EFFECT OF INCREASING RUMINAL PROPIONATE ON FEED INTAKE AND GLUCOSE METABOLISM IN STEERS FED A FINISHING DIET

By

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Abstract: Two experiments were conducted to investigate the effects of increased propionate supply to steers fed a feedlot finishing diet on dry matter intake, feeding behavior, glucose clearance rate, insulin response and hepatic gene expression. In experiment 1, Holstein steers (n = 15) were allocated by body weight to receive: 0 g/d Ca propionate (CON), 100 g/d (LOW), or 300 g/d (HIGH) with a finishing diet, ad libitum. Blood samples were collected on d 0, 7, and 21, and BW recorded on d 0, 14, and 28. A glucose tolerance test was conducted on d 14 and 28 of the trial. Liver biopsies were collected for gene expression. Blood samples were analyzed for glucose, lactate, NEFA and insulin concentrations. The CON treatment had greater (P < 0.01) DMI than LOW and HIGH. Glucose concentrations tended (P = 0.09) to be higher on d 21 than d 0 and 7. Nonesterified fatty acid concentrations were lower (P = 0.05) for CON than other treatments, and greater (P = 0.002) on d 0 than d 7 and 21. HIGH had greater insulin response than other treatments (P = 0.02). There was no treatment ($P \geq 0.16$) or day effect ($P \geq 0.36$) on glucose peak, plateau, or clearance rate. HIGH had greater (P = 0.05) hepatic expression of *SLC16A1*. In experiment 2, ruminally-cannulated Holstein steers (n = 6) were randomly assigned to the same treatments, describes in experiment 1, in a 3 × 6 Latin rectangle to be administered directly into the rumen. Weekly blood samples and body weight were collected and single glucose tolerance test and liver biopsies were conducted similarly to experiment 1. Additionally, rumen fluid samples were collected. All samples were analyzed the same as experiment 1. Dry matter intake, meal size, and number of meals per day was decreased (P < 0.049) in HIGH steers. There was no treatment effect ($P \geq 0.13$) on weekly body weight, plasma glucose, NEFA, lactate, or insulin, rumen fluid lactate or pH, or glucose clearance peak, plateau and rate. These data indicate that increased propionate may decrease DMI and alter feeding behavior.

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CHAPTER I

REVIEW OF LITERATURE

INTRODUCTION

It is well known that glucose is one of the primary energy sources for most tissues. Ruminants rely on gluconeogenesis to maintain blood glucose levels in both fed and fasted states (Fahey Jr and Berger, 1993). Volatile fatty acids (VFA) are estimated to provide 60-80% of the metabolizable energy intake for ruminants (Bergman, 1990). The microbial fermentation of ingested carbohydrates to VFA in the rumen makes the ruminant dependent on acetate, propionate, and butyrate for energy, and propionate is the predominant glucose precursor. Propionate flux to the liver helps to initiate gluconeogenesis and to maintain blood glucose homeostasis (Aschenbach et al., 2010); however, propionate production is dependent on diet composition (Bauman et al., 1971). Ruminants that receive a high concentrate diet tend to have a greater propionate production in the rumen (Sutton et al., 2003).

Feed intake in ruminants can be controlled through many different variables, from the physical aspects of the diet such as fiber and starch content or metabolic response to the diet such as volatile fatty acids produced in the rumen to oxidation of fuels by the liver. The hepatic oxidation theory has been used to describe the role of the liver in feed

intake in ruminants. The overall reliance of ruminants on gluconeogenesis to keep blood glucose homeostasis leads to the need for a greater understanding of the relationship between feed intake and the resulting glucose production.

VFA PRODUCTION IN THE RUMEN

Volatile fatty acids are produced in the rumen as a product of microbial fermentation of carbohydrates and amino acids. Bacteria quickly hydrolyze starch into maltose and glucose, and the resulting glucose is rapidly fermented by the present Saccharolytic bacteria, producing pyruvate. Additionally, cellulose fermentation ends in pyruvate production, though through a more complex process with cellulases. Pyruvate is the common intermediate of carbohydrate fermentation before being converted to VFA (Fahey Jr and Berger, 1993). The main VFA produced from pyruvate are acetate, propionate, and butyrate (Bergman, 1990).

