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The Influence of Chromium Supplementation on the Insulin Pathway, Carcass Traits, and Meat Quality of Feedlot Steers

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THE INFLUENCE OF CHROMIUM SUPPLEMENTATION ON THE INSULIN PATHWAY, CARCASS TRAITS, AND MEAT QUALITY OF FEEDLOT STEERS

BY

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This thesis is approved as a credible and independent investigation by a candidate for the Master of Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABBREVIATIONS

ADFI	Average daily feed intake
ADG	Average daily gain
Akt	Protein kinase B
BF	12th rib backfat thickness
Biopsy184	*Longissimus* muscle biopsy on d 184
Biopsy200	*Longissimus* muscle biopsy on d 200
BSA	Bovine serum albumin
BW	Body weight
C	Celsius
cDNA	Complementary deoxyribonucleic acid
cm	Centimeter(s)
Cr300	300 ppb supplemental Chromium propionate
Cr400	400 ppb supplemental Chromium propionate
Cr	Chromium Supplementation
CrPrp	Chromium propionate
CON	0 ppb supplemental Chromium propionate
d	Day(s)
DP	Dressing percent
Exp.	Experiment
g	grams
G:F	Gain to feed ratio
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase

GLUT1	Glucose transporter 1
GLUT4	Glucose transporter 4
GTF	Glucose tolerance factor
GTT	Glucose tolerance test
h	hour(s)
HCW	Hot carcass weight
IGF	Insulin-like growth factor
IMPS	Institutional meat purchase specifications
IR	Insulin receptor
IRS	Insulin receptor substrate
kDa	KiloDalton
kg	Kilogram(s)
km	Kilometer(s)
KPH	Kidney, pelvic, and heart fat
LM	*Longissimus* muscle
LMA	*Longissimus* muscle area
LMWCr	Low molecular weight chromium binding substance
MGA	Melengesterol acetate
mg	Miligram(s)
min	Minute
mL	Milliliter(s)
mM	Millimolar
mTOR	Mammalian target of rapamycin

mTORC1	Mammalian target of rapamycin complex 1
n	Number
ng	Nanogram(s)
NEFA	Non-esterified fatty acids
OBB	Odyssey Blocking Buffer
p70-S6K	Ribosomal protein S6 kinase 1
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline with Tween 20
PCR	Polymerase chain reaction
PI3K	Phosphotidylinositide 3-kinase
PPARγ	Peroxisome proliferator activated receptor γ
ppb	Parts per billion
ppm	Parts per million
PVDF	Polyvinylidene difluoride
RAC	Ractopamine hydrochloride
RNA	Ribonucleic acid
rpm	Revolutions per minute
s	Second(s)
SEM	Standard error of the mean
SDS	Sodium dodecyl sulfate
TBS	Tris buffered saline
TBST	Tris buffered saline with Tween 20
TPN	Total parenteral nutrition

TSC	Tumor sclerosis complex
VFA	Volatile fatty acids
V	Volts
vol	Volume
vol/vol	Volume per volume
YG	Yield grade
WBSF	Warner-Bratzler shear force
wt	Weight
wt/vol	Weight per volume
βAA	Beta-adrenergic agonists
βAR	Beta-adrenergic receptors
µg	Micrograms(s)
µL	Microliter(s)

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ABSTRACT

THE INFLUENCE OF CHROMIUM SUPPLEMENTATION ON THE INSULIN PATHWAY, CARCASS TRAITS, AND MEAT QUALITY OF FEEDLOT STEERS

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Chromium supplementation (Cr) can increase efficiency of growth in livestock by amplifying insulin signaling. Previous research showed Cr increases nutrient utilization and changes in muscle growth and fat deposition. Therefore, the objective of this research was to determine how Cr affects carcass traits, meat quality, and the insulin pathway in feedlot steers. Calf-fed steers were allotted to 0 ppb (CON) or 400 ppb Cr propionate (Cr400) for 210d in experiment 1. Two *longissimus* muscle (LM) biopsies were collected to examine protein abundance and gene expression after adaptation to a terminal implant and after adaption to ractopamine hydrochloride (RAC). A follow up study, Experiment 2, was conducted with yearling steers supplemented 0 ppb (CON) or 300 ppb Cr propionate (Cr300) for 138d. Cattle were transported to a commercial packing plant for slaughter. Carcass data was collected as well as strip loins for shear force and proximate analysis in both experiments. Carcass composition was estimated using 9-10-11 rib dissections with subsequent proximate analysis. Biopsies were used to analyze peroxisome proliferator activated receptor γ (PPARγ), protein kinase B (Akt), phospho-Akt, and ribosomal protein S6 kinase 1 (p70S6K) abundance and glucose transporters (GLUT) 4 and 1 gene expression. Experiment 1 showed an increase in dressing percentage (DP), KPH, and shear force, but a decrease in marbling for Cr400 steers. Final BW, HCW, 12th rib backfat (BF), LMA, yield grade (YG), or carcass composition were

not different between treatments. Also, there was no difference in abundance of Akt, phospho-Akt, or p70S6K after the terminal implant, but PPARγ tended to be higher for CON. No differences were observed in protein abundance after RAC supplementation. Expression of GLUT4 and GLUT1 was not different after either biopsy. In experiment 2, there was no difference in final BW, HCW, BF, LMA, YG, DP, marbling score, chemical fat, or shear force. However, percent KPH tended to increase for CON. Variation between studies may be attributed to differences in duration of Cr and the animal model used. In conclusion, earlier and longer Cr supplementation can positively increase DP, but may have negative impacts on beef quality with calf-fed steers.

CHAPTER 1

LITERATURE REVIEW

Introduction

Since 1975, the U.S. cattle herd has shrunk from 140 million head to 92 million head as recently reported by the United States Department of Agriculture (USDA) (Meyer, 1997; Koontz, 2010; USDA, 2016). The decline in the cattle herd is partially attributed to a decrease in red meat consumption, because consumer demand for beef is half of what it was in 1980 (Andersen et al., 2002; Koontz, 2010). More recently in 2013, a significant portion of the decline was attributed to drought that caused ranches to liquidate cattle number (USDA, 2016). Also, cattle producers now have to compete with ethanol production for corn, which has caused increases in feed costs (Lawrence et al., 2008). All of these factors coupled with the opportunities to sell land to capture equity, lack of interest from the younger generation, and increasing input costs have all played a role in ranchers exiting the industry never to reinvest (Field, 2010). This has left the remaining ranchers to produce more beef with fewer cattle (Field, 2010; Koontz, 2010). Nonetheless, the beef industry has managed to increase production by making strides in genetics, nutrition, health, and reproduction, as well as utilizing technologies such as anabolic implants and beta-agonists to produce more beef (Koontz, 2010). This has increased production per cow by 30% compared to cow production in 1980 (Koontz, 2010) satisfying U.S. beef demand and export markets (Field, 2010).

However, there is an expected 9 billion people to feed in the coming generation, and there will be fewer resources available for animal agriculture (Field, 2010; Godfray et al., 2010). The majority of undeveloped land is expected to go to social and culture

needs due to the population growth, leaving less productive land for agricultural production (Godfray et al., 2010). Due to these expected production limitations, there is a need for continued research to discover areas for improvement in beef production. The following will be a review of the literature describing current feedlot cattle production in an attempt to discover possible areas for improvements.

Anabolic Implants

Anabolic implants are utilized to alter metabolism from a state of catabolism towards anabolism resulting in increased protein synthesis, improved feed conversions, and possibly a reduction in carcass fatness (Lone, 1997). Currently, there are five FDA approved growth promoting steroids used in implants: estradiol (in the form of 17β-E_2 or estradiol benzoate), progesterone, testosterone propionate, trenbolone acetate (TBA), and zeranol (Kolok and Sellin, 2008). Melengesterol acetate (MGA), a progestin, is another growth promoting steroid used as a feed additive to restrict females from cycling, as the estrus cycle causes disruptions in growth and loss of efficiency (Lone, 1997). The mode of action of implants and combination implants are well reviewed in Lone (1997), Webb et al. (2002), and Sillence (2004). Implants have a direct effect on skeletal muscle accretion by altering protein synthesis and degradation, as well as indirectly increasing growth hormone and insulin-like like factor (IGF) concentrations, which lead to increases in muscle accretion (Lone, 1997; Webb et al., 2002; Sillence, 2004). Also, implants increase satellite cell recruitment and proliferation towards skeletal muscle (Webb et al., 2002). Increased skeletal muscle growth with the use of implants translates to increased hot carcass weights (HCW) and *longissimus* muscle area (LMA) while increasing average daily gain (ADG) and feed efficiency compared to nonimplanted cattle (Johnson

et al., 1996; Bruns et al., 2005). Other than improvements in carcass traits, implants have improved growth rates in cattle (Bruns et al., 2005; Parr et al., 2011). Moreover, it is well understood implants have the capability of negatively altering quality grade, skeletal maturity, the occurrence of dark cutting beef (Webb et al., 2002) as well as tenderness (Nichols et al., 2002; Webb et al., 2002). However, the effect implants have on these traits is highly variable depending on the type and aggressiveness of implants, breed, environmental conditions, stress, and timing of implant in relation to slaughter (Morgan, 1997).

Beta Adrenergic Agonists

Even though implants have been proven effective, researchers discovered another physiological mechanism to increase beef production through the use of beta-adrenergic agonists (βAA). Beta-adrenergic agonists mimic naturally occurring catecholamines: epinephrine, norepinephrine, and isoproterenol (Mersmann, 1998; Rathmann et al., 2012; Johnson et al., 2014a). Beta-adrenergic agonists work by attaching to beta-adrenergic receptors (βAR) located on the cell membrane, which activates a cascade of phosphorylation of numerous proteins (Johnson et al., 2014a). The overriding response is an increase in protein synthesis, a decrease in protein degradation (only associated with a few βAA), an increase in lipolysis, and a decrease in fatty acid biosynthesis (Mersmann, 1998; Johnson et al., 2014a).

Ractopamine hydrochloride, Optaflexx®, and Zilpaterol hydrochloride, Zilmax®, are the two FDA approved βAA for cattle in the United States. Ractopamine hydrochloride has a higher affinity for βeta 1-Adrengenic receptors and causes an increase in protein synthesis (Mersmann, 1998). Zilpaterol hydrochloride, however, has a

higher affinity for βeta 2-Adrengenic receptors and elicits an increase in protein synthesis and decrease in protein degradation (Scramlin et al., 2010). Therefore, zilpaterol hydrochloride has a higher protein accretion rate resulting in heavier HCW and increased ADG compared to RAC (Johnson et al., 2014b). However, over recent years RAC is the only βAA being used by the cattle industry, because zilpaterol hydrochloride has been removed from the market because of animal welfare concerns (Johnson et al., 2014b).

Nonetheless, typical results with the use of RAC include increased ADG (Gruber et al., 2007; Scramlin et al., 2010; Bohrer et al., 2014), feed conversion (Gruber et al., 2007; Scramlin et al., 2010; Bohrer et al., 2014; Bittner et al., 2016), HCW (Gruber et al., 2007; Scramlin et al., 2010; Bohrer et al., 2014; Bittner et al., 2016; Edenburn et al., 2016), and LMA (Bittner et al., 2016). The magnitude of change for each trait with RAC is dependent on the dosage as discussed in Bittner et al. (2016). Nonetheless, the optimum dose for RAC supplementation is 200 mg of RAC/steer daily, regardless of supplement duration (Bittner et al., 2016).

AS previously discussed, RAC has been shown to be a beneficial technology to meet beef demands. However, consumers have become wary of the technologies used in beef production, and a less invasive means of growth promotion has become more important. The addition of grains into diets of finishing cattle has been proven as a way to hasten growth compared to forage finished cattle.

Grain finishing

Grain finishing started growing in popularity between 1945 – 1972 when there was a surplus of cheap grain (Field, 2010). Incorporating grains into finishing diets has continued because of the increased efficiencies of gain (Oltjen et al., 1971; Waldo, 1973;

Crouse et al., 1984), increased carcass weights (Crouse et al., 1984; Mandell et al., 1998; Berthiaume et al., 2006), and reduction of time on feed to reach market weights (Oltjen et al., 1971; Crouse et al., 1984; Muir et al., 1998). Grain is generally more digestible versus forages, because there are a lower proportion of structural components (Church, 1988). Structural components in plant matter reduce the production of ruminal volatile fatty acids (VFA), and VFAs are the main substrates the ruminant animal uses to produce energy (Church, 1988). Furthermore, grains have higher amounts of starch, which is more readily available to the animal, and this results in more energy available for growth verses maintenance (Church, 1988)..

Even though the use of grain in finishing diets has increased efficiencies, there is a growing evidence of insulin resistance developing in feedlot cattle. Recent data has shown increased insulin resistance the longer cattle are on a finishing diet (Kneeskern et al., 2016). As well, corn-based diets increased insulin resistance compared to diets consisting of either grass hay or dry distillers grain as the main energy sources (Radunz et al., 2012). The development in insulin resistance is thought to be similar to obesity-induced insulin resistance occurring in human health. Therefore, an understanding of insulin function and insulin resistance is necessary to determine if growth can be augmented in feedlot cattle.

Insulin Function and Resistance

Insulin is a major metabolic regulator, as it stimulates nearly all the anabolic pathways; of glucose uptake, the synthesis and storage of carbohydrates, fats, and proteins (Sherwood et al., 2013). Furthermore, the presence of insulin conserves substrates from catabolism. The overall function of insulin is to promote storage when

nutrients are adequately supplied, however impairment to the insulin pathway can develop.

Insulin resistance can be defined as a less than normal biological response to normal concentrations of insulin (Kahn, 1978). Insulin resistance can be due to decreased insulin sensitivity or responsiveness of the tissue or a combination of both. A reduction in insulin sensitivity means more insulin is required to elicit the same response, whereas reduced insulin responsiveness is a decrease in the maximum biological response to the same concentrations of insulin (Kahn, 1978; Muniyappa et al., 2008). Insulin resistance can develop because of two general reasons. One reason is because the body is sparing glucose for higher priority tissues, such as the brain or fetus. This scenario is commonly observed in gestational diabetes (Vernon et al., 1990; Kerestes, 2009). Secondly, insulin resistance can develop because the cell is saturated with glycogen and is limiting the amount of nutrients coming into the cell. This case is observed in a high-energy state and has been related to obesity-induced type 2 diabetes (Kahn et al., 2006). This review will focus on the latter scenario, as it is the logical cause of developing insulin resistance in feedlot steers.

Insulin signaling propagates by binding to the extracellular α-subunit of the insulin receptor (IR), which leads to autophosphorylation of its β-subunit (Patti and Kahn, 1998). As insulin phosphorylates itself it creates an active tyrosine kinase on the β-subunit allowing phosphorylation

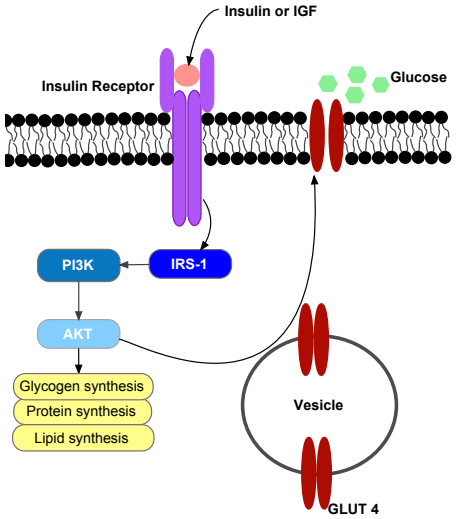


Figure 1.1. Insulin signaling and cellular affects downstream. Adapted from Vincent (2010).

of tyrosine residuals located on IR substrate proteins (IRS) (Vincent and Bennett, 2007). Activation of IRS proteins leads to the proliferation of the signaling cascade between IRS –> phosphotidylinositide 3-kinase (PI3K) –> protein kinase B (Akt) ultimately leading to increased glucose uptake because of the translocations of glucose transporter 4 (GLUT4) to the cell membrane (Shepherd and Kahn, 1999). This is shown in Figure 1.1 along with the cellular effects downstream of Akt.

