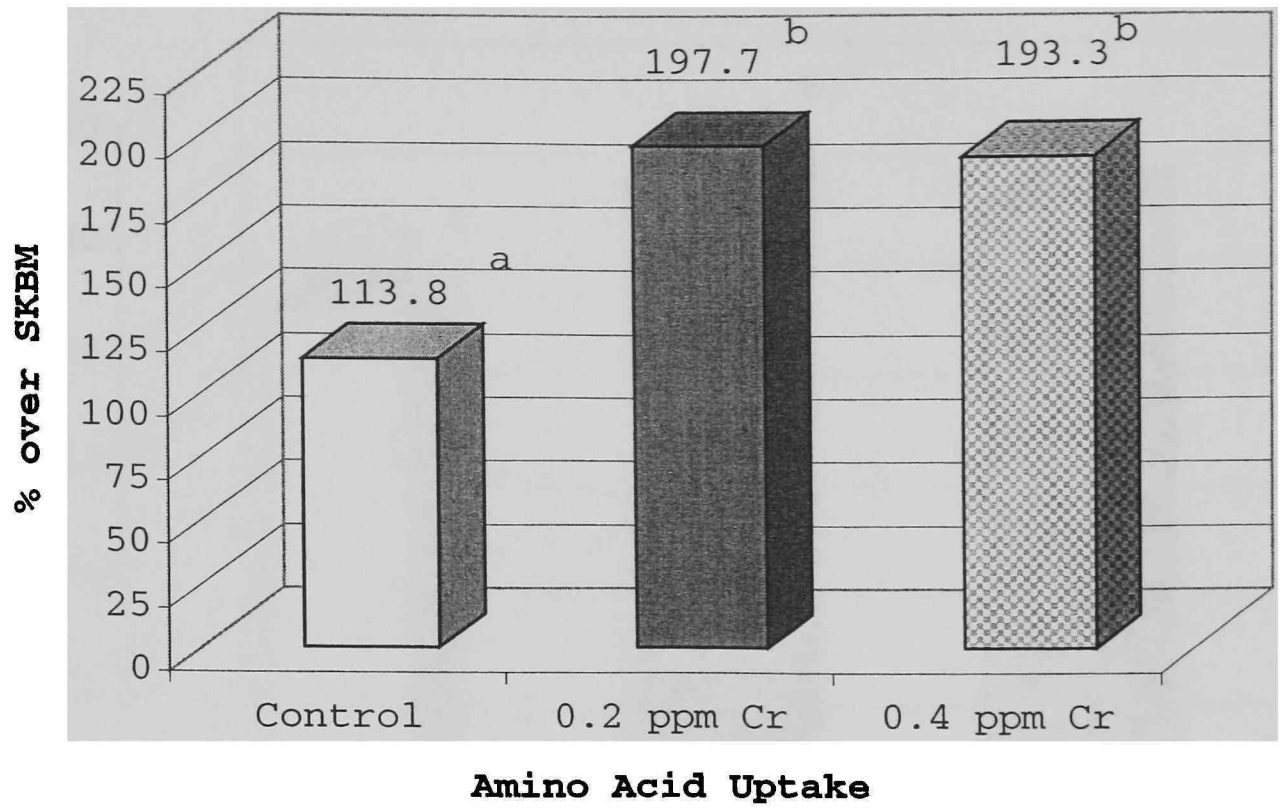

Figure 5.1. Effects of serum from Cr supplemented steers on amino acid uptake of primary bovine myoblasts (SEM = 20.37). [1]Means with different superscripts differ (P < .05).