The diet has a large impact on the ratio of VFA concentrations produced in the rumen (Bergman, 1990). High roughage diets tend to shift VFA ratios toward greater acetate proportion, with propionate and butyrate decreasing in proportions. As concentrates increase as a percentage of the diet, acetate proportions tend to decrease while propionate and butyrate increase in proportions (Annison and Armstrong, 1970).

Acetate

Acetate is a two-carbon fatty acid produced through the pyruvate-formate lyase pathway or the pyruvate-ferredoxin oxidoreductase pathway (Fahey Jr and Berger, 1993). Both pathways convert pyruvate to acetyl-CoA and then to acetate, depending on the intermediate bacteria present (Fahey Jr and Berger, 1993). It has been reported that acetate production tends to increase with increased proportions of forage in the diet

(Davis, 1967). After being absorbed through the rumen epithelium, a majority of acetate is used by the smooth muscle and adipose tissue (Bergman, 1990). This is in part due to the low activity of acetyl-CoA synthase in the liver leading to little or no hepatic oxidation of acetate (Bergman, 1990). Kristensen and Harmon (2004) also saw no metabolism of acetate by the rumen epithelium.

Propionate

Propionate is the three-carbon VFA converted from pyruvate through the succinate (randomizing) pathway or the acrylate pathway (Fahey Jr and Berger, 1993). In the succinate pathway, pyruvate is converted to oxaloacetate (OAA) by phosphoenolpyruvate carboxykinase (PEP) or pyruvate carboxylase (PC). Oxaloacetate is converted then to succinate, followed by propionyl-CoA via methylmalonyl-CoA. Finally, propionyl-CoA is converted to propionate. The secondary acrylate pathway converts pyruvate to lactate, then acrylyl-CoA, reduces lactate to propionyl-CoA, which is then converted to propionate (Fahey Jr and Berger, 1993). The acrylate pathway is more predominate in ammonia-producing species of bacteria (Fahey Jr and Berger, 1993).

Production of propionate in the rumen tends to increase as the diet shifts from high forage to high concentrate. Sutton et al. (2003) demonstrated a shift if propionate production when dairy cows were fed a low roughage diet compared to a normal lactating diet. Propionate production was significantly higher in the low roughage diet compared to the normal ration. This same trend has been seen repeatedly by others (Judson et al., 1968; Bauman et al., 1971; Wang et al., 2020). Once propionate is produced in the rumen, it is absorbed by the rumen epithelium. During this process 2-5% of the

propionate absorbed is converted to lactate and the remaining enters the portal vein and sent to the liver (Elliot, 1980). Once in the liver, a majority of propionate is converted to glucose via gluconeogenesis.

Butyrate

Butyrate is the four-carbon VFA and is produced in lesser quantities (~12% of total VFA production) compared to both acetate (46%) and propionate (42%) in high concentrate diets. Butyrate synthesis is primarily described as β-oxidation reversal where pyruvate is reduced to acetyl-CoA. Two acetyl-CoA molecules are then bound together to form acetoacetyl-CoA, which is reduced to butyryl-CoA and then butyrate.

Microbial production of butyrate appears to be highest when forage and concentrates are balanced in ratio, although it still tends to be the least abundant of the three major VFA (Plöger et al., 2012). Several studies have found that butyrate promotes epithelial cell growth in the rumen and intestinal tissue (Sakata and Engelhardt, 1983; Kripke et al., 1989). In the rumen epithelium, a majority of butyrate is converted to ketones, including β-hydroxybutyric acid (βHBA), acetoacetate, and acetone (Kristensen et al., 1998). Additionally, βHBA is utilized for fatty acid production in adipose and mammary gland tissue and oxidized in cardiac and skeletal muscle (Fahey Jr and Berger, 1993).