Insulin resistance in obese subjects develops as a result of increased non-esterified fatty acids, glycerol, and pro-inflammatory adipokines released from adipose tissue causing interruptions in insulin signaling (Kahn et al., 2006). The reduced insulin signaling leads to less GLUT4 translocation to the cell membrane and less glucose absorbed by the cell. The elevated levels of blood glucose elicit the pancreatic β-cells to sequester more insulin in order to compensate, leading to hyperinsulimia (Kahn et al., 2006). Unless the insulin resistance is alleviated, then this cycle will continue until the pancreatic β-cells are unable to keep up with insulin demands, then the β-cells become dysfunctional resulting in reduced mass and secretion of the cells (Rutter et al., 2015). This is when non-insulin dependent diabetes mellitus (Type 2) transpires. However, cattle on feed are not diagnosed as type 2 diabetic most likely because they are on high-energy diets during a time of rapid growth, and cattle are slaughtered before damage to the pancreatic β-cells occurs. Nonetheless, as stated previously, cattle are becoming more insulin resistant the longer they are on feed, and necessitate the need for research towards improvements. Thus, the research area of Cr supplementation has grown in livestock animals to determine if insulin resistance can be mitigated.

Chromium Supplementation

Chromium is a mineral proposed to be essential for normal carbohydrate metabolism as there have been observations of Cr potentiating insulin signaling (Anderson, 1997). Chromium was first proposed an essential mineral in rats by Mertz and Schwarz (1959) and later in humans by Jeejeebhoy et al. (1977), and because of this research Cr was classified as essential. However, conclusions from original Cr research is concerning, because the lack of a "low" or deficient diet (Vincent and Bennett, 2007). Plus, Cr assays were proven to have contained significant Cr contamination (Vincent and Bennett, 2007). Further, the original Cr molecule, glucose tolerance factor (GTF), proved to be an artifact from acid hydrolysis of low molecular weight Cr binding substance (LMWCr) (Sumrall and Vincent, 1997). These issues contribute to the debate among researchers whether Cr is essential or not. Nonetheless, supplemental Cr has gained attention because of beneficial results related to glucose and nutrient metabolism, which will be discussed below.

Chromium bioavailability and tissue accumulation

Chromium is a common element in the earth and is found primarily in two forms: trivalent Cr (+3) and hexavalent Cr (+6) (Cefalu and Hu, 2004). Trivalent Cr is the form behind carbohydrate metabolism and has a very low toxicity versus Cr (6+) (Cefalu and Hu, 2004). The form of Cr supplementation has proven to have different bioavailabilities. Lindemann et al. (2008) demonstrated in growing pigs that Cr tripicolinate had the highest bioavailability in comparison to Cr methionine, Cr yeast, and Cr propionate (CrPrp) with a linear decrease in availability among the listed sources. Also, Cr will accumulate in liver, kidney, spleen, and bones, but there is no appreciable accumulation

of Cr in muscle (Wallach, 1985; Anderson et al., 1996; Lindemann et al., 2008). Anderson et al. (1996) and Lindemann et al. (2008) demonstrated different bioavailabilities of organic sources of Cr, but overall Cr absorption is generally low at 0.4 – 2.0% (Anderson and Kozlovsky, 1985).

Proposed Biological Actions of Chromium

When Cr is absorbed, it enters the blood stream, binds with transferrin, and is transported to the insulin dependent tissue (Vincent, 1999). Through endocytosis the transferrin-Cr complex is taken into the cell. Chromium is released from transferrin and then is available to bind to the apo-, or inactive, LMWCr, also known as chromodulin (Vincent, 1999). Four trivalent Cr molecules will bind to LMWCr resulting in the active

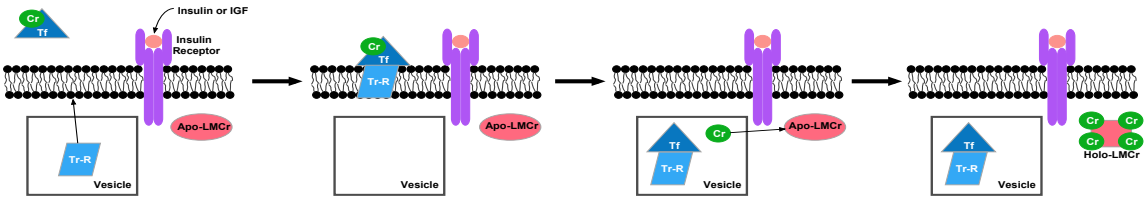


Figure 1.2. The proposed mechanisms of LMWCr with the insulin receptor. Adapted from Vincent (2000).

form, or holo-LMWCr (Vincent, 1999), which is shown in figure 2. Once the LMWCr is activated, it will bind and increase phosphorylation of the IR (Vincent, 1999; Vincent, 2000; Wang et al., 2005) as well as increasing phosphorylation of IRS (Wang et al., 2005). It has already been discussed above how insulin stimulates the IR leading to the cascade between IRS-PI3K-Akt. Insulin or IGF-1 signaling resulted in phosphorylation of both Threonine 308 and Serine 473 on Akt (Alessi et al., 1996). Both sites were required for full activation of Akt, but these two sites are not dependent on each other for phosphorylation (Alessi et al., 1996). Phosphorylation at Thr308 is accomplished by Akt co-localizing with phosphoinositide-dependent kinase 1 (PDK1), which is recruited to the

cell membrane by PI3K (Alessi et al., 1997). Phosphorylation on Ser473 is proposed to be caused by various proteins in addition to insulin (Song et al., 2005). Chromium supplementation in obese, insulin resistant mice resulted in increased phosphorylation at Ser473 when stimulated with insulin (Chen et al., 2009a). Once fully active, Akt is responsible for either inhibiting or activating several proteins associated with metabolism, proliferation, growth, angiogenesis, glucose uptake, and cell survival (Manning and Cantley, 2007).

Downstream of Akt lies the mammalian target of rapamycin (mTOR) signaling protein and the mTOR complex (mTORC1), which is responsible for the integration of cellular signaling and the transduction of further responses (Laplante and Sabatini, 2009). Protein kinase B (Akt) inhibits the tumor sclerosis complex (TSC), which in turn reduces TSC's inhibitory regulation on mTOR (Inoki et al., 2002). Ribosomal protein S6 kinase 1 (p70S6K), is involved in regulation of protein synthesis, and is phosphorylated by Akt because of increased insulin signaling (Inoki et al., 2002). Ribosomal S6 kinase 1 is also activated by mTORC1 (Laplante and Sabatini, 2009). This demonstrates mTOR and mTORC1 are downstream targets of insulin, and could be a potential target of Cr supplementation.

Further evidence of Cr influencing insulin signaling and responsiveness has been observed through the interaction with glucose transporters. The IRS-PI3K-Akt pathway causes GLUT4 to move from the cytoplasm to the cell membrane (Gomperts et al., 2004). GLUT4 will then translocate glucose from the blood stream into the cell to be utilized for metabolism (Hadley and Levine, 2007). Increases in membrane-associated GLUT4 were observed with Cr picolinate after insulin tests in obese rats (Cefalu et al.,

2002), as well as in cell culture with adipocytes (Chen et al., 2006). Furthermore, Cr has increased GLUT4 translocation to the cell membrane by a cholesterol induced mechanism (Pattar et al., 2006; Sealls et al., 2011). Sterol regulatory element-binding protein (SREBP) was upregulated with Cr supplementation, which is responsible for controlling cellular cholesterol balance (Pattar et al., 2006). Sterol regulatory element-binding protein is regulated by the mTORC1 complex (Laplante and Sabatini, 2009). Therefore, Cr could be modifying cholesterol balance by increased activation of IRS and/or Akt leading to increased activation of mTORC1 and resulting in increased SREBP in addition to increased GLUT4 translocation.

Other note worthy actions of insulin include increased PI3K activity, which also results in increased absorption of amino acids and lipids (Gomperts et al., 2004). As well, insulin results in up regulation of lipoprotein lipase and GLUT4 transcription (Gomperts et al., 2004). Lipoprotein lipase is responsible for cleaving fatty acids from chylomicrons, which transport fatty acids from the liver to target tissues, allowing for the fatty acids to be utilized for storage or energy metabolism in the cell (Hadley and Levine, 2007).

Thus, Cr supplementation could lead to more active LMWCr allowing for amplification of insulin signaling, ultimately resulting in a faster and more efficient use of metabolites. Therefore, Cr research has increased as its role in normal physiology and the potential for improving or alleviating metabolic disorders has been identified.

Chromium supplementation in Human Health

The case for Cr being essential for humans was stemmed from a patient receiving total parenteral nutrition (TPN). The patient started showing severe signs of diabetes, weight loss, and hyperglycemia, but symptoms were completely reversed when

supplemented Cr (Jeejeebhoy et al., 1977). This study, as well as other TPN studies (Freund et al., 1979; Brown et al., 1986; Tsuda et al., 1998), suggests Cr is necessary for proper insulin signaling. This has lead to research examining if Cr could improve impaired or normal glucose metabolism.

Human studies have generally consisted of type 2 diabetics, because they are insulin resistant and this is a major health concern in the US. Also, studies have included the elderly as they have impaired glucose metabolism, and adults with normal insulin metabolism. The majority of research shows Cr can improve aspects of glucose metabolism related to insulin resistance as well as age (Glinsmann and Mertz, 1966; Nath et al., 1979; Offenbacher and Pi-Sunyer, 1980; Roeback et al., 1991; Anderson et al., 1997). Chromium has also decreased blood insulin levels and total cholesterol levels in patients with normal insulin metabolism (Urberg et al., 1988; Wang et al., 1989; Press et al., 1990; Lefavi et al., 1993; Anderson et al., 1997). However, there are studies that are inconsistent with these, as they reported no effects of supplemental Cr improving glucose metabolism with Type 2 Diabetes Mellitus (Sherman et al., 1968; Rabinowitz et al., 1983; Thomas and Gropper, 1996), or in adults with normal metabolism (Glinsmann and Mertz, 1966; Thomas and Gropper, 1996). Anderson (1998) suggested the variation between studies is due to varying degrees of glucose intolerance, diet, duration, and type and amount of Cr supplementation. Additionally, response to Cr is reduced by stress (Kegley et al., 1996; Anderson, 1998) and absorption is reduced by simple sugars in the diet (Kozlovsky et al., 1986). All of these issues add variation to human studies making it difficult to form a conclusion about Cr supplementation, but Cr appears to be beneficial in improving impaired glucose metabolism.

Cr supplementation in Livestock

The majority of research leading to the theory Cr is an essential nutrient was conducted on rodents. From the results, it led researchers to question if Cr is adequately supplied to livestock species, and whether or not production would improve with supplementation.

Cr supplementation in swine

Chromium research among livestock species was first conducted in swine production. There has been mixed results in live performance as Matthews et al. (2003; 2006) did not see differences of CrPrp supplementation at 200ppb on ADG, average daily feed intake (ADFI), and gain to feed (G:F) when fed to growing-finishing pigs. Page et al. (1993) reported improved G:F and an increase in ADFI with Cr picolinate at 200 ppb. However, Lindemann et al. (2008) used four different Cr supplements (Cr tripicolinate, CrPrp, Cr methionine, and Cr yeast) and reported improved G:F during the middle and late phases of finishing; as well as improved ADG in the late phase of growth when supplemented 5,000 μg of Cr/kg. Kornegay et al. (1997) observed improved dry matter digestibility and nitrogen absorption when supplementing 200 ppb Cr picolinate, but no other improvements in live animal performance. Amoikon et al. (1995) supplemented 200 μg of Cr/kg of diet as Cr and observed no differences in growth performance, but observed increased insulin sensitivity as glucose disappearance rates were increased with supplementation. Furthermore, blood metabolites were altered, as there were decreased levels of non-esterified fatty acids (NEFA) and increased fasting cholesterol levels with Cr supplementation. However, Page et al. (1993) observed mixed results in ADG between three experiments as Exp. 1 had increased ADG, Exp. 2 ADG was decreased,

and Exp. 3 there was no difference. Furthermore, Page et al. (1993) observed no difference between experiments on blood metabolites or serum insulin levels.

Investigators have also examined the effects of supplemental Cr on carcass performance with the same variability observed in live animal performance. Lindemann et al. (2008) observed no difference in LMA or BFT, but this is contrary to Matthews et al. (2003; 2006) and Mooney and Cromwell (1995; 1997). Kornegay et al. (1997) observed no changes in dressing percentage or backfat thickness at the last rib or 10^{th} rib, however LMA was increased with Cr supplementation. Page et al. (1993) and Lindemann et al. (1995) observed reduced backfat and increased LMA. Moreover, percent muscle was increased (Page et al., 1993; Mooney and Cromwell, 1995; Jackson et al., 2009) and percent carcass fat was decreased (Mooney and Cromwell, 1995) with Cr supplementation.

There have been indications of increased water holding capacity with Cr supplementation. Lindemann et al. (2008) reported a decrease in cooler shrink in hogs supplemented with Cr supplementation. Furthermore, there has been decreases in purge loss (Matthews et al., 2006), thaw loss (Matthews et al., 2003), and cook loss (Shelton et al., 2003) in loin chops from Cr supplemented animals.

Other pork quality traits have been measured, but with more variability in results. Minolta a* and b* values were decreased at day 1 in Lindemann et al. (2008), but by day 5 these color differences diminished. Furthermore, Matthews et al. (2003); (2006) did not see any differences in color. Matthews et al. (2003); (2006); Lindemann et al. (2008) found no differences in pH between Cr supplementation and control. Matthews et al. (2006) did not report differences in marbling score, but Matthews et al. (2003) reported

Cr supplementation increased subjective marbling. Percent intramuscular fat was not measured in Matthews et al. (2003), but percent moisture was decreased with Cr supplementation. The researchers suggested this confirms the subjective marbling difference, because marbling and moisture are inversely related.

As it can be inferred from the previous literature, there is considerable variability in growth, carcass, and meat quality traits in swine when given Cr supplementation. This lead Sales and Jancik (2011) to conduct a meta-analysis of the existing research. The results from the analysis concluded Cr supplementation decreases fat deposition, increases muscle deposition, and improves gain to feed, but had no affect on meat quality (Sales and Jancik, 2011).

Cr supplementation in Cattle

As a result of the discoveries of Cr supplementation in rodents, there has been an interest to determine if cattle would have improved growth. The majority of Cr related research has been performed on beef calves during the receiving period or with dairy cattle. Little research has been conducted on finishing cattle to determine if there would be similar alterations in carcass tissues as noticed in swine production.

Glucose tolerance tests provided indications that Cr could have an affect on insulin sensitivity in cattle. In a study conducted on Angus and Angus x Simmental heifers, data revealed CrPrp supplementation increased insulin sensitivity (Spears et al., 2012). Heifers supplemented with CrPrp in this study had lower serum insulin concentrations, lower molar insulin to glucose ratio, and the area under the glucose response curve was lower compared to controls (Spears et al., 2012). In a study with Holstein bull calves, Cr supplementation lowered plasma glucose concentrations after

intravenous infusion of insulin, indicating an improved insulin response with Cr (Kegley et al., 1997). Improved insulin responsiveness is also supported by a study with pregnant Angus and Simmental cows. Cows supplemented with Cr had lowered plasma glucose, insulin, and NEFA concentrations after glucose tolerance testing (Stahlhut et al., 2006). Furthermore, Sumner et al. (2007) also observed improved glucose utilization by growing dairy heifers with Cr supplementation. When it comes to glucose tolerance tests in feedlot cattle, it has been shown cattle become more insulin resistant with increased days on feed (Radunz et al., 2012; Kneeskern et al., 2016). However, this developing insulin resistance was not mitigated by Cr supplementation in previous research (Kneeskern et al., 2016). Nonetheless, this is the only glucose tolerance test (GTT) data for feedlot cattle supplemented Cr and this area of research warrants further investigation.

As stated previously, there is little research on Cr supplementation in feedlot cattle. Within the published literature, live growth performance has been similar with Cr supplementation in feedlot cattle (Chang et al., 1992; Guifen et al., 2011; Bohrer et al., 2014; Sánchez-Mendoza et al., 2014; Edenburn et al., 2016; Kneeskern et al., 2016). Yet, Pollard et al. (2002) observed reductions in G:F and ADG with 0.4 ppm Cr yeast supplementation compared to control and 0.2 ppm Cr, but no difference between control and 0.2 ppm.

