EFFECTS OF CHROMIUM PROPIONATE AND ITS INTERACTION WITH CORN GRAIN CONSERVATION METHOD ON PRODUCTIVE PERFORMANCE AND METABOLISM OF PERIPARTURIENT DAIRY COWS

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ABSTRACT

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Our objective was to determine the effects of chromium propionate (CrPr) supplementation throughout the peripartum period on productive performance and metabolism of dairy cows and to determine the effects of ruminal starch fermentability on production responses to chromium propionate supplementation in the postpartum period. Forty-eight multiparous dairy cows were used in a randomized block design with a 2x2 factorial arrangement of treatments of control or 8 mg chromium (Cr) as CrPr and dry ground corn (DC) or high-moisture corn (HMC). Chromium treatment began 28±3 d before expected parturition and continued until 28±3 d postpartum (PP), and DC and HMC were fed in rations from parturition to 28±3 d PP. A common diet was fed from 28±3 d PP to 84±3 d PP. There were no effects of treatment on dry matter intake (DMI) from parturition to 28±3 d PP, but an interaction among Cr, corn, and d PP was detected ($P = 0.06$) when cows were fed the common diet. A Cr, corn, and time interaction was also detected for 3.5% fat-corrected milk (FCM, $P = 0.07$) from parturition until 28±3 d PP which continued after treatment ceased ($P = 0.07$).

Although CrPr improved productive performance, no evidence of increased insulin sensitivity was observed. Postpartum plasma NEFA increased numerically andmaximum NEFA was higher during the glucose challenge with CrPr supplementation. Supplementation of CrPr throughout the periparturient period interacted with starch source in PP diets and d PP to affect production responses after treatment application ceased.

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ABBREVIATIONS

Cr – Chromium

CrPr – Chromium propionate

DMI – Dry-matter intake

NEFA – Non-esterified fatty acids

DM – Dry matter

TMR – Total mixed ration

CrPic – Chromium picolinate

CrMet – Chromium methionine

CrCl – Chromium chloride

NE_L – Net energy for lactation

BW – Body weight

FCM – Fat-corrected milk

d PP – Days postpartum

CrY – Cr yeast

ECM – Energy corrected milk

BCS – Body condition score

BHBA – Beta-hydroxybutyric acid

TG – Triglyceride

GTT – Glucose tolerance test

AUC – Area under the curve

GTF – Glucose tolerance factor

LMWCr- Low molecular weight chromium binding substance or chromodulin

VFA – Volatile fatty acids

NDF – Neutral detergent fiber

CP – Crude protein

FCE – Feed conversion efficiency

CR – Clearance rate

SNF – Solids non-fat

SCM – Solids corrected milk

MUN – Milk Urea Nitrogen

NFC – Non-fiber carbohydrate

CHAPTER 1
COMPREHENSIVE LITERATURE REVIEW

INTRODUCTION

Biological responses to chromium have been studied since the late 1950's (Mertz and Schwarz, 1955) and despite over a half-century of research, full understanding of chemical form, intake, absorption, transport, and biochemical mechanism is elusive. Chromium (Cr)supplementation for dairy cattle did not begin until the early 1990's (Burton et al., 1993), but since has been reported to increase insulin sensitivity in both ruminants (Subiyatno et al., 1996; Hayirli et al., 2001; Sumner et al., 2007) and non-ruminants (Matthews et al., 2001; Clodfelder et al., 2005; Cefalu et al., 2010). Increased insulin sensitivity by Cr supplementation may decrease lipolysis through the transition period of the dairy cow benefiting both feed intake and milk yield (McNamara and Valdez, 2005). In 2009, chromium propionate (CrPr) was approved by the U.S. Food and Drug Administration Center for Veterinary Medicine for use in dairy cattle bycommercial dairy farms.

The periparturent periodis a time of extreme physiologic, metabolic, and nutritional changes for the dairy cow. The shift from maintenance or positive energy balance in the dry period, to severe negative energy balance at the onset of milk production can affect peak milk yield and total milk over the lactation. The increased energy requirementis met by increasing energy density of the diet while maximizing dry-matter intake (DMI).However, the addition of a highly fermentable starch to increase energy of the ration can reduce intake (Allen, 2000). Increased fermentability of starch increases production of volatile fatty acids, specifically propionate. Although propionate is the primary gluconeogenic precursor in dairy cowsand glucose demand is high for cows in the postpartum period, propionate can stimulate hepatic

oxidation of acetyl CoA and depress feed intake (Allen et al., 2009). Hypoinsulinemia and insulin resistance increases mobilization of stored adipose as non-esterified fatty acids (NEFA) in the periparturient period. Feed intake is likely depressed by uptake and oxidation of NEFA by the liver (Allen et al., 2009) and propionate is more hypophagic for cows with elevated hepatic acetyl CoA concentrations (Stocks and Allen, 2011. If CrPr increases insulin sensitivity and decreases lipolytic state of cows, we expect to detect an interaction between starch fermentability and CrPr supplementation in which CrPr would ameliorate the decrease in DMI observed when cows are fed a highly fermentable diet in the postpartum period by reducing the supply of NEFA and acetyl CoA concentration in the liver.

CHROMIUM

Chromium chemistry

In 1959, Klaus Schwarz and Walter Mertz found that Cr was necessary to maintain normal glucose tolerance in rats. Following that discovery, Cr was shown to improve glucose tolerance in humans, which provided a prospective treatment for Type II diabetes (Glinsmannand Mertz, 1966). Despite these early results, technology and biological misunderstandings hindered progress in Cr research. Over fifty years later, Cr is still disputed as an essential micromineral(Wang and Cefalu, 2010). Conflicting data regarding Cr intake, method of absorption, biological activity, and metabolism leave Crsupplementation in question.

The first Cr studies in mammals were a result of glucose intolerance caused by liver necrosis (Schwarz and Mertz, 1957). Selenium alleviated the liver necrosis, but chromium was named “glucose tolerance factor” to treat the glucose intolerance (Schwarz and Mertz; 1959a). Schwarz and Mertz (1959b) used fourteen different Cr compounds to determine which were

most biologically active. They found that Cr in a trivalent state in a hexagonal complex bound to nitrogen, oxygen, or sulfur were metabolically most relevant. Five of the compounds in the trial included Cr(VI). Today it is widely recognized that Cr in its hexavalent oxidation state is not only toxic, but carcinogenic and mutagenic when inhaled or absorbed. Naturally occurring Cr, mined as chromite ore contains both Cr(III) and Cr(VI); the ratio determined by pH and the oxidative properties of the environment (Kotas and Stasicka, 2000).

Chromium must be extracted from the ore. During commercial processing Cr exists in oxidation states ranging from negative two to positive six, with positive two, three, and six being most common(Bailey, 2002). There are alsothree stable isotopes and 19 radioisotopes (Georges, 2003). Oxidation states of 0, I, and II are toxic and unstable. Cr(IV) is most often found in the form of Cr(IV) oxide, a synthetic magnetic material used in magnetic tape. Cr(V) generally exists only as an intermediate to hexavalent Cr. The carcinogenic properties of Cr(VI) are due to its strong oxidative potential at a neutral pH (or less), which includes the internal environment of most organisms (Dayan and Paine, 2001).

When in the oxidation state III, not only is Cr stable, it can be metabolically active. Brewer's yeast (Saccharomyces cerevisiae) was the biologically active source of Cr used in Schwarz and Mertz (1959b). They also found liver and kidney tissue to possess significant concentrations of Cr. Unbeknownst at the time, the liver and kidney are central organs in Cr metabolism. Since the late 1950's many other chromium compounds have been manufactured and used to treat glucose intolerance (Subiyatno et al., 1996), improve immune function (Burton et al., 1995) and even build muscle in pigs (Zhang et al., 2011) and humans.

Chromium in the diet

Chromium deficiency is very uncommon in healthy individuals. Humans require only about 30μg per day based on adequate intake values set by the National Academy of Sciences (Institute of Medicine, 2001). Adequate intake is unknown in other species (NRC, 2001); little research has been conducted as normal diets were thought, until recently, to supply sufficient chromium.

Diet analysis for Cr is challenging. Feedstuffs are normally low in Cr, but measuredlevels can be impacted by contamination from metal coatingson harvest equipment, conservation method, and sample processing. Stainless steel is generally coated with a Cr alloy. In 2005, Li et al.tested for heavy metals of Wisconsin dairy feed components. The feeds ranged from 0.33mg Cr/kg of dry matter (DM) in corn grain to 0.91mg Cr/kg DM in alfalfa haylage. The lower Cr concentration in the grain is consistent with a study showing feedstuffs higher in non-structural carbohydratesare lower in chromium (Kozlovsky et al., 1986). Lloyd et al. (2010) found Cr concentration in a dairy cattle total mixed ration (TMR) to be 0.48mg/kg DM in a prepartum diet and 0.38mg/kg in a higher starchpostpartum diet, consistent with Kozlovsky's findings. Actual chromium concentrations in forages and concentrates vary based on the Cr levels present in soils.

Chromium Bioavailability and Gastrointestinal Absorption

Bioavailability of naturally occurring Cr remains undetermined.It is widely recognized that Cr is cleared by the kidneys and the amount absorbed equals the amountexcreted in the urine. The form of Cr greatly influences rate of absorption and concentration in the urine. When rats were supplemented with sodium chromate, less than 3% Cr was excreted in the urine, the rest was collected in the feces (MacKenzie et al., 1959), which indicates that 97% of Cr intake remained unabsorbed. Feeding rats Cr^{51} labeled alfalfa reportedly provided over 30% Cr

bioavailability and yet only 3% was measured in the urine (Starich and Blincoe, 1983). With about 60% recovered in the feces, approximately 27% was unaccounted for. Starich and Blincoe, (1983) suggested that the ligand in which Cr is bound in the forage will breakdown to form inorganic Cr when pH of the gut is low, decreasing absorption. Inorganic Cr, such as Cr chloride, reacts with hydroxyl ions, which polymerize to form high molecular weight compounds causing the complex to precipitate, reducing absorption.

Artificial supplements currently marketed are meant to mimic the natural ligand binding to increase absorption. Picolinic acid, nicotinic acid, ascorbic acid, and amino acids such as phenylalanine, methionine, and histidine form these ligands. The absorption rate for Cr picolinic and nicotinic acid in rodents ranges between 0.5-2.0% depending on the amount supplemented (Clodfelder et al., 2004). This issimilar to the absorption of Cr in an unsupplemented high-quality diet, implying the supplements provide little benefit. When bound to propionate, however, Cr is absorbed with greater than 40 and 60% efficiency at high and low doses, respectively (Clodfelder et al., 2004). Cr absorption of other ligands is poorly characterized.

Chromium Transport and Storage

If Cr is absorbed, transferrin,the known ferric iron-carrier, likely transports Cr through the blood (Clodfelder et al., 2004). When Cr(III) was administered orally or by injection, Cr appeared in the transport protein. Transferrin is maintained on average about 30% loaded, therefore, it has the potential to carry other metals (Williams and Moreton, 1980). Insulin stimulates transferrin receptors to move from cytosolic vesicles to the cell's surface (Krandor, 1999). Once bound, the transferrin complex undergoes endocytosis and the metal contents are released into a new vesicle. Both high or low plasma insulin can cause Cr deficiency (Clodfelder

et al., 2004). In addition, when iron levels are high, transferrin can become saturated with iron as the protein has a higher affinity foriron than Cr.

The most common sources of chromium are complexes that do not bind to transferrin. The mode of absorption through the intestinal epithelial cells and through the blood may reflect the molecule to which the Cr is bound. Chromium picolinate (CrPic) is very stable in gastric juice and remains intact throughout the jejunum (Gammelgaard et al., 1999) and likely migrates to cells in intact (Clodfelder et al., 2004). As a result, the ligands alter the redox potential and can be reduced to Cr(II), which reacts with oxygen to form hydroxyl radicals (Sun et al., 2000).In contrast, propionate passively diffuses through the rumen wall of dairy cows and flows to the liver, where it is extracted and metabolized.

In vivo Cr concentrations vary widely among tissues and species. Concentrations of Cr in the dairy cow are higher in the kidney and liver than other tissues with or without supplementation (Lloyd et al., 2010). The high levels of Cr in the kidney are likely due to clearance via urine (Anderson et al., 1982). Higher liver Cr concentrations may bea result of the mechanism by which the Cr compound is metabolized. Hepatocytes have transferrin receptors and iron is stored by the liver. If Cr is carried by transferrin, it is reasonable to conclude that Cr concentrations would be higher in liver tissue. Liver Crrepresents only 1% ofCr quantity supplemented in mice (MacKenzie et al., 1959). Any remaining Cr isfurther metabolized and excreted. No studiescompared effects of different Cr sources for concentration of Cr in dairy cow tissues. In pigs, as supplement levels of CrPic, chromium methionine (CrMet), and chromium chloride (CrCl) increased, tissue Cr concentration increased proportionately. However,CrPr was the only Cr supplement that did not result in elevated tissue concentrations (Lindemann et al., 2008).

Chromium concentration in blood plasma is a combination of both dietary Cr(III) and inhalation or absorption of hexavalent Cr, where as Cr measured in red blood cells represents exposure to Cr(VI)(Miksche and Lewalter, 1997). Cr(VI) is capable of crossing cell membranes, but once inside the cell it is reduced to Cr(III) and can no longer leave the cell (Miksche and Lewalter, 1997; Ryan et al., 2000). Data from the U.S. Environmental Protection Agency reveals Cr concentrations of 0.2 to 70 ng/ml in plasma and concentrations of 5 to 54 ng/ml in red blood cells of non-occupationally exposed human populations (World Health Organization, 1996).

All of the data compiled for absorption, transport, and biochemical function for Cr is in laboratory animals, and remains ambiguous. Therefore, application to other species, specifically production species is only theoretical.

Production and Performance

One reason to supplement Cr in dairy cows is to enhance feed intake and reduce negative energy balance. Just as the effects of Cr are controversial in rodents and humans, so are they in dairy cows. Few studies address supplementing cattle diets with Cr and even fewer with transition dairy cow diets. In addition, chromium supplements vary widely across these studies.

After demonstrating a positive response of Cr in enhancing growth and preventing stress related diseases in beef calves, Burton et al. (1993) conducted the first chromium study in lactating dairy cows. The purpose was to determine the effects of chromium on immune response of transition cows (Burton et al., 1993). Six weeks prior to parturition, cows were provided 0.5 ppm Cr by Cr chelate. The study showed significant and beneficial results on a

number of immunological parameters. It was then postulated by Burton et al. (1993) that Cr could ameliorate the risk of stress related diseases in periparturient cows.

Three years later the first two studies concerned with performance, metabolites, and hormones were conducted (Subiyatno et al., 1996; Yang et al., 1996). Total mixed rations for both primiparous and multiparous cows were top-dressed with amino acid chelated chromium. Chromium supplementation increased milk yield up to 13.2% for primiparous cows and did not affect DMI (Yang et al., 1996). Multiparous cows, however, did not respond to chromium treatment (Yang et al., 1996).This was accredited to a decreased stress response by primiparous cows. Cortisol increases glucose metabolism and can cause Cr loss by excretion in the urine (Andersen et al., 1991); chromium supplemented to the multiparous cows might have been lost if cortisol was elevated.

The lack of production response to Cr treatment by multiparous cows might have been because of low dietary net energy for lactation (NE_L) of the postpartum diet. Chromium also failed to affect milk yield of cows onpasture-based diets, which are low in energy. (Peterson, 2000; Bryan et al., 2004).Low energy diets may have limited milk yield in some studies.

An incorrect statistical model used by Yang et al. (1996) may also have preventeda multiparous cow response from being detected. The linear regression model employed in this study assumes that all data points are independent. As more than one sample was taken over time from the same cow, each point is related to the first. A mixed model ANOVA with repeated measures would have been more appropriate.

Accurateconclusionsaboutthe effects of Cr on dairy cows is hindered by variation acrossCr studies. Chromium form, dose, ration, stage of lactation, and other factors can influence results. Two studies that supplemented CrMet had similar results, however. From

three weeks prepartum to four weeks postpartum cows received 0, 0.03, and 0.06 mg Cr/kg $BW^{0.75}$, by bolus in both studies (Hayirli et al., 2001; Smith et al., 2005) and one also provided 0.12 mg Cr/kg $BW^{0.75}$(Hayirli et al., 2001). Postpartum DMI, milk yield, and 3.5% fat-corrected milk (FCM)increased with increasing CrMet supplementation up to 0.06 mg Cr/kg$BW^{0.75}$ (Hayirli et al., 2001; Smith et al., 2005), but the largest dose caused a decrease in milk yield and 3.5% FCM (Hayirli et al., 2001). Even supplementing the same form of chromium does not always yield the same results. CrMet increased prepartum DMI in one study (Hayirli et al., 2001), with no effect on another (Smith et al., 2005). Sadri et al. (2009) also supplemented CrMet at a rate of 0 or 0.8 mg Cr/kg $BW^{0.75}$to periparturient cows, but found only an increase in milk yield and DMI when barley was used as the dietary grain source and but not when corn grain was used.

The form of Cr effects absorption (Clodfelder et al., 2004), so it is not surprising that results vary across form. McNamara and Valdez (2005) supplemented 10mg Cr/day from three weeks prepartum to five weeks postpartum and continued to monitor performance until 90days postpartum (d PP). Although there was no effect of CrPrduring supplementation, CrPr increased DMI and milk yield after treatment ceased. The increase in DMI began in the fifth week and continued and milk production followed by week eight. According to the authors, decreased lipolysis would permit increased intake and milk yield. However, the authorsdid not adequately address the fact that the significant results were carryover effects and not while animals were being treated.

Chromium supplementation as a treatment of heat stress in dairy cows is another variation of chromium studies.Pairing metabolic and physiological stress with environmental

stress exacerbates lactational challenges. The studies discussed below reported ambient temperatures ranging between 24° and 35°C for the duration of the studies. Heat stress lowers DMI and milk yield (West, 1999). Chromium supplementation by CrPic, CrMet, and Cr yeast (CrY) all mitigated these issues to varying degrees.Cows treated with 0, 3.6, 7,2, or 10 mg Cr/head daily byCrPic, beginning 21 days postpartum had linear increases in DMI and milk yield as the Cr amount increased (AnQiang et al., 2009). Feed intake and milk yield also increased when mid-lactation cows were supplemented with CrY at a rate of 4 g CrY/head daily (Al-Saiady et al., 2004). Soltan (2010) also supplemented heat stressed cows with 0.6 mg Cr/head/day with CrY from three weeks prior to parturtion to twelve weeks postpartum. DMI and milk yield increased 10% and 12.1%, respectively, over the twelve weeks postpartum, despite ambient temperatures of 35°- 40°C persisting throughout the entire trial. Production and DMI intake improved with Cr supplementation in all three studies, despite differences in Cr source and dosages.However, none of these heat stress studies used repeated measures in the statistical model. Nikkhah et al., (2011) did use repeated measures in a heat stress study and found no effect of Cr on milk yield, but an increase in energy corrected milk (ECM) yield and DMI, when cows were supplemented with CrMet for nine weeks starting 38±6 d PP. Despite possible issues with statistical analysis, Cr supplementation likely abated the consequences of heat stress.

In addition to improving milk yield and DMI, chromium affected milk components. Chromium increased fat and lactose yield (Hayirli et al., 2001; Nikkhah et al., 2011) and increased protein and total solids yield (Nikkhah et al., 2011). Chromium supplementation did not affect milk components in other studies (Al-Saiady et al., 2004; AnQiang et al., 2009; Bryan et al., 2004; Pechová et al., 2002; Peterson, 2000; Smith et al., 2005; Soltan, 2010; Yang et al.,

1996) and in one study milk fat percentage and yield decreased (McNamara and Valdez, 2005). Inconsistent results can be related to numerous factors, including Cr source and diet. When paired with a barley ration, Cr increased protein and total solids yield, but not with corn (Sadri et al., 2009). Further research is required to definitively resolve the effects of Cr on milk components.

The sudden energy demand of lactation and depressed intake cause negative energy balance in the postpartum period resulting in mobilization of fat reserves and a loss in body weight and body condition loss. Chromium is thought to increase glucose uptake by insulin sensitive tissues thereby slowing lipolysis. Hayirli et al. (2001) reported a decrease in body condition score (BCS) loss over time with no effect on BW, while Smith et al. (2005) found increased prepartum BCS and postpartum BW. Both studies supplemented the same doses of CrMet. Despite these statistically significant treatment effects, the small magnitude of change with no treatment effect on calculated net energy balance led Smith et al. (2005) to conclude that the Cr effects were minimal. Other studies support this conclusion, as Cr did not affect BW or BCS (Al-Saiady et al., 2004; McNamara and Valdez, 2005; Subiyatno et al., 1996; Yang et al., 1996).