GLUCONEOGENESIS

Due to the microbial fermentation of carbohydrates in the rumen, very little glucose is available to be directly absorbed (Aschenbach et al., 2010), making ruminants reliant on gluconeogenesis for their primary source of glucose. Glucose availability tends to decrease even more as starch or soluble carbohydrate concentration increases

(Bergman, 1990), as commonly seen in feedlot diets. Volatile fatty acids are the primary end products of microbial fermentation, but propionate, valerate, and isobutyrate are the only ones that can enter gluconeogenesis (Bergman, 1990), of which propionate is produced in the greatest quantity of the three (Reynolds et al., 2003). Although some glucose will be absorbed from the gastrointestinal tract, it has been estimated that 30-40% of absorbed glucose is metabolized by the enterocytes, never reaching the bloodstream (Larsen and Kristensen, 2009). In the case that starch does pass the rumen, the small intestine is the most prominent site of glucose absorption, predominantly via sodium-dependent glucose transport-1 (Huntington and Reynolds, 1986). For glucose to be absorbed in the small intestine, high starch content feeds have to make it to the small intestine without being fermented by rumen microbes first (Taylor and Allen, 2005; Larsen et al., 2009). The liver is the primary organ for gluconeogenesis in the ruminant (Aschenbach et al., 2010). Most of the glucose produced in peripheral (non-hepatic) tissues is done by the kidneys (Bergman, 1976) via amino acids like alanine and glutamine.

In the ruminant animal, gluconeogenesis is controlled by the availability of precursors, normally increasing after a meal and decreasing during a period of fasting. Propionate, as a precursor, accounts for 60-74% of the glucose produced from hepatic gluconeogenesis, with L-lactate, alanine, valerate, isobutyrate, glycerol, and other amino acids making up the other 26-40% (Reynolds et al., 2003). Of precursors utilized by the liver, initial metabolism in the portal-drained viscera will decrease the proportions that actually reach the liver (Kristensen et al., 1998). In a lipolytic state, where a deficit of

exogenous glucogenic precursors exists, ruminants will pull glucogenic carbon from peripheral tissues, such as lactate release from skeletal muscle (Aschenbach et al., 2010).

For entry to gluconeogenesis in the liver, precursors are converted to mitochondrial oxaloacetate (OAA). Lactate and alanine are converted to pyruvate then to OAA via mitochondrial pyruvate carboxylase (PC). Propionate is converted to OAA by mitochondrial propionyl-CoA carboxylase (PCoAC) and methylmalonyl-CoA mutase (MCM) to succinyl-CoA for entry to the tricarboxylic acid (TCA) cycle. Phosphoenolpyruvate carboxykinase (PEPCK) converts OAA to phosphoenolpyruvate (PEP) and then on to glucose. The activity of PEPCK in the cytosol (PEPCK-C) and mitochondria (PEPCK-M) regulates gluconeogenesis by controlling the entry point of lactate, alanine, and propionate (Aschenbach et al., 2010). Propionate entry can also be regulated by PCoAC and MCM; however, little is known about the regulation of the enzyme transcription (Aschenbach et al., 2010). It has been seen that propionate can positively regulate PCK1 expression, which codes for the PEPCK enzyme in the liver cytosol (Koser et al., 2008). The relationship demonstrated between propionate and *PCK1* supports the idea of increased intake will increase gluconeogenesis (Aschenbach et al., 2010). Greenfield et al. (2000) also reported an increase in *PCK1* expression with increased feed intake in dairy cows.

REGULATION OF FEED INTAKE IN RUMINANTS

Feed intake in ruminants can be regulated by both physical, metabolic, and hormonal mechanisms. The intake of low digestible, high fiber, low energy feeds are often controlled by rumen physical fill and digesta passage rate. On the other hand, intake

of highly digestible, low fiber, high energy feeds are controlled by energy demand and supply of metabolic fuels (NASEM, 2016).