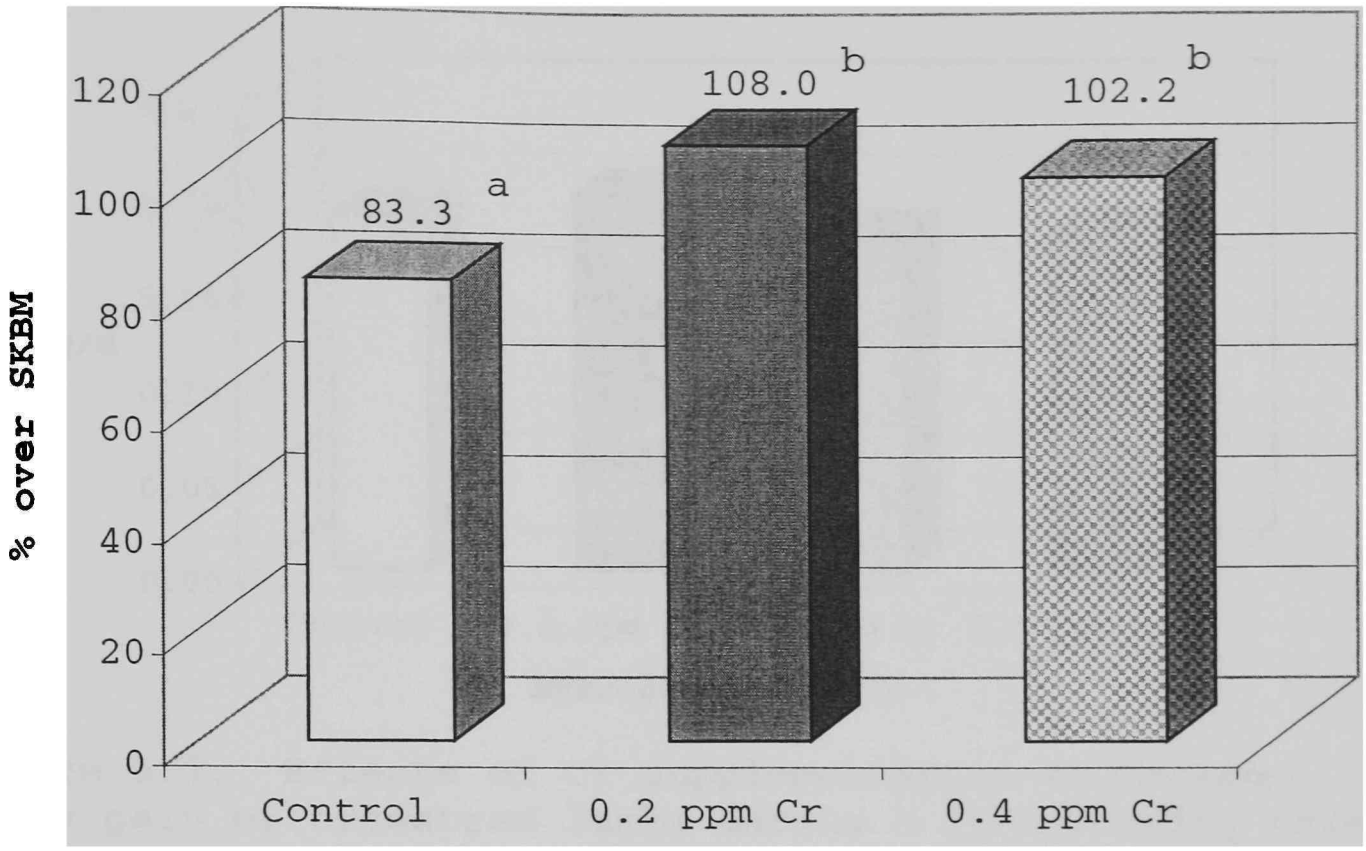

Figure 5.2. Effects of serum from Cr supplemented steers on glucose uptake of primary bovine myoblasts (SEM = 7.20). [1]Means with different superscripts differ (P < .09).