Effects of Cr on Hormones and Metabolites

Non-esterified fatty acid concentrations are elevated in the postpartum period when cows are in a lipolytic state, andsupplementation with Cr may decrease lipolysis by increasing insulin sensitivity.Some studies reported lower NEFA prepartum, with no effect of treatment postpartum (Hayirli et al., 2001; Bryan et al., 2004), while others using chelated chromium sources, reported decreased NEFA postpartum (Nikkhah et al., 2011; Yang et al., 1996). Nikkhah et al. (2011) reported a decrease in NEFA when cows were supplemented with 0.05mg Cr/kg $BW^{0.75}$, but not

at 0.1mg/kg $BW^{0.75}$during peak lactation, while Yang et al. (1996), demonstrated a treatment effect only in parity three or greater. However, most studies reported no effect of Cr treatment on serum NEFA (Besong et al., 1996; McNamara and Valdez, 2005; Pechová et al., 2002; Smith et al., 2008; Williams et al., 2004, Yang et al., 1996). The inconsistent results may be due to diurnal variation in plasma NEFA concentrations (Blum et al., 2000) or variation in metabolism among cows as is seen in human studies (Wang and Cefalu, 2010).

If lipolysis decreased as a result of Cr treatment, beta-hydroxybutyric acid (BHBA) from NEFA metabolism should also decrease. Consistent with NEFA findings, BHBA was not affected by Cr treatment in most studies (Bryan et al., 2004; Hayirli et al., 2001; Nikkhah et al., 2011; Pechová et al., 2002; Smith et al., 2008). Yang et al., (1996) reported lower BHBA in cows in parity three or greater with Cr treatment, which was consistent with NEFA concentrations. Whereas Besong et al., (1996) reported no significance difference in NEFA concentrations, but lower BHBA with CrPic supplementation.

Few transition cow studies report plasma triglyceride (TG), but Cr supplementation decreased TG in both small ruminants (Uyanik, 2001; Besong et al., 2001) and non-ruminants (Lee et al., 1994; Sun et al., 1999).In the fewdairy cattle Cr studies that reported TG, there were no main effects of Cr supplementation, butthere was a treatment by time interaction in both studies (Pechová et al., 2002; Nikkhah et al., 2011). When supplemented with CrY, plasma TGdecreased during weeks four and five postpartum (Pechová et al., 2002).However, Cr supplementation increased triglyceride concentration during a postpartum glucose challenge(Subiyatno et al., 1996).

Effects of Cr supplementation on concentrations of glucose, glucagon, and insulin are also inconsistent within, and across, all species. In rats and humans, Cr has been shown to

decrease plasma glucose and insulin concentrations in some trials (Anderson et al., 1997; Cefalu et al., 2002; Martins et al., 2006) and not in others (Gunton et al., 2005; Kleefstra et al., 2007;Iqbal et al., 2009).In dairy cows, CrMet supplementation generally decreased postpartum plasma glucoseover time in one study (Smith et al., 2008), but most dairy cow studies report no effect of Cr treatment on plasma glucose concentrations postpartum (Al-Saiady et al., 2004; Bryan et al., 2004; Hayirli et al., 2001; McNamara and Valdez, 2005; Nikkhah et al., 2011; Smith et al., 2008; Soltan, 2010; Williams et al., 2004). In some studies, Cr supplementation actually increased plasma glucose concentrations, but this might be attributed to increased DMI (Pechová et al., 2002; AnQiang et al., 2009). The response of glucose to Cr supplementation may be influenced by lactation and the use of glucose by the mammary gland.

Effects of Cr supplementation on insulin and glucagon concentrations are as inconsistent as glucose concentration. Even use of the same Cr source yielded opposing results. CrMet decreased insulin concentration with increasing Cr supplementation up to 0.06 mg Cr/kg $BW^{0.75}$ CrMetin two studies (Hayirli et al., 2001; Nikkhah et al., 2011), but 0.03 mg Cr/kg $BW^{0.75}$CrMet did not affect insulin dosein another (Smith et al., 2008). In addition,CrMet increased glucagon concentrations when insulin concentration was not affected (Smith et al., 2008), butCrMet did not affect glucagon when insulin was decreased. Postpartum supplementation of CrPic also decreased serum insulin in heat stressed cows (AnQiang et al., 2009), while otherstudies reported no effect of various Cr treatments on insulin (Peterson, 2000; Williams et al., 2004). The inconsistent results are likely a reflection of variation in metabolic state of cows within and among studies.

Effects of Cr on Response to a Glucose Challenge

A glucose tolerance test (GTT) indirectly measures insulin resistance and hypoinsulemia by quantifying glucose entry into insulin sensitive tissues after inundating the blood with glucose. This method was employed to test the effectiveness of Cr supplementation on insulin resistance(Subiyatno et al., 1996;Hayirli et al., 2001). The GTTs were administered once prepartum and once postpartum in each experiment. Both studies found no differencepre- or postpartum of basal glucose concentrations with respect to treatment (Subiyatno et al., 1996; Hayirli et al., 2001), but during the postpartum challenge,Hayirli et al. (2001) reported decreased peak glucose concentration and clearance rate with increasing supplementation up to 0.06 mg/kg $BW^{0.75}$. CrMet, also, decreased basal insulin prepartum and decreased insulin half-life and area under the curve (AUC) (Hayirli et al, 2001). Decreased insulin to glucose ratio andincreased insulin clearance rate to glucose clearance rate postpartum as well as decreased basal NEFA and NEFA AUC prepartum with CrMet may indicate improved insulin sensitivity. However, differences in blood metabolite concentrations may reflect increased utilization, decreased milk production or both. By removing variables of milk production and pregnancy,studies with growing heifers were able to show increased insulin sensitivity through GTTs (Bunting et al., 1994; Sumner et al., 2007; Spears et al., 2010). Heifers supplemented with CrPr had increased glucose clearance rates (Sumner et al., 2007) and decreased basal insulin and insulin to glucose ratio during following glucose infusion (Spears et al., 2010). Sumner et al. (2007) also showed that long-term chromium supplementation produced lower basal insulin in heifers.

Mechanism of Chromium Action

It is widely accepted that chromium functions to potentiate insulin action (Mertz, 1992; Vincent, 2001; Cefalu, 2010), but the mechanism has been disputed. Schwarz and Mertz (1957,

1959) discoveredglucose tolerance factor (GTF), a compound derived from Brewer's yeast and acid-hydrolyzed porcine kidney powder that reversed liver degeneration in rats. The active ingredient was determined to be Cr (Schwarz and Mertz, 1959). When given by stomach tube GTF restored normal glucose metabolism in Cr deficient rats. The methods for preparation were not provided, so the amount of chromium that corresponded to GTF remains unknown. Terminology in the years following led to confusion regarding GTF as a biologically active molecule. Mertz and Schwarz (1962) equate GTF and trivalent Cr, but GTF was also the term later used to describe the organic biomolecules that interact with Cr. Efforts to determine the chemical structure of "GTF", the Cr(III) complex, in yeast has yielded numerous contradictory results, delaying progress in the discovery of the mechanism of Cr action (Vincent, 2001).

Recently, low molecular weight chromium binding substance (LMWCr) or chromodulin, an oligopeptide isolated in liver and kidney in a number of mammalian species (Davis and Vincent, 1997; Yamamato et al., 1987; Sumrall and Vincent, 1997) has exhibited many of the characteristics of GTF described by Mertz and Schwarz (1962). It is composed of 4 amino acid residues, glycine, cysteine, glutamate, and aspartate and weighs about 1.5 kDa (Yamanoto et al., 1987; Davis and Vincent, 1997).Chromodulin is stored in its apo-form in the cytosol (Yamamoto et al., 1989) and Cr is thought to have a high binding constant to apochromodulin and subsequently binds four ions, producing holochromodulin (Sun et al., 2000). The holoproteinbinds to the insulin receptor and maintains the active conformation to enhance insulin signaling. Insulin must first bind to the α-subunit of the insulin receptor, which causes autophosphorylation of tyrosine residues of the β-subunit (Davis et al, 1997). Phosphorylation enhances insulin receptor tyrosine kinase activity and eventually increases translocation of GLUT 4 transporters to the cell membrane (Cefalu et al., 2002). The compound binds to the

insulin receptor and maintains the active conformation to enhance insulin signaling. This may occur by inhibition of phosphotyrosine phosphatase, which terminates the insulin-signaling pathway (Goldstein et al., 2001). When the signal is disrupted Cr does not disassociate, but is excreted as the holoproteinin urine. Chromium excretion increases after carbohydrate intake, which is consistent with turnover of the holochromodulin (Anderson et al., 1982; Kovlovsky et al., 1986).Others have attempted to replicate the original amino acid sequence and have been unsuccessful due the inability to release Crfrom the holo-oligopeptide (Chen et al., 2011). A synthesized peptide that tightly binds Cr, similar to chromodulin, was recently created to help characterize the peptide (Chen et al., 2011).

STARCH

Starch Characteristics

Carbohydrates are the major source of energy in dairy cow diets. Starch, a non-structural carbohydrate, predominates in cereal grains (NRC, 2001). Concentrations range from 45% in oats to about 70% in corn, on a DM basis and starch is also supplied by some forages such as corn and small grain silages. Starch can represent a substantial portion of the diet, especially for high producing lactating cows.

Just as the concentration of starch varies by grain type, its ruminal digestibility also varies (McCarthy et al., 1989). In the rumen, 80-90% of barley, wheat, and oat starch is digested, while only 50-70% of corn and sorghum starch is digested, meaning a greater portion reaches the small intestine (Nocek and Tamminga, 1991). This is a direct reflection of endosperm cell structure. Barley and wheat endosperm are primarily floury; the starch granules are only loosely associated in the protein matrix allowing bacterial penetration and rapid

digestion. The endosperm of corn and sorghum, however, are comprised of both floury and more vitreous or flinty endosperm in which the amylose and amylopectin are encapsulated in a matrix with less soluble protein. This structure is highly resistant to proteolytic bacterial digestion. Vitreousness is affected by cultivar, and is negatively correlated to starch digestion in the rumen (Philippeau, 1999).

Conservation method and processing of starch increase rumen availability and degradability (Knowlton et al., 1998). Whole grain with an intact pericarp is resistant to microbial degradation (McAllister and Cheng, 1996).Grinding, for example, increases the surface area and disrupts the structure, allowing rumen microbes to penetrate. In a meta-analysis, grinding increased apparent total tract digestibility of starch compared to dry-rolling in corn-based diets (Firkins et al., 2001). Processing by ensiling facilitates anaerobic microbial digestion of the protein matrix, thereby increasing starch fermentability (Kotarski et al., 1992). Whole tract digestibilityof corn ranges from 91-99% across different processed forms (Huntington, 1997). Ruminal digestibility, however, ranges from ~50% with dry ground corn (McCarthy et al., 1989) to ~90% with high moisture corn (Stock et al., 1987). The less-vitreous endosperm of high-moisture corn (28-32% moisture) facilitates microbial fermentation in the rumen. Unlike dry corn, further processing of high-moisture corn only marginally improves digestibility (Firkins et al., 2001). Despite some variability in ruminal digestion of dry and high-moisture corn (Knowlton et al., 1998; McCarthy et al., 1989; Stock et al., 1987) it is clear that ensiling results in increased ruminal starch fermentation compared with dry corn.

Ruminal starch fermentation influences microbial metabolism. Greater rumen digestibility of starch is associated with increased microbial N flow to the duodenum (Reynolds, 1997; Theurer, 1999; Firkins, 2001). Microbial efficiency is also positively correlated with

starch passage rate and negatively related to rate of starch digestion (Oba and Allen, 2003a; Voelker and Allen, 2003). Cows fed HMC had lower microbial efficiency than cows fed dry ground corn, but efficiency was unaffected by dietary starch concentration (Oba and Allen, 2003a).

Site of starch digestion influences efficiency of feed utilization (Huntington, 1997). When starch is fermented by rumen microbes, volatile fatty acids (VFA) are produced and partially absorbed across the ruminal epithelium. These glucose precursors are the major energy supply for milk production. Starch that escapes the rumen into the duodenum is degraded by pancreatic amylase permitting glucose absorption. A greater net flux of lactate than glucose in the portal drained viscerasuggests that enterocytes metabolize glucose to lactate (Reynolds et al., 2003). Lactate is released into circulation and used for gluconeogenesis in the liver and kidney. There is a linear relationship between starch escaping the rumen and compensatory intestinal digestion(Nocek and Tamminga, 1991).As non-ruminally fermentable starch intake increases, postruminal digestion also increases. Whether grain is more efficiently utilized when digested in the rumen or the intestine is disputed (Huntington, 1997; Firkins et al., 2001).

Ruminal starch degradability can affect DMI(Allen, 2000). Three of ten studies reported decreased DMI with increased ruminal starch fermentation as a percentage of DM or of total starch, as reviewed by Allen (2000). Variation among studies may have been a result of other dietary characteristics and temporal conditions.

Fermentability and grain source

Barley is more fermentable in the rumen than corn, but has lower starch concentration (Huntington, 1997). Rolled barley depressed intake compared to cows receiving ground corn

during early lactation (Casper et al., 1990; McCarthy et al., 1989). Grings et al., (1992) fed the same starch sources in the same stage of lactation and reported no difference in DMI between diets. There was also no effect on DMI when both barley and corn were coarsely ground (Khorasani et al., 2001). In another early lactation study, intake was greater for cows fed corn diets rather than barley, but the grain processing method was not noted (Khorasani et al., 1994).

Effects of starch source on milk yield and components were also inconsistent across studies. Grain source did not affect milk yield in most cases (Casper et al., 1990; Grings et al., 1992; Khorasani et al., 1994). Fat yield and 4% FCM increased when cows were fed a corn-based diet compared to barley in two trials (Casper et al., 1990; Khorasani et al., 1994) By separating primiparous from multiparous cows, Khorasani et al. (2001) found that coarse ground corn increased milk yield, but did not affect fat yield and 4% FCM compared to coarse ground barley in primiparous cows. McCarthy et al. (1989) reported similar results in multiparous cows.

Fermentability: processing and particle size

Processing and particle size influence DMI and milk yield. Firkins et al., (2001) concluded in a meta-analysis that grinding dry corn increases milk production without affecting DMI compared to dry rolling. A fine grind also increases milk production, but depresses intake and milk fat percentage compared to a coarser grind (Firkins et al., 2001). As particle size is poorly documented, a continuous response of particle size could not be determined. Steam-flaked and high-moisture corn supported higher milk production without decreasing DMI more than 0.5 kg/d, with little effect of particle size, compared to dry ground corn (Firkins et al., 2001).

High-moisture corn vs. dry ground corn

High-moisture corn is highly fermentable in the rumen. Interest in high-moisture corn as a concentrate began in the early 1970s as a solution to crop loss at the end of the growing season (Clark et al., 1973). Proper storage was the primary objective in these early trials. The original studies comparing HMC to dry ground corn had conflicting results (Chandler et al., 1975; Clark et al., 1973). One reported no effect of conservation method on DMI (Clark et al., 1973), while the other found depressed intake in all but late lactation cows when fed HMC compared to DC (Chandler et al., 1975). Twenty years later high-moisture corn was analyzed for its affects on rumen bacteria (Aldrich et al., 1993) and optimal rumen digestion (Knowlton et al., 1998). HMC slightly depressed DMI (Aldrich et al., 1993; Knowlton et al., 1998). Oba and Allen (2003b) reported a 1.7 kg/d reduction in feed intake when early lactation cows were fed high-moisture corn with greater ruminal starch digestion compared to dry ground corn in a high starch diet. HMC also decreased intake in high producing mid-lactation dairy cows (Bradford and Allen, 2007). Depressed intake is likely a result of the 19-24% increase in ruminal digestibility of HMC compared to dry ground corn (Knowlton et al., 1998; Oba and Allen, 2003b). Increasing ruminally degradable starch increases the contribution of VFAs, specifically propionate, (Allen and Oba, 2003b; Sutton et al., 2003) produced by rumen microbes (Bergman, 1990), relative to lactate from the intestines.

High-moisture cornalso has varying effects on milk production. Cows fed HMC had higher productivity compared to cows fed dry ground corn according to Clark et al. (1973) and Oba and Allen (2003b). This is supported by the 2001 meta-analysis by Firkins et al. concluding that HMC supported higher milk production. Bradford and Allen (2004) showed that HMC increased yield of milk for high producing cows, but decreased yield of milk and milk fat for low

producing cows. The inconsistent results among studies are likely influenced by multiple factors including physiological state of cows, diet starch concentration, and other diet characteristics.

Propionate and depression of feed intake

High-producing dairy cows rely on gluconeogenesis to maintain glucose supply for the mammary gland (Reynolds et al., 1988). Propionate is the primary glucogenic precursor in ruminants and contributes to at least half of glucose requirements (Bergman, 1990). A highly fermentable diet will produce a greater concentration of propionate during and directly following meals than a less fermentable diet (Oba and Allen, 2003b). Removal of propionate by the liver exceeds 70% of the total supply with little variation in percentages between late gestation and early lactation (Reynolds et al., 2003). Propionate production can vary 4-fold depending on ruminal starch digestion (Oba and Allen, 2003b; Sutton et al., 2003). When flooded with propionate, hepatocytes can oxidize the fatty acid in the TCA cycle (Allen et al., 2009). Black et al., (1966) demonstrated that 31% of propionate taken up by the liver is oxidized in lactating dairy cows. However, Pocius and Herbein, (1986) reported a 60% increase in gluconeogenesis with equal increase in oxidation when lactating cows were treated with growth hormone. The rate of oxidation, therefore, is not constant. The ratio of glucose production to oxidation likely depends on regulation of enzymes (Knapp et al., 1992). Observed increases in mRNA abundance for PEPCK and glucose-6-phosphatase from pre- to postpartum are evidence of gluconeogenic upregulation (Graber et al., 2010).

A sudden influx of propionate from the rumen can cause depression of feed intake (Anil and Forbes, 1980; Elliot et al., 1985, Sheperd and Combs, 1998). Although propionate has a

higher energy content than acetate, metabolizable energy intake decreased linearly with propionate compared to acetate when iso-osmotically infused into the rumen of lactating dairy cows (Oba and Allen, 2003). The reduction in metabolizable energy intake exceeded the energy supplied by the infusions, which was caused by a reduction in meal size and frequency. Compared to iso-energetic infusions of mixed VFAs (Hurtaud et al., 1993) or acetate (Sheperd and Combs, 1998) propionate decreased feed intake. Satiety may be linked to hepatic oxidation stimulated by propionate. The near absence of acetyl CoA synthetase in the ruminant liver (Ricks and Cook, 1981) allows little net metabolism of acetate (Reynolds et al., 1995). Propionate, therefore, is likely a primary satiety signal (Allen, 2000).

Propionate enhances oxidation of acetyl CoA, the oxidative product of not only carbohydrate-derived propionate, but also amino acids and fatty acids. Acetyl CoA accumulates in the liver of cows in a lipolytic state and its oxidation quadruples ATP production, compared to the export of ketones. This flux of ATP likely stimulates a satiety signal (Allen et al., 2009).

The hypophagic effects of propionate and a high starch diet are further exacerbated in a postpartum cow. For several weeks after parturition, energy requirements for milk production are greater than intake, creating negative energy balance or a lipolytic state (Allen and Bradford, 2006). Mobilization of fat stores is caused by low plasma insulin concentrations accompanied by reduced insulin sensitivity of adipose tissue (Doepel et al., 2002). Hepatic uptake and oxidation of NEFA results in a build-up of acetyl CoA and the oxidation of fatty acids and generation of ATP may restrict feed intake at the onset of lactation (Allen et al., 2009). When hepatic acetyl CoA concentrations are elevated from lipolysis, propionate from the highly fermentable diet is even more hypophagic (Stocks and Allen, 2011), further potentiating the cycle.

Supplementation of Cr may decrease the hypophagic effects of a highly fermentable starch source. By increasing insulin sensitivity and decreasing lipolysis, Cr may reduce the pool of acetyl CoAfrom oxidation of NEFA in the periparturient period (Allen et al., 2009). Therefore, oxidation of a smaller pool of acetyl CoA would lessen ATP production upon propionate stimulation within a meal, reducing its hypophagic effects.Alternatively, propionate might also decrease lipolysis by stimulating insulin release and decrease ß-oxidation by inhibiting carnitine palmitoyltransferase and transport of NEFA into the mitochondria decreasing oxidation and increasing feed intake (Friedman et al., 1986).

Although Cr was used in a variety of lactational studies, only one study paired the effects of Cr with the effects of starch source during the transition period as discussed above (Sadri et al., 2009). To assess the effects of Cr and the effects of corn conservation method, the following experiment was designed and conducted.The objectives of this experiment were to determine the effects of KemTRACE® chromium propionate supplementation through the transition period on milk yield and components, metabolism and energy balance of dairy cows and also, to determine effects of starch fermentability of the fresh cow diet on responses to KemTRACE® chromium propionate supplementation. We hypothesized that feed intake and milk production would improve as a result of increased insulin sensitivity and decreased lipolysis from chromium propionate supplementation and that the benefit would be greater for high moisture corn compared to dry corn. This interaction of treatments was expected because greater propionate production from high moisture compared with dry corn is more hypophagic when hepatic acetyl CoA concentration is elevated (Stocks and Allen, 2011), and chromium supplementation is expected to decrease lipolysis and subsequently concentration of acetyl CoA in the liver.