Unlike the live performance, Cr supplementation has resulted in changes in carcass and meat quality traits, however the data is highly variable. Pollard et al. (2002) observed the heaviest HCW for 0.2 ppm Cr, the lightest HCW for 0.4 ppm Cr, and control was intermediate. Control and 0.2 ppm Cr were not different for DP, yield grade, or marbling. However, 0.4 ppm Cr had lower dressing percent and lower marbling score,

but higher yielding carcasses (Pollard et al., 2002). The difference in yield grade is presumed to be due to the decrease in HCW as there was no difference between LMA and backfat thickness was not reported. Sánchez-Mendoza et al. (2014) reported larger LMA as well as trends for both a decrease in backfat thickness and higher retail yields with 500 ppb Cr yeast. However, Chang et al. (1992) found no difference in HCW, DP, LMA, marbling, KPH, or 9-10-11 rib composition estimates with 0.2 mg Cr yeast/kg. Similarly, carcass data from Guifen et al. (2011) and Danielsson and Pehrson (1998) were unchanged with Cr supplementation. Yet, Cr resulted in a decrease in the percent of high-grade cuts using China's quality grading system (Guifen et al., 2011).

Of the data discussed above, only the studies by Bohrer et al. (2014); Edenburn et al. (2016); and Kneeskern et al. (2016) use CrPrp, which is the only approved form of supplemental Cr for beef cattle in the US. Bohrer et al. (2014) and Edenburn et al. (2016) have the only published data using RAC and CrPrp. The CrPrp treatment occurred during the last 30 – 60 d prior to slaughter with the RAC supplement. Whereas, Kneeskern et al. (2016) started with 246 kg steers supplemented for 134 – 174 d, depending on when target backfat thickness was reached, and RAC was not used in this study.

Nonetheless, all three studies found no difference in live animal performance, HCW, LMA, backfat thickness, or yield grade. Yet, Kneeskern et al. (2016) observed a 1.3% increase in dressing percentage. It should be noted statistical difference was at $P \leq 0.10$ in Edenburn et al. (2016). Bohrer et al. (2014) observed a trend for a decrease in the percentage of carcasses grading Low Choice in steers supplemented CrPrp, and Edenburn et al. (2016) reported a trend for an increase in the percent of carcasses grading Select from steers supplemented CrPrp. Furthermore, Bohrer et al. (2014) and Edenburn et al.

(2016) observed no differences in WBSF, cook loss, pH, a*, or b* results. Chromium supplementation, however did increase L*(lightness of muscle), but information on how Cr could affect L* is unknown (Edenburn et al., 2016).

Conclusion

There is not enough literature with fed cattle to conclude if Cr could be a beneficial nutritional supplement, especially with current production practices utilized today. The variation that exists in observations can be attributed to various forms of supplement, dose, duration, stress (metabolic and/or environmental), growth stage when animals started supplement, inherent level of Cr in the diet, and the biological need of Cr by the animal. Other than type, dose, and duration, the other factors are uncontrollable, and contribute to the biological variation innate with animal studies. Thus, more research is needed to determine whether Cr is a beneficial nutritional supplement. Furthermore, implants and beta-agonists alter normal physiology and the influence of Cr with these technologies is yet to be determined. Thus, we chose to conduct the following studies in fed cattle supplemented with Cr to determine if Cr altered the signaling pathways downstream of insulin in muscle tissue.

CHAPTER 2

THE INFLUENCE OF CHROMIUM SUPPLEMENTATION ON THE INSULIN PATHWAY, CARCASS TRAITS, AND MEAT QUALITY OF FEEDLOT STEERS

INTRODUCTION

Chromium (Cr) had been accepted as an essential nutrient for animals (Mertz and Schwarz, 1959) and humans (Jeejeebhoy et al., 1977) because Cr is proposed to be essential for normal insulin metabolism (Anderson, 1997). Research has demonstrated supplemental Cr can improve insulin sensitivity in some, but not all, human patients exhibiting insulin resistance (Anderson, 1998). Cattle have a lower insulin response in insulin-dependent tissues and therefore are more insulin resistant compared to monogastrics (Koster and Opsomer, 2013). Improving insulin sensitivity could lead to increased utilization of nutrients by insulin dependent tissues, possibly translating to increased growth of carcass tissues. There is limited research focusing on Cr supplementation in finishing beef cattle and the effect on beef carcass traits. Barajas et al. (2008) and Song et al. (2013) observed increases in HCW in steers supplemented Cr as Cr methionine. Kneeskern et al. (2016) reported increased dressing percentage (DP) with Cr propionate, and Sánchez-Mendoza et al. (2014) observed an increase in *longissimus* muscle area (LMA) in steers supplemented Cr yeast. Additionally, a cell culture study by Tokach et al. (2015) indicated Cr supplementation improved adipogenesis of intramuscular adipocytes (IMF) when cells were exposed to Cr propionate, and indicated Cr could increase marbling in finishing beef cattle. Thus, we hypothesized Cr

supplementation would increase tissue utilization of nutrients due to amplified insulin signaling resulting in improvements in HCW, DP, LMA, and marbling. Therefore, our objective was to determine the effect Cr supplementation on carcass characteristics, meat quality, and signaling factors downstream of the insulin pathway.

MATERIALS AND METHODS

South Dakota State University Animal Care and Use Committee approved all procedures involving live animals in this study.

Exp. 1

Animal Care and Use

Angus steers from a single source (n = 28; BW = 289 kg ± 12 kg) were received at the Ruminant Nutrition Center (Brookings, SD). Steers were allotted to a pen by BW and randomly assigned a treatment resulting in 2 pens/treatment with 7 steers/pen. Treatment diets consisted of 0 ppb added Cr (CON) or 400 ppb added Cr supplemented as Cr propionate (Cr400, KemTRACE 0.4% Chromium Propionate; Kemin Industries, Des Moines, IA). Receiving protocol and basal diet is recorded in Smith (2015). Supplementation of Cr400 began on d 4 of the trial and continued until slaughter. Supplement formulation is provided in Table 1 and the composition of diets fed for the finishing phase are provided in Table 2. Cattle received Synovex-Plus as the terminal implant (Zoetis, Florham Park, NJ) on d 123. A subset of cattle (n = 5/pen) closest to the mean ADG for each pen was selected for muscle biopsies. Following the terminal implant, the first LM biopsy was collected on d 184 (biopsy184) posterior to the 13th rib and approximately 5.08 cm ventral to the spine on the right side. Lidocaine® was

administered as a local anesthetic followed by a 2.54 cm incision. Jackson uterine biopsy forceps (Jorgenson Labs, Loveland, CO) were used to collect muscle tissue. Tissue samples were snap frozen in liquid nitrogen and stored at -80°C. Steers started 200 mg/hd/d ractopamine hydrochloride (RAC; Optaflexx 45®; Zoetis, Florham Park, NJ) on d 185. During supplementation of RAC, the second LM biopsy was collected on d 200 (biopsy200) following the same procedure but approximately 5.08 cm posterior to biopsy184 location. Two steers were removed from the study because of foot rot resulting in 12 steers in the CON and 14 steers in Cr400.

Carcass data and sample collection

Cattle were weighed off test on d 210, and then transported approximately 240 km to a commercial packing facility (Tyson Foods, Inc. Dakota City, NE). Cattle were tracked throughout harvest to maintain identity. Following carcass chilling (approximately 28 h), LMA, 12th rib back fat (BF), HCW, KPH, marbling score, skeletal maturity, and lean maturity were collected on the left side of the carcass by trained personnel. The average of two objective color score measurements (Minolta colorimeter, Konica Minolta, Ramsey, NJ) were obtained within the LM for L*, a*, and b* values after approximately one hour of bloom time. A subset of carcasses (n = 3/pen) closest to the mean ADG for each pen was selected for carcass composition estimation. Rib sections were marked for rib-plate separation as outlined for 9-10-11 rib dissection in Hankins and Howe (1946). Strip loins (n = 26, IMPS 180) and wholesale ribs (n = 12, IMPS 103) from the left side were tracked through fabrication, and transported under refrigeration to South Dakota State University Meat laboratory for further processing. Carcass and quality data were collected from a dark cutter in the CON treatment, but this

sample was excluded from LM quality measurements, 9-10-11 rib composition, WBSF, and cook loss analyses. Approximately 3 d postmortem, strip loins were denuded and the first steak was removed for proximate analysis and pH determination. Steaks were frozen at -20°C until analyses. Three steaks (2.54 cm) were cut starting from the anterior end of each strip loin, and were allotted sequentially for Warner-Bratzler shear force (WBSF) at 6, 14, and 21 d postmortem. Steaks were refrigerated (1.7 - 4.4°C) until the designated aging period was reached and then frozen at -20°C until WBSF could be performed. Wholesale ribs were fabricated into 9-10-11 rib sections according to Hankins and Howe (1946) and weighed. Then soft tissue was separated from bone and cartilage and weighed separately. Soft tissue was homogenized using a bowl chopper (Model CM-14, Manica, St. Louis, MO), vacuumed packaged, and stored at -20°C for proximate analysis according to Mohrhauser et al. (2015).

Warner-Bratzler Shear Force and Cook loss

All steaks were thawed at 4°C for 12 h and then tempered to room temp for 12 h. Steaks were weighed prior to cooking, cooked to an internal temperature of 71°C at the geometric center using clamshell grills (George Forman, Model GR2144P, Middleton, WI), and weighed after cooking. Peak internal temperature was recorded using calibrated hand held digital thermometers (Atkins Aqua Tuff thermometers, Cooper-Atkins Cooperation, Middlefield, CT). Steaks were allowed to cool in refrigeration until internal temperatures were below 4°C. Six cores (1.27 cm-diameter) were removed running parallel to the muscle fibers and sheared perpendicular to fiber orientation with a Warner-Bratzler shear machine (G-R Electric Manufacturing Company, Manhattan, KS) equipped with a Mecmesin 500N basic force gauge (Mecmesin Ltd., West Sussex, United

kingdom). Peak force (kg) was recorded for each core and an average was calculated using the values of 6 cores for each steak. Cook loss was determined as the percent difference between fresh and cooked weight using the following equation.

$$Cook\ loss, \% = \frac{(Fresh\ weight, g\ -\ cooked\ weight, g)}{fresh\ weight, g} \times 100$$

Proximate Analysis and Carcass Composition Estimation

The first steak from each strip loin was designated for ether extraction. Steaks were allowed to thaw slightly, minced, frozen in liquid nitrogen, and powered in a Waring commercial blender (Waring Products Division, New Hartford, CT). Powdered samples were stored at -20°C for subsequent extraction. Approximately 5 g of sample was dried in pre-weighed aluminum tins and filter paper in a drying oven (Thelco Laboratory Oven, Precision Scientific, Winchester, VA) at 100°C for 24 h. Samples were reweighed, and were extracted for fat with petroleum ether in a side-arm soxhlet (AOAC, 1990) for 60 h. Following extraction excess ether was allowed to evaporate under a fume hood for approximately an hour. Then samples were dried an additional 24 h at 100°C before final weighing. Fat content was calculated as the difference between dried and extracted weight.

Proximate analysis was performed on the soft tissue portion from the 9-10-11 rib sections. Water and fat content were determined using the same procedure outlined for ether extraction. Protein content was determined by weighing approximately 200 mg of sample into nitrogen free foil tins and placed in an Elementar nitrogen analyzer (Rapid N III, Hanau, Germany). Ash content was determined by placing approximately 3 g of sample into pre-weighed crucibles, dried at 100°C for 24 h, and heated in a muffle furnace (Isotemp Programmable Muffle Furnace, Fischer Scientific, Waltman, MA) at

500°C for 16 h. All samples were repeated in duplicate. The chemical data from proximate analysis of 9-10-11 ribs was used to estimate carcass soft tissue composition using equations in Hankins and Howe (1946). Estimated soft tissue composition was then used to calculate total carcass composition for bone and soft tissue (Hankins and Howe, 1946).

pH Determination

Ten grams from the powdered steak samples were homogenized in 90 mL of distilled water using an Ultra Turrax homogenizer (IKA, Model T25DS1, Germany). Ultimate postmortem pH was determined using a Thermo Orion PerpHecT LogRmeter model 330 (Thermo Scientific, Beverly, MA) as described by Kern et al. (2013).

Real-Time PCR

Muscle biopsy samples were thawed and approximately 50 mg of tissue was removed under sterile conditions. Samples were placed in bead beater tubes containing 700 µL of QIAzol Lysis Reagent provided in the Qiagen miRNeasy mini kit (Hilden, Germany). RNA extraction was completed according to the methodology and buffers provided in the Qiagen miRNeasy mini kit, and the method is outlined below. Samples were homogenized using a PowerLyzer® 24 (Mo-Bio Laboratories, Inc., Carlsbad, CA) for 45 s, 30 s dwell, and 45 s at 3500 x *g*. Homogenized tissue lysates were held at room temperature for approximately 5 min to promote disassociation of nucleoprotein complexes. 140 µL of chloroform was added to samples, shaken vigorously for 15 s, then held at room temperature for approximately 2 min. Samples were centrifuged at 12,000 x g at 4°C for 15 min to achieve phase separation; upper RNA aqueous phase, white interphase, and lower red organic phase. The RNA aqueous phase, approximately 450

μL, was transferred to a new collection tube and mixed with 1.5 volumes of ethanol. Then the RNA aqueous phase sample was pipetted into a filter tube and centrifuged at 8,000 x *g* for 15 s at room temperature. 700 μL of Buffer RWT was added to the samples and filtered by centrifuging at 8,000 x *g* for 15 s at room temperature. Followed by pipetting 500 μL of Buffer RPE to the samples and filtered by centrifuging at 8,000 x g for 15 s at room temperature. Samples were washed again with Buffer RPE and centrifuged at 8,000 x *g* for 2 min at room temperature. RNA was eluted from filter tube with 50 μL of RNase-free water and centrifuging at 8,000 x *g* for 1 min at room temperature. Filtrated RNA sample was pipetted into a RNA free micro-centrifuge tube, and total nucleic acid content was estimated using a Nanodrop 2000 spectrophometer (Thermo Scientific, Wilmington, DE). Samples were diluted to 200 ng/μL RNA with RNase-free water and stored at -80°C.

RNA was converted to cDNA using a high capacity cDNA reverse transcriptase kit (Applied Biosystems, Foster City, CA) and PCR was performed using a MyCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA). Real-time quantitative PCR was performed using SYBER Green real time PCR, and samples were ran in triplicate. Primers used were published in Takahashi et al. (2014) and Kern et al. (2014) and listed with primer sequences and accession numbers in Table 3. Primers were diluted in 400 μL of RNase-free water. A master mix was made containing SYBER Green, forward primer, reverse primer, and RNase-free water. Twenty-five μL of master mix was added to each well resulting in 12.5 μL of SYBER Green, 9.5 μL water, 1 μL forward primer, and 1 μL reverse primer. Real time polymerase chain reaction was conducted using a Stratagene Mx3005P (Agilent Technologies, Santa Clara, CA) with cycle settings in three segments,

Segment 1: 1 cycle of 95°C for 10 min; Segment 2: 40 cycles of 95°C for 30 s, 55°C for 1 min, and 72°C for 1 min; Segment 3: 1 cycle of 95°C for 1 min, 55°C for 30 s, and 95°C for 30 s.

Western Blot

Muscle biopsy samples were thawed and approximately 75 mg of tissue was removed, placed in 50 mL centrifuge tube, and held in -20°C while further extractions continued. Homogenizing solution [5 mM NaH_2PO_4, 1 mM $(Na_2HPO_4)7H_2O$, 69 mM SDS] was added at 25 times the tissue weight followed 60 s of homogenization using Ultra Turrax homogenizer (IKA, Model T25DS1, Germany). Homogenized samples were held at -20°C while remaining samples was homogenized. Samples were warmed prior to centrifuging at 1,500 x *g* for 15 min at room temperature. The supernatant was transferred to new 15 mL tube, and stored -80°C while protein concentration was being determined using the RC/DC Protein assay (based on Lowry's assay, Bio-Rad Laboratories, Hercules, CA). Samples were then diluted to 2.5 mg/mL with distilled water. Equals parts of sample loading buffer [150 mM Tris-HCl (pH 6.8), 20% (vol/vol) glycerol, 0.004% (wt/vol) bromophenol blue] and diluted samples were added followed by denaturing the sample on a heat blocker at 100°C for 5 min. Samples were then stored at -80°C until western blotting. Mercaptoethanol was added at 6% to samples followed by subsequent heating at 100°C for 5 min.