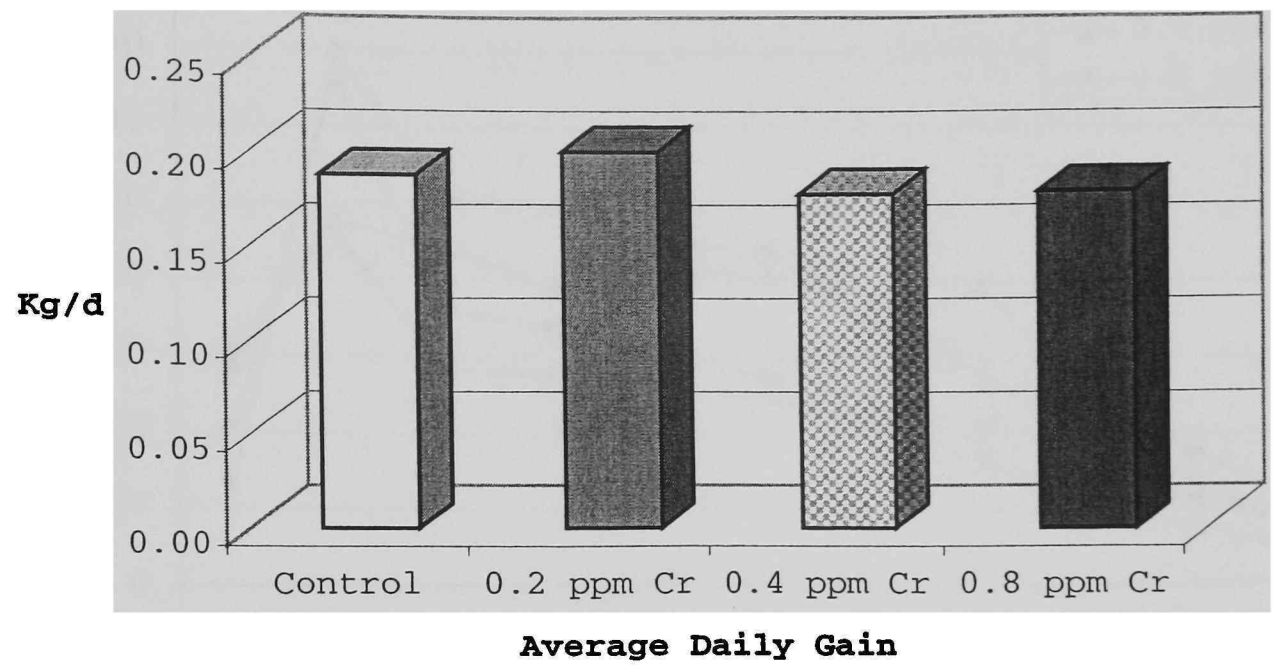

Figure 5.3. Effects of Cr supplementation on average daily gain of crossbred lambs during a 21-d feeding trial (SEM = 0.09). [1]Means without superscripts do not differ (P > .10).