CHAPTER 2

EFFECTS OF CHROMIUM PROPIONATE SUPPLEMENTATION THROUGHOUT THE PERIPARTURIENT PERIOD OF DAIRY COWS AND ITS INTERACTION WITH STARCH FERMENTABILITY POSTPARTUM ON PRODUCTIVE PERFORMANCE AND METABOLISM

ABSTRACT

Holstein cows (n=48) entering second or later lactation were used in a randomized block design experiment with a 2x2 factorial arrangement of treatments to determine production, dry matter intake (DMI), and metabolic responses to chromium propionate supplementation throughout the periparturient period and starch source in the postpartum (PP) diet. Treatments were chromium propionate (KemTRACE® Chromium Propionate, Kemin Industries, CrPr, 8 mg Cr/cow/d) or control (Con, ground corn) top-dressed (20 g/d) daily at feeding from 28±3 d before expected parturition until 28±3 d PP, and dry corn (DC) or high moisture corn (HMC) in diets fed from parturition until 28±3 d PP. Cows were fed a common diet from 28±3 d to 84±3 PP. No effects of treatment were detected for daily DMI from parturition until 28±3 d PP, but an interaction of chromium, corn and d PP was detected (P = 0.06) when cows were fed a common diet. An interaction among chromium supplementation, starch source, and week was detected for 3.5% fat-corrected milk (FCM, *P* = 0.07) from parturition until 28±3 d PP. The CrPr/HMC treatment tended to increase FCM by 28±3 d PP compared to Con/DC and Con/HMC (57.4 vs. 48.6/48.5, *P* = 0.10) andsignificantly increased FCM by 42±3 d PP compared to Con/DC (59.2 vs. 44.8 kg, *P* = 0.001). CrPralso tended to increase milk yield (55.4 vs. 51.9 kg/d, *P* = 0.09) and FCM (52.0 vs. 48.3 kg/d, *P* = 0.108) from 28±3 d to 84±3 d PP after treatment ceased. Although CrPr improved production performance, there was no evidence of increased insulin sensitivity

with CrPr supplementation; postpartum plasma NEFA concentration was numerically higher and plasma NEFAconcentration before the glucose tolerance test was significantly increased by the CrPr treatment. Glucose and NEFA AUC were unaffected by CrPr. Supplementation of CrPr throughout the periparturient period interacted with starch source in PP diets and d PP to affect production responses that were also affected after treatment application ceased.

INTRODUCTION

Chromium supplementation might benefit feed intake and milk yieldthrough the transition period by decreasing lipolysis (McNamara and Valdez, 2005). Chromium supplementation has reduced plasma NEFA concentration and improved lactational performance of dairy cows and heifers (Subiyatno et al., 1996; Hayirli et al., 2001; Sumner et al., 2007). The improvement in feed intake might be because uptake and oxidation of NEFA depress feed intake of cows in a lipolytic state (Allen et al., 2009). However, effects of Cr on feed intake during this period are inconsistent among Cr studies with dairy cows. This might be a result of variation in starch fermentability of rations. Fermentability of starch sources fed to dairy cattle is highly variable and affects feed intake of lactating cows because propionate produced from ruminally-fermented starch is hypophagic (Allen, 2000). The hypophagic effects of short-term propionate infusions are greaterfor cows in a lipolytic state (Oba and Allen, 2003) likely because propionate stimulates oxidation of the pool of acetyl CoA in the liver (Stocks and Allen, 2011). The benefits of reduced lipolysis from Cr supplementation through the transition period might be altered by increased starch fermentability of rations several ways. Decreased lipolysis might have greater effects on DMI when a ration with highly fermentable starch is fed by decreasing the supply of acetyl CoA that is readily oxidized by the rapid flux of propionate to the liver within the

timeframe of meals, causing satiety. Alternatively, increased insulin concentration by the more fermentable starch source might reduce lipolysis further, decreasing hepatic oxidation of NEFA resulting in increased DMI. Lastly, greater propionate production might decrease ß-oxidation by inhibiting carnitine palmitoyltransferase and transport of NEFA into the mitochondria, decreasing ß-oxidation and increasing DMI. Therefore, we hypothesized that production response to CrPr supplementation through the periparturient period is dependent upon starch source varying in ruminal fermentability. The objectives of this experiment were to determine the effects of KemTRACE® chromium propionate supplementation through the transition period on feed intake and metabolic and production responses and to determine effects of starch fermentability of the fresh cow diet on responses to KemTRACE® chromium propionate supplementation.

MATERIALS AND METHODS

Animals, treatment, and experimental design All experimental procedures involving animals were approved by The Institutional Animal Care and Use Committee at Michigan State University. Forty-eight multiparous Holstein cows from the Michigan State University Dairy Cattle Teaching and Research Center were randomly assigned to 1 of 4 treatments in a randomized block design with a 2x2 factorial arrangement of treatments with 12 cows per treatment. Cows were blocked by calving date, body condition score, and 305-ME from the previous lactation. Cows were housed in individual tie stalls for the duration of the experiment.

Treatments were chromium as chromium propionate (CrPr) or control (Con) supplemented from 28±3 d before expected parturition until 28±3 d postpartum (PP), and dry ground corn (DC) or high moisture corn (HMC) in diets fed from parturition until 28±3 d PP.

KemTRACE® chromium propionate (0.04% chromium, KeminAgriFoods North America, DesMoines, IA) and dry ground corn control were supplemented by top-dressing the common base ration prepartum and the treatment rations postpartum at a rate of 20 g/head/d. The CrPr treatment supplied 8mg Cr/cow per day, the maximum permitted by the U.S. Food and Drug Adminstration Center for Veterinary Medicine.

Beginning 28±3 days before expected parturition, cows were provided a prepartum diet. Diet changes occurred on the same day each week. The postpartum corn treatment diets began on the day of parturition or the next feeding, depending on parturition time of day. Cows were fed once daily (1000 h) at 110% of expected intake. From 28±3 to 84±3 days, cows were offered a common diet. All diets were formulated to meet or exceed predicted requirements for minerals and vitamins (NRC, 2001) and water was available in each stall. Ingredient and nutrient composition of the four diets fed throughout the experiment are listed in Table 1. Feed offered and orts were weighed and recorded daily.

Data and sample collection Dry matter of fermented feeds was determined twice per week and concentrations of individual ingredients in the TMRs were adjusted accordingly. Forages and feeds with variable composition were sampled weekly, composited, and analyzed biweekly. Concentrates were sampled weekly, composited, and analyzed monthly. Ingredients were dried at 55°C for 72 h. All samples were ground with a Wiley mill (1-mm screen; Arthur H. Thomas, Philidelphia, PA). Samples were analyzed by wet chemical methods for neutral detergent fiber (NDF; Van Soest et al., 1991, method A), crude protein (CP; Hach et al., 1987), lignin (Van Soest, 1973), starch, and ether extract. Starch was measured by an enzymatic method, after samples were gelatinized with sodium hydroxide (Karkalas, 1985). Glucose concentration was

determined with a glucose oxidase method (Sigma Chemical Co., St. Louis, MO). Starch digestibility was estimated by a 7-h in vitro fermentation (Goering and VanSoest, 1970) with rumen fluid collected from a lactating cow on a high starch diet. Ether extract concentration was determined using a modified Soxhlet apparatus (AOAC, 1990). Ash concentration was determined after 5 hours of oxidation at 500°C in a muffle furnace. Corn grain was dry sieved through a sieve shaker (Sieve apertures: 4750, 2360, 1180, 600, 300, 150, 75μm bottom pan; model RX-86; W.S. Tyler Inc., Gastonia, NC) for approximately 20 min until the bottom pan weight was constant and mean particle size of corn grain was calculated. Corn grain was also wet sieved manually through the same set of sieves (ASAE, 1968).

A composite of each feed ingredient was taken to determine chromium concentration of the diet. Samples were analyzed by electrothermal atomic absorption spectrophotometry at North Carolina State University Raleigh, NC according to Lloyd et al. (2010).

Body condition score of each animal was recorded 35±3 days prior to expected parturition to block cows. Body weights and BCS were recorded on day 28±3 days prepartum then biweekly until 28±3 days postpartum and every 28±3 days until 84±3 days. Body condition was scored on a 5-point scale (Wildman et al., 1982) by 3 trained investigators.

After parturition cows were milked twice daily at 0400 and 1400. Milk yield was recorded at each milking until 84±3 d PP. Milk samples were collected at each milking on one day per week, from parturition until 28±3 days postpartum and then biweekly until 84±3 d PP. Samples were analyzed for fat, true protein, lactose, solids not fat, somatic cell count, and milk urea nitrogen by Michigan Dairy Herd Information Association (AOAC, 1997). Feed conversion efficiency (FCE) was determined by dividing 3.5% FCM by DMI.

Blood samples were collected by coccygeal venipuncture approximately three hours before feeding and six hours after feeding weekly from -28±3 to 28±3 days relative to parturition. Blood was collected into two Vacutainer® tubes (Becton Dickinson, Franklin Lakes, NJ), one containing potassium EDTA and another containing potassium oxalate with sodium fluoride as a glycolytic inhibitor. Samples were centrifuged at 2000 x g for 20 min; plasma was harvested and frozen at -20°C for later analysis. Plasma aliquots from the potassium EDTA tube were stored with 0.5M benzamidine, a protease inhibitor. Plasma samples were analyzed using commercial kits for concentration of insulin (Coat-A-Count, Siemens Healthcare Diagnostics, Deerfield, IL), glucagon (kit #GL-32K, Millipore, Billerica, MA), FFA (NEFA HR kit, Wako Chemicals USA, Richmond, VA), beta-hydroxybutryic acid (BHBA, #2240, Stanbio Laboratory, Boerne, TX), and triglyceride (L-Type TG M, Microtiter Procedure, Wako Chemicals USA, Richmond, VA). Plasma glucose concentration was analyzed using a glucose oxidase method (Sigma Chemical Co., St. Louis, MO).

Whole blood and plasma samples were prepared for chromium determination by diluting 200 uL of sample with 5mL of a solution containing 0.5% EDTA and Triton X-100, 1% ammonia hydroxide, 2% propanol, and 20 ppb of scandium, rhodium, indium and bismuth as internal standards. Samples were frozen until -20°C until analysis. Chromium concentration was determined by the Michigan State University Diagnostic Center for Population and Animal Healthwith an InductivelyCoupled Plasma – Mass Spectrometer (Agilent 7500ce, Aligent Technologies, Santa Clara, CA) according to manufacturer's specifications.

Liver samples were collected by needle biopsy (Bradford and Allen, 2005) at -7, 7, 14, and 21 (all ±3) days relative to parturition. Samples were snap-frozen in liquid nitrogen and

stored at until analysis for TG and glycogen concentrations. Triglyceride concentration was determined according to Bligh and Dyer (1959) as modified by Rice et al. (2010). Absorbance was determined with a micro-plate reader (SpectraMax 340; Molecular Devices Corp., Sunnyvale, CA). Liver glycogen was analyzed according to Hawk and Bergeim (1926) as modified by Bernal-Santos et al. (2003).

A glucose tolerance test (GTT) was conducted on all cows 7±3 days after parturition (Bradford and Allen, 2007). All cows were fitted with a single jugular catheter 2 days prior to the GTT. Cows were blocked from feed at 0800 on the day of the test and not allowed access to feed until the GTT was completed. Sterile solutions of 50% dextrose (wt/vol) were administered via jugular bolus at a dose of 1.67 mmol glucose/kg of BW over 5 to 10 minutes. Samples were collected from the jugular catheter 10 min prior to infusion, immediately before infusion, every 10 min through 120 min, and at 150 min and 180 min post-infusion. Catheters were flushed with sterile 4.2% Na citrate after infusion and after each blood sample. Samples were processed and analyzed for glucose, insulin, NEFA, and BHBA using previously described methods. Measurements included basal concentration, maximum and/or minimum, area under the curve (AUC), and clearance rate (CR) of glucose and insulin. AUC was calculated by the trapezoidal rule using the mean of the -10 and 0 min time points as the baseline value for each test. Clearance rate was calculated according to Hayirli et al. (2001).

Statistical analysis

Data were analyzed according to stage of lactation: prepartum, postpartum, and peak. Daily DMI and milk yield, weekly milk components and plasma metabolites and hormones, and biweekly or monthly body weights and BCS values were used for analysis. One cow was removed from study due to injury before the 28±3 d PP after feeding blood sample and data was

excluded for postpartum cumulative DMI and cumulative milk yield. All other postpartum data were included. ANOVA was conducted using the GLM procedure of SAS (Version 9.2, SAS Institute, 2008) to test assumptions of normality, homoscedasticity, and to identify potential outliers.

Using the MIXED procedure of SAS (2008), ANOVA was conducted using a randomized block design with repeated measures and an autoregressive covariance structure based upon the Bayesian Information Criterion. Sattherwaite degrees of freedom adjustment was used to account for heterogeneous variances. Block and cow within block were included as random effects. The effect of treatments on prepartum performance, and metabolic responses were analyzed with the following linear model:

$$Y_{ijm} = \mu + B_i + C(B_iK_k)_j + K_k + T_m + K_kT_m + J + e_{ijm}$$

where μ = overall mean, B_i = random effect of block (i= 1 to 12), $C(B_iK_k)_j$ = random effect of cow (j = 1 to 4) within block and chromium treatments, K_k = fixed effect of chromium treatment (k = 1 to 2), T_m = fixed effect of week (m = 1 to 4), K_kT_m = interaction of chromium and week, J = random effect of Julian date, and e_{ijm}= residual. The effect of treatment for postpartum and peak, milk yield, body weight and condition and milk production were analyzed with the same model, but with the addition of S_l= fixed effect of corn (l = 1 to 2) and its interactions:

$$Y_{ijm} = \mu + B_i + C(B_iK_kS_l)_j + K_k + S_l + K_kS_l + T_m + K_kT_m + S_lT_m + K_kS_lT_m + J + e_{ijm}.$$

Effects of treatment on GTT measurements, cumulative DMI and milk yield, plasma and whole blood chromium, and plasma calcium were analyzed using a linear model without repeated measures.

$$Y_{ijm} = \mu + B_i + C(B_iK_kS_l)_j + K_k + S_l + K_kS_l + J + e_{ijm}$$

Treatment effects were declared significant at $P < 0.05$ and tendencies for treatment effects at $P < 0.10$. Interactions were declared significant at $P < 0.10$ and tendencies or treatment effects at $P < 0.15$.

RESULTS

Prepartum period

Chromium propionate tended to decrease daily DMI prepartum (11.8 vs. 12.7 kg/d, P=0.06), but no effect of treatment was detected for cumulative DMI for the 14 days before parturition (Table 2). No effect of treatment was detected for BW or BCS or for plasma concentrations of insulin or NEFA either before feeding or after feeding. Chromium propionate decreased plasma concentration of BHBA after feeding (6.95 vs. 7.58 mg/dl, P = 0.05) but increased plasma concentration of BHBA as parturition approached (treatment x time interaction P < 0.001). Chromium propionate tended to increase plasma glucagon concentration before feeding (91.9 vs. 85.9 pg/ml, P = 0.10), but the difference in glucagon concentrations between treatments decreased as parturition approached (P=0.02). Plasma glucagon concentration after feeding was not affected by treatment. Treatment did not affect plasma glucose concentration before feeding, but CrPr increased glucose concentration after feeding as parturition approached (P = 0.05).

Postpartum period

High moisture corn increased yields of milk (42.8 vs. 39.2 kg, P = 0.02) and lactose (2.11 vs. 1.94, P = 0.05),and cumulative milk yield for the first 28±3 d PP (1196 vs. 1099, P = 0.02)compared to DC, but no effect of CrPr was observed for milk yield (Table 3a; 3b). High

moisture corn tended to decrease protein concentration in milk (3.11 vs. 3.00, P=0.10) and tended to increase concentration of lactose in milk (4.69 vs. 4.60, P = 0.07) compared with DC. Treatment did not affect concentrations of fat or solids non-fat (SNF) in milk. Chromium propionate and corn interacted over time to affect yields of FCM (P = 0.07), SCM (P = 0.09), ECM (P = 0.11), and SNF (P = 0.09). The CrPr/HMC and Con/DC treatments resulted in consistent and higher (54.9 kg/d) and lower (49.3 kg/d) FCM, respectively, throughout the period compared to the other treatments in which FCM decreased over time (Figure 1). Similar effects of treatments were observed for yields of solids corrected milk (SCM), and ECM. Yields of fat and protein were not affected by treatment.

Daily DMI, cumulative DMI over 28±3 d PP, and FCE were not affected by treatment. High moisture corn tended to increase BW compared with DC (728 vs. 695 kg, P = 0.09), but the difference decreased with increasing days PP (P = 0.10). Cows treated with chromium tended to have higher BW the week of parturition, but BW did not differ between treatments by 14 d PP (interaction P = 0.13). Body condition score was not affected by treatment.

Chromium propionate increased plasma Cr concentration measured at 28±3 d PP (4.56 vs. 3.53 ng/ml, P = 0.002, Table 4a; 4b) but an interaction between CrPr and corn source was detected (P = 0.03) for Cr concentration in whole blood; CrPr increased Cr concentration for DC (9.16 vs. 7.17 ng/ml) and decreased Cr concentration for HMC (5.31 vs. 10.35 ng/ml). No effects of treatment on plasma insulin, or glucose concentrations were observed either before feeding or after feeding, on plasma NEFA concentration before feeding, or on plasma BHBA concentration after feeding. However, HMC tended to decrease plasma NEFA concentration at a greater rate over time PP compared with DC after feeding (P = 0.12) and CrPr tended to increase plasma BHBA concentration over time PP compared with control before feeding (P = 0.09).

Chromium propionate tended to increase plasma glucagon concentration compared to control before feeding (P = 0.11) but the difference between treatments decreased by 28±3 d PP (interaction P < 0.01) and no effect of CrPr treatment was observed after feeding. Interactions of corn and week were detected for plasma glucagon concentration both before feeding (P = 0.002) and after feeding (P = 0.04) with plasma glucagon concentration initially higher for DC compared with HMC but increased by HMC over time with greater concentration compared with DC by week 3. Treatment interacted with time for plasma TG concentration both before feeding and after feeding; CrPr increased TC concentration over time for HMC and decreased TG concentration over time for DC, while plasma TG concentration was consistently high for HMC and low for DC for control through week 4 (interaction of CrPr x corn x week, P = 0.14 for before feeding and 0.01 for after feeding). Chromium propionate decreased plasma TG concentration compared to control before feeding (2.41 vs. 6.68, P = 0.02) and HMC increased plasma TG concentration compared to DC both before feeding (3.30 vs. 2.45, P = 0.04) and after feeding (3.35 vs. 2.33, P = 0.02).

Chromium propionate and corn source interacted to affect maximum plasma glucose concentration during the GTT (P < 0.0001, Table 5); CrPr decreased maximum concentration for DC (117.6 vs. 120.3 mg/dl) but increased maximum concentration for HMC (120.7 vs. 119.4 mg/dl). No effects of treatment were observed for AUC, clearance rate, or basal glucose concentration. Chromium propionate and corn source also tended to interact to affect maximum plasma glucose concentration during the GTT (P = 0.11); CrPr increased maximum concentration for DC (109 vs. 83 mg/dl) but reduced maximum concentration for HMC (90 vs. 108 mg/dl). Chromium propionate and corn source interacted to affect AUC for insulin response to the GTT (P = 0.08); CrPr increased AUC for DC (350 vs. 253 μIU*min/ml) but reduced AUC

for HMC (296 vs. 349 μIU*min/ml). Maximum plasma NEFA concentration was increased by CrPr compared with control (1,000 vs. 775μeq/L, P = 0.03) and HMC compared withDC (1,002 vs. 773μeq/L, P = 0.03) and HMC tended to reduce plasma NEFA to a greater extent over time compared withDC (AUC: -2,294 vs. -1,488 μeq*min/L, P=0.10) but did not affect minimum plasma NEFA concentration. No effect of treatment was detected for BHBA response during the GTT.

Chromium propionate tended to increase liver glycogen concentration (3.34% vs. 2.79%, P = 0.06) compared with control, which was not affected by corn source (Table 6). Liver TG concentration was not affected by treatment but liver TG concentration was increased from d -7±3 to d 14±3 by CrPr compared with control (3.51% vs. 2.00%, P = 0.04) and by HMC compared with DC (3.49% vs. 2.02%, P = 0.04).

Carryover effects of treatment on production

Chromium propionate and corn treatments interacted over time to affect daily DMI (P = 0.06, Table 7a; 7b); DMI was generally higher for CrPr/HMC, lower for Con/DC with intermediate DMI for CrPr/DC and Con/HMC throughout the post-treatment collection period from 28±3 to 84±3 d PP (Figure 2). High moisture corn tended to increase cumulative DMI (25.5 vs. 24.4 kg/d, P = 0.09) when cows were offered a common ration.