PPAR γ

Samples (10 µL) were loaded onto a 15% tris-hydrochloride gels (Criterion Precast gels, Bio-Rad Laboratories, Hercules, CA) exposed to SDS-gel electrophoresis for 90 min at 200 V using the Bio-Rad Criterion Cell system (Bio-Rad Laboratories,

Hercules, CA). Gels were equilibrated in transfer buffer [25 mM Tris (pH 8.3), 192 mM glycine, 15% vol/vol methanol] for approximately 30 min and then transferred onto polyvinylidene difluoride (PVDF) membranes for 60 min at 90 V using a Criterion blotter system (Bio-Rad Laboratories, Hercules, CA). Membranes were equilibrated in Tris buffer saline [50 mM Tris-HCl (pH 7.6), and 150 mM NaCl] (TBS) and blocked in a solution containing equals parts TBS and Odyssey Blocking Buffer/TBS (OBB, Li-Cor Biosciences, Lincoln NE) one hour at room temperature. Membranes were then incubated for one hour at room temperature in primary antibody solution containing 1:100 dilution of anti-PPARγ goat (Santa Cruz Biotechnology, sc-1984), 1:8,000 dilution of anti-actin rabbit (Sigma Aldrich, A5060) in equal parts TBST [TBS + 0.1% Tween-20] and OBB while rocking. After incubation, membranes were rinsed with 20 mL TBST followed by 5 min washes with 10 mL TBST repeated 4 times. Membranes were labeled with secondary antibody solution containing 1:20,000 dilution of donkey-anti-goat and donkey-anti-rabbit (Li-Cor Biosciences, Lincoln, NE) in TBST:OBB for one hour at room temperature while rocking. Membranes were rinsed with 20 mL TBST followed by four 5 min washes with 10 mL TBST.

Akt and phospho-Akt (Ser473)

Samples (10 μL) were loaded onto a 4-20% tris-hydrochloride gel (Criterion Precast gels, Bio-Rad Laboratories, Hercules, CA) exposed to SDS-gel electrophoresis for 90 min at 200 V using the Bio-Rad Criterion Cell system (Bio-Rad Laboratories, Hercules, CA). Gels were equilibrated in transfer buffer for approximately 30 min and then transferred onto either nitrocellulose (Akt) or PVDF membranes (phospho-Akt) for 90 min at 90 V using a Criterion blotter system (Bio-Rad Laboratories, Hercules, CA).

Membranes were dried for an hour, rewetted in TBS for 15 min, and then blocked in TBS:OBB for one hour at room temperature. Membranes were then incubated overnight at 4°C in primary antibody solution containing either 1:100 dilution of anti-akt1/2/3 (H-136) rabbit or anti p-Akt (ser473) rabbit (Santa Cruz Biotechnology, sc-8312 or sc-7985 respectively), and 1:8,000 dilution of anti-actin mouse (Sigma Aldrich, A4700) in TBST:OBB while rocking. After incubation, membranes were rinsed with 20 mL TBST followed by 5 min washes with 10 mL TBST repeated 4 times. Membranes were labeled with secondary antibody solution contained 1:20,000 dilution of goat-anti-rabbit and goat-anti-mouse (Li-Cor Biosciences, Lincoln, NE) in TBST:OBB for one hour at room temperature while rocking. Membranes were rinsed with 20 mL TBST followed by four 5 min washes with 10 mL TBST.

p70S6K

Samples (10 μL) were loaded onto a 4-20% tris-hydrochloride gel (Criterion Precast gels, Bio-Rad Laboratories, Hercules, CA) exposed to SDS-gel electrophoresis for 90 min at 200 V using the Bio-Rad Criterion Cell system (Bio-Rad Laboratories, Hercules, CA). Gels were equilibrated in transfer buffer for approximately 30 min and then transferred onto nitrocellulose membranes for 90 min at 90 V using a Criterion blotter system (Bio-Rad Laboratories, Hercules, CA). Membranes were dried for an hour, rewetted in TBS for 15 min, then blocked in TBS + 5% bovine serum albumin (BSA) (Sigma Aldrich, A3059) for one hour at room temperature. Membranes were then incubated overnight at 4°C in primary antibody solution containing 1:1000 dilution of anti-p70S6K rabbit (Cell Signaling Technology, 9202) in TBST + 5% BSA while rocking. Rinsed with 20 mL TBST followed by 5 min washes with 10 mL TBST repeated

4 times. Labeled with secondary antibody solution contained 1:20,000 dilution of goat-anti-rabbit in TBST + 5% BSA. Membranes were rinsed with 20 mL TBST followed by four 5 min washes with 10 mL TBST. Membranes were then scanned before incubation of actin antibody. Following the initial scan membranes were then incubated with primary antibody solution with 1:8,000 dilution of anti-actin mouse (Sigma Aldrich, A4700) in TBST + 5% BSA for one hour at room temperature while rocking. After incubation, membranes were rinsed with 20 mL TBST followed by 5 min washes with 10 mL TBST repeated 4 times. Membranes were labeled with secondary antibody solution contained 1:20,000 dilution of goat-anti-mouse in TBST + 5% BSA for one hour at room temperature while rocking. Membranes were rinsed with 20 mL TBST followed by four 5 min washes with 10 mL TBST.

Quantification of Western Blots

Proteins were visualized and quantified using a Li-Cor Odyssey scanner (Lincoln, NE). Relative abundance of target proteins was determined by normalizing relative intensities of target protein to relative intensities of actin. Then to account for differences in transfer efficiency, relative abundance of target proteins were then standardized to a reference sample, which was run on each gel.

Exp. 2

Animal Care and Carcass Collection

The study was comprised of 256 heavy yearling steers (BW = 405 kg) divided into pens by BW, and then allocated to a dietary treatment of either 0 ppb Cr (CON) or 300 ppb supplemented Cr (Cr300; KemTRACE, 0.4% Chromium Propionate; Kemin Industries, Des Moines, IA) resulting in 16 pens of 8 steers/treatment. Supplement

formulation is provided in Table 4 and the composition of diets fed in the finishing phase are provided in Table 5. Cattle were implanted with Synovex Choice on d 1 and Cr300 started on d 9. Cattle were re-implanted with Synovex Choice on d 77, and started RAC on d 105 at a target of 300 mg/hd/d. Cattle were weighed off test the morning of d 138 and shipped to a commercial packing facility (240 km; Tyson Foods, Inc. Dakota City, NE). A subset of steers (1 steer / pen; n = 32) closet to the ADG pen mean were chosen as the subsample to collect carcass data and strip loins. Animal identity was tracked through harvest and chilling. After chilling (approximately 28 h), LMA, BF, HCW, KPH, marbling score, skeletal maturity, and lean maturity were collected on the left side of the carcass by trained personnel. Carcass and quality data were collected from a dark cutter in the Cr300 treatment, but is not represented in quality measurements from the LM, WBSF, or cook loss. Strip loins (n = 30, IMPS 180) were tracked through fabrication and transported under refrigeration to South Dakota State University meat laboratory for further processing. Strip loins were denuded and the first steak was removed for proximate analysis. Four steaks (2.54 cm) were cut starting from the anterior end and were allotted sequentially for Warner-Bratzler shear force (WBSF) at 7, 14, 21, and 28 d postmortem. Steaks were held in refrigeration (1.7 – 4.4°C) until designated aging period was reached and then frozen at -20°C until WBSF could be performed. Shear force and proximate analysis of LM was performed as previously described.

Statistical Analysis

Carcass data, pH, proximate analysis of LM, and 9-10-11 rib data were analyzed as a completely randomized design in PROC MIXED (SAS Inst. Inc., Cary, NC) with diet as the main effect. Shear force and western blot data were run as a repeated measure

in PROC MIXED using the Toeplitz covariance structure. Diet, age period, and diet x age were the main effects for Exp. 1 WBSF. Peak internal temperature was added as a covariate for Experiment 2 WBSF and percent cook loss in both experiments. Gene expression was analyzed using REST 2009 (Relative Expression Software Tool v2.0, Corbett Research and M. Pfaffl, Technical University Munich) by normalizing target genes to housekeeping genes. Animal was the experimental unit for all analyses and significance was considered at $P < 0.05$ and trends at $P < 0.10$.

RESULTS

Exp. 1

The purpose of Exp. 1 was to evaluate the effects of Cr supplementation on beef carcass traits and cellular mechanisms related to the insulin pathway. Therefore, the live animal data presented in Table 6 is for characterization of the animals in the experiment, not to determine the effects of Cr on performance. Carcass and meat quality data is reported in Table 7. There were no differences in final BW or HCW ($P > 0.10$) between treatments. However, Cr400 steers had numerically lighter final body weights and numerically heavier carcasses leading to an increase in DP (61.8% vs. 63.3%, CON vs. Cr400 respectively; $P = 0.03$). There was no difference in BF, LMA, YG, or maturity. CON steers had a 0.4% decrease in percent KPH compared to Cr400 ($P = 0.03$). Additionally, Cr400 treated steers had decreased marbling scores ($P = 0.046$) and a trend for a decrease in percent IMF ($P = 0.07$). No difference was observed in pH or color scores ($P > 0.10$) of LM steaks between treatments. The Hankins and Howe (1946) 9-10-11 rib procedure revealed there were no difference of the estimated carcass composition (percent bone, fat, protein, or moisture) or estimated composition of the edible portion

(percent fat, protein, and moisture) as denoted in Tables 8 and 9, respectively. Warner Bratzler shear force values were increased for steaks from Cr400 compared to CON (P = 0.02; Table 10). Also, Cr400 steaks had an increase in cook loss vs. CON (P = 0.01). *Longissimus* muscle biopsies observed no difference in relative abundance of Akt, phospho-Akt, or p70S6K at biopsy184 (P > 0.10), while growth was promoted with the terminal implant, or at biopsy200 when RAC was supplemented (P > 0.10; Table 11). However, the protein related to adipogenesis, PPARγ, had a tendency to be higher in CON steers vs. Cr400 steers at biopsy184 (P = 0.09), but this trend was diminished by biopsy200 (P > 0.10). In conjunction, gene expression of GLUT4 or GLUT1 was unchanged at either biopsy time point (P > 0.10) and is depicted in Figure 2.1.

Exp. 2

Experiment 2 was conducted as a follow up study to Exp.1. A subsample of steers were chosen at the end of an ongoing feeding trial to determine if differences in carcass traits and shear force data would parallel results from Exp. 1. There was no difference in final BW, HCW, DP, BF, LMA, YG or maturity between CON and Cr300 (P > 0.10; Table 7). There was a trend for percent KPH to be higher for CON than Cr300 (P = 0.10). Marbling score and IMF were not different between treatments (P > 0.10). Shear force and percent cook loss were not different (P > 0.10; Table 10). However, there was an interaction for percent cook loss between treatment and aging period (P = 0.04), which is depicted in Figure 2.2.

DISCUSSION

Of the literature with Cr supplemented to feedlot cattle, there is limited information on how Cr could influence carcass traits. Original Cr studies with cattle focused on steers during the receiving period, and conclude Cr generally alleviates the detrimental effects of stress (Moonsie-Shageer and Mowat, 1993; Borgs and Mallard, 1998). Blood metabolite studies showed Cr improved insulin sensitivity during receiving (Sumner et al., 2007; Spears et al., 2012). From this information others have continued Cr through finishing to evaluate if the increased insulin sensitivity would translate to increased growth. However, there is a wide variation in experimental designs between studies. Two of the main differences are the duration of Cr (35d – 222d) and starting BW (240 kg – 527 kg). This has caused inconsistency among carcass traits, because when significance of a trait or traits is reported it is not repeated in other studies (Chang et al., 1992; Valdes-Garcia et al., 2011; Song et al., 2013; Sánchez-Mendoza et al., 2014). Similarly, the changes in DP, KPH, WBSF, and marbling for Exp.1 were not repeated in Exp. 2. Therefore, if Cr is improving insulin sensitivity, it has not consistently increased growth of carcass tissues.

The lack of repeatability between the two experiments in this research could be attributed to the difference in animal model (calf-feds vs. yearlings) when starting Cr trials as well as duration of Cr supplementation as indicated above. Yearlings are older in age and have heavier body weights when starting in the feedlot compared to calf-feds, which means they differ in maturity and are at different points in their growth curve. Yearlings are placed on grass for several months during their growing phase and have a slower rate of growth (Coleman et al., 1993). This slow rate of growth causes restriction

of nutrients to lower priority tissues (i.e. fat depots). Nonetheless, once yearling cattle start consuming the high-energy diet, fat will have an exponential rate of growth (Dolezal et al., 1993). In contrast, calf feds are placed in the feedlot throughout the growing and finishing phases; this allows for unrestricted development of all tissues. Generally this results in calf-feds having lighter final BW and HCW because they reach a common backfat thickness earlier compared to yearlings (Dolezal et al., 1993). Thus, calf-feds were at a different point of development when Cr supplementation began. Also, tissue demand for nutrients is different at different stages of growth. Insulin regulates the cell metabolism, substrate uptake, and anabolic growth (Saltiel and Kahn, 2001), thus Cr could affect insulin sensitive tissues differently between calf-feds and yearlings. Furthermore, Cr has shown to increase HCW with longer supplementation (Song et al., 2013). This could be another explanation why there were more differences in Exp. 1 vs. 2 since animals were supplemented for 72 d longer in Exp. 1.

Significant increases in HCW has been observed in Pollard et al. (2002), Barajas et al. (2008), and Song et al. (2013). Although not significant, the trend for numeric improvements in HCW among the remaining literature is noteworthy (Valdes-Garcia et al., 2011; Sánchez-Mendoza et al., 2014; Bibber et al., 2015; Kneeskern et al., 2016), as well as in both of our experiments. Bohrer et al. (2014) and Edenburn et al. (2016) are the only studies reporting numeric decreases in HCW, but the focus of these studies was determining if there was an additive effect of Cr and RAC during the last 30 – 60 days of finishing. The authors indicated Cr did not heighten the effects of RAC on carcass traits. Overall, the small numbers used in Cr research, including our studies, could have be a limitation to determine whether Cr supplementation can increase carcass weights.

As stated previously, data between experiments were not consistent, which holds true among the literature. Kneeskern et al. (2016) reported Cr increased DP, which agrees with the increased DP in Exp. 1. This 1.5% increase in DP in Cr400 steers is partially due to of the 0.4% increase in KPH. Yet, the increased KPH does not completely account for the increase in DP. This indicates Cr supplementation increased tissue accumulation in calf-feds that was not identified by carcass composition measurements, yield grade, or carcass data. The effect of Cr400 on KPH in Exp. 1 conflicts with Exp. 2. Instead, KPH tended to decrease in Cr300 steers in Exp. 2. The only reported difference in KPH has been for a linear decrease in KPH with increasing Cr dosage (Valdes-Garcia et al., 2011) agreeing with the latter of our experiments. Therefore, the effect Cr has on KPH is inconclusive, but the increase in DP supports the potential for increases in tissue accumulation.

Despite the 0.5 kg increase in shear force from steaks from Cr400 steers, mean WBSF values for all treatments in both experiments were below 4.4 kg. This is the threshold consumers consider a steak tender (Platter et al., 2003). Moreover, treatments in both experiments had mean WBSF values less than 3.9 kg qualifying steaks for the marketing claim *Certified Very Tender* (International, 2011). It should also be noted aging period was significant to WBSF, in both experiments, demonstrating steaks were normal as tenderness increased during the aging period. Previous literature has not reported a change in WBSF from beef steaks (Bohrer et al., 2014; Edenburn et al., 2016), lamb chops (Arvizu et al., 2011), or pork chops (Sales and Jancik, 2011) from animals supplemented Cr or not supplemented. Therefore, the difference in Exp. 1 indicates Cr supplementation with calf-fed steers throughout growing and finishing may decrease

tenderness, but supplementing Cr to yearling steers resulted in no difference in tenderness.