Physical Regulation

Physical regulation of feed intake is generally seen by distension of the reticulorumen (Allen, 1996; Forbes, 2007). In the muscles of the reticulorumen, stretch receptors are stimulated and send signals to the brain to initiate the end of a meal (Forbes, 1996). Dado and Allen (1995) found that approximately 88% physical fullness is the threshold to induce the distention required to end a meal. The NDF content of feed has a major role in the physical regulation of feed intake as it has been seen that DMI is negatively correlated with NDF when rumen fill limits intake in the case of high forage diets (Mertens, 1994). In the case of high grain diets, NDF is less likely to limit feed intake by distension; rather DMI will decrease with an excess of metabolic fuels (Allen, 2000). After the initial filling effect of diet on intake, digestion and passage of feed from the reticulorumen has a large impact on intake as well. Factors such as size and density of feed particles, rumen motility, and rate of abomasum emptying control the passage rate of digesta through the gastrointestinal tract (Allen, 1996). An increased density and decreased particle size can increase DMI by altering rumination times (Allen, 2000). A decrease in active rumination can lead to a decrease in passage, this will in turn decrease DMI due to feed not being broken down for digestion (Allen, 1996). In high concentrate diets, such as in a feedlot diet, physical fill is less likely a controlling mechanism of feed intake compared to metabolic and hormonal regulation.

Metabolic Regulation - Hepatic Oxidation Theory

The hepatic oxidation theory (HOT) explains how feed intake can be controlled by signals sent from the liver to the brain in response to the presence of oxidative fuels. These fuels include non-esterified fatty acids (NEFA), lactate, VFA (mainly propionate), glycerol and amino acids. The signals traveling from the liver to the brain via the vagus nerve are thought to be both inhibitory and stimulatory in terms of satiety and hunger (Friedman, 1997). Increased oxidation in the liver appears to stimulate satiety by decreasing the firing rate of the vagus nerve; and alternatively, a decrease in oxidation will increase the firing rate and stimulate hunger (Friedman, 1997). With the almost constant fermentation of nutrients in the rumen, oxidation of fuels in the liver can change minute by minute depending on the need and efficiency of the liver to metabolize the substrates (Allen, 2020).

Due to the limited amount of glucose absorbed from the gastrointestinal tract by ruminants, glucose must be produced through gluconeogenesis. The near-constant glucose production by the ruminant liver creates a large draw tricarboxylic acid (TCA) cycle intermediates. Hepatic oxidation is controlled by importing the previously mentioned metabolites and exporting TCA cycle products (Allen, 2020).

Propionate is quickly produced, easily absorbed into the bloodstream, and extracted by the liver. Bergman and Wolff (1971) reported that about 88% of propionate was removed from the portal vein by hepatic circulation, with only 12 μ*M* propionate appearance present in the arterial blood. This rapid uptake by the liver has shown that propionate leads to satiety within the timeframe of a meal having a larger role in decreasing meal size (Allen, 2000). A decrease in meal size with increased propionate supply and an overall decrease in dry matter intake (DMI) has been seen in lactating

cows (Farningham and Whyte, 1993; Oba and Allen, 2003c, b; Stocks and Allen, 2012). Anil and Forbes (1988) reported that denervation of the liver prevented the hypophagic effects seen with propionate infusion, supporting the role of propionate in the HOT. Although it is understood that propionate will decrease feed intake, it is likely not due to the increased energy content of the VFA. Propionate infusion was seen to decrease metabolizable energy (ME) intake by 10.5 Mcal, in excess of the 6.2 Mcal that was being supplied by the propionate infusion (Oba and Allen, 2003c).

When starch production shifts to post-ruminal digestion, an increase in lactate production occurs, with a decrease in propionate production in the rumen (Reynolds et al., 2003). In contrast to propionate, this increased lactate production tends to increase feed intake. Reynolds et al. (2003) suggested that since the time it takes for the lactate to be taken up by the liver is increased, post-ruminal starch digestion has a lesser impact on hepatic oxidation than propionate from digestion in the rumen. A dramatic increase in starch fermentation in the rumen however, can cause a large increase in lactate, leading to lactic acidosis in the rumen. Lactic acidosis can cause a drop in intake due to a decrease in rumen pH (Valente et al., 2017).

Circulating NEFA concentrations are negatively correlated with energy balance in cows, often demonstrated around the time of parturition (Canfield and Butler, 1991). Receiving calves are in a lipolytic state as they enter the feedlot, which could be in part due to the increased stress hormones elevating lipolysis (Gupta et al., 2005), as well as the feed restriction during transportation. In support of HOT, Allen et al. (2009) proposed that increased blood NEFA concentrations of animals in a lipolytic state could instead be

causing the decrease in DMI, as it is providing an additional oxidative substrate for the liver as NEFA.