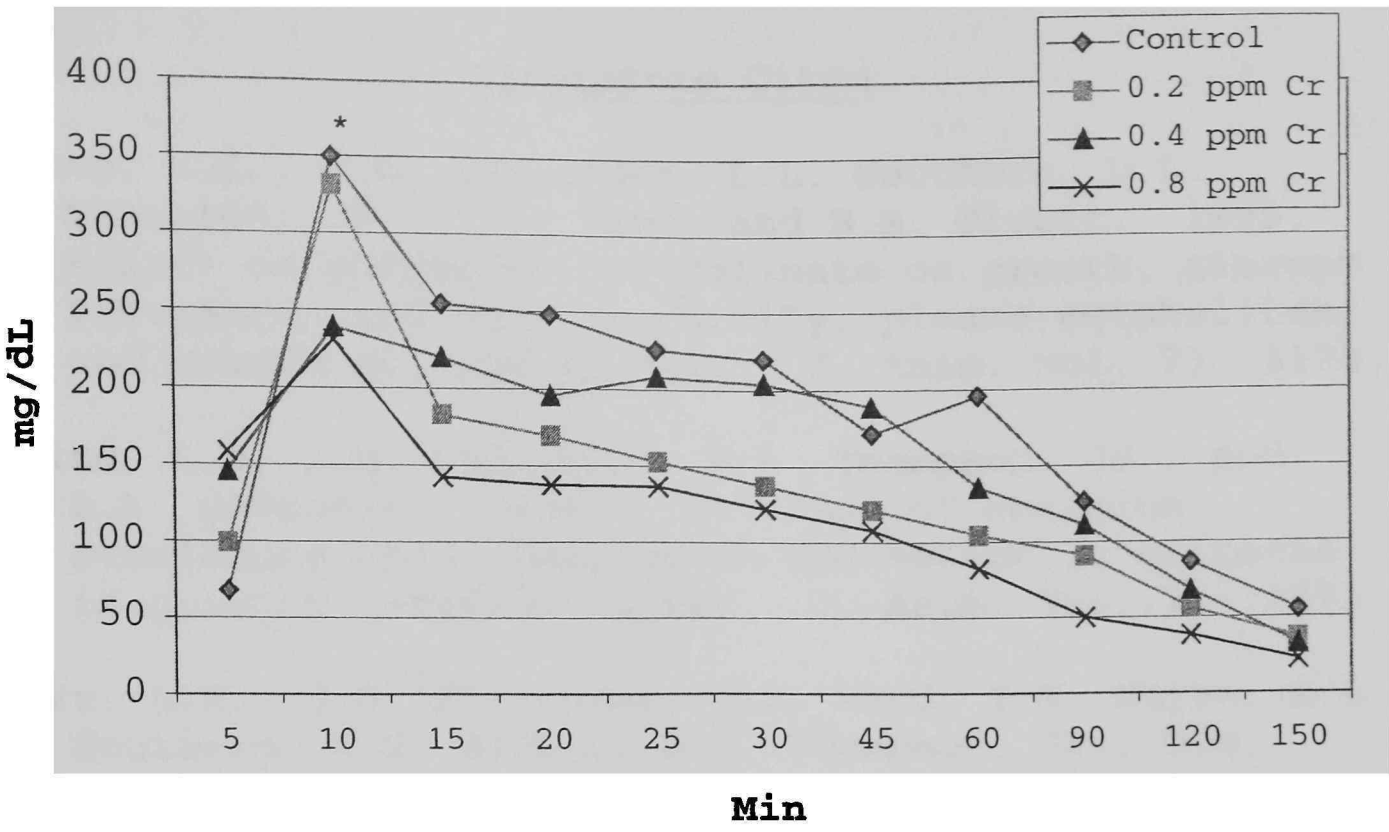

Figure 5.4. Effects of Cr supplementation on glucose clearance of crossbred lambs during a 21-d feeding trial. *Control and Cr 0.2 ppm means differ ($P < .05$) from 0.4 ppm Cr and 0.8 ppm Cr.

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CHAPTER VI

INTEGRATED SUMMARY

The effects of trace minerals in ruminant animal diets have been investigated for nearly 100 years. During this time, their effects on reproduction, immunity, growth, and proper development have been delineated. However, it has only been in the last 15 years that one of the more recently discovered essential trace minerals, chromium, has been evaluated for use in beef cattle diets.

This dissertation examined the effects of organic chromium on feedlot performance, carcass characteristics, growth, and glucose clearance by ruminants. Steers receiving organic chromium at 0.2 ppm did not differ from control steers for any performance measurements. However, feeding 0.4 ppm chromium decreased ADG, DMI, and GF. Inclusion of organic chromium into steer diets increased longissimus muscle area over that of control steers. Additionally, chromium at 0.4 ppm also decreased final yield grade, decreased marbling score, and decreased carcass weight. Chromium did not affect serum cortisol concentrations or liver abscesses of feedlot

steers. Cell culture techniques were used to examine the effects of serum from steers fed organic chromium on muscle cell protein synthesis and glucose uptake. Cultured muscle cells treated with serum from chromium-fed steers had higher levels of amino acid uptake, an indicator of increased levels of protein synthesis. Glucose utilization also was increased by serum from organic chromium-fed steers, over that of muscle cells treated with control serum. Glucose clearance, using sheep as a model, following a 21-day feeding period, also was increased by organic chromium. Sheep receiving organic chromium diets had lower glucose levels initially, and took less time to return to normal levels. It is concluded that organic chromium affects carcass measurements of feedlot steers through changes in energy utilization (glucose uptake) and amino acid uptake (protein synthesis). These effects might be caused by alterations of endogenous hormone concentrations and other factors found in serum, as a result of increased cell sensitivity from glucose tolerance factor chromium.