As previously mentioned, peak internal temperature was used as a covariant for cook loss in Exp. 1 and 2, because peak internal temperature contributes significantly to cook loss (Brewer and Novakofski, 1999). Previous literature has reported no difference in cook loss of beef steaks from steers supplemented Cr (Edenburn et al., 2016). There were indications of increased water holding capacity when pigs were supplemented Cr as cooler shrink was decreased for carcasses (Lindemann et al., 2008), and thaw loss (Matthews et al., 2003), purge loss (Matthews et al., 2006), and cook loss (Shelton et al., 2003) were decreased in loin chops. However, a meta-analysis of pigs supplemented Cr did not observe these traits to be different (Sales and Jancik, 2011). Thus, variation in degree of doneness of steaks could been the reason for increased cook loss with Cr400 in Exp. 1 and the interaction of treatment and aging period in Exp. 2.

Most Cr studies have not included the use of an implant strategy coupled with RAC during supplementation, which are two common technologies utilized within US feedlots to enhance efficiency of gain and pounds of red meat produced per animal. The two LM biopsies were collected to evaluate how Cr could be influencing growth when current technologies are applied, and how mechanisms downstream of insulin are involved. A mechanism through which implants are proposed to increase lean growth is by increasing circulating insulin-like growth factor (IGF) concentrations as well as increasing IGF production within skeletal muscle (Hossner, 2005). Insulin-like growth factor can mediate its effect through the same pathway as insulin, and the result is an increase in muscle hypertrophy (Delavaud et al., 2002; Du and McCormick, 2009).

Ractopamine hydrochloride, a beta-agonist, is considered a “repartitioning agent”. Ractopamine hydrochloride is fed for the last 28-42 d of finishing, when animals have reduced lean growth and rapid fat development (Johnson et al., 2014a). Feeding RAC dramatically increases demand for nutrients by skeletal muscle at the expense of adipose (Mersmann, 1998). This shift causes an increase in protein synthesis and lipolysis and a decrease in lipogenesis (Mersmann, 1998; Johnson et al., 2014a). The use of these technologies cause dramatic changes to the animal’s physiology; thus, Cr effects on insulin signaling could be diminished by these potent technologies.

Proteins downstream of insulin were measured at each biopsy to determine if there is a difference in insulin signaling with the use of implants or RAC. It is well understood phosphorylation of Akt is a necessary for continuing intracellular signaling of insulin (Alessi et al., 1996), and Cr supplementation has shown to increase phosphorylation of Akt at Serine 473 (Chen et al., 2009b), which is a site required for complete activation of Akt (Alessi et al., 1996). Activation of Akt leads to translocation of GLUT4 to the cell membrane for glucose absorption (Shepherd and Kahn, 1999), and causes increased activity of p70S6K, which is responsible for regulating protein synthesis (Inoki et al., 2002). Lastly, GLUT4 and 1 gene expression were measured, because insulin signaling leads to increased GLUT4 gene transcription (Gomperts et al., 2004). In addition, GLUT1 is responsible for basal glucose uptake and is higher in ruminants verses other species (Duhlmeier et al., 2005). Representative western blots for relative protein abundance determination are depicted in Figures 2.3, 2.4, and 2.5. Since there was no difference observed with the proteins and genes measured indicates Cr did not

have an additive effect on proteins related to insulin signaling or genes related to glucose transporters when steers were given growth promoters.

Tokach et al. (2015) evaluated gene expression coupled with protein abundance in cell cultures of bovine satellite cells (muscle). Supplementation increased myotube number and diameter in satellite cells compared to control. Also, Cr led to no changes in GLUT4 mRNA expression, which agrees with our data. But there was a decrease in the ratio of protein abundance between GLUT4 and GAPDH (glyceraldehyde 3-phosphate dehydrogenase) with Cr supplementation (Tokach et al., 2015). The authors stated the decrease in GLUT4:GAPDH combined with the changes in myotube number and diameter indicates the muscle cells are more efficient at glucose absorption, because there was increased growth with less glucose receptors present at the cell membrane. Also, a technical report by Kemin Industries (2016) observed Cr increased internalization of GLUT4 ($P = 0.01$) from LM biopsies in conjunction with a numeric increase in HCW. They concluded the increased internalization of GLUT4 leads to less GLUT4 at the cell membrane to absorb glucose. The researchers made the similar conclusion as Tokach et al. (2015) that Cr increases gain through more efficient absorption of glucose. This theory could explain why Cr400 increased DP with no difference in proteins related to the insulin pathway in Exp.1. This data provides the basis that Cr could be potentiating basal glucose uptake independent of insulin signaling and translocation of GLUT4.

Adipogenesis, the development of fat, is regulated by PPARγ (Tontonoz and Spiegelman, 2008). Cell culture work showed Cr increased PPARγ expression in bovine intramuscular adipocytes (Tokach et al., 2015). This work indicated Cr supplementation could increase IMF and subsequently garner premiums for increased beef quality grades.

However, only numeric increase in marbling scores have only been reported in Exp. 2 and in Sánchez-Mendoza et al. (2014). Marbling scores have been significantly decreased with Cr400 in Exp. 1. As well, 400 ppm Cr decreased marbling score compared to CON or 200 ppb Cr in Pollard et al. (2002) and tended to decrease in (Kneeskern et al., 2016). Noteworthy, is the 16.4% increase of USDA Select steers reported in Edenburn et al. (2016) in steers supplemented Cr. Furthermore, the first muscle biopsy indicated a trend for decreased PPARγ in the LM of Cr400 steers. Since marbling development occurs during the growing phase and PPARγ regulates adipogenesis, the timing of the biopsy may not have been optimal to measure Cr effects on PPARγ *in vivo*. Moreover, it is presumed IMF and connective tissue may be in competition for the limited pool of progenitor cells during development (Du et al., 2013). If connective tissue development is activated, then progenitor cells would be destined for this cell line, and this could leave a smaller pool of progenitor cells for IMF development (Du et al., 2013). This could explain the decrease in marbling along with the increase in toughness observed in Exp. 1. However, it is unknown what caused the increased WBSF values, because the amount of connective tissue or other traits known to affect tenderness were not measured in these studies. Nonetheless, supplementing Cr could potentially cause an increase in connective tissue at the expense of intramuscular adipogenesis.

Researchers have established carcass tissues have a different response to Cr. Intramuscular adipocytes increased GLUT4 mRNA, GLUT4 abundance, and PPARγ mRNA with Cr (Tokach et al., 2015). Subcutaneous (SC) adipocytes did not differ in PPARγ or GLUT4 expression or GLUT4 abundance. The authors concluded IMF is more sensitive to Cr than SC fat. In the live animal, IMF is not in competition with SC fat for

glucose, because SC fat utilizes more acetate than glucose as carbon sources (Smith and Crouse, 1984), but IMF is competing for glucose with muscle. Insulin stimulation of muscle causes a 7-10 times higher absorption of glucose into muscle versus fat (Shulman et al., 1990; Perseghin et al., 1996), so muscle has a higher affinity for glucose over fat when stimulated by insulin. Therefore, Cr supplementation could be increasing muscle glucose absorption at the expense of IMF, which could explain the increased DP and decreases in marbling observed in this data and previous fed cattle research.

CONCLUSION

The hypothesis of our research was Cr would increase accretion of economically important carcass traits: HCW, DP, and marbling. However, this was not proven within our research. Even though DP was increased for Exp. 1, it was not replicated in Exp. 2. In the live animal, marbling is likely reduced with Cr since marbling and skeletal muscle are in competition for glucose. Overall, the increased DP and decreased marbling in Exp. 1 could be a residual effect of Cr increasing muscle growth at the expense of fat during the growing phase. An additive effect with Cr was not observed with the utilization of terminal implants or RAC as measured by proteins related to anabolic muscle growth. Therefore, any affects of Cr could potentially be negated by these potent technologies. Nonetheless, Cr research should continue in cattle designated towards alternative marketing programs that do not allow the use of implants or beta-agonists. There is the potential for Cr to be a natural growth-promoting supplement allowing for beef producers to more efficiently produce beef under these marketing programs.

Table 1. Pelleted supplement formulations to include chromium and ractopamine hydrochloride in cattle diets for Exp. 1[a]

	d 4 - 183		d 184 - 210	
S1480 Supplement	CON[b]	Cr400[b]	CON[b]	Cr400[b]
Wheat Middlings, kg	453.5	453.5	–	453.5
Ground Corn, kg	453.5	449.4	–	449.4
Chromium Propionate,[c] kg	–	4.1	–	4.1
Optaflexx supplement				
Corn, kg	–	–	612	612
Wheat middlings, kg	–	–	272	272
Binder, kg	–	–	7.3	7.3
Premix				
- Optaflexx 45,[d] kg	–	–	7.56	7.56
- Corn, kg	–	–	7.84	7.84

[a] As Is basis

[b] CON = 0 ppb chromium propionate; Cr400 = 400 ppb chromium propionate

[c] KemTRACE 0.4% Chromium Propionate

[d] 99 g of ractopamine hydrochloride per kg of Optaflexx 45

Table 2. Percent of feedstuffs of the finishing diets and calculated crude protein and net energy of gain for Exp. 1[a]

	d 89 - 146	**d 147 - 183**		**d 184 - 210**	
	All treatments	CON[b]	Cr400[b]	CON[b]	Cr400[b]
Corn silage, %	–	11	11	11	11
HMC,[c] %	83.9	35	35	35	35
DDGS,[c] %	9.6	–	–	–	–
DRC,[c] %	–	34.75	32.75	32.75	30.75
mDGS,[c] %	–	15	15	15	15
S1480,[d] %	2.0	–	2.0	–	2.0
Liquid Supplement,[e] %	4.5	4.25	4.25	4.25	4.25
Optaflexx Supplement,[f] %	–	–	–	2.0	2.0
CP,[c] %	13	13	13	13	13
NE_G,[c] Mcal/kg	1.34	1.41	1.41	1.41	1.41

[a] on a dry matter basis
[b] CON = 0 ppb chromium propionate; Cr400 = 400 ppb chromium propionate
[c] High moisture corn (HMC), Dried Distillers Grains with Solubles (DDGS), Dry Rolled Corn (DRC), Modified Distillers Grains with Solubles (mDGS), Crude Protein (CP), Net Energy for gain (NE_g)
[d] pelleted carrier for chromium propionate
[e] supplement contained vitamins and minerals to meet or exceed NRC requirements
[f] pelleted carrier for ractopamine hydrochloride

Table 3. Primer sequences for house-keeping and genes of interest for *longissimus* muscle biopsy

Gene		Primer Sequence	Accession Number
EEF1A2[*a]	forward	5' – GGTACTGGACAAGCTGAAGG – 3'	NM_001037464
	reverse	5' – GCGTCGATGATGGTGATGTA – 3'	
SF3A1[*b]	forward	5' – GCCCGTGGTGGGTATTATTTA – 3'	NM_001081510
	reverse	5' – TGTTGATCTCGTTCTGTCGTATC – 3'	
GLUT 1[c]	forward	5' – GATGATGCGGGAGAAGAAGG	NM_174602.2
	reverse	5' – TGAAGGCTGTGTTGACGATG	
GLUT 4[d]	forward	5' – AGTGGCTGGGAAGGAAGAGG	NM_174604.1
	reverse	5' – TAGCACCTGGGCGATTAGGA	

[*] Housekeeping Gene

[a] Eukaryotic Translation Elongation Factor 1, alpha 2; [b] Splicing Factor 3, subunit 1

[c] Glucose Transporter 1, bovine; [d] Glucose Transporter 4, bovine

Table 4. Pelleted supplement formulations to include chromium and ractopamine hydrochloride in cattle diets for Exp. 2[a]

	d 1 – 105		d 106 - 138	
S1580 Supplement	CON[b]	Cr300[b]	CON[b]	Cr300[b]
Soy hulls, kg	320	315	386	381
Soybean meal, kg	587	588	735	735
Chromium Propionate,[c] kg	–	4.06	–	5.08
Optaflexx 45,[d] kg	–	–	13.70	13.70

[a] As Is basis

[b] CON = 0 ppb chromium propionate; Cr300 = 300 ppb chromium propionate

[c] KemTRACE 0.4% Chromium Propionate

[d] 99 g of ractopamine hydrochloride per kg of Optaflexx 45

Table 5. Percent of feedstuffs of the finishing diets and calculated crude protein and net energy of gain for Exp. 2[a]

	d 8 - 120	**d 121 - 138**
Corn silage, %	12.25	–
Grass Hay, %	–	8.15
HMC,[b] %	34.02	35.19
DRC,[b] %	32.18	33.56
mDGS,[b] %	14.83	16.38
S1580,[c] %	1.95	1.99
Liquid Supplement,[d] %	4.78	4.75
CP,[b] %	13	13
NE_G,[b] Mcal/kg	1.41	1.43

[a] on a dry matter basis

[b] High moisture corn (HMC), Dry Rolled Corn (DRC), Modified Distillers Grains with Solubles (mDGS), Crude Protein (CP), Net Energy for gain (NE_g)

[c] pelleted carrier for chromium propionate and ractopamine hydrochloride

[d] supplement contained vitamins and minerals to meet or exceed NRC requirements

Table 6. Live animal performance data from steers supplemented chromium propionate for both Exp. 1 and Exp. 2

	Exp. 1[a]				**Exp. 2**[a]			
	CON[b]	Cr400[b]	SEM	*P*-value	CON[b]	Cr300[b]	SEM	*P*-value
	n = 2	n = 2			n = 16	n = 16		
Initial BW, kg	288	287	4	0.88	406	412	7	0.60
Final BW, kg	660	655	6	0.63	674	691	15	0.44
ADG,[c] kg	1.81	1.79	0.07	0.81	1.94	2.02	0.07	0.44
Total days of Cr supplementation	0 d	210 d			0 d	130 d		

[a] Exp. 1 = 150 days on finishing diet; Exp. 2 = 138 days on finishing diet

[b] CON = 0 ppb chromium propionate; Cr400 = 400 ppb chromium propionate; Cr300 = 300 ppb chromium propionate

[c] ADG calculated over the finishing phase

Table 7. Carcass data and quality data of *longissimus* muscle from steers supplemented chromium propionate for both Exp. 1 and Exp. 2[a]

	Exp. 1					Exp. 2				
	CON[b]	SEM	Cr400[b]	SEM	*P*-value	CON[b]	SEM	Cr300[b]	SEM	*P*-value
	n = 12		n = 14			n = 16		n = 16		
HCW, kg	396	6	402	6	0.46	407	10	419	10	0.42
Dressing Percent,[c] %	61.77	0.47	63.26	0.43	**0.03**	62.23	0.50	62.52	0.50	0.68
12th Rib Backfat, cm	1.24	0.13	1.37	0.12	0.44	1.09	0.10	1.33	0.10	0.11
LMA,[d] cm^2	90.3	1.9	91.5	1.7	0.65	92.6	2.1	96.5	2.1	0.20
KPH, %	1.79	0.12	2.18	0.11	**0.02**	1.94	0.09	1.72	0.09	**0.10**
Yield Grade[e]	2.89	0.19	3.11	0.17	0.39	2.77	0.17	2.88	0.17	0.63
Lean Maturity[f]	153	18	130	17	0.37	144	13	165	13	0.26
Skeletal Maturity[f]	105	3	106	3	0.87	143	5	153	5	0.15
	n = 11		n = 14			n = 15		n = 14		
Marbling Score[g]	512	19	459	17	**0.046**	432	16	449	16	0.45
IMF,[h] %	6.20	0.45	5.09	0.40	**0.07**	5.02	0.36	5.18	0.35	0.76
IMM,[h] %	71.23	0.35	71.94	0.31	0.12	71.93	0.28	71.85	0.27	0.83
pH	5.54	0.03	5.51	0.03	0.46	–	–	–	–	–
L*	43.68	0.54	43.26	0.48	0.57	–	–	–	–	–
a*	26.41	0.32	27.03	0.29	0.17	–	–	–	–	–
b*	11.86	0.20	12.21	0.18	0.22	–	–	–	–	–

[a] Least square means

[b] CON = 0 ppb supplemental chromium propionate; Cr400 = 400 ppb chromium propionate; Cr300 = 300 ppb chromium propionate

[c] HCW / Final body weight with 3% percent shrink

[d] *Longissimus* muscle area

[e] calculated based on equations from USDA (2016)

[f] A = 100, B = 200

[g] Marbling Score: Small = 400, Modest = 500

[h] Intramuscular fat (IMF), Intramuscular moisture (IMM)

Table 8. Estimated carcass composition using the 9-10-11 rib procedure for steers from Exp. 1[ab]