Insulin has been identified as a satiety hormone, and propionate has shown to cause an increase in insulin concentrations (Allen et al., 2009). However, the hypophagic effects seen with propionate have occurred without the presence of insulin indicating that propionate can alter feed intake independently of insulin (Frobish and Davis, 1977; Farningham and Whyte, 1993). Following the HOT, it is hypothesized that the role of insulin in initiating the uptake of nutrients by the liver leads to an increase in available oxidative fuels, which could cause satiety (Allen et al., 2005). It could also be explained as the accelerated uptake of nutrients to the liver could cause hunger as well. After intravenous infusions of propionate at varying concentrations, Stern et al. (1970) concluded that propionate is not a major regulator of insulin, but glucose itself is.

Hormonal Regulation

In non-ruminants, insulin stimulates the uptake of glucose from the blood to the liver, muscle and adipose tissues for glycogen synthesis after a meal (Woods et al., 2006). However, in ruminants, insulin tends to have more inconsistent effects on intake, partially thought to be due to the metabolic effects explained above (Allen et al., 2009). Leptin has been shown to have an active role in feed intake regulation, as leptin tends to be positively regulated by energy intake in cows, but not correlated with plasma insulin (Delavaud et al., 2002). Glucagon caused an increase of insulin release from the pancreas and increased blood glucose, as seen by Deetz and Wangsness (1981). It was then suggested that the increase of glucagon and propionate in sheep may have increased hepatic gluconeogenesis and plasma insulin, all resulting in increased insulin can lead to

a decrease in feed intake (Deetz and Wangsness, 1981). Ghrelin is the only hormone found to initiate feed intake in ruminants (Allen, 2014). Wertz-Lutz et al. (2006) has stated that ghrelin is an indicator of energy insufficiency and its plasma concentrations increase when cattle are fasted and decreased after feeding. It is thought that the greater ghrelin concentrations can indicate greater appetite and DMI when animals are fed ad libitum (Foote et al., 2016).

METHODOLOGY TO EVALUATE GLUCOSE METABOLISM

Several approaches to measure glucose metabolism and insulin sensitivity have been developed. The "gold standard" of insulin sensitivity testing is the hyperinsulinemic-euglycemic clamp (HEC). An HEC test involves elevating plasma insulin concentrations by continuous insulin infusion, and at the same time, blood glucose concentrations are held constant by infusing glucose. Insulin sensitivity is measured as the quantity of glucose required to reach a steady-state of glucose concentration (DeFronzo et al., 1979). This method allow researchers to evaluate the action of insulin on glucose metabolism as opposed to the insulin release in response to a glucose stimulus.

An intravenous glucose tolerance test (IVGTT) uses a single infusion (or bolus dose) of glucose followed by repeated blood sampling to measure glucose and insulin concentrations as they are cleared from the blood (De Koster et al., 2016). Area under the curve (AUC) and glucose clearance rate are calculated based on the glucose concentrations as the IVGTT progresses. With an IVGTT, insulin resistant animals would have a slower glucose clearance rate and potentially a greater insulin response to the standard glucose dose (De Koster et al., 2016). The disadvantage of the IVGTT method is

that it does not directly measure the action of insulin, but more the amount of insulin released. The glucose clearance rate can give some indication of insulin sensitivity, but it is confounded by the differences in insulin release by individual animals. Similar to an IVGTT, an intravenous insulin challenge test (IVICT) utilizes a single infusion of insulin followed by serial blood sampling. The IVICT concentrates on measuring the whole-body glucose response to an insulin flux.