Chromium propionate tended to increase daily yield of milk (P = 0.09) as well as cumulative daily milk yield from 28±3 d to 84±3 d PP (3142 vs. 2852 kg, P = 0.10, Table 5). However, chromium propionate and corn treatments tended to interact with time to affect daily milk yield (P = 0.11, Figure 3). Concentration of milk fat decreased following termination of treatment when cows were offered a common ration but the decrease was faster for DC compared with HMC (interaction P = 0.01) and no effect of CrPr was detected. Following

termination of treatment, yields of FCM and fat decreased greatly for HMC compared with DC (interaction $P < 0.001$) and chromium propionate and corn treatments interacted over time to affect yields of FCM ($P = 0.07$, Figure 1) and fat ($P = 0.09$, Table 5). By the time treatments were terminated at 28±3 d PP, the CrPr/HMC treatment combination tended to increase FCM by ~8.9 kg/d compared with both Con/DC and Con/HMC ($P \leq 0.10$). Two weeks after treatments were terminated the CrPr/HMC treatment combination increased FCM compared with Con/DC by 14.4 kg/d ($P = 0.001$) and HMC increased FCM by 10.4 kg/d compared with DC for the control treatment ($P = 0.03$). However, treatment effects on yields of milk and milk components were no longer different by 56±3 d PP.

High moisture corn decreased milk protein concentration compared with DC (2.77 vs. 2.85%, $P = 0.05$) but an interaction among CrPr, corn, and time was observed ($P = 0.09$); Con/DC and CrPr/HMC consistently increased and decreased milk protein concentration, respectively, but the remaining treatments were intermediate and varied over time. Yield of protein increased for several weeks following termination of treatment but an interaction among CrPr, corn and time was detected ($P = 0.01$); CrPr/HMC increased, and Con/DC decreased protein yield for the first few weeks only with little difference among treatments for the remainder of the period. High moisture corn increased lactose concentration compared with DC (4.83 vs. 4.71%, $P = 0.02$) and CrPr tended to decrease lactose concentration compared with Con (4.81 vs. 4.72%, $P = 0.06$). Yield of lactose was higher with Con/HMC compared to other treatments week 6 and then decreased overtime (interaction $P = 0.06$). Yield of lactose remained lowest with CrPr/DC overtime. Chromium propionate decreased SNF concentration compared with Con (8.21 vs. 8.35%, $P = 0.04$) but tended to interact with corn and time ($P = 0.11$). There also tended to be an interaction among CrPr, corn, and time ($P = 0.11$). Percent SNF remained

lowest for CrPr/DC overtime, while Con/HMC increased and decreased percent SNF every two weeks. A tendency for an interaction of CrPr, corn and time was also detected for yield of SNF ($P = 0.12$). Yield of SNF tended to increase with CrPr/HMC overtime compared to Con/DC (interaction $P = 0.12$). High-moisture corn increased milk urea nitrogen (MUN) compared to DC (16.4 vs. 15.3 mg/dl, $P = 0.03$). Interactions among CrPr, corn and time were detected for yield of SCM and ECM ($P < 0.05$) with patterns of treatments over time similar to FCM (Figure 1).

Body weight was consistently greater for CrPr/HMC compared to the other treatments but an interaction was detected among CrPr, corn, and time ($P = 0.05$) due to variation among treatments over time. High moisture corn tended to decrease BCS over time compared withDC (interaction $P = 0.12$), but BCS was not affected by CrPr. Feed conversion efficiency decreased following termination of treatment but an interaction was detected between corn and time ($P = 0.01$) with a greater decrease between 28±3 d and 56±3 d PP for DC compared with HMC.

DISCUSSION

The CrPr treatment increased plasma Cr concentration nearly 30% in the present experimentwhen Cr intake from the CrPr supplement was between 47 and 74% of the Cr intake from the basal diet. Although several experiments have supplemented organic chromium to dairy cows, only Subiyatno et al., (1996) reported plasma concentration, which was lower in cows supplemented with chelated Cr. Reasons for this are unclear and not discussed by the authors. No studies report Cr concentration of whole blood. Chromium supplementation may interact with corn source to affect Cr excretion, which might explain the interaction of starch source and CrPr on Cr concentration of whole blood in this experiment. One potential mechanism for effects of Cr on increased insulin sensitivity is that four Cr ions are incorporated into a holo-oligopeptide and the holoenzyme is excreted in the urine following dephosphorylation of the insulin receptor (Chen et al., 2011). Kozlovsky et al. (1986) show that Cr excretion in the urine is greater with a high carbohydrate diet in mice and ruminal fermentability of starch might affect Cr excretion in the urine. Determination of Cr excretion in urine might help increase our understanding of this interaction. To our knowledge, chromium concentrations of urine and feces have not been reported in dairy cows.

Requirements for chromium have not been established (NRC, 2001) partly because it had been assumed that adequate levels of this trace element were supplied by diets without supplementation. However, favorable responses to chromium supplementation have been demonstrated in several studies with cattle (Burton et al., 1993; Hayirli et al., 2001; AnQiang et al., 2009) despite that the amount of chromium supplemented was less than the amount supplied by the basal diet. This is likely because the inorganic forms of chromium in most feedstuffs have low bioavailability compared to organic forms of supplements. In rats and humans, less than 2%

of dietary chromium is absorbed, but CrPr was absorbed with greater than 60% efficiency when supplemented to the diet (Clodfelder et al., 2004).It is likely that bioavailability of chromium varies among organic sources as well, but comparisons are lacking.

Chromium propionate tended to decrease daily DMI prepartum and did not affect cumulative DMI either pre or postpartum or daily DMI postpartum. These responses were not expected; we hypothesized that Cr would increase DMI through the transition period by increasing insulin sensitivity and decreasing lipolysis. We thought that decreased lipolysis might reduce hypophagia in the transition period by decreasing the supply of NEFA to the liver according to the hepatic oxidation theory of the control of feed intake (Allen et al., 2009). Chromium supplementation increased DMI of dairy cows both pre and postpartum in a study reported by Hayirli et al. (2001). However, effects of Cr on DMI in the transition period have been inconsistent. Supplementation of Cr did not affect DMI in other experiments either prepartum (Yang et al., 1996; McNamara and Valdez, 2005; Smith et al., 2005; Sadri et al., 2009) or postpartum (McNamara and Valdez, 2005; Sadri et al., 2009; Soltan, 2010).

We were unable to detect a reduction in plasma NEFA concentration or any improvement in metabolic status by CrPr treatment. We sampled blood both before and after the largest meal of the day (at feeding) to enhance our chances of detecting a response of Cr on plasma metabolites. There was no effect of Cr on plasma NEFA concentration prepartum when measured either before or after feeding and a numerical increase in plasma NEFA concentration postpartum for the CrPr treatment both before and after feeding. In addition, Cr increased the initial plasma NEFA concentration (MAX) prior to the glucose challenge and did not affect AUC for NEFA or glucose. Therefore, no evidence was provided for improved insulin sensitivity or metabolic status by CrPr either by the concentrations of blood metabolites or responses to a

glucose challenge in this experiment. These results were also unexpected. We expected Cr to increase insulin sensitivity through the transition period, decreasing lipolysis and NEFA supply to the liver because Cr has been reported to reduce plasma NEFA concentration in cattle (Subiyatno et al., 1996; Hayirli et al., 2001; Sumner et al., 2007) and other species (Matthews et al., 2001; Clodfelder et al., 2005; Cefalu et al., 2010). However, effects of Cr on plasma NEFA concentration has been inconsistent among experiments with cows in the prepartum period with a decrease in plasma NEFA concentration when measured in blood sampled before feeding (Soltan, 2010) but no effect when measured in blood sampled after feeding (Smith et al., 2008). Effects of Cr supplementation were also inconsistent for plasma NEFA concentration postpartum with no effect (Hayirli et al., 2001; McNamara and Valdez, 2005) or decreased NEFA concentration (Soltan, 2010) when measured before feeding. Smith et al., (2008) measured plasma NEFA concentration after feeding throughout the postpartum period and detected an interaction between chromium supplementation and diet non-fiber carbohydrate (NFC) concentration in prepartum diets; increasing amounts of chromium methionine decreased plasma NEFA concentration for cows fed the low NFC diet and increased plasma NEFA concentration for cows fed the high NFC diet in the prepartum period.

The reason for the interaction among CrPr, starch fermentability and time for plasma TG observed in this experiment is unknown. Few studies report TG concentration in serum or plasma in periparturient dairy cows, because circulating TG is low (Tyburczy et al., 2008) and is generally from the diet (Wadsworth, 1968). However, no effects of treatment on DMI were detected in the postpartum period.

If CrPr had increased insulin sensitivity and decreased lipolysis in the transition period, we expected cows to lose less BW and body condition. However, we detected no effect of CrPr

on either BW or BCS prepartum or postpartum in this experiment consistent with other studies for BW (McNamara and Valdez, 2005; Smith et al., 2005; Soltan, 2010) and BCS (McNamara and Valdez, 2005). Smith et al. (2005) reported that Cr-Met increased BCS linearly but they concluded there was little biological significance in the values as the change was very small and there were no effects of treatment on calculated energy balance.

Contrary to our hypothesis, there were no effects of starch source or interaction with CrPr on daily or cumulative DMI in the postpartum period in this experiment. Highly fermentable starch sources can decrease intake by lactating cows (Allen, 2000) and propionate produced from ruminal fermentation of starch has been shown to be hypophagic in several experiments possibly through stimulation of hepatic oxidation (Allen, 2000). If CrPr decreased plasma NEFA concentration in the peripartum period as expected, we expected to detect an interaction among starch fermentability and CrPr supplementation. Propionate was more hypophagic when infused in cows in the postpartum period compared to mid-lactation (Oba and Allen, 2003) and a more fermentable starch source tended ($P = 0.11$) to decrease DMI (% of BW) of lactating cows compared to a less fermentable source in the postpartum period (Dann et al., 1999). Decreased NEFA oxidation would reduce the pool of acetyl CoA available for oxidation when propionate flux to the liver increases within a meal. Greater hypophagic effects of propionate for cows in a lipolytic state might be because propionate stimulates oxidation of the pool of acetyl CoA in the liver; hypophagia from propionate infusion was linearly related to liver acetyl CoA concentrations (Stocks and Allen, 2011). Alternatively, propionate might also decrease lipolysis by stimulating insulin release and decrease ß-oxidation by inhibiting carnitine palmitoyltransferase and transport of NEFA into the mitochondria decreasing oxidation and increasing feed intake. Chromium interacted with starch fermentability, increasing prepartum

DMI and tending to increase postpartum DMI when cows were fed a ration containing barley, but not corn (Sadri et al., 2009). The lack of interaction of starch source with CrPrfor DMI in the present experiment might be because CrPr failed to reduce plasma NEFA concentration or metabolic profile. While this could be because propionate is an insulin secretagogue and increased insulin concentration might decrease lipolysis and NEFA supply to the liver diminishing the short-term effects of propionate on DMI over time, we were unable to detect an effect of starch source on plasma concentrations of either insulin or NEFA in the postpartum period when measured either before or after feeding.

Although there were no effects of treatment on daily or cumulative DMI in the postpartum period, CrPr interacted with starch source over time to affect DMI after treatment ceased and when cows offered a common diet from week 4 to 12 postpartum. Carryover effects of treatment have not generally been measured for experiments evaluating effects of chromium supplementation to dairy cows. However, a carryover effect was reported in a previous experiment in which CrPr supplementation increased DMI after treatment ceased at 35 d postpartum, with a numerical increase in milk yield (McNamara and Valdez, 2005). In the present experiment, differences among treatments for FCM yield (Figure 1) were consistent with the differences among treatments for DMI (Figure 2).

Treatment did not affect liver TG concentration consistent with other experiments (Hayirli et al., 2001; McNamara et al., 2005; Smith et al., 2008). However, both CrPr and greater starch fermentability increased concentration of TG in the liver from -7±3 d prepartum to 14±3 d postpartum. It is noteworthy that the ranking for this increase in liver TG concentration among treatments (Table 6) is similar to the ranking for effects of treatment on DMI (Figure 2) and yield of FCM (Figure 1). The effects of treatment on partitioning of NEFA cleared by the

liver between storage as TG and ß-oxidation is consistent with effects of treatment on DMI according to the hepatic oxidation theory (Allen et al., 2009). However, it appears that effects of treatment on milk yield preceded effects on DMI in this experiment; effects of treatment on FCM yield began during treatment in the postpartum period and continued for several weeks after cows were offered a common diet. There appears to be merit in evaluating effects of Cr supplementation further into lactation. While most experiments have focused on supplementing Cr through the periparturient period, initiation of chromium supplementation after lactation is initiated increased DMI and milk yield (Al-Saiady et al., 2004) and increased DMI, but not yield of milk or FCM (Nikkhah et al. 2011) for cows that were heat-stressed. In addition, longer-term supplementation through lactation might have potential to affect partitioning of energy to milk. Chromium supplementation decreased plasma insulin concentration when supplemented for longer periods than control groups (Sumner et al., 2007). A reduction in plasma insulin concentration might increase energy partitioned to milk if insulin sensitivity is not increased by Cr treatment.

The reason(s) for the effects of treatment on milk yield in this experiment are unclear but might be related to effects of treatment on plasma glucagon concentration which was affected by both CrPr and starch source over time. Chromium supplementation was reported to increase conversion of propionate to glucose in a study with young rams (Sano et al., 1997). Although treatment effects on plasma glucose concentration were not detected, stimulation of gluconeogenesis by elevated glucagon concentration might have resulted in increased glucose supply to the mammary gland, increasing milk yield.

Table 1. Ingredients and nutrient composition of experimental diets (% of dietary DM).

	Prepartum diet	Postpartum diet		Peak diet
Item		DC	HMC	
Ingredient				
DC[1,4,5]		23.3		7.1
HMC[2,4,5]			23.3	6.6
Corn silage	43.6	25.0	25.0	32.3
Alfalfa silage		19.2	19.2	8.9
Grass hay	24.3			5.2
Alfalfa hay		11.8	11.8	2.0
Whole cottonseeds				5.9
Soy hulls				8.9
Soybean meal	17.5	12.9	12.9	14.6
Soy Chlor	1.8			
Mineral and Vitamin Mix	12.8	7.8	7.8	8.1
Nutrient Composition				
DM	49.2	50.3	48.0	53.2
OM	92.6	92.6	92.6	93.2
Starch	14.2	26.4	26.5	27.9
NDF	38.5	31.4	31.1	32.4
% NDF from forage	90.7	87.1	88.6	63.1
CP	15.7	16.2	16.2	16.6
Ether Extract	3.0	3.9	3.8	4.32
Cr[3], mg/kg of DM	1.44	0.68	0.67	0.37

[1]DC = dry ground corn; [2]HMC = high-moisture corn; [3] Cr = chromium

[4]mean particle size (μm): dry sieve (DC = 1397; HMC =3758); wet sieve (DC = 1450; HMC = 3945)

[5]starch digestibility: 7-h starch in vitro starch digestibility (DC = 29%; HMC = 67%)

Table 2. Effects of CrPr supplementation on prepartum intake and plasma metabolites and hormones

	Treatment			*P* value	
Item	Con	CrPr[4]	SE	Cr[5]	Cr*Time
DMI kg/d[1]	12.7	11.8	0.42	0.06	0.95
Cumulative DMI, kg	173.6	163.2	5.62	0.18	NA
BCS[3]	3.34	3.44	0.15	0.35	0.49
BW, kg [3]	764.2	796.5	21.2	0.16	0.69
Plasma-Before feeding[2]					
Triglyceride, mg/dl	16.10	14.48	0.88	0.18	0.14
NEFA, µeq/L	208.9	207.4	17.52	0.99	0.67
BHBA, mg/dl	5.49	5.73	0.24	0.35	>0.001
Glucose, mg/dl	57.79	57.10	1.84	0.68	0.23
Glucagon, pg/ml	85.86	91.94	3.13	0.10	0.02
Insulin, µIU/ml	2.61	2.60	[8]	0.90	0.54
Plasma-After feeding[2]					
Triglyceride, mg/dl	14.79	13.44	0.98	0.27	0.27
NEFA, µeq/L	192.0	184.5	20.86	0.73	0.87
BHBA, mg/dl	7.58	6.95	0.25	0.05	0.69
Glucose, mg/dl	59.15	58.77	1.88	0.81	0.05
Glucagon, pg/ml	89.67	94.17	3.12	0.18	0.17
Insulin, µIU/ml	3.74	3.79	0.46	0.91	0.79

[1] = recorded daily

[2] = recorded weekly

[3]= recorded biweekly

[4]CrPr = chromium propionate supplementation

[5]Cr = effect of CrPr supplementation

[6]Cr*Time = interaction of CrPr and time[1,2,3]

[7]= Cumulative DMI was calculated from -14 d PP to -1 d PP.

[8] = data was transformed for analysis [xtrans = logx]. LS Means are back-transformed. 95% confidence intervals are Con: 3.28 - 2.07 µIU/ml U/L; CrPr: 3.22 – 2.03 µIU/ml U/L.

Table 3a. Effects of CrPr supplementation and corn grain conservation method on postpartum productivity

	Control		CrPr[4]		
Item	DC[6]	HMC[7]	DC	HMC	SE
Milk Yield, kg/d[1]	38.5	41.4	39.9	44.1	1.65
FCM (3.5%), kg/d[2]	49.3	51.1	50.8	54.9	2.80
SCM, kg/d[2]	43.8	45.4	44.6	48.4	2.38
ECM, kg/d[2]	47.9	49.5	48.9	52.9	2.61
Fat, kg/d[2]	1.95	1.99	2.00	2.17	0.13
Protein, kg/d[2]	1.27	1.32	1.28	1.36	0.06
Lactose, kg/d[2]	1.92	2.06	1.95	2.15	0.10
SNF, kg/d[2]	3.48	3.69	3.53	3.79	0.17
Cumulative Milk, kg	1079	1158	1118	1233	47.3
Fat %	4.83	4.59	4.77	4.80	0.21
Protein %	3.15	3.01	3.06	2.98	0.07
Lactose %	4.64	4.70	4.57	4.68	0.05
SNF %	8.49	8.42	8.33	8.38	0.09
MUN, mg/dL[2]	14.0	14.4	14.1	14.1	0.66
SCC, x 1000[2]	21.3	32.0	24.9	25.3	[13]
DMI, kg/d[1]	18.1	18.6	18.5	18.8	0.67
Cumulative DMI, kg	506.8	521.4	519.0	526.6	19.7
FCE[2]	2.68	2.70	2.59	2.83	0.14
BW, kg[3]	692.2	708.1	697.9	748.1	21.8
BCS[3]	2.82	2.70	2.78	2.90	0.18

[1-4]see Table 2;[6]DC = dry ground corn;[7]HMC = high-moisture corn;[13]= data was transformed for analysis [xtrans = logx].
LS Means are back-transformed. 95% confidence intervals are (all x 1000) Con/DC: 42.5 – 10.7 U/L; Con/HMC: 63.8-16.0 U/L Con/HMC: 63.8-16.0 U/L; CrPr/DC: 49.8 – 12.5 U/L; CrPr/HMC; 50.5 – 12.7 U/L.