	CON[c]	SEM	Cr400[c]	SEM	*P*-value
	n = 5		n = 6		
Bone	13.65%	0.29	13.42%	0.27	0.56
Protein	13.32%	0.24	13.46%	0.22	0.67
Fat	27.10%	1.43	25.93%	1.30	0.56
Moisture	45.66%	0.91	46.71%	0.83	0.42

[a] Least Square Means

[b] 9-10-11 rib procedure Hankins and Howe, 1946

[c] CON = 0 ppb chromium propionate, Cr400 = 400 ppb chromium propionate

Table 9. Estimated composition of edible portion using the 9-10-11 rib procedure for steers from Exp. 1[ab]

	CON[c]	SEM	Cr400[c]	SEM	*P*-value
	n = 5		n = 6		
Protein	15.43%	0.31	15.55%	0.28	0.78
Fat	31.36%	1.55	29.95%	1.42	0.52
Moisture	52.89%	1.19	53.96%	1.08	0.52

[a] Least Square Means

[b] 9-10-11 rib procedure Hankins and Howe, 1946

[c] CON = 0 ppb chromium propionate, Cr400 = 400 ppb chromium propionate

Table 10. Warner-Bratzler shear force and cook loss of *Longissimus* steaks from steers supplemented chromium propionate for both Exp. 1 and Exp. 2[a]

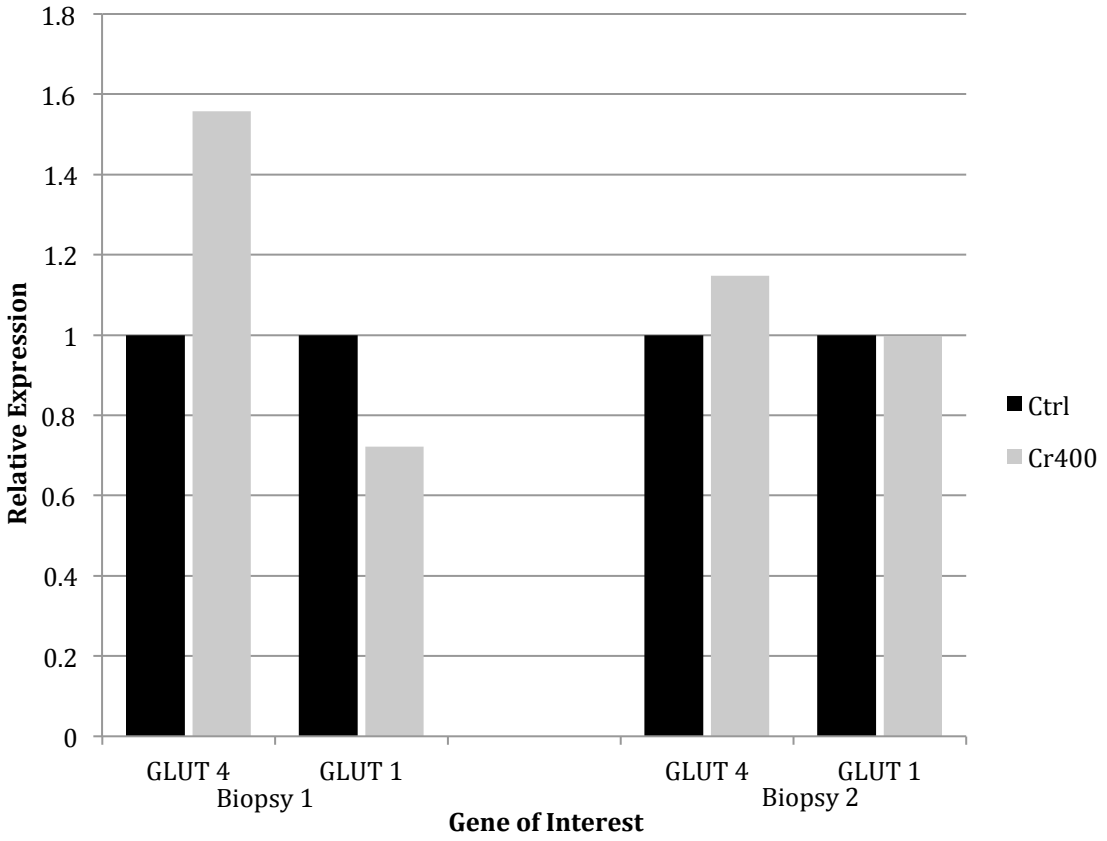
	Treatment				Age					*P*-value		
Exp. 1	CON[b]	SEM	Cr400[b]	SEM	6 d	14 d	21 d		SEM	Trt	Age	Trt x Age
	n = 11		n = 14		n = 25	n = 25	n = 25					
WBSF,[c] kg	2.56	0.12	2.95	0.11	3.17^{x}	2.78^{xy}	2.32^{y}	–	0.20	**0.02**	**0.0003**	0.98
Cookloss,[d]* %	19.88	0.29	20.90	0.26	22.02^{x}	20.93^{y}	18.22^{z}	–	0.47	**0.01**	**< 0.0001**	0.13
Exp. 2	CON[b]	SEM	Cr300[b]	SEM	7 d	14 d	21 d	28 d	SEM	Trt	Age	Trt x Age
	n = 15		n = 15		n = 30	n = 30	n = 30	n = 30				
WBSF,* kg	2.70	0.07	2.83	0.07	3.12^{x}	2.86^{x}	2.58^{y}	2.49^{y}	0.10	0.20	**< 0.0001**	0.40
Cookloss,* %	19.85	0.33	19.27	0.33	20.13	19.53	19.01	19.59	0.81	0.23	0.41	**0.04**

[a] Least Square Means
[b] CON = 0 ppb chromium propionate; Cr400 = 400 ppb chromium propionate; Cr300 = 300 ppb chromium propionate
[c] Warner-Bratzler shear force
[d] Cookloss = ((fresh weight – cooked weight) / fresh weight)*100
[xyz] means with different superscripts differ ($P \leq 0.05$)
* peak eternal temperature used as a covariant

Table 11. Western blot relative protein abundance within *Longissimus* muscle biopsies between steers supplemented chromium and between biopsies for Exp. 1[ab]

	CON[b]	SEM	**Cr400**[c]	SEM	*P*-value
Biopsy184[d]	n = 9		n = 10		
PPARγ[e]	0.68	0.13	0.37	0.12	**0.09**
AKT[f]	0.027	0.004	0.036	0.004	0.22
AKT(Ser473)[g]	1.25	0.18	1.21	0.17	0.87
p70-S6K[h]	0.046	0.004	0.056	0.004	0.11
Biopsy200[i]	n = 10		n = 10		
PPARγ	0.32	0.12	0.30	0.12	0.92
AKT	0.042	0.004	0.043	0.004	0.96
AKT(Ser473)	1.25	0.16	0.99	0.17	0.27
p70-S6K	0.054	0.004	0.053	0.004	0.90
	Biopsy184	SEM	**Biopsy200**	SEM	*P*-value
	n = 19		n = 20		
PPARγ	0.52	0.09	0.31	0.09	**0.095**
AKT	0.031	0.003	0.043	0.003	**0.03**
AKT(Ser473)	1.22	0.12	1.12	0.12	0.53
p70-S6K	0.056	0.003	0.053	0.003	0.56

[a] Least Square Means
[b] Target protein intensity was normalized to Actin intensity then standardized to a reference sample
[c] CON = 0 ppb chromium propionate, Cr400 = 400 ppb chromium propionate
[d] *Longissimus* muscle biopsy taken after adaptation to terminal implant
[e] Peroxisome proliferator-activated receptor gamma
[f] Protein kinase B
[g] Protein kinase B phosphorylated at serine 473
[h] Ribosomal protein S6 kinase 1
[i] *Longissimus* muscle biopsy taken after adaptation to ractopamine hydrochloride



	Biopsy 1			Biopsy 2	
	GLUT 4	GLUT 1		GLUT 4	GLUT 1
CON	1	1		1	1
Cr400	2.633	1.221		1.463	1.273
P-value	0.152	0.601		0.285	0.334

Figure 2.1. Influence of chromium on gene expression of glucose transporter 4 and 1 in longissimus muscle from steers supplemented either 0 ppb (CON) or 400 ppb chromium propionate (Cr400) in Exp. 1. Biopsy 1 was taken after adaption to terminal implant (n=9 CON; n=10 Cr400), and biopsy 2 was taken after adaption to ractopamine hydrochloride (n=10 per treatment). Relative expression is reported on a base gene expression level of 1 for the CON treatment. No differences were observed in relative expression of genes between treatments ($P > 0.05$).

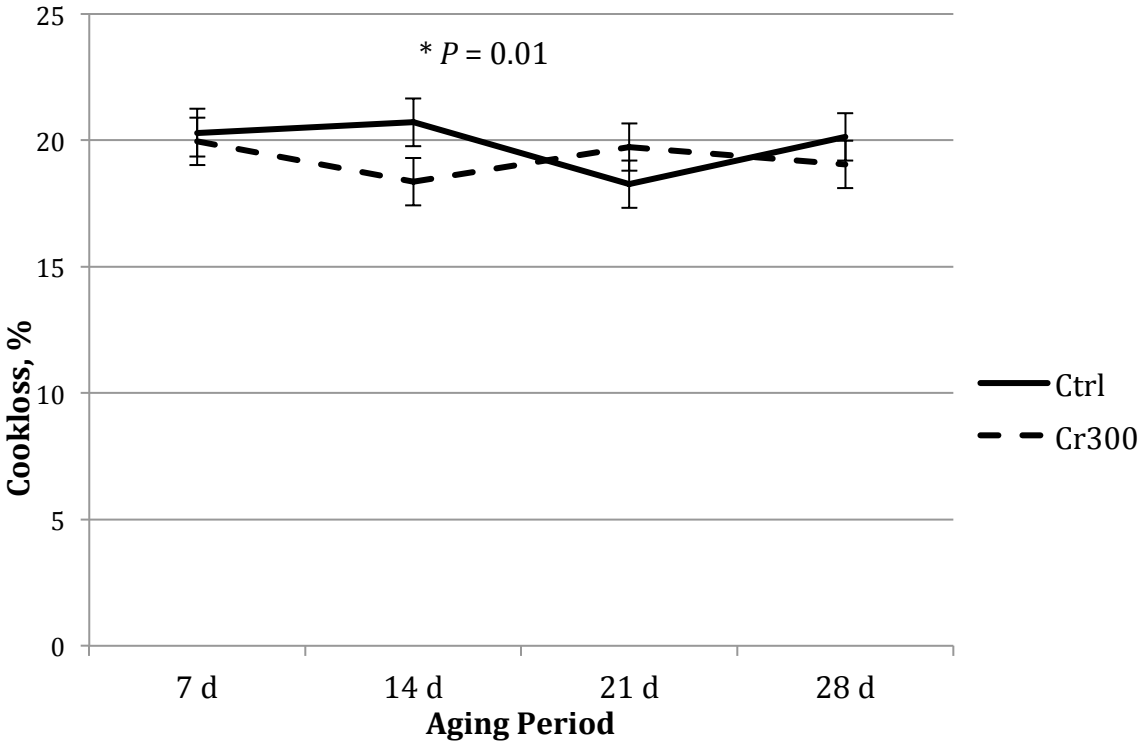


Figure 2.2. Percent cook loss interaction between treatment and aging period of longissimus steaks from steers supplemented either 0 ppb (CON) or 300 ppb chromium propionate (Cr300) aged for 7, 14, 21, and 28 d in Exp. 2. Main effects of treatment and aging period were not significant ($P > 0.05$). Peak internal temperature contributed variation to cook loss ($P < 0.001$) and used a covariant. Error bars reflect the standard error of the mean and n = 16 steaks for each treatment at each aging period.

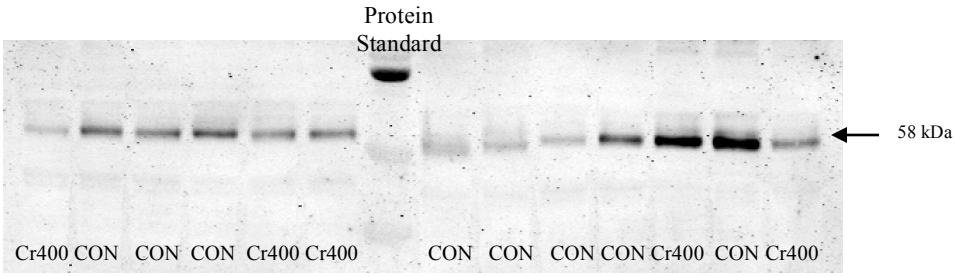
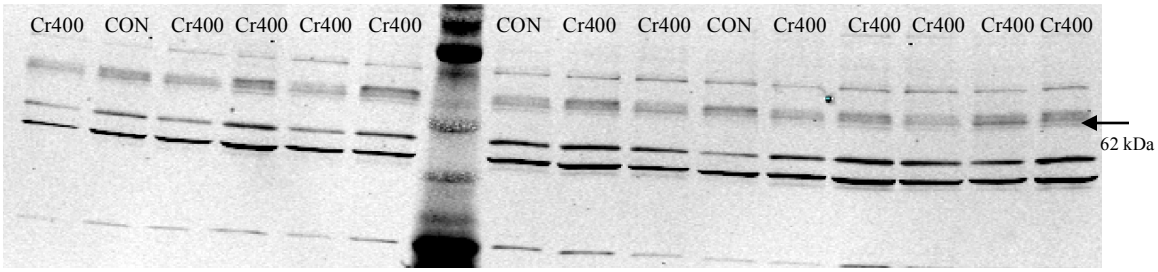


Figure 2.3. Representative western blot of peroxisome proliferator-activated receptor γ (PPARγ) from bovine longissimus muscle biopsies from steers supplemented either 0 ppb (CON) or 400 ppb chromium propionate (Cr400) in Exp. 1. Blot prepared from 15% Tris-HCl gels transferred to a polyvinylidene difluoride membrane and labeled with anti-PPARγ antibody (Santa Cruz Biotechnology, sc-1984).

a) Akt



b) phospho-Akt(ser473)

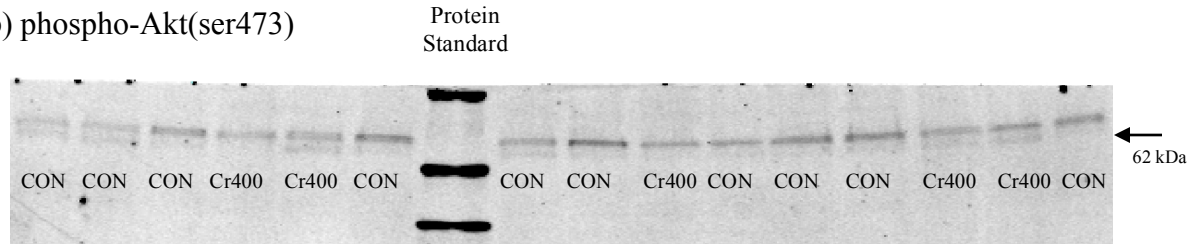


Figure 2.4. Representative western blot of a) protein kinase B (Akt) and b) Akt phosphorylated at Serine 473 from bovine longissimus muscle biopsies from steers supplemented either 0 ppb (CON) or 400 ppb chromium propionate (Cr400) in Exp. 1. Blots prepared from 4 – 20% Tris-HCl gels transferred to nitrocellulose membranes and labeled with anti-Akt and anti-phospho Akt(Ser473; Santa Cruz Biotechnology, sc-8312 and sc-7985, respectively).

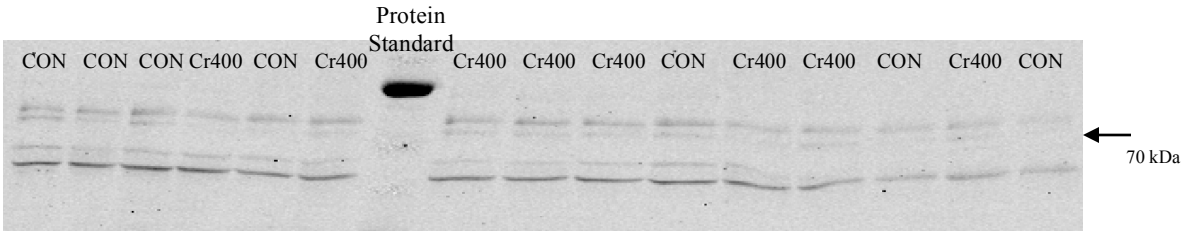


Figure 2.5. Representative western blot of ribosomal protein S6 kinase 1 (p70S6K) from bovine longissimus muscle biopsies from steers supplemented either 0 ppb (CON) or 400 ppb chromium propionate (Cr400) in Exp. 1. Blots prepared from 4 – 20% Tris-HCl gels transferred to nitrocellulose membranes and labeled with anti-p70S6K rabbit (Cell Signaling Technology, 9202).