Recently, surrogate indices for insulin sensitivity have been developed to measure insulin sensitivity with a single fasting time point in humans. The most commonly used indices are the homeostasis model of insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI) and revised quantitative insulin sensitivity check index (RQUICKI). The HOMA-IR and QUICKI both use fasting glucose and insulin concentrations to calculate insulin sensitivity. The RQUICKI includes fasting glucose, insulin, and NEFA concentrations (Muniyappa et al., 2008). The inclusion of NEFA is thought to be more accurate for non-obese individuals (Perseghin et al., 2001). As HOMA-IR values increase, insulin resistance is thought to increase (Bonora et al., 2002), and as QUICKI and RQUICKI values decrease, insulin resistance increases (Katz et al., 2000). De Koster et al. (2016) has worked to validate these surrogate indices in dry dairy cattle compared to both the HEC and IVGTT. Correlations between the surrogate indices and traditional HEC and IVGTT were not strong in dairy cattle (De Koster et al., 2016). Due to the differences in glucose metabolism seen between ruminants and non-ruminants, it is likely that a single fasting time point is not enough to predict insulin resistance in ruminants. Additionally, creating a fasting state in ruminants would require about 4 days withholding feed, which has shown to cause compensatory effects in glucose, insulin and

NEFA to make up for the lack of feed intake (Bradford and Allen, 2007a; Schoenberg et al., 2012).

CONCLUSION

Understanding feed intake in ruminants is required for being able to predict if diets will meet requirements for growth and performance. However, due to the microbial fermentation of nutrients ingested, an additional understanding is needed of the impact if fermentation products, like VFA, have on the growth and performance typically impacted by intake.

The overall control of feed intake in ruminants can be highly variable depending on physical, metabolic, and hormonal factors. Due to the large requirement for gluconeogenesis to provide energy to ruminants, the hepatic oxidation of fuels seem to have greater impact on feed intake. As propionate is the primary precursor of gluconeogenesis, hepatic oxidation likely controls intake to a greater extent in cattle fed a high concentrate diet due to the increased availability of oxidative fuels from fermentation. The increased production of propionate in high concentrate diets and the role of propionate in hepatic gluconeogenesis, leads it to be highly involved in the control of intake via HOT. However, the extent of increased propionate supply when production quantities are already elevated in concentrate diets is variable.

Much of the current research regarding the impact of propionate on production in cattle has focused in dairy cattle receiving a high forage, lactation diet. Due to the increase in propionate production seen with high concentrate diets, it can be inferred that performance is altered from that of dairy cows. The following chapters investigate the

impact how increased propionate supply can alter DMI, metabolic and endocrine factors in steers fed a high concentrate diet.

CHAPTER II

EFFECTS OF INCREASING PROPIONATE IN A FINISHING DIET ON DRY MATTER INTAKE AND GLUCOSE METABOLISM

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ABSTRACT: The objective of this experiment was to determine if increasing propionate alters dry matter intake (DMI), glucose clearance rate, blood metabolite, and insulin concentrations and hepatic gene expression in steers fed a finishing diet. Holstein steers (n = 15) were individually fed a finishing diet ad-libitum. Steers were allocated by body weight (BW) to receive: no Ca propionate (**CON**), 100 g/d (**LOW**), or 300 g/d (**HIGH**) in the diet. Orts were collected and weighed daily to determine DMI. Blood samples were collected on d 0, 7, and 21, and BW recorded on d 0, 14, and 28. A glucose tolerance test was conducted on d 14 and 28 of the trial. Liver biopsies were collected on d 33 for gene expression. Blood samples were analyzed for whole blood glucose and lactate, plasma NEFA and insulin concentrations. Data were analyzed using a mixed model with treatment, day and their interaction included, with day and minute as a repeated measure.

The CON treatment had greater ($P < 0.01$) DMI than LOW and HIGH. BW was greater for CON throughout the experiment and all treatments had an increased BW on day 28 ($P = 0.03$ for the interaction). Glucose concentrations tended ($P = 0.09$) to be higher on d 21 than d 0 and 7, but was not affected by treatment ($P = 0.58$). NEFA concentrations were lower ($P = 0.05$) for CON than other treatments, and greater ($P = 0.002$) on d 0 than d 7 and 21. Lactate concentrations were greater ($P = 0.05$) on d 7, than d 0 and 21, but not effected by treatment ($P = 0.13$). HIGH had greater insulin response than other treatments ($P = 0.02$). There was no treatment ($P \geq 0.16$) or day effect ($P \geq 0.36$) on glucose peak, plateau, or clearance rate. HIGH had greater expression of *SLC16A1* ($P = 0.05$) and tended to have greater expression of *SLC2A2* ($P = 0.07$). These data indicate that increased propionate may decrease DMI and insulin sensitivity.