Table 3b. Effects of CrPr supplementation and corn grain conservation method on postpartum productivity

	P-value					
Item	Cr[5]	Corn[8]	Cr *Corn[9]	Cr *Time[10]	Corn*Time[11]	Cr *Corn*Time[12]
Milk Yield (kg/d)[1]	0.15	0.02	0.66	0.31	0.95	0.44
FCM (3.5%) kg/d[2]	0.25	0.20	0.60	0.19	0.86	0.07
SCM kg/d[2]	0.31	0.16	0.56	0.12	0.81	0.09
ECM kg/d[2]	0.30	0.18	0.58	0.11	0.73	0.11
Fat kg/d[2]	0.28	0.33	0.53	0.30	0.82	0.16
Protein kg/d[2]	0.63	0.28	0.72	0.18	0.75	0.19
Lactose kg/d[2]	0.50	0.05	0.72	0.12	0.79	0.13
SNF kg/d[2]	0.62	0.13	0.83	0.20	0.95	0.09
Cumulative Milk, kg	0.16	0.02	0.64	NA	NA	NA
Fat %	0.70	0.62	0.51	0.47	0.90	0.36
Protein %	0.42	0.10	0.66	0.18	1.00	0.77
Lactose %	0.33	0.07	0.55	0.88	0.94	0.28
SNF %	0.26	0.81	0.51	0.30	1.00	0.82
MUN, mg/dL[2]	0.93	0.71	0.73	0.40	0.86	0.02
SCC, x 1000[2]	0.90	0.54	0.55	0.06	0.66	0.06
DMI, kg/d[1]	0.63	0.53	0.81	0.24	0.40	0.74
Cumulative DMI, kg	0.60	0.51	0.83	NA	NA	NA
FCE[2]	0.81	0.34	0.42	0.31	0.40	0.60
BW, kg[3]	0.25	0.09	0.37	0.13	0.10	0.18
BCS[3]	0.56	0.99	0.30	0.82	0.40	0.17

[1-3]see Table 2; [5]Cr = effect of CrPr supplementation; [8]Corn = effect of corn grain conservation method;
[9]Cr*Corn = interaction between CrPr supplementation and corn grain conservation method;
[10]Cr*Time = interaction of CrPr supplementation and time; [11]Corn*Time = interaction of corn and time;
[12]Cr*Corn*Time = 3-way interaction among CrPr, corn, and time

Table 4a. Effects of CrPr supplementation and corn grain conservation method on postpartum plasma metabolites and hormones

Item	Control		CrPr[4]		SE
	DC[6]	HMC[7]	DC	HMC	
Plasma-AM[2]					
Triglyceride, mg/dl	2.14	4.54	2.76	2.05	0.46
NEFA, μeq/L	613.0	702.6	705.7	739.8	59.6
BHBA, mg/dl	9.29	9.30	9.06	9.77	0.82
Glucose, mg/dl	51.85	52.64	51.18	50.39	2.17
Glucagon, pg/ml	84.62	84.75	90.26	91.37	4.34
Insulin, μIU/ml	2.78	2.36	2.98	2.66	0.39
Plasma-PM[2]					
Triglyceride, mg/dl	1.91	4.14	2.75	2.55	0.46
NEFA, μeq/L	498.1	561.4	568.9	647.7	52.4
BHBA, mg/dl	12.41	11.38	10.88	12.02	1.03
Glucose, mg/dl	50.07	50.04	48.46	47.20	1.81
Glucagon, pg/ml	85.60	87.26	87.90	91.76	3.85
Insulin, μIU/ml	3.01	3.20	3.23	2.75	0.45
Cr Whole Blood,ng/ml	7.18	10.35	9.16	5.31	1.57
Cr Plasma, ng/ml	3.78	3.27	4.38	4.73	0.34

[1-3] see Table 2; [4]CrPr = chromium propionate; [5]Cr = effect of CrPr supplementation;[6]DC = dry ground corn;

[7]HMC = high-moisture corn; [8] Corn = effect of corn grain conservation method;

[9]Cr*Corn = interaction between CrPr supplementation and corn grain conservation method;

[10]Cr*Time = interaction of CrPr supplementation and time;

[11]Corn*Time = interaction of corn grain conservation method and time;

[12]Cr*Corn*Time = 3-way interaction among CrPr, corn, and time

Table 4b. Effects of CrPr supplementation and corn grain conservation method on postpartum plasma metabolites and hormones

Item	P-value					
	Cr[5]	Corn[8]	Cr *Corn[9]	Cr *Time[10]	Corn*Time[11]	Cr *Corn*Time[12]
Plasma-AM[2]						
Triglyceride, mg/dl	0.02	0.04	>0.001	0.45	0.01	0.14
NEFA, µeq/L	0.22	0.25	0.61	0.62	1.00	0.62
BHBA, mg/dl	0.92	0.69	0.73	0.09	0.94	0.73
Glucose, mg/dl	0.41	0.98	0.66	0.42	0.36	0.39
Glucagon, pg/ml	0.11	0.90	0.89	0.01	0.002	0.24
Insulin, µIU/ml	0.54	0.35	0.88	0.89	0.21	0.55
Plasma-PM[2]						
Triglyceride, mg/dl	0.36	0.02	0.006	0.41	0.02	0.01
NEFA, µeq/L	0.12	0.15	0.91	0.28	0.12	0.77
BHBA, mg/dl	0.64	0.94	0.42	0.75	0.17	0.27
Glucose, mg/dl	0.14	0.74	0.74	0.97	0.24	0.32
Glucagon, pg/ml	0.34	0.42	0.78	0.57	0.04	1.00
Insulin, µIU/ml	0.75	0.73	0.45	0.29	0.50	0.90
Cr Whole Blood,ng/ml	0.33	0.83	0.03	NA	NA	NA
Cr Plasma, ng/ml	0.002	0.80	0.17	NA	NA	NA

[1] = recorded daily; [2] = recorded weekly;[3] = recorded biweekly

[5]Cr = effect of CrPr supplementation;

[8] Corn = effect of corn grain conservation method

[9]Cr*Corn = interaction between CrPr supplementation and corn grain conservation method

[10]Cr*Time = interaction of CrPr supplementation and time

[11]Corn*Time = interaction of corn grain conservation method and time

[12]Cr*Corn*Time = 3-way interaction among CrPr, corn, and time

Table 5. Effects of CrPr and corn grain conservation method on glucose metabolism

	Control		CrPr[1]			P-value		
Item	DC[3]	HMC[4]	DC	HMC	SE	Cr[2]	Corn[5]	Cr *Corn[6]
Glucose								
AUC, mg.min/dl[7]	298.3	282.9	301.5	310.6	21.2	0.47	0.88	0.57
Max, mg/dL[8]	120.3	119.4	117.6	120.7	0.75	0.03	0.003	<.0001
CR, %/min	1.02	1.28	0.94	1.57	0.08	0.24	0.008	0.77
Basal, mg/dL	57.35	54.54	55.61	54.65	2.35	0.69	0.38	0.68
Insulin								
AUC, µIU.min/ml	253.4	349.2	349.8	295.5	42.4	0.59	0.62	0.08
Max, µIU/ml	83.00	108.24	108.56	89.50	13.56	0.78	0.81	0.11
CR, %/min	4.01	4.46	4.39	4.28	0.40	0.79	0.65	0.47
Basal, µIU/ml	5.54	5.29	7.29	4.79	1.20	0.57	0.21	0.31
NEFA								
AUC,µeq.min/L	-1484	-2455	-1492	-2132	504.0	0.73	0.10	0.69
Max, µeq/L	734.6	815.9	812.1	1187.7	117.0	0.03	0.03	0.18
Min, µeq/L	193.8	226.3	239.0	208.6	35.12	0.66	0.98	0.34
50, µeq/L	254.6	246.1	244.9	360.6	55.23	0.27	0.30	0.27
BHBA								
AUC, mg.min/dL	-42.19	-54.19	-50.58	-34.77	10.58	0.54	0.83	0.14
Max, mg/dL	13.02	13.67	14.79	14.58	1.72	0.31	0.70	0.93
Min, mg/dL	2.68	3.01	3.22	3.90	0.82	0.20	0.38	0.76
Basal, mg/dL	9.14	10.15	11.04	11.87	1.38	0.11	0.44	0.94
50, mg/dL	6.85	6.02	5.50	6.96	1.30	0.83	0.77	0.34

[1]CrPr = chromium propionate; [2]Cr = effect of Cr-Pr supplementation; [3]DC = dry ground corn; [4] HMC = high-moisture corn; [5] Corn = effect of corn grain conservation method; [6]Cr*Corn = interaction of CrPr and corn conservation method; [7]AUC = area under the curve; [8]Max = maximum concentration; [9]Min = minimum concentration; [10]Basal = average concentration of ten minutes and one minute prior to infusion; [11] 50 = concentration at 50 min after glucose infusion

Table 6. Effects of CrPr supplementation and corn grain conservation method on postpartum liver glycogen and triglyceride

Item	Control		CrPr[1]		SE	P-value					
	DC[3]	HMC[4]	DC	HMC		Cr[2]	Corn[5]	Cr*Corn[6]	Cr*Wk[7]	Corn*Wk[8]	Cr*Corn *Wk[9]
Glycogen, %	2.86	2.91	3.68	3.22	0.41	0.06	0.45	0.37	0.72	0.51	0.70
Triglyceride, %	2.70	2.80	2.37	3.72	0.57	0.58	0.18	0.28	0.31	0.32	0.70
Δ Glycogen -7 to 14, %	0.53	0.86	0.95	1.66	0.58	0.15	0.22	0.65	NA	NA	NA
Δ TG -7 to 14, %	1.29	2.71	2.75	4.27	0.71	0.04	0.04	0.95	NA	NA	NA

[1]CrPr = chromium propionate

[2]Cr = effect of CrPr supplementation

[3]DC = dry ground corn

[4]HMC = high-moisture corn

[5]Corn = effect of corn grain conservation method

[6]Cr*Corn = interaction between CrPr supplementation and corn grain conservation method

[7]Cr*Time = interaction of CrPr supplementation and time

[8]Corn*Time = interaction of corn grain conservation method and time

[9]Cr*Corn*Time = 3-way interaction among CrPr, corn, and time

Table 7a. Carryover effects of CrPr supplementation and corn grain conservation method on peak productivity

Item	Control		CrPr[4]		
	DC[6]	HMC[7]	DC	HMC	SE
Milk Yield, kg/d[1]	50.7	53.2	54.0	56.8	2.20
FCM (3.5%), kg/d[2]	46.87	49.91	51.13	53.71	2.30
SCM, kg/d[2]	42.93	45.54	46.06	48.54	1.99
ECM, kg/d[2]	47.02	49.49	50.90	52.45	2.15
Fat, kg/d[2]	1.56	1.70	1.72	1.83	0.10
Protein, kg/d[2]	1.44	1.45	1.51	1.53	0.06
Lactose, kg/d[2]	2.40	2.55	2.52	2.68	0.11
SNF, kg/d[2]	4.23	4.43	4.42	4.66	0.18
Cumulative Milk, kg	2882	3022	3061	3222	127
Fat %	3.14	3.27	3.22	3.28	0.14
Protein %	2.88	2.78	2.81	2.75	0.04
Lactose %	4.76	4.86	4.65	4.79	0.05
SNF %	8.35	8.35	8.17	8.24	0.07
MUN, mg/dL[2]	15.22	16.14	15.30	16.67	0.54
SCC, x 1000[2]	12.57	16.18	12.33	16.03	[13]
DMI, kg/d[1]	24.1	25.2	24.7	25.7	0.62
Cumulative DMI, kg	1366	1430	1406	1459	35.0
FCE[2]	2.01	2.03	2.11	2.20	0.09
BW, kg[3]	642.4	647.8	650.2	681.3	18.1
BCS[3]	2.23	2.05	2.30	2.19	0.18

[1-3]see Table 2; [4]CrPr = chromium propionate; [6]DC = dry ground corn; [7]HMC = high-moisture corn;

[13]= data was transformed for analysis [xtrans = logx]. LS Means are back-transformed. 95% confidence intervals are (all x 1000) Con/DC: 42.50 – 10.68 U/L; Con/HMC: 63.82-16.03 U/L; CrPr/DC: 49.81 – 12.51 U/L; CrPr/HMC; 50.45 – 12.67 U/L.

Table 7b. Carryover effects of CrPr supplementation and corn grain conservation method on peak productivity

	P-value					
Item	Cr[5]	Corn[8]	Cr *Corn[9]	Cr *Time[10]	Corn*Time[11]	Cr *Corn*Time[12]
Milk Yield, kg/d[1]	0.09	0.18	0.93	0.95	0.38	0.11
FCM (3.5%), kg/d[2]	0.11	0.20	0.91	0.81	>0.001	0.07
SCM, kg/d[2]	0.11	0.18	0.97	0.84	0.001	0.05
ECM, kg/d[2]	0.09	0.32	0.82	0.46	>0.001	0.04
Fat, kg/d[2]	0.11	0.18	0.87	0.79	>0.001	0.09
Protein, kg/d[2]	0.15	0.80	0.94	0.70	0.02	0.01
Lactose, kg/d[2]	0.22	0.14	0.95	0.88	0.12	0.06
SNF, kg/d[2]	0.20	0.19	0.90	0.94	0.27	0.12
Cumulative Milk, kg	0.10	0.18	0.92	NA	NA	NA
Fat %	0.74	0.42	0.78	0.96	0.01	0.34
Protein %	0.19	0.05	0.70	0.56	0.87	0.09
Lactose %	0.06	0.02	0.68	0.34	0.44	0.78
SNF %	0.04	0.60	0.58	0.13	0.47	0.11
MUN, mg/dL[2]	0.54	0.03	0.66	0.97	0.20	0.92
SCC, x 1000[2]	0.97	0.56	0.99	0.35	0.37	0.01
DMI, kg/d[1]	0.33	0.09	0.85	0.76	0.02	0.06
Cumulative DMI, kg	0.31	0.09	0.87	NA	NA	NA
FCE[2]	0.11	0.53	0.69	0.66	0.01	0.21
BW, kg[3]	0.20	0.27	0.41	0.15	0.40	0.05
BCS[3]	0.42	0.31	0.88	0.96	0.12	0.75

[1-3]see Table 2; [5]Cr = effect of CrPr supplementation; [8] Corn = effect of corn grain conservation method; [9]Cr*Corn = interaction betweenCrPr and corn conservation method; [10]Cr*Time = interaction of CrPr supplementation and time; [11]Corn*Time = interaction of corn grain conservation method and time; [12]Cr*Corn*Time = 3-way interaction among CrPr, corn, and time

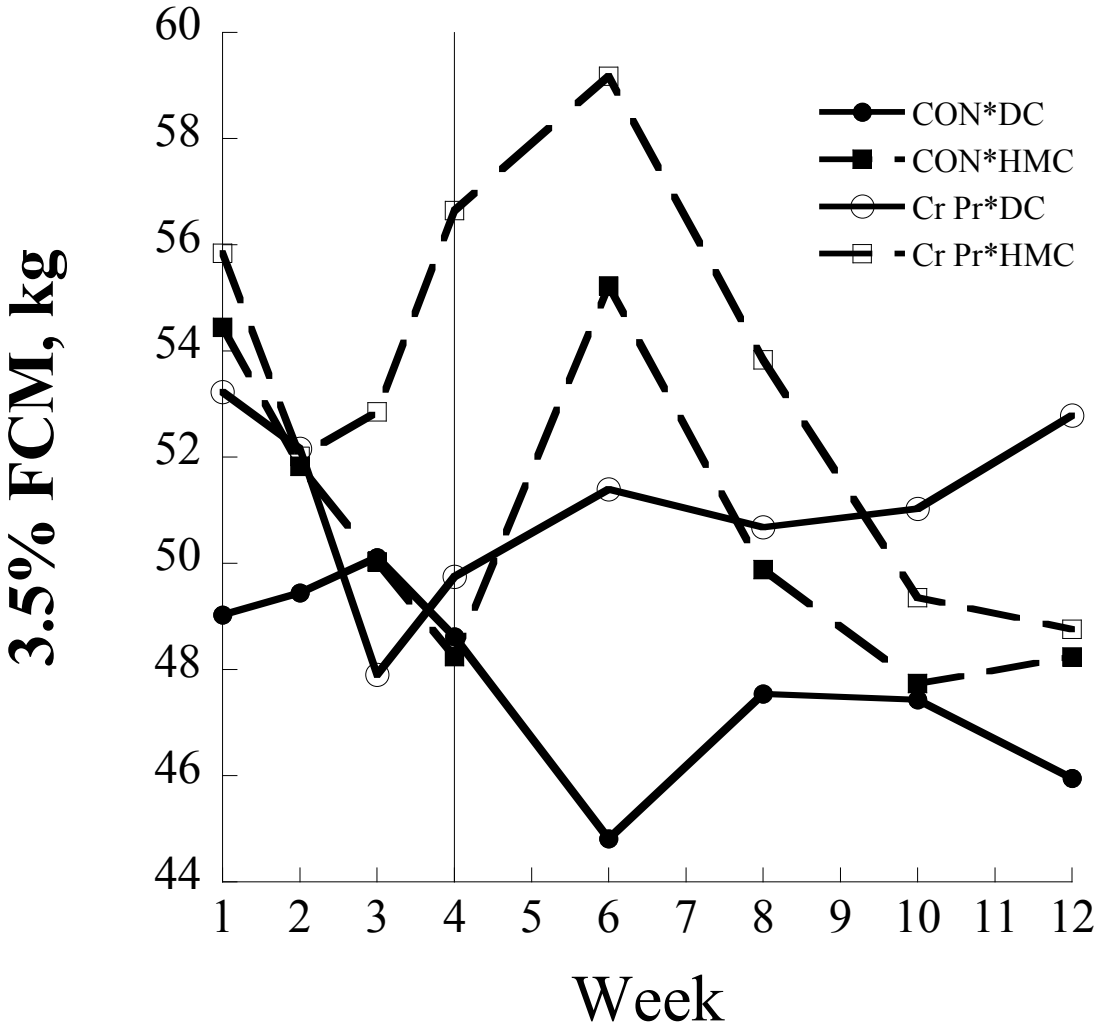

Figure 1. Interaction between chromium and corn over time on 3.5% fat-corrected milk from week 1 to 12. The vertical line is the end of the treatment period at 28±3 d PP.

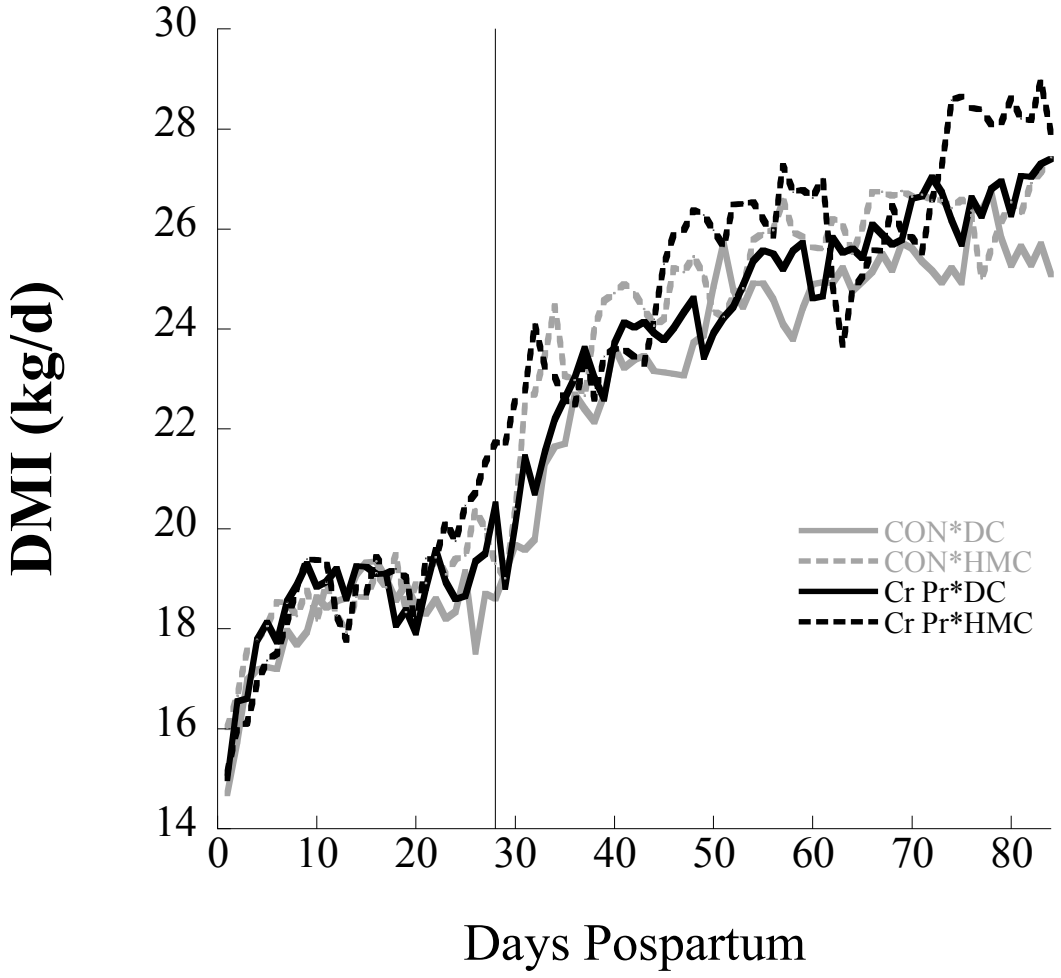

Figure 2. Interaction of chromium and corn over time on DMI from 0-84 days. The vertical line delineates the end of the treatment periodat 28±3 d PP.