LITERATURE CITED

Alessi, D. R., M. Andjelkovic, B. Caudwell, P. Cron, N. Morrice, P. Cohen, and B. A.Hemmings. 1996. Mechanism of activation of protein kinase B by insulin and IGF-1. The EMBO journal 15: 6541 - 6551.

Alessi, D. R., S. R. James, C. P. Downes, A. B. Holmes, P. R. Gaffney, C. B. Reese, and P. Cohen. 1997. Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Bα. Current Biology 7: 261-269.

Amoikon, E. K., J. M. Fernandez, L. S. L, J. D. L. Thompson, T. L. Ward, and B. M. Olcott. 1995. Effect of Chromium Tripicolinate on Growth, Glucose Tolerance, Insulin Sensitivity, Plasma Metabolites, and Growth Hormone in Pigs. Journal Animal Science 73: 1123-1130.

Andersen, M. A., S. C. Blank, T. LaMendola, and R. J. Sexton. 2002. California's cattle and beef industry at the crossroads. California Agriculture 56: 152-156.

Anderson, R. A. 1997. Chromium as an Essential Nutrient for Humans. REGULATORY TOXICOLOGY AND PHARMACOLOGY 26: S35-S41.

Anderson, R. A. 1998. Chromium, Glucose Intolerance and Diabetes. Journal of American College of Nutrition 17: 548-555.

Anderson, R. A., N. A. Bryden, M. M. Polansky, and K. Gautschi. 1996. Dietary chromium effects on tissue chromium concentrations and chromium absorption in rats. The Journal of Trace Elements in Experimental Medicine 9: 11-25.

Anderson, R. A., N. Cheng, N. A. Bryden, M. M. Polansky, N. Cheng, J. Chi, and J. Feng. 1997. Elevated Intakes of Supplemental Chromium Improve Glucose and Insulin Variables in Individuals With Type 2 Diabetes. Diabetes 46: 1786-1791.

Anderson, R. A., and A. S. Kozlovsky. 1985. Chromium intake, absorption and excertion of subjects consuming self-selected diets. The American Journal of Clinical Nutrition 41: 1177-1183.

Arvizu, R., I. Domínguez, M. Rubio, J. Bórquez, J. Pinos-Rodríguez, M. González, and G. Jaramillo. 2011. Effects of genotype, level of supplementation, and organic chromium on growth performance, carcass, and meat traits grazing lambs. Meat Science 88: 404-408.

Barajas, R., B. J. Cervantes, E. A. Velazquez, J. A. Romo, F. Juarez, and P. J. Rojas. 2008. Chromium methionine supplementation on feedlot performance and carcass characteristics of bulls: I. Results during cool season in the northwest of Mexico. Proceedings of western Section of American Society of Animal Science 59: 383 - 386.

Berthiaume, R., I. Mandell, L. Faucitano, and C. Lafreniere. 2006. Comparison of alternative beef production systems based on forage finishing or grain-forage diets with or without growth promotants: 1. Feedlot performance, carcass quality, and production costs. J Anim Sci 84: 2168-2177.

Bibber, C. L. V., J. E. Axman, J. M. Gonzalez, and J. S. Drouillard. 2015. Dietary chromium propionate combined with yeast minimally affects growth performance and carcass traits of finishing steers, Kansas Agricultural Experiment Station Research Reports.

Bittner, C. J., G. I. Crawford, L. L. Berger, S. Holt, R. H. Pritchard, W. J. Platter, M. T. V. Koevering, N. A. Pyatt, and G. E. Erickson. 2016. Effect of ractopamine hydrochloride (Optaflexx) dose and duration. J Anim Sci 94.

Bohrer, B. M., B. M. Edenburn, D. D. Boler, A. C. Dilger, and T. L. Felix. 2014. Effect of feeding ractopamine hydrochloride (Optaflexx) with or without supplemental zinc and chromium propionate on growth performance, carcass characteristics, and meat quality of finishing steers. J Anim Sci 92: 3988-3996.

Borgs, P., and B. A. Mallard. 1998. Immune-endocrine interactions in agricultural species: chromium and its effect on health and performance. Domestic Animal Endcrinology 15: 431 - 438.

Brewer, M. S., and J. Novakofski. 1999. Cooking rate, pH and final endpoint temperature effects on color and cook loss of a lean ground beef model system. Meat Science 52: 443-451.

Brown, R. O., S. Forloines-Lynn, R. E. Cross, and W. D. Heizer. 1986. Chromium deficiency after long-term total parenteral nutrition. Digestive diseases and sciences 31: 661-664.

Bruns, K. W., R. H. Pritchard, and D. L. Boggs. 2005. The effect of stage of growth and implant exposure on performance and carcass composition in steers. J Anim Sci 83: 108-116.

Cefalu, W., and F. B. Hu. 2004. Role of Chromium in Human Health and in Diabetes. Diabetes Care 27: 2741-2751.

Cefalu, W. T., Z. Q. Wang, X. H. Zhang, L. C. Baldor, and J. C. Russell. 2002. Oral chromium picolinate improves carbohydrate and lipid metabolism and enhances

skeletal muscle Glut-4 translocation in obese, hyperinsulinemic (JCR-LA corpulent) rats. the Journal of Nutrition 132: 1107-1114.

Chang, X., D. N. Mowat, and G. A. Spiers. 1992. Carcass characteristics and tissue-mineral contents of steers fed supplemental chromium. Canadian Journal of Animal Science 72: 663 - 669.

Chen, G., P. Liu, G. R. Pattar, L. Tackett, P. Bhonagiri, A. B. Strawbridge, and J. S. Elmendorf. 2006. Chromium activates glucose transporter 4 trafficking and enhances insulin-stimulated glucose transport in 3T3-L1 adipocytes via a cholesterol-dependent mechanism. Molecular Endocrinology 20.

Chen, W. Y., C. J. Chen, C. H. Liu, and F. C. Mao. 2009a. Chromium supplementation enhances insulin signalling in skeletal muscle of obese KK/HlJ diabetic mice. Diabetes, Obesity, and Metabolism 11: 293-303.

Chen, W. Y., C. J. Chen, C. H. Liu, and F. C. Mao. 2009b. Chromium supplementation enhances insulin signalling in skeletal muscle of obese KK/HlJ diabetic mice. Diabetes Obes Metab 11: 293-303.

Church, D. C. 1988. The ruminant animal. Digestive physiology and nutrition. Prentice Hall.

Coleman, S. W., B. C. Evans, and J. J. Guenther. 1993. Body and carcass composition of Angus and Charolais steers as affected by age and nutrition. J Anim Sci 71: 86 - 95.

Crouse, J. D., H. R. Cross, and S. C. Seideman. 1984. Effects of a grass of grain diet on the quality of three beef muscles. Journal Animal Science 58: 619 - 625.

Danielsson, D. A., and B. Pehrson. 1998. Effects of chromium supplementation on the growth and carcass quality of bulls fed a grain-based diet during the finishing period Journal of Veterinary Medicine and Science 45: 219-224.

Delavaud, C., A. Ferlay, Y. Faulconnier, F. Bocquier, G. Kann, and Y. Chilliard. 2002. Plasma leptin concentraiton in adult cattle. J Anim Sci 80: 1317 - 1328.

Dolezal, H. G., J. D. Tatum, and J. F. L. Williams. 1993. Effects of feeder cattle frame size, muscle thickness, and age class on days fed, weight, and carcass composition. J Anim Sci 71: 2975 - 2985.

Du, M., Y. Huang, A. K. Das, Q. Yang, M. S. Duarte, M. V. Dodson, and M. J. Zhu. 2013. Meat Science and Muscle Biology Symposium: manipulating mesenchymal progenitor cell differentiation to optimize performance and carcass value of beef cattle. J Anim Sci 91: 1419-1427.

Du, M., and R. J. McCormick. 2009. Applied muscle biology and meat science. CRC Press.

Duhlmeier, R., A. Hacker, A. Widdel, W. von Engelhardt, and H. P. Sallmann. 2005. Mechanisms of insulin-dependent glucose transport into porcine and bovine skeletal muscle. Am J Physiol Regul Integr Comp Physiol 289: R187-197.

Edenburn, B. M., S. G. Kneeskern, B. M. Bohrer, W. Rounds, D. D. Boler, A. C. Dilger, and T. L. Felix. 2016. Effects of supplementing zinc or chromium to finishing steers fed ractopamine hydrochloride on growth performance, carcass characteristics, and meat quality. Journal Animal Science.

Field, T. 2010. Raising beef in a first world country: science, media and politics. In: Proceedings of the Beef Improvement Federation Annual Research Conference. p 39-45.

Freund, H., S. Atamian, and J. E. Fischer. 1979. Chromium deficiency during total parenteral nutrition. Jama 241: 496-498.

Glinsmann, W. H., and W. Mertz. 1966. Effect of trivalent chromium on glucose tolerance. Metabolism 15: 510-520.

Godfray, H. C., J. R. Beddington, I. R. Crute, L. Haddad, D. Lawrence, J. F. Muir, J. Pretty, S. Robinson, S. M. Thomas, and C. Toulmin. 2010. Food security: the challenge of feeding 9 billion people. Science 327: 812-818.

Gomperts, B. D., I. M. Kramer, and P. E. R. Tatham. 2004. Signal Transduction. Elsevier Academic Press, London, UK.

Gruber, S. L., J. D. Tatum, T. E. Engle, M. A. Mitchell, S. B. Laudert, A. L. Schroeder, and W. J. Platter. 2007. Effects of ractopamine supplementation on growth performance and carcass characteristics of feedlot steers differing in biological type. Journal Animal Science 85: 1809-1815.

Guifen, L., W. Fachun, S. Enliang, L. Xiaomu, T. Xiuwen, and L. Zhenshan. 2011. Effects of chromium picolinate supplementation on the growth, carcass quality and gene expression of beef during the finishing period. Molecular Biology Reports 38: 4469-4474.

Hadley, M. E., and J. E. Levine. 2007. Endocrinology. Pearson Prentice Hall, Upper Saddle River, NJ.

Hankins, O. G., and P. E. Howe. 1946. Estimation of the composition of Beef Carcasses and Cuts. USDA Technical Bulletin 926: 1 - 20.

Hossner, K. L. 2005. Hormonal regulation of farm animal growth. CABI.

Inoki, K., Y. Li, T. Zhu, J. Wu, and K. L. Guan. 2002. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. Nat Cell Biol 4: 648-657.

International, A. 2011. ASTM F 2925-11. Standard specification for tenderness marketing claims associated with meat cuts derived from beef.

Jackson, A. R., S. Powell, S. L. Johnston, J. O. Matthews, T. D. Bidner, F. R. Valdez, and L. L. Southern. 2009. The effect of chromium as chromium propionate on growth performance, carcass traits, meat quality, and the fatty acid profile of fat from pigs fed no supplemented dietary fat, choice white grease, or tallow. Journal Animal Science 87: 4032-4041.

Jeejeebhoy, K. N., R. Chu, E. Marliss, G. R. Greenberg, and A. Bruce-Robertson. 1977. Chromium deficiency, glucose intolerance, and neuropathy reversed by chromium supplementation, in a patient receiving long-term total parenteral nutrition. The American Journal of Clinical Nutrition 30: 531-538.

Johnson, B. J., P. T. Anderson, J. C. Meiske, and W. R. Dayton. 1996. Effect of combined trenbolone acetate and estrodial implant on feedlot performance, carcass charateristics, and carcass compostition of feedlot steers. Journal Animal Science 74.

Johnson, B. J., S. B. Smith, and K. Y. Chung. 2014a. Historical Overview of the Effect of beta-Adrenergic Agonists on Beef Cattle Production. Asian-Australas Journal of Animal Science 27: 757 - 766.

Johnson, M. D., D. P. Anderson, J. E. Sawyer, T. A. Wickersham, and J. W. Richardson. 2014b. The economic impact of beta agonist removal from beef production. Annu. Meet. Southern Agricultural Association: 1-4.

Kahn, C. R. 1978. Insulin resistance, insulin insensitivity, and insulin unresponsiveness: a necessary distinction. Metabolism 27: 1893-1902.

Kahn, S. E., R. L. Hull, and K. M. Utzschneider. 2006. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 444: 840-846.

Kegley, E. B., J. W. Spears, and T. T. Brown. 1996. Immune response and disease resistance of calves fed chromium nicotinic acid complex or chromium chloride. Journal of dairy science 79: 1278-1283.

Kegley, E. B., J. W. Spears, and J. H. Eisemann. 1997. Performance and Glucose Metabolism in Calves Fed a Chromium-Nicotinic Acid Complex

or Chromium Chloride. Journal of dairy science 80: 1744 - 1750.

Kemin Industries, I. 2016. Effects of KemTRACE Chromium on blood parameters, GLUT4, and muscle fiber charateristics of finishing steers, http://www.kemin.com.

Kerestes, Á. M. 2009. Role of insulin in the development of metabolic and reproductive malfunctions of periparturient dairy cows. Ph.D. thesis, Szent István University.

Kern, S. A., T. D. Jennings, D. S. Kohls, H. L. Doering-Resch, S. M. Scramlin, K. R. Underwood, and A. D. Weaver. 2013. Inclusion of Hydro-Lac preslaughter increases glycolytic potential and shelf life of beef11Salaries and research support provided by state and federal funds appropriated to South Dakota State University. The Professional Animal Scientist 29: 443-448.

Kern, S. A., R. H. Pritchard, A. D. Blair, S. M. Scramlin, and K. R. Underwood. 2014. The influence of growth stage on carcass composition and factors associated with marbling development in beef cattle. Journal Animal Science 92: 5275-5284.

Kneeskern, S. G., A. C. Dilger, S. C. Loerch, D. W. Shike, and T. L. Felix. 2016. Effects of chromium supplementation to feedlot steers on growth performance, insulin sensitivity, and carcass characteristics. Journal Animal Science 94: 217-226.

Kolok, A. S., and M. K. Sellin. 2008. The Environmental Impact of Growth-Promoting Compounds Employed by the United States Beef Cattle Industry: History, Current Knowledge, and Future Directions Reviews of Environmental Contamination and Toxicology. p 1-30. Springer New York.

Koontz, S. R. 2010. Economic Factors Impacting the Cattle Industry, the Size of the Beef Cow Herd, and Profitability and Sustainability of Cow-Calf Producers, Colorado State University Extension.

Kornegay, E. T., Z. Wang, C. M. Wood, and M. D. Lindemann. 1997. Supplemental chromium picolinate influences nitrogen balance, dry matter digestibility, and carcass traits in growing-Finishing pigs. Journal Animal Science 75.

Koster, J. D. D., and G. Opsomer. 2013. Insulin Resistance in Dairy Cows. The veterinary clinics of North America - food animal practice 29: 299-322.

Kozlovsky, A. S., P. B. Moser, S. Reiser, and R. A. Anderson. 1986. Effects of diets high in simple sugars on urinary chromium losses. Metabolism 35: 515-518.

Laplante, M., and D. M. Sabatini. 2009. mTOR signaling at a glance. J Cell Sci 122: 3589-3594.

Lawrence, J., J. Mintert, J. D. Anderson, and D. P. Anderson. 2008. Feed Grains and Livestock: Impacts on Meat Supplies and Prices. Choices 23: 11.

Lefavi, R. G., G. D. Wilson, R. E. Keith, R. A. Anderson, D. L. Blessing, C. G. Hames, and J. L. McMillan. 1993. Lipid-lowering effect of a dietary chromium (III)—Nicotinic acid complex in male athletes. Nutrition Research 13: 239-249.

Lindemann, M. D., G. L. Cromwell, H. J. Monegue, and K. W. Purser. 2008. Effect of chromium source on tissue concentration of chromium in pigs. Journal Animal Science 86: 2971-2978.