Key words: cattle, finishing diet, glucose metabolism, glucose tolerance test, propionate

INTRODUCTION

Gluconeogenesis is one of the primary sources of energy for ruminants, as the potential glucose from ingested carbohydrates are often metabolized by microbes. Microbial digestion of glucose results in less than 10% of the required glucose being directly absorbed from the gastrointestinal tract (Yost et al., 1977). Volatile fatty acids produced by microbes are often the main source of glucose precursors, with propionate making up 60-74% of the substrates for hepatic gluconeogenesis (Aschenbach et al., 2010). It is well documented that cattle fed a high concentrate diet produce a greater concentration of propionate than cattle fed a high forage diets (Bauman et al., 1971;

Wang et al., 2020). Much of the current research regarding increasing propionate in cattle has been done in lactating or prepartum dairy cows on a high forage, lactating ration.

Results have been somewhat inconsistent regarding dry matter intake (DMI) with a negative impact on DMI in late lactation dairy cows ruminally infused with propionate (Oba and Allen, 2003c) but no impact when fed to early lactating cows (DeFrain et al., 2005; Liu et al., 2010). It has been suggested that the constant and rapid production and absorption of VFA in the rumen has some control in signaling satiety in ruminants (Allen et al., 2009). The decreased DMI could potentially be explained by the presence of increased energy content associated with propionate, but as seen by Oba and Allen (2003c), propionate infusion linearly decreased metabolizable energy (ME) intake from the diet seen from a decrease in meal size.

The impact of increasing propionate supply on nutrient metabolism as also been inconsistent. In some cases plasma glucose and non-esterified fatty acids have not been effected by an increase in propionate supply (DeFrain et al., 2005; McNamara and Valdez, 2005; Ferreira and Bittar, 2011). Other cases have seen a linear increase of plasma glucose and insulin with increased propionate supply (DiCostanzo et al., 1999; Oba and Allen, 2003a; Liu et al., 2010). A decrease in plasma NEFA has been seen in lactating cows supplied with an increase in calcium propionate, likely due to the increase in energy provided by propionate in turn decreasing the need for mobilized fatty acids (DiCostanzo et al., 1999; Liu et al., 2010). In general, a decrease in DMI tends to result in increased plasma NEFA. However, the elevated energy requirements of a lactating cow already result in an increase in NEFA, making decreased NEFA with increased propionate logical.

The hepatic oxidation theory (HOT) has been used to explain the role of the ruminant liver in controlling feed intake through oxidative fuels like NEFA, lactate, and propionate (Allen, 2014). The oxidation of propionate in the tricarboxylic acid cycle (TCA) is much greater compared to acetate and butyrate in the liver (Allen and Piantoni, 2013). Propionate is likely a point of regulation in the liver as denervation of the liver has shown a lack of hypophagic response to propionate (Anil and Forbes, 1988). In feedlot animals the high starch content of diets regularly used has been seen to decrease DMI without gut distension, implying propionate could have a larger effect on feed intake through HOT (Allen et al., 2009).

Little research has looked at increased propionate supply in a feedlot finishing setting and how it may impact the performance and metabolism of steers. What has been done has shown no change in dry matter intake in steers fed calcium propionate (Zhang et al., 2015b); however, the effects of propionate feeding on alterations on metabolic and endocrine factors have not been investigated. Therefore, the objective of this experiment was to determine if increasing propionate alters DMI, glucose clearance rate, basal blood metabolite, insulin response, and hepatic gene expression in steers fed a finishing diet. It was hypothesized that increasing propionate supply would decrease DMI and decrease insulin sensitivity in steers.

MATERIALS AND METHODS

All animal procedures were approved by the Oklahoma State University Institutional Animal Care and Use Committee (Protocol #19-77).

Animal Management

Fifteen Holstein steers (average initial BW = 243 ± 3.62 [SEM] kg) were individually housed in the Oklahoma State University Nutrition and Physiology Research Facility (Stillwater, OK) for the duration of the trial in individual pens (1.8 × 2.4 m pen with a 1.2 × 1.8 m rubber mat) with automatic waterers. Steers were fed a finishing diet (Table 2.1) for 14 d prior to initiating the experiment. The basal finishing diet was mixed at the Oklahoma State University Willard Sparks Beef Research Center (Stillwater, OK) and transported to the Nutrition and Physiology Barn as needed in 454 kg batches.