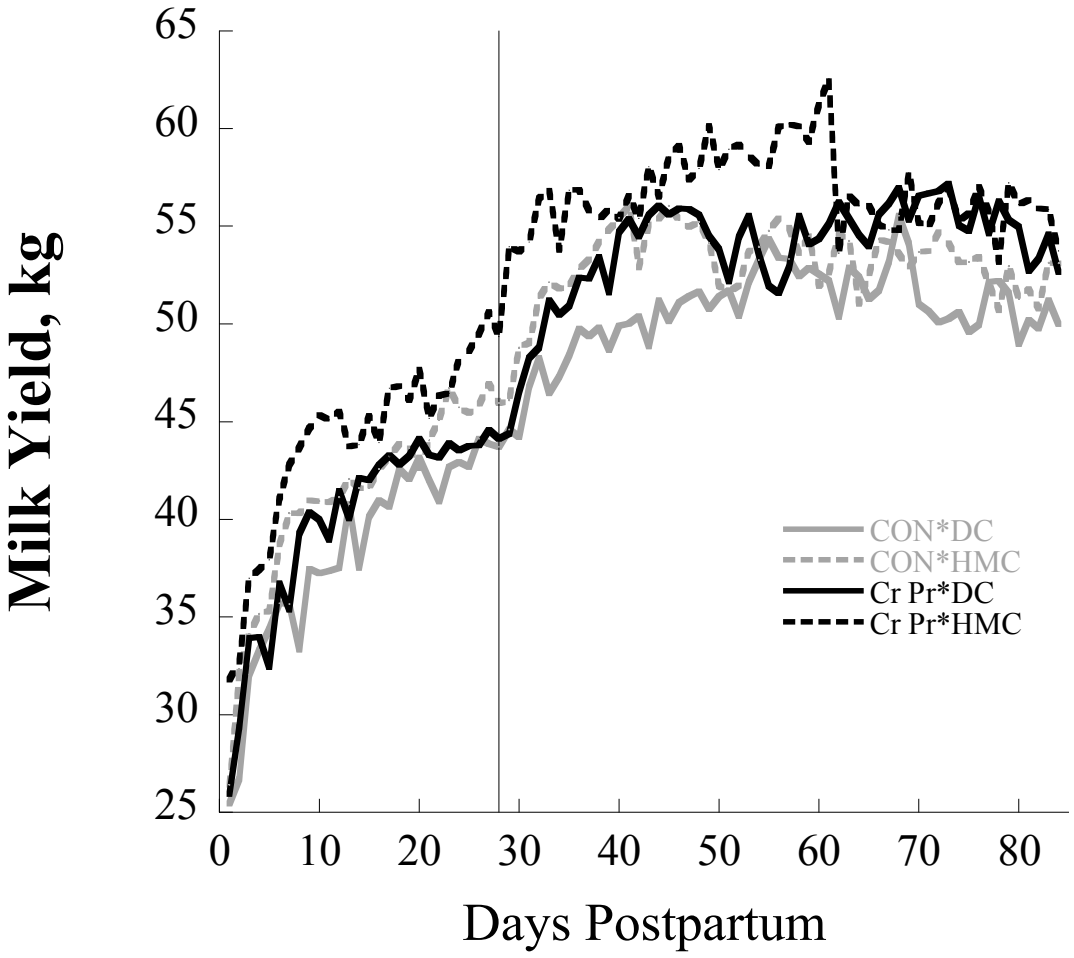

Figure 3. Interaction of chromium and corn over time on daily milk yield from 0-84 days. The vertical line delineates the end of the treatment periodat 28±3 d PP.

CHAPTER 3

EPILOGUE

There are still no concrete answers regarding the mechanism of action of chromium for its effects on metabolism and milk yield of dairy cows. Practical application is limited by our minimal understanding.

In this study, Cr had the most significant effect after the treatment period ended between 28 and 84 d PP. Fat-corrected milk peaked at 42 d PP with the CrPr/HMC diet and decreased to the level of the Con/DC diet by 56 d PP. Continuing treatment past 28 d PP might have extended the effects of treatment on milk yield and DMI. Nikkhah et al. (2011) began Cr treatment at 38±6 d PP. Although they reported increased DMI, there was no increase in milk yield or FCM. The Cr treatment was hypothesized to treat heat stress, which added a variable that may have influenced the results. The treatment may have begun too late as well. Our peak production was 42 d postpartum when we observed the greatest effects of treatment on milk yield. Beginning Cr supplementation just before peak lactation may not allowed enough time for Cr to effect milk yield. Also, some cows began treatment after 42 days and likely missed the benefit of Cr supplementation. Heat stressed cows receiving Cr starting 120-130 d PP, however, had increased DMI and milk yield (Al-Saiady et al., 2004).

Sumner et al., 2007 showed the potential benefit of long-term Cr supplementation. In a study consisting of four periods, in which heifers were randomly assigned to three periods for which to receive Cr and one period as a control. The control cows of the fourth period that received Cr for the first three periods had lower basal insulin than the other three control groups. If Cr did not

elicit an effect by supplementing starting at 28 d PP, it would demonstrate the benefit of long-term supplementation.

The relationship between Cr and starch source should also be considered further. One study fed a high grain diet of high-moisture corn ration supplemented with Cr to young rams (Sano et al., 1997). The high-moisture corn increased propionate production (measured by isotope infusion) in rams. Chromium supplementation increased conversion of propionate to glucose. This might be a mechanism by which Cr and starch fermentation interacted in this experiment and should be evaluated further.

Determination of Cr excretion by total collection might provide useful information. Chromium concentrations of urine and feces have not been measured in dairy cows. The popular mechanism suggests that four Cr ions are incorporated into a holo-oligopeptide and following dephosphorylation of the insulin receptor the entire holoenzyme is excreted in the urine (Chen et al., 2011). Unabsorbed Cr is excreted in the feces (Starich and Blincoe, 1983). In addition, Kozlovsky et al. (1986) show that Cr excretion in the urine is greater with a high carbohydrate diet in mice. A ration of HMC may increase Cr excretion in the urine compared to a DC ration. Chromium supplementation may interact with corn source to affect Cr excretion.

Although results are inconsistent and little is known about the mechanism of Cr action, application of CrPr in a dairy cow diet may be advantageous. There is enough evidence of improved production and potential health benefits to merit the minimal cost of the Cr supplement.

APPENDIX

APPENDIX

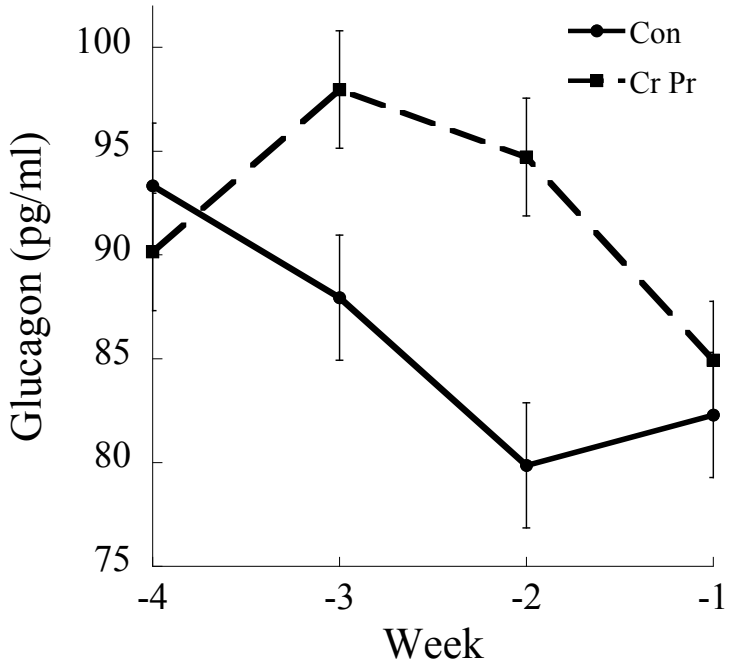

Figure A1. Effects of CrPr on prepartum glucagon over time.

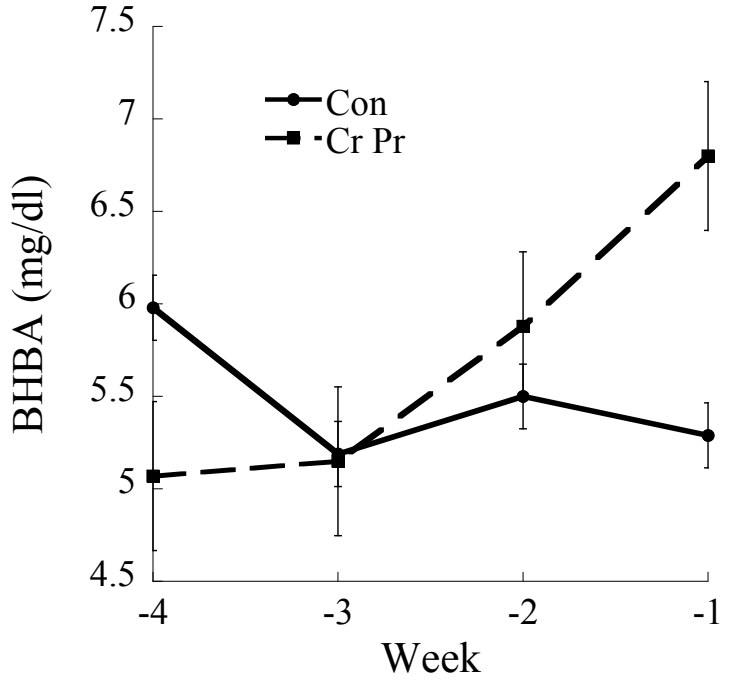

Figure A2. Effects of CrPr on prepartum BHBA over time.

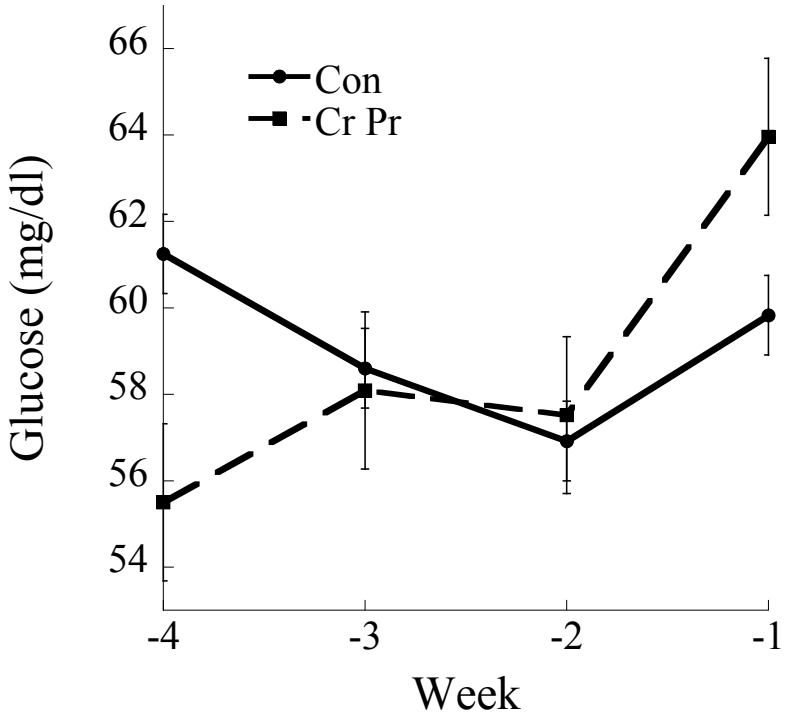

Figure A3. Effects of CrPr on prepartum glucose over time.

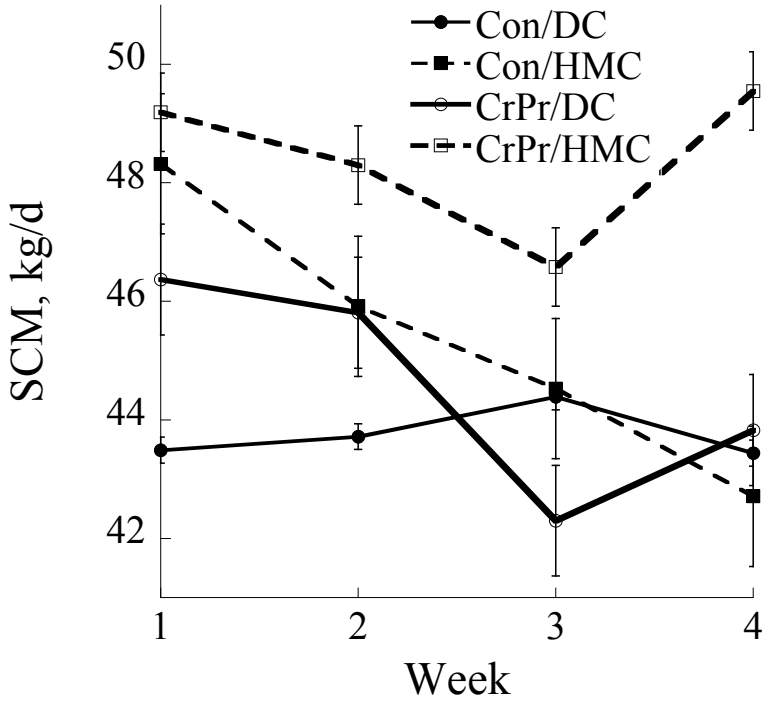

Figure A4. Interaction of CrPr and corn source over time on postpartum SCM.

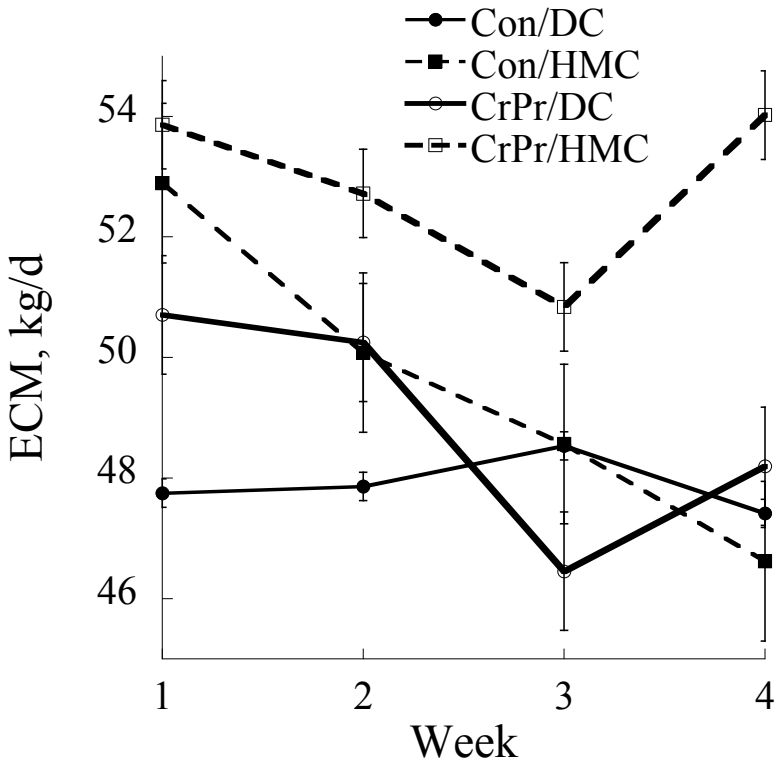

Figure A5. Interaction of CrPr and corn source over time on postpartum ECM.

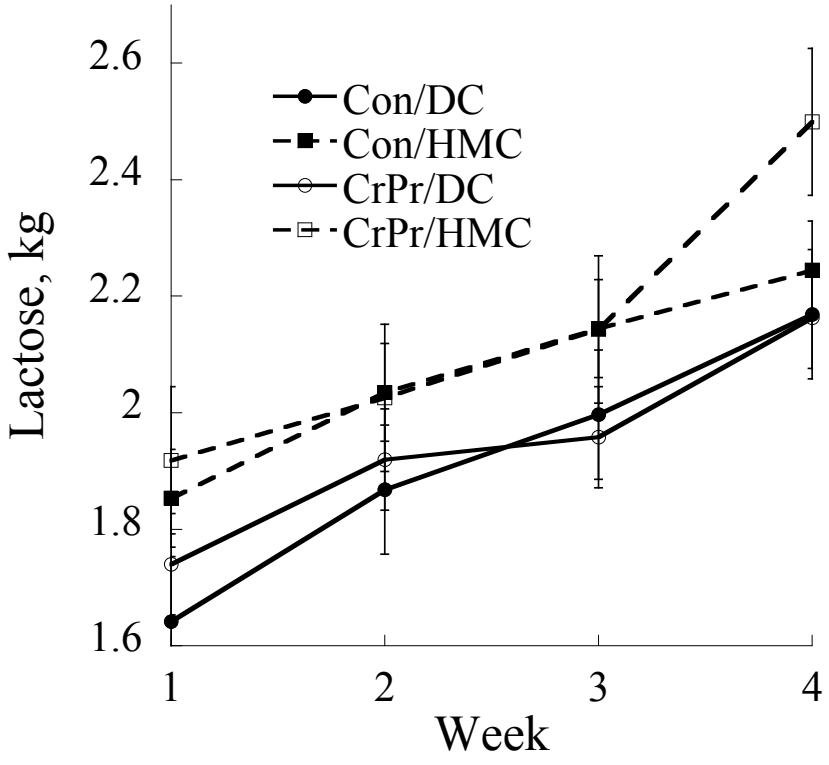

Figure A6. Interaction of CrPr and corn source over time on postpartum yield of lactose.

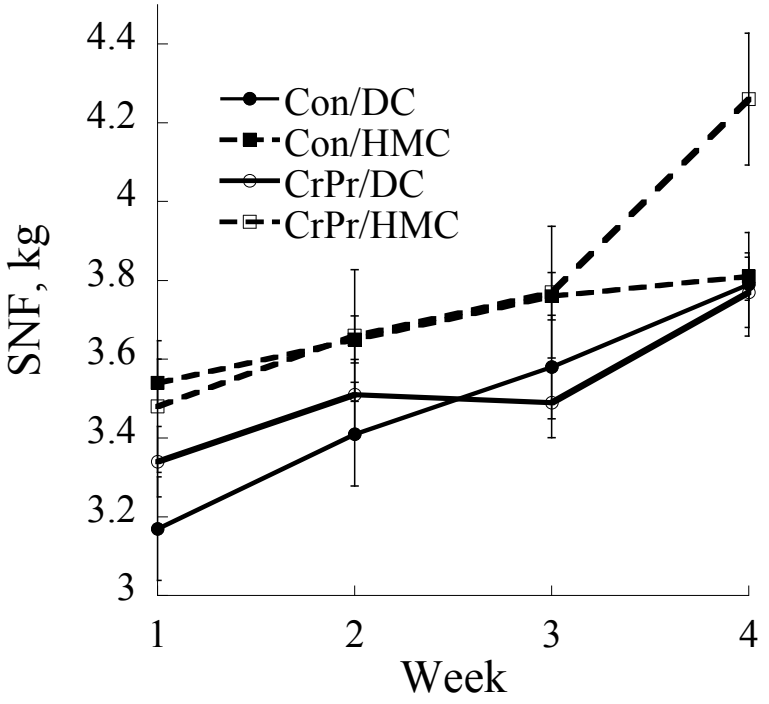

Figure A7. Interaction of CrPr and corn source over time on postpartum SNF.

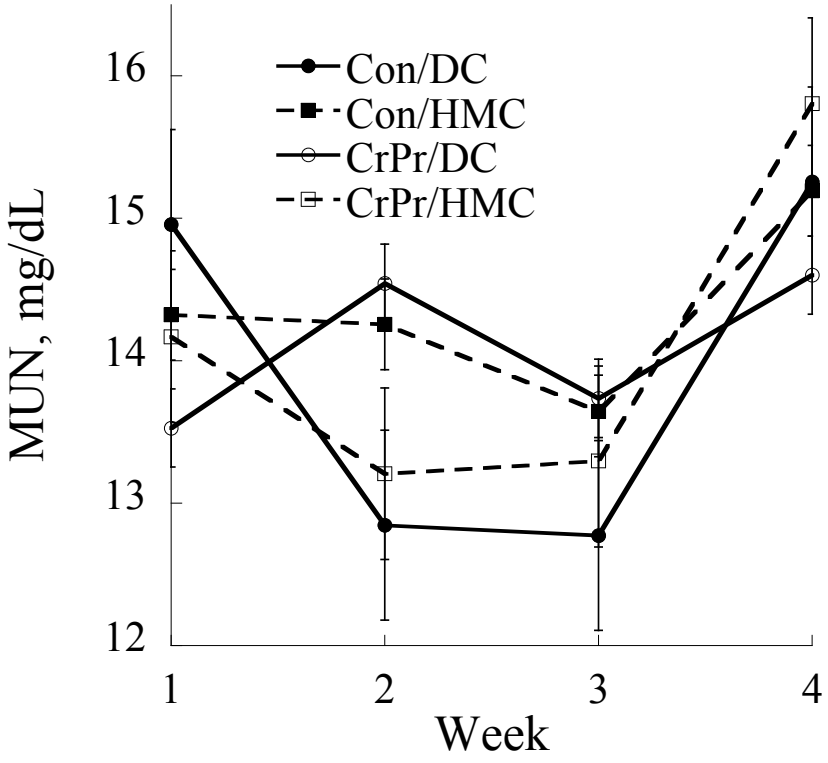

Figure A8. Interaction of CrPr and corn source over time on postpartum MUN.

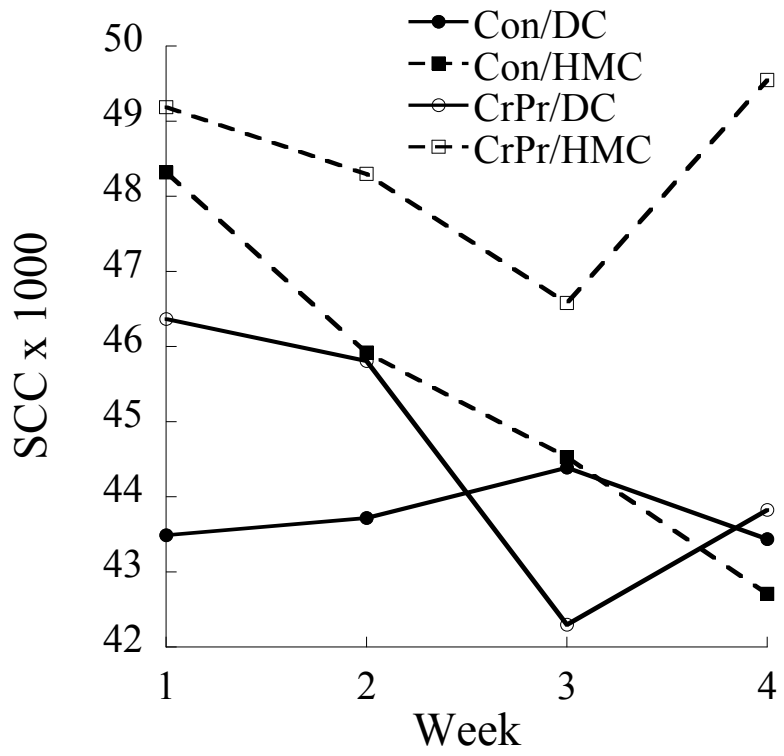

Figure A9. Interaction of CrPr and corn source over time on postpartum SCC.