Lindemann, M. D., C. M. Wood, A. F. Harper, E. T. Kornegay, and R. A. Anderson. 1995. Dietary chromium picolinate additions improve gain:feed and carcass characteristics in growing-finishing pigs and increase litter size in reproducing sows. Journal Animal Science 73: 457-465.

Lone, K. P. 1997. Natural sex steroids and their xenobiotic analogs in animal production: growth, carcass quality, pharmacokinetics, metabolism, mode of action, residues, methods, and epidemiology. Critical Review Food Science Nutrition 37: 93-209.

Mandell, I. B., J. G. Buchanan-Smith, and C. P. Campbell. 1998. Effects of Forage vs Grain Feeding on Carcass Characteristics, Fatty Acid Composition, and Beef Quality in Limousin-Cross Steers When Time on Feed Is Controlled. Journal Animal Science 76: 2619-2630.

Manning, B. D., and L. C. Cantley. 2007. AKT/PKB signaling: navigating downstream. Cell 129: 1261-1274.

Matthews, J. O., A. C. Guzik, F. M. LeMieux, L. L. Southern, and T. D. Bidner. 2006. Effects of chromium propionate on growth, carcass traits, and pork quality of growing-finishing pigs. Journal Animal Science 84: 858-862.

Matthews, J. O., A. D. Higbie, L. L. Southern, D. F. Coombs, T. D. Bidner, and R. L. Odgaard. 2003. Effect of chromium propionate and metabolizable energy on growth, carcass traits, and pork quality of growing-finishing pigs. Journal Animal Science 81: 191-196.

Mersmann, H. J. 1998. Overview of the Effects of b-Adrenergic Receptor Agonists on Animal Growth Including Mechanisms of Action. Journal Animal Science 76: 160-172.

Mertz, W., and K. Schwarz. 1959. Relation of glucose tolerance factor to impaired intravenous glucose tolerance of rats on stock diets. American Journal of Physiology--Legacy Content 196: 614-618.

Meyer, L. 1997. Marketing beef cattle. Kentucky Beef Book, ID-108. Section 10.

Mohrhauser, D., A. Taylor, M. Gonda, K. Underwood, R. Pritchard, A. Wertz-Lutz, and A. Blair. 2015. The influence of maternal energy status during mid-gestation on beef offspring tenderness, muscle characteristics, and gene expression. Meat Science 110: 201-211.

Mooney, K. W., and G. L. Cromwell. 1995. Effects of Dietary Chromium Picolinate Supplementation on Growth, Carcass Characteristics, and Accretion Rates of Carcass Tissues in Growing-Finishing Swine. Journal Animal Science 73 3351-3357.

Mooney, K. W., and G. L. Cromwell. 1997. Efficacy of Chromium Picolinate and Chromium Chloride as Potential Carcass Modifiers in Swine. Journal Animal Science 75: 2661-2671.

Moonsie-Shageer, S., and D. N. Mowat. 1993. Effect of Level of Supplemental Chromium on Performance, Serum Constituents, and Immune Status of Stressed Feeder Calves. J Anim Sci.

Morgan, J. B. 1997. Implant program effects on USDA beef carcass quality grade traits and meat tenderness.

Muir, P. D., J. M. Deaker, and M. D. Bown. 1998. Effects of forage‐ and grain‐based feeding systems on beef quality: A review. New Zealand Journal of Agricultural Research 41: 623-635.

Muniyappa, R., S. Lee, H. Chen, and M. J. Quon. 2008. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. American Journal of Physiology-Endocrinology and Metabolism 294: E15-E26.

Nath, R., J. Minocha, V. S. Lyall, V. Kumar, S. Kapoor, and K. Dhar. 1979. Assessment of chromium metabolism in maturity onset and juvenile diabetes using chromium-51 and therapeutic response of chromium administration on plasma lipids, glucose tolerance and insulin levels. Developments in nutrition and metabolism.

Nichols, W. T., M. L. Galyean, D. U. Thomson, and J. P. Hutcheson. 2002. Review: Effects of Steroid Implants on the Tenderness of Beef. The Professional Animal Scienctist 18.

Offenbacher, E. G., and F. X. Pi-Sunyer. 1980. Beneficial effect of chromium-rich yeast on glucose tolerance and blood lipids in elderly subjects. Diabetes 29: 919-925.

Oltjen, R. R., T. S. Rumsey, and P. A. Putnam. 1971. All-Forage Diets for Finishing Beef Cattle. Journal Animal Science 32: 327 - 333.

Page, T. G., L. L. Southern, T. L. Ward, and J. D. L. Thompson. 1993. Effect of Chromium Picolinate on Growth and Serum and Carcass Traits of Growing-Finishing Pigs. Journal Animal Science 71: 656-662.

Parr, S. L., K. Y. Chung, M. L. Galyean, J. P. Hutcheson, N. DiLorenzo, K. E. Hales, M. L. May, M. J. Quinn, D. R. Smith, and B. J. Johnson. 2011. Performance of finishing beef steers in response to anabolic implant and zilpaterol hydrochloride supplementation. Journal Animal Science 89: 560-570.

Pattar, G. R., L. Tackett, P. Liu, and J. S. Elmendorf. 2006. Chromium picolinate positively influences the glucose transporter system via affecting cholesterol homeostasis in adipocytes cultured under hyperglycemic diabetic conditions. Mutation Research 610.

Patti, M.-E., and C. R. Kahn. 1998. The insulin receptor-a critical link in glucose homeostasis and insulin action. Journal of basic and clinical physiology and pharmacology 9: 89-110.

Perseghin, G., T. B. Price, K. F. Petersen, M. Roden, G. W. Cline, K. Gerow, D. L. Rothman, and G. I. Shulman. 1996. Increased glucose transport–phosphorylation and muscle glycogen synthesis after exercise training in insulin-resistant subjects. New England Journal of Medicine 335: 1357-1362.

Platter, W., J. Tatum, K. Belk, P. Chapman, J. Scanga, and G. Smith. 2003. Relationships of consumer sensory ratings, marbling score, and shear force value to consumer acceptance of beef strip loin steaks. J Anim Sci 81: 2741-2750.

Pollard, G. V., C. R. Richardson, and T. P. Karnezos. 2002. Effects of supplemental organic chromium on growth, feed efficiency and carcass characteristics of feedlot steers. Animal Feed Science and Technology 98: 121-128.

Press, R. I., J. Geller, and G. W. Evans. 1990. The effect of chromium picolinate on serum cholesterol and apolipoprotein fractions in human subjects. Western Journal of Medicine 152: 41.

Rabinowitz, M. B., H. C. Gonick, S. R. Levin, and M. B. Davidson. 1983. Clinical trial of chromium and yeast supplements on carbohydrate and lipid metabolism in diabetic men. Biological trace element research 5: 449-466.

Radunz, A. E., F. L. Fluharty, A. E. Relling, T. L. Felix, L. M. Shoup, H. N. Zerby, and S. C. Loerch. 2012. Prepartum dietary energy source fed to beef cows: II. Effects on progeny postnatal growth, glucose tolerance, and carcass composition. Journal Animal Science 90: 4962-4974.

Rathmann, R. J., B. C. Bernhard, R. S. Swingle, T. E. Lawrence, W. T. Nichols, D. A. Yates, J. P. Hutcheson, M. N. Streeter, J. C. Brooks, M. F. Miller, and B. J. Johnson*. 2012. Effects of zilpaterol hydrochloride and days on the finishing diet on feedlot performance, carcass characteristics, and tenderness in beef heifers. Journal Animal Science 90.

Roeback, J. R., K. M. Hla, L. E. Chambless, and R. H. Fletcher. 1991. Effects of chromium supplementation on serum high-density lipoprotein cholesterol levels

in men taking beta-blockers: a randomized, controlled trial. Annals of internal medicine 115: 917-924.

Rutter, G. A., T. J. Pullen, D. J. Hodson, and A. Martinez-Sanchez. 2015. Pancreatic beta-cell identity, glucose sensing and the control of insulin secretion. Biochemical journal 466: 203-218.

Sales, J., and F. Jancik. 2011. Effects of dietary chromium supplementation on performance, carcass characteristics, and meat quality of growing-finishing swine: a meta-analysis. Journal Animal Science 89: 4054-4067.

Saltiel, A. R., and C. R. Kahn. 2001. Insulin signaling and the regulation of glucose and lipid metabolism Nature 414: 799 - 806.

Sánchez-Mendoza, B., A. Montelongo-Terriquez, A. Plascencia, N. Torrentera, R. A. Ware, and R. A. Zinn. 2014. Influence of feeding chromium-enriched enzymatically hydrolyzed yeast on growth performance, dietary energetics and carcass characteristics in feedlot cattle under conditions of high ambient temperature. Journal of Applied Animal Research 43: 390-395.

Scramlin, S. M., W. J. Platter, R. A. Gomez, W. T. Choat, F. K. McKeith, and J. Killefer. 2010. Comparative effects of ractopamine hydrochloride and zilpaterol hydrochloride on growth performance, carcass traits, and longissimus tenderness of finishing steers. Journal Animal Science 88: 1823-1829.

Sealls, W., B. A. Penque, and J. S. Elmendorf. 2011. Evidence that chromium modulates cellular cholesterol homeostasis and ABCA1 functionality impaired by hyperinsulinemia--brief report. Arterioscler Thromb Vasc Biol 31.

Shelton, J. L., R. L. Payne, S. L. Johnston, T. D. Bidner, L. L. Southern, R. L. Odgaard, and T. G. Page. 2003. Effect of chromium propionate on growth, carcass traits, pork quality, and plasma metabolites in growing-finishing pigs. Journal Animal Science 81: 2515-2524.

Shepherd, P. R., and B. B. Kahn. 1999. Glucose transporters and insulin action: implications for insulin resistance and diabetes mellitus. The New England Journal of Medicine 341: 248 - 257.

Sherman, L., J. Glennon, W. Brech, G. Klomberg, and E. Gordon. 1968. Failure of trivalent chromium to improve hyperglycemia in diabetes mellitus. Metabolism 17: 439-442.

Sherwood, L., H. Klandorf, and P. H. Yancey. 2013. Animal Physiology From Genes to Organisms 2ed. Brooks/Cole Cengage Learning Belmont, CA.

Shulman, G. I., D. L. Rothman, T. Jue, P. Stein, R. A. DeFronzo, and R. G. Shulman. 1990. Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. New England Journal of Medicine 322: 223-228.

Sillence, M. N. 2004. Technologies for the control of fat and lean deposition in livestock. The Veterinary Journal 167: 242-257.

Smith, D. R., and J. Crouse. 1984. Relative contributions of acetate, lactate, and glucose to lipogenesis in bovine intramuscular and subcutaneous adipose tissue. Journal of Nutrition 114: 792-800.

Smith, Z. K. F. 2015. The evaluation of chromium propionate supplementation on insulin responsiveness in finishing steers and energy status in newly weaned steer calves, South Dakota State University

Song, G., G. Ouyang, and S. Bao. 2005. The activation of Akt/PKB signaling pathway and cell survival. Journal of Cellular and Molecular Medicine 9: 59 - 71.

Song, Y. S., J. G. Nejad, S. J. Ohh, B. H. Lee, H. S. Kim, and K. I. Sung. 2013. Effects of Chromium-Methionine Chelate Feeding for Different Duration on Growth and Carcass Characteristics of Holstein Steers in the Late Fattening Stage. Annals of Animal Resource Sciences 24: 38-43.

Spears, J. W., C. S. Whisnant, G. B. Huntington, K. E. Lloyd, R. S. Fry, K. Krafka, A. Lamptey, and J. Hyda. 2012. Chromium propionate enhances insulin sensitivity in growing cattle. Journal of dairy science 95: 2037-2045.

Stahlhut, H. S., C. S. Whisnant, K. E. Lloyd, E. J. Baird, L. R. Legleiter, S. L. Hansen, and J. W. Spears. 2006. Effect of chromium supplementation and copper status on glucose and lipid metabolism in Angus and Simmental beef cows. Animal Feed Science and Technology 128: 253-265.

Sumner, J. M., F. Valdez, and J. P. McNamara. 2007. Effects of chromium propionate on response to an intravenous glucose tolerance test in growing Holstein heifers. Journal of dairy science 90: 3467-3474.

Sumrall, K. H., and J. B. Vincent. 1997. Is glucose tolerance factor an artifact produced by acid hydrolysis of low-molecular-weight chromium-binding substance? Polyhedron 16: 4171-4177.

Takahashi, H., K. Sato, T. Yamaguchi, M. Miyake, H. Watanabe, Y. Nagasawa, E. Kitagawa, S. Terada, M. Urakawa, M. T. Rose, C. D. McMahon, K. Watanabe, S. Ohwada, T. Gotoh, and H. Aso. 2014. Myostatin alters glucose transporter-4 (GLUT4) expression in bovine skeletal muscles and myoblasts isolated from double-muscled (DM) and normal-muscled (NM) Japanese shorthorn cattle. Domestic Animal Endocrinology 48: 62-68.

Thomas, V. L., and S. S. Gropper. 1996. Effect of chromium nicotinic acid supplementation on selected cardiovascular disease risk factors. Biological trace element research 55: 297-305.

Tokach, R. J., F. R. Ribeiro, K. Y. Chung, W. Rounds, and B. J. Johnson. 2015. Chromium Propionate Enhances Adipogenic Differentiation of Bovine Intramuscular Adipocytes. Frontiers in Veterinary Science 2: 26.

Tontonoz, P., and B. M. Spiegelman. 2008. Fat and beyond: the diverse biology of PPARgamma. Annu Rev Biochem 77: 289-312.

Tsuda, K., Y. Yokoyama, M. Morita, Y. Nakazawa, and S. Onishi. 1998. Selenium and chromium deficiency during long-term home total parenteral nutrition in chronic idiopathic intestinal pseudoobstruction. Nutrition (Burbank, Los Angeles County, Calif.) 14: 291-295.

Urberg, M., J. Benyi, and R. John. 1988. Hypocholesterolemic effects of nicotinic acid and chromium supplementation. The Journal of family practice 27: 603-606.

USDA. 2016. Overview of the United States Cattle Industry In: N. A. S. Service (ed.).

Valdes-Garcia, Y. S., J. I. Aguilera-Soto, A. Barreras, A. Estrada-Angulo, A. Gomez-Vazquez, A. Plascencia, F. G. Rios, J. J. Reyes, J. Stuart, and N. G. Torrentera.

2011. Growth performance and carcass characteristics in finishing feedlot heifers fed different levels of chromium-enriched live yeast or fed zilpaterol hydrochloride. Cuban Journal of Agricultural Science 45: 361 - 368.

Vernon, R., A. Faulkner, W. Hay, D. Calvert, and D. Flint. 1990. Insulin resistance of hind-limb tissues in vivo in lactating sheep. Biochemical journal 270: 783-786.

Vincent, J. B. 1999. Mechanisms of chromium action: low-molecular-weight chromium-binding substance. Journal of the American College of Nutrition 18: 6-12.

Vincent, J. B. 2000. The Biochemisty of Chromium. the Journal of Nutrition: 715-718.

Vincent, J. B., and R. Bennett. 2007. Potential and purported roles for chromium in insulin signaling: The search for the holy grail. In: J. B. Vincent (ed.) The Nutitional Biochemistry of Chromium (III) p139 - 158. Elsevier, Amesterdam.

Waldo, D. 1973. Extent and partition of cereal grain starch digestion in ruminants. J Anim Sci 37: 1062-1074.

Wallach, S. 1985. Clinical and biochemical aspects of chromium deficiency. Journal of the American College of Nutrition 4: 107-120.

Wang, H., A. Kruszewski, and D. L. Brautigan. 2005. Cellular Chromium Enhances Activation of Insulin Receptor Kinase. Biochemistry 44.

Wang, M. M., E. A. Fox, B. J. Stoecker, C. E. Menendez, and S. B. Chan. 1989. Serum cholesterol of adults supplemented with brewer's yeast or chromium chloride. Nutrition Research 9: 989-998.

Webb, A. S., R. W. Rodgers, and B. J. Rude. 2002. Review: Androgenic, Estrogenic, and Combination Implants: Production and Meat Quality in Beef. The Professional Animal Scienctist 18.