Dietary Treatments

Steers were allocated by BW to one of three treatments: Control (CON) receiving no supplemental propionate; Low Propionate (LOW) receiving 100 g calcium propionate/d; or High Propionate (HIGH) receiving 300 g/d of calcium propionate (CaP; Niacet CrystalPro Calcium Propionate; Ingredi, Wilkes-Barre, PA). Steers were individually fed twice daily (0630 and 1730 h) with adjustments made to insure ad libitum intake with constant access to water. When feeding, steers were initially given the respective treatment of propionate in 15% of the basal diet for 1-h. Following the initial hour the remaining allotment of feed adequately mixed together with any remaining treatment. This feeding procedure has previously been used by Zhang et al. (2015b). The previous day's orts were weighed and sampled prior to the morning feeding and composited weekly. Additionally, a 100 g daily feed subsample was collected each day (composited weekly) and a 500 g batch sample was collected for every diet batch mixed.

Body weights were collected on d 0, 14, and 28 of the experiment and used to calculate average daily gain and dosing volumes for glucose tolerance tests on d 14 and 28.

Blood Sample Collection and Analysis

Jugular blood samples were collected on d 0, 7, and 21 prior to the morning feeding via jugular venipuncture (9 mL neutral Sarstedt Monovette, Sarstedt AG & Co. KG, Nümbrecht, Germany) with K_2EDTA added at 1.5 mg/mL, inverted, and immediately placed on ice.

On d 0, 7, and 21 whole blood glucose and L-lactate was immediately analyzed after collection using an immobilize glucose oxidase enzymatic system (YSI Biochemistry Analyzer 2900, YSI Inc., Yellow Springs, OH), then centrifuged for 20 minutes at 3,500 × g at room temperature. Plasma was collected and stored at -20°C in 2 mL aliquots until further analysis.

A glucose tolerance test (GTT) was conducted on d 14 and 28 for each steer following a 12 hour fast using the methods of Joy et al. (2017). A temporary indwelling jugular catheter (14-gauge x 5.08 cm; TERUMO Surflo, Leuven, Belgium) was placed in each steer about 1 h prior to sampling with a 76.2-86.4 cm catheter extension set (Oasis, Mettawa, IL). A 2.78 *M* glucose solution was infused at 7.57 *mmol*/kg $BW^{0.75}$ via the jugular catheter at a continuous rate over 2 minutes. Blood samples were collected at -10, 0, 5, 10, 15, 20, 25, 30, 45, 60, 90, and 120 minutes after the glucose infusion and immediately placed on ice. Catheters were flushed with 10 mL of heparinized physiological saline (10 IU/mL; Thermo Fisher Scientific Chemicals, Inc., Ward Hill, MA) immediately after each blood collection. Blood glucose was immediately analyzed as described above and plasma was collected and stored as described. After completion of each GTT, steers were fed in small meals to prevent digestive upset.

Plasma Nonesterified fatty acids (NEFA) were analyzed using a modified protocol of the NEFA-HR (2) kit (Wako Pure Chemical Corporation, Osaka, Japan) based on the acyl-CoA synthetase-acyl-CoA oxidase method. Samples were analyzed in duplicate in flat-bottom 96-well polystyrene plates on a microplate reader (Biotek EPOCH, Biotek Instruments Inc., Winooski, VT) at 550 nm. The intraassay and interassay CV were 3.50% and 8.21%, respectively.

Plasma insulin was analyzed using a commercially available porcine insulin radioimmunoassay (RIA) kit (Millipore Corporation, Billerica, MA) with insulin from bovine pancreas (Sigma-Aldrich Inc., St. Louis, MO) used to construct a standard curve. The RIA kit had a sensitivity of 0.045 ng/mL with a sample size of 100 µL, and 90% specificity to bovine insulin. Samples were prepared for analysis in 12 × 75 mm glass culture tubes and counted in duplicate