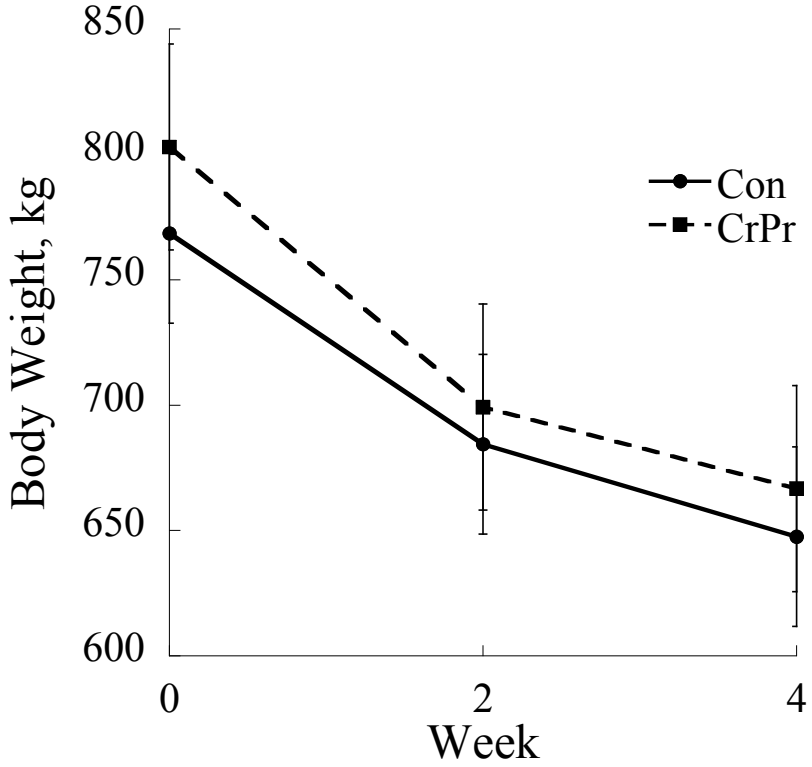

Figure A10. Effects of CrPr on postpartum BW over time.

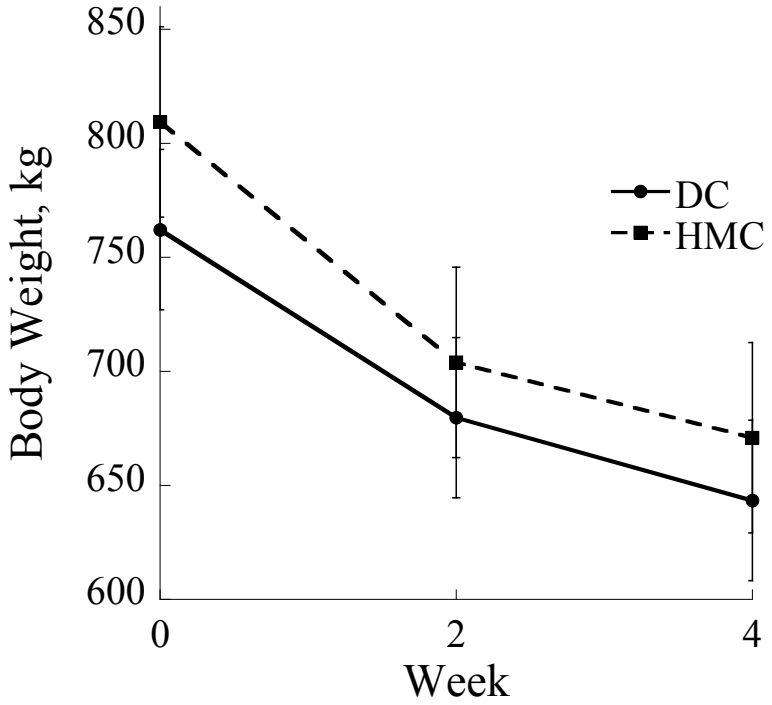

Figure A11. Effects of corn source on postpartum BW over time.

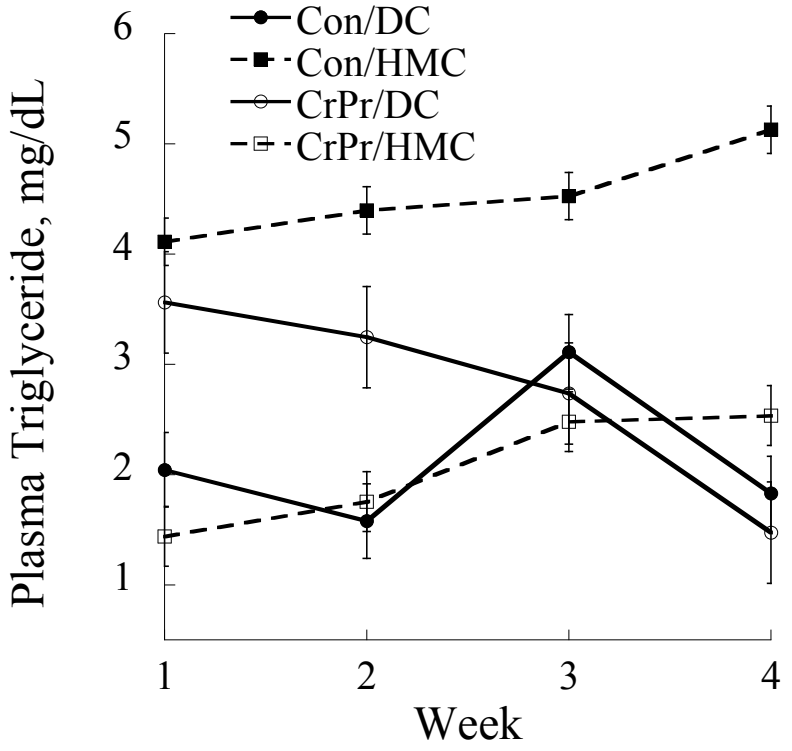

Figure A12. Interaction of CrPr and corn source over time on postpartum plasma TG before feeding.

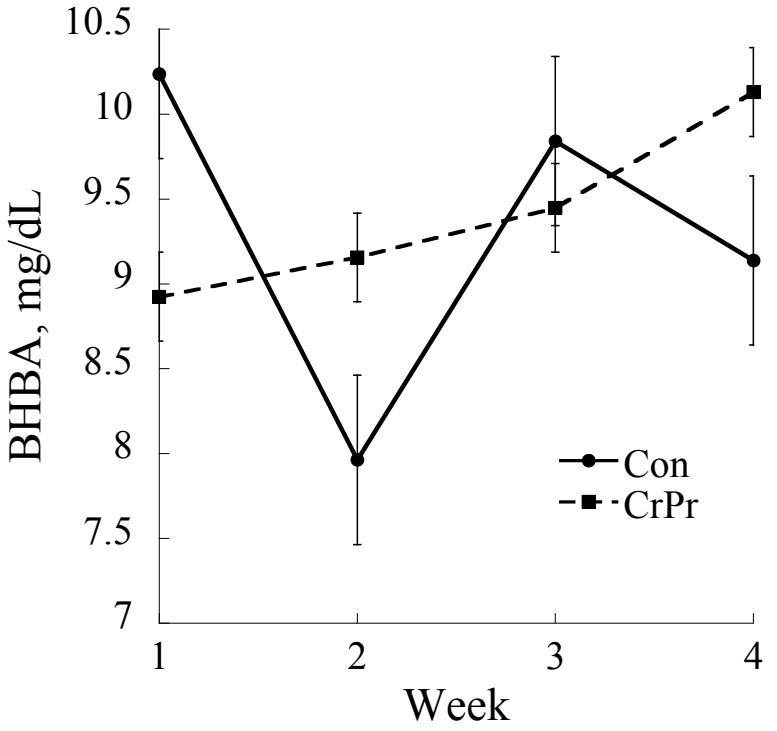

Figure A13. Effects of CrPr over time on postpartum plasma BHBA before feeding.

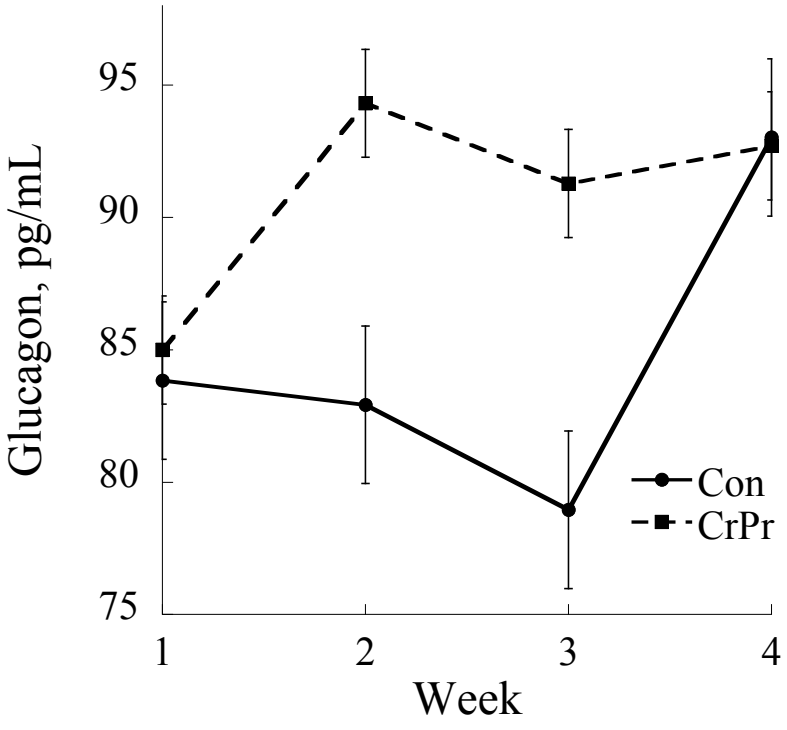

Figure A14. Effects of CrPr over time on postpartum plasma glucagon before feeding.

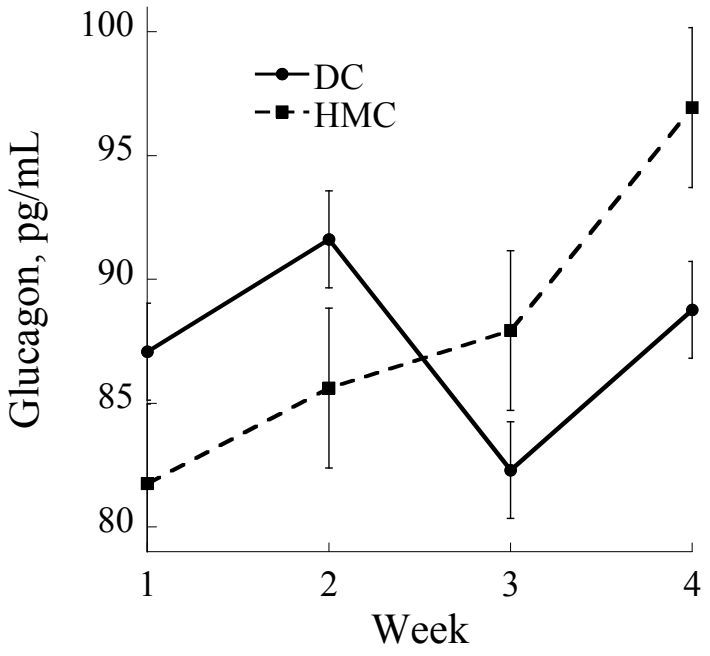

Figure A15. Effects of corn source over time on postpartum plasma glucagon before feeding.

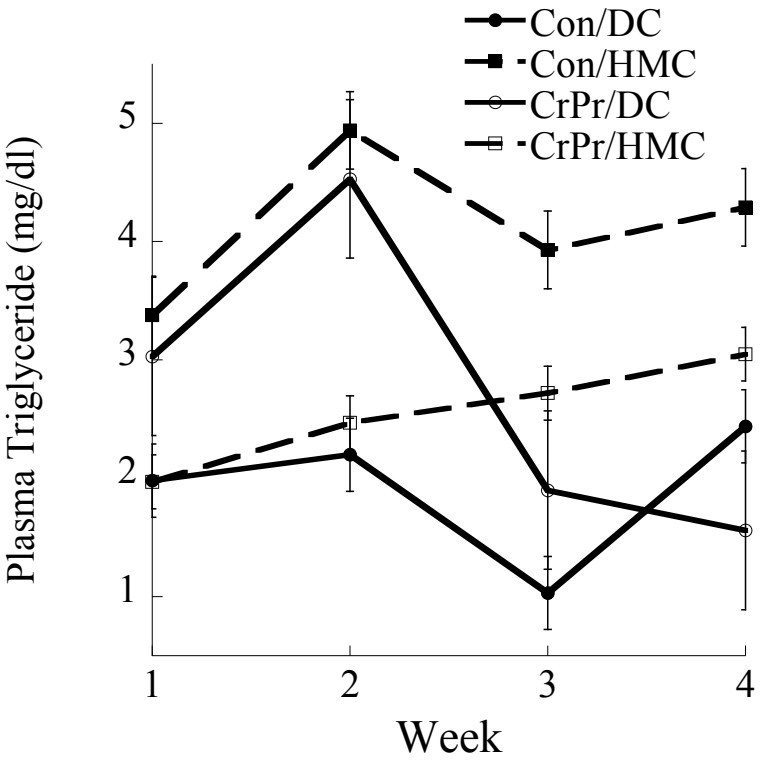

Figure A16. Interaction of CrPr and corn source over time on postpartum plasma TG after feeding.

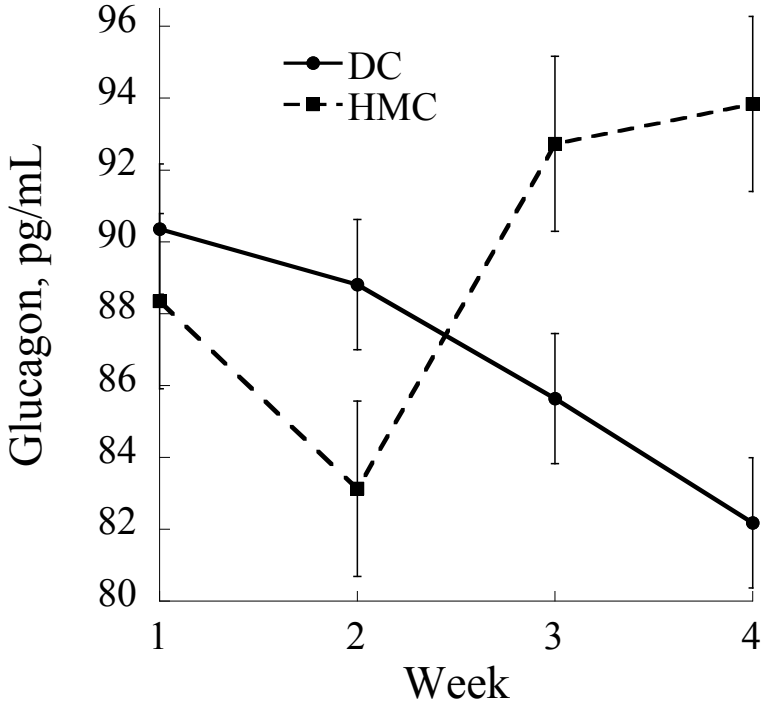

Figure A17. Effect of corn source over time on postpartum plasma glucagon after feeding.

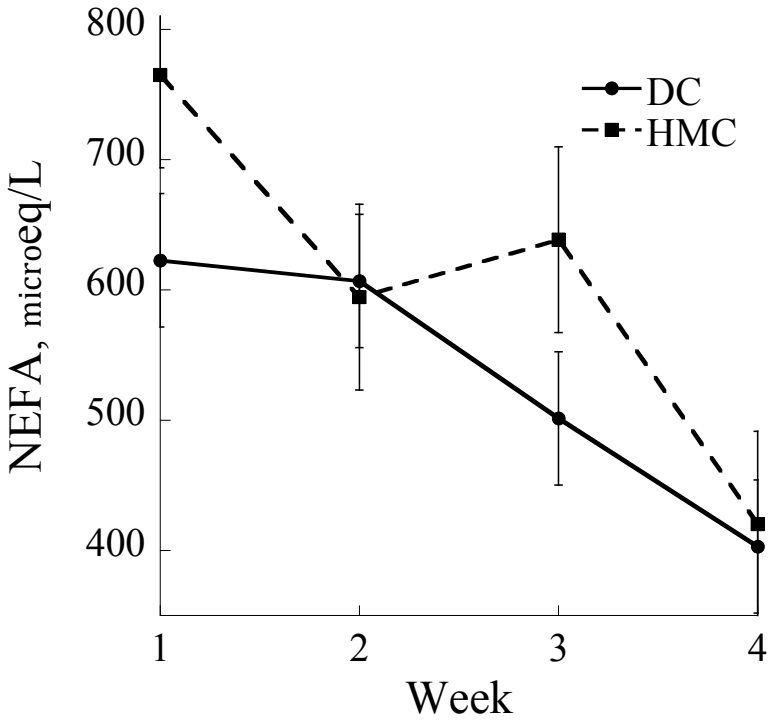

Figure A18. Effect of corn source over time on postpartum plasma NEFA after feeding.

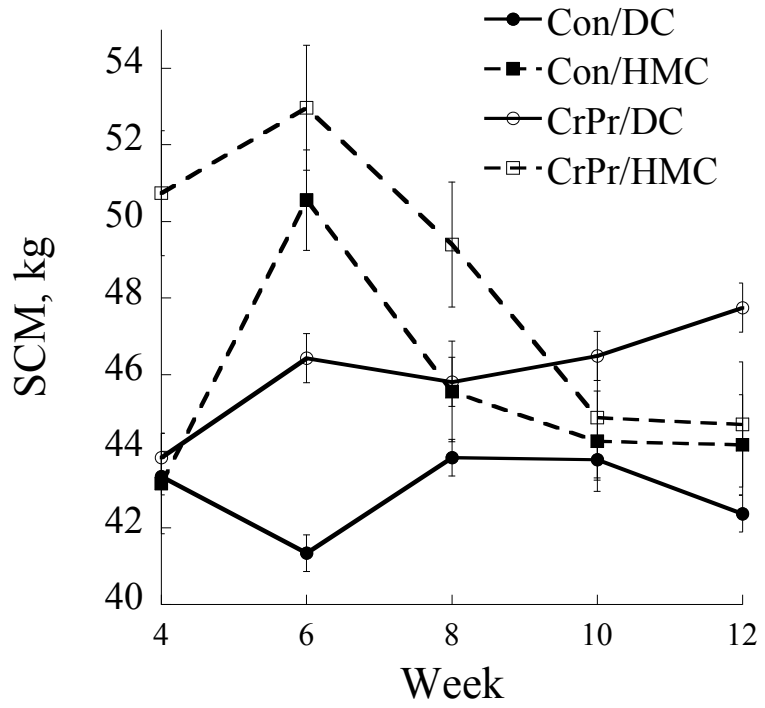

Figure A19. Interaction of CrPr and corn source over time on SCM after treatment ceased.

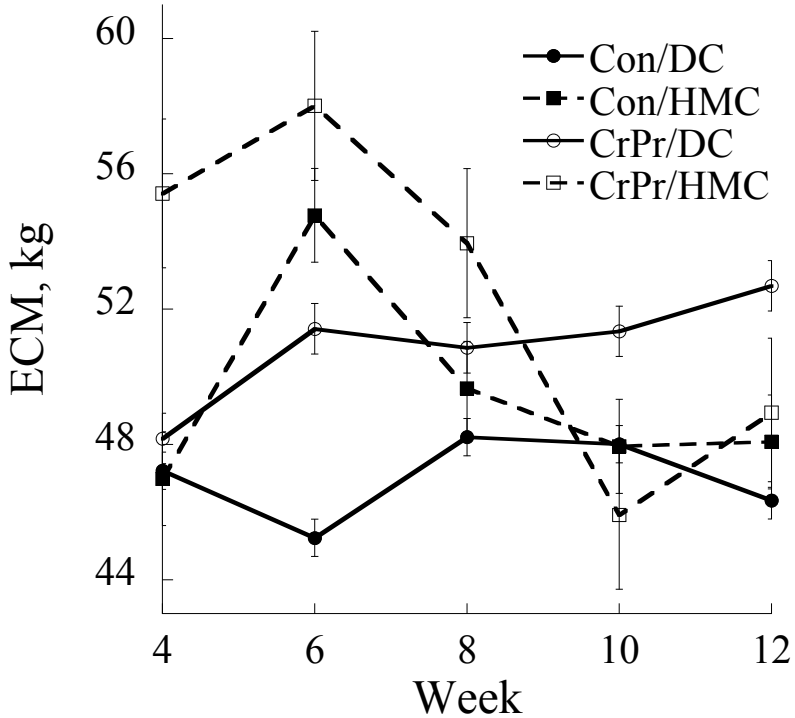

Figure A20. Interaction of CrPr and corn source over time on ECM after treatment ceased.

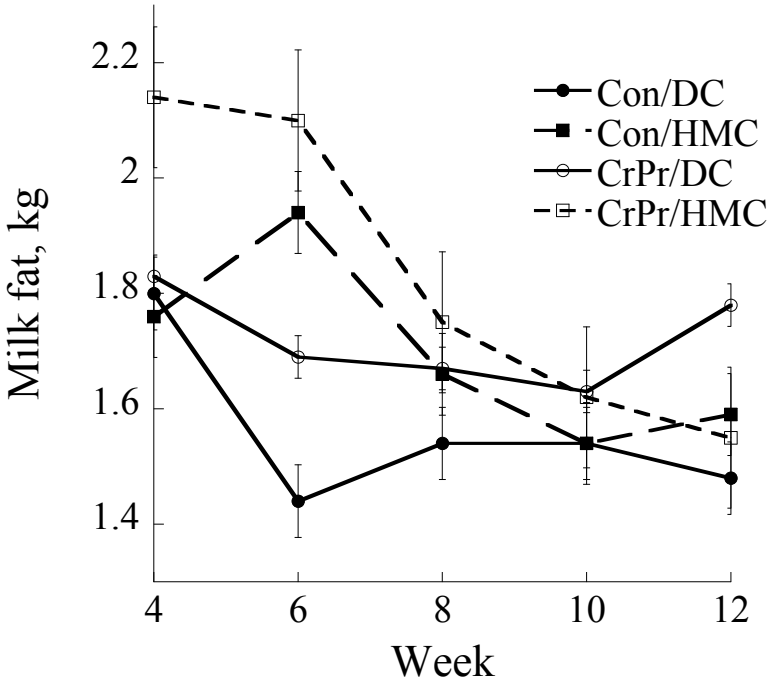

Figure A21. Interaction of CrPr and corn source over time on yield of milk fat after treatment ceased.

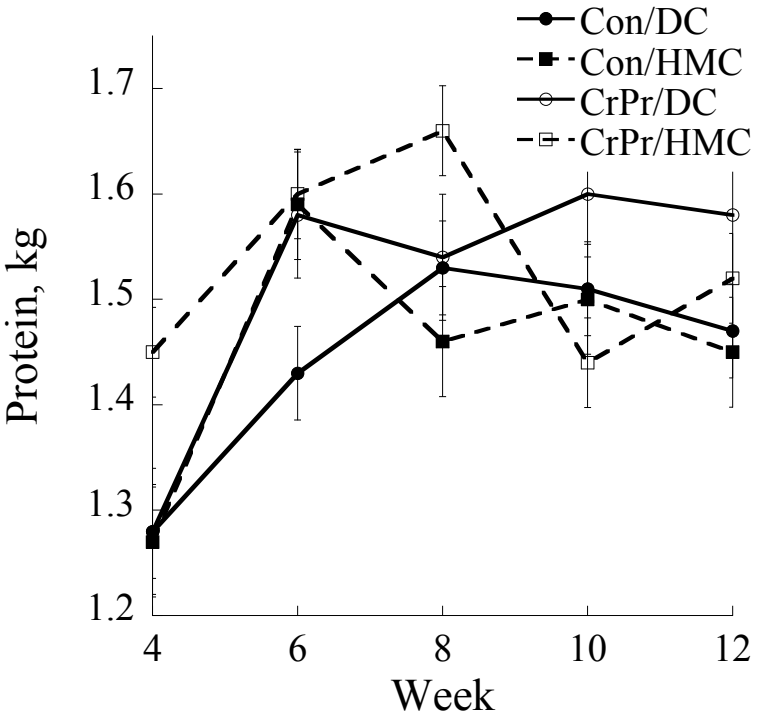

Figure A22. Interaction of CrPr and corn source over time on yield of milk protein after treatment ceased.

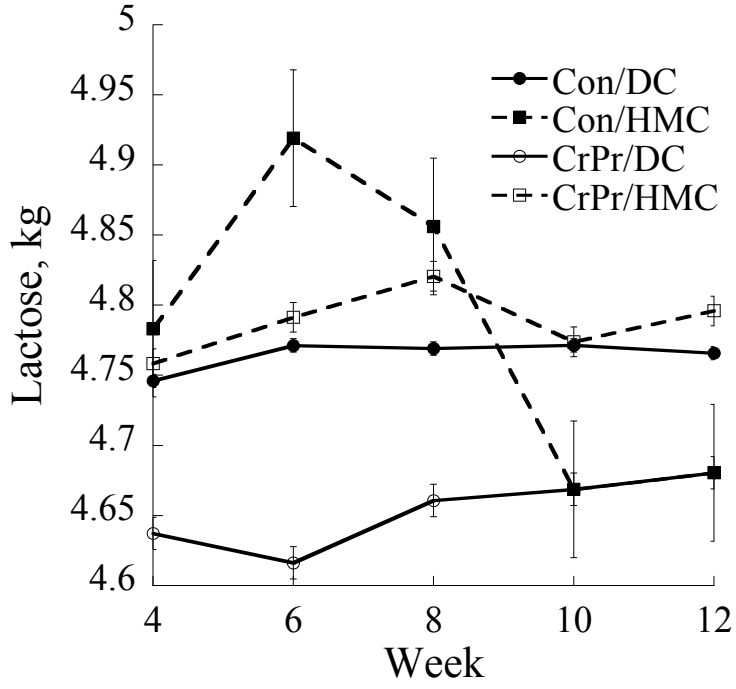

Figure A23. Interaction of CrPr and corn source over time on yield of milk lactose after treatment ceased.

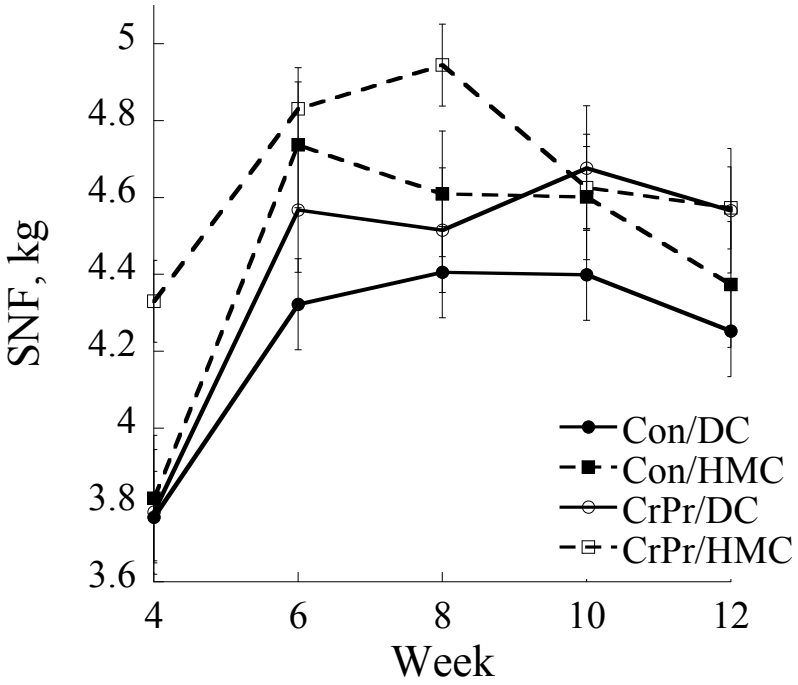

Figure A24. Interaction of CrPr and corn source over time on yield of milk solids non-fat after treatment ceased.

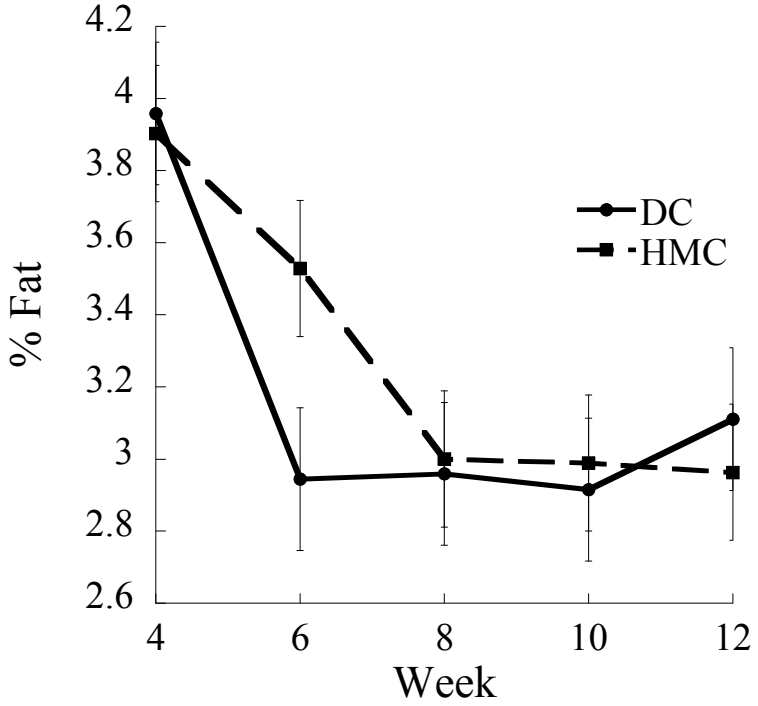

Figure A25. Interaction of corn source over time on percentage of milk fat after treatment ceased.

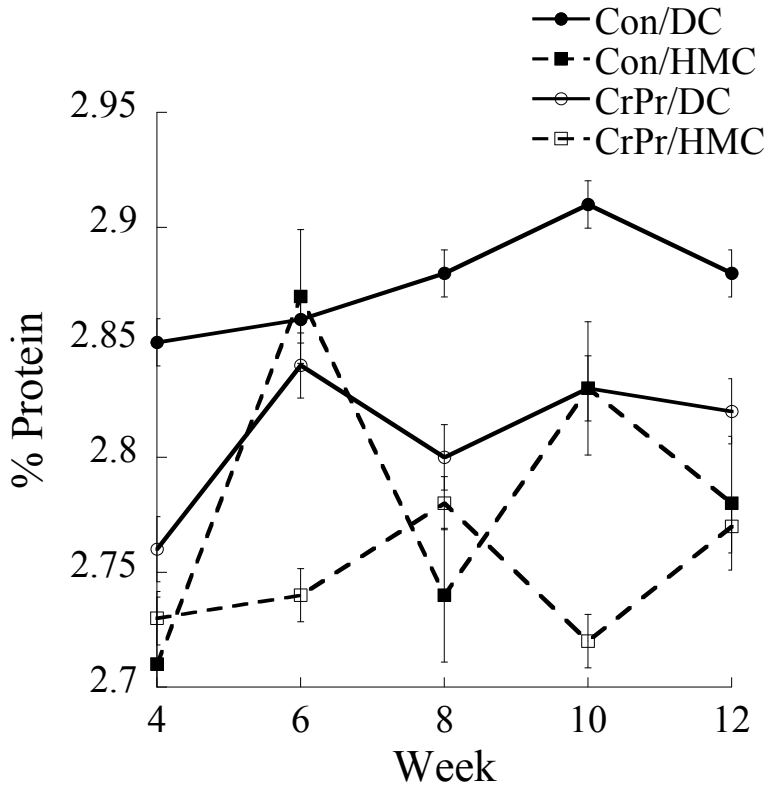

Figure A26. Interaction of CrPr and corn source over time on percentage of milk protein after treatment ceased.

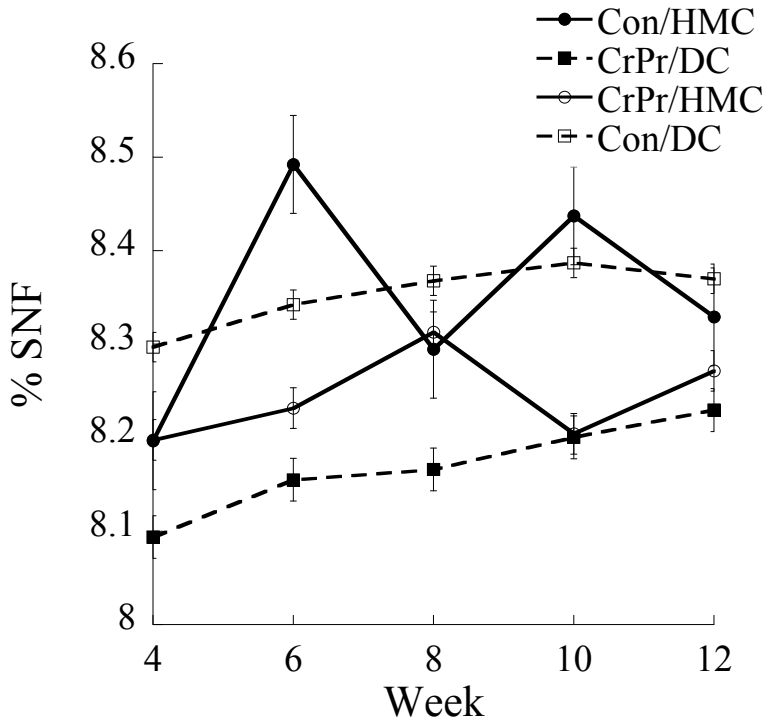

Figure A27. Interaction of CrPr and corn source over time on percentage of milk SNF after treatment ceased.

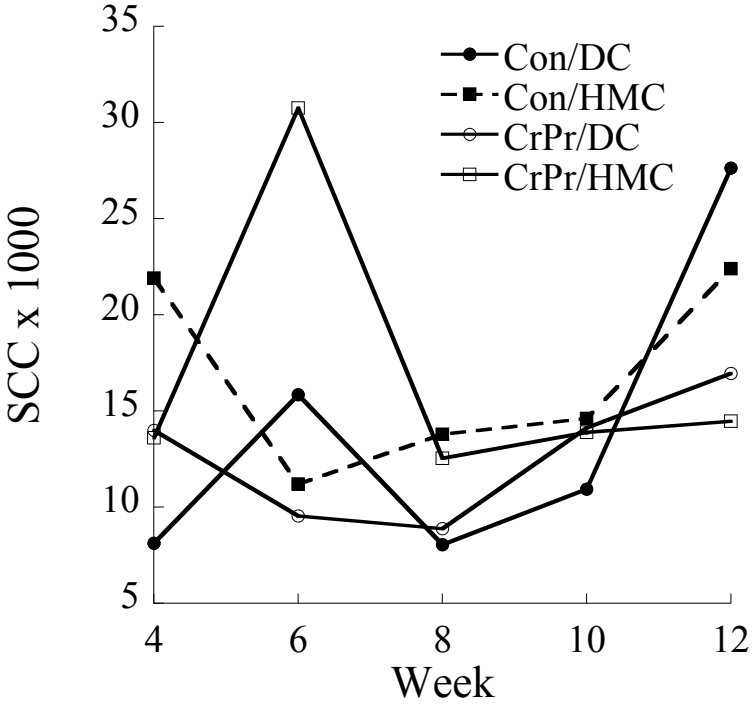

Figure A28. Interaction of CrPr and corn source over time on SCC after treatment ceased.

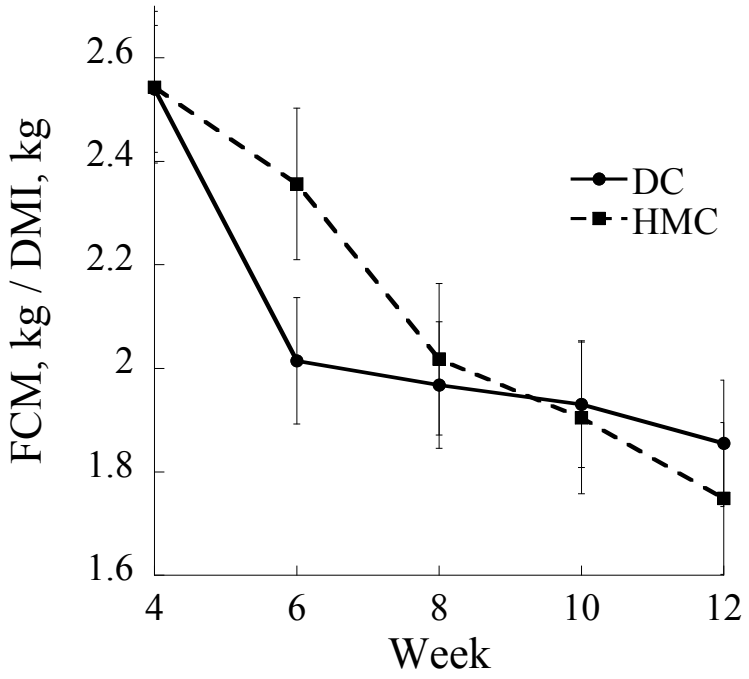

Figure A29. Effect of corn source over time on feed conversion efficiency after treatment ceased.

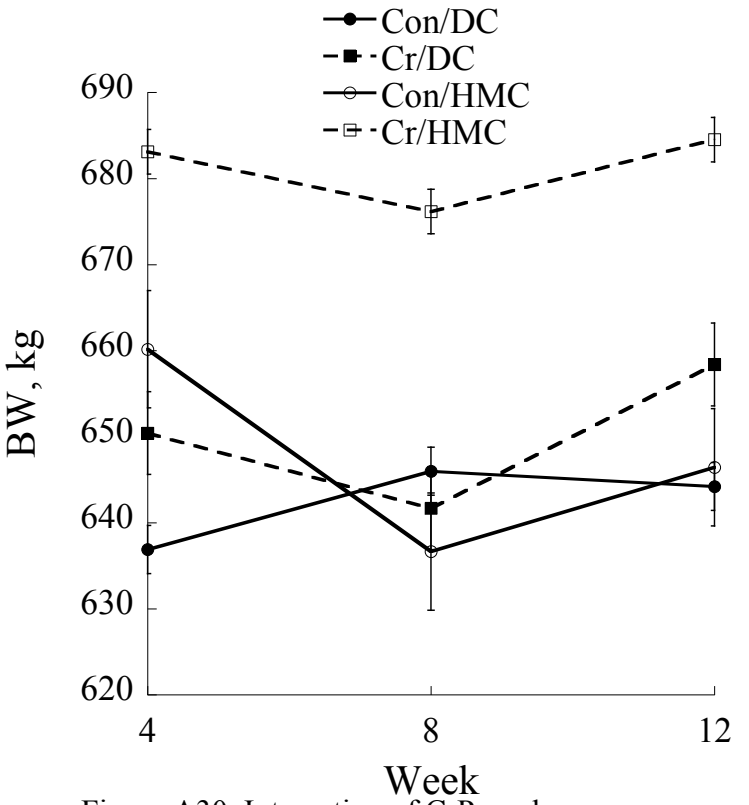

Figure A30. Interaction of CrPr and corn source over time on body weight after treatment ceased.

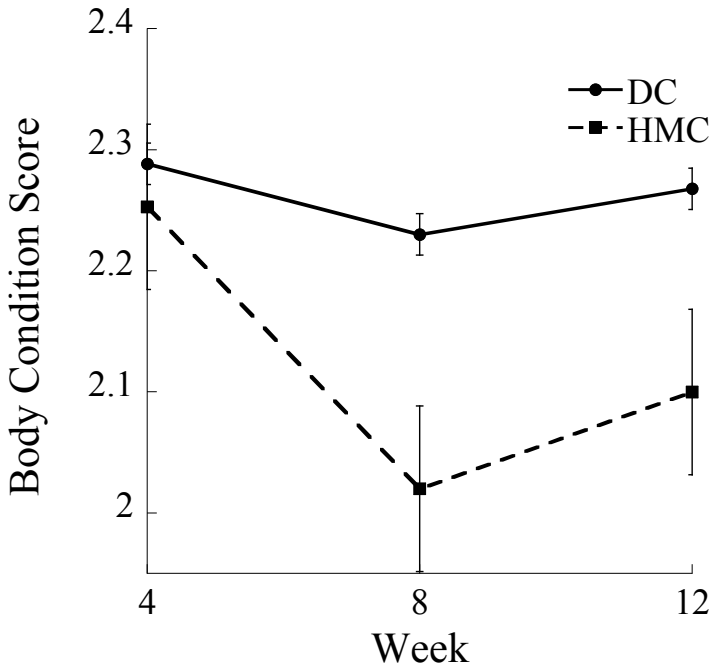

Figure A31. Effects of corn source over time on body condition score after treatment ceased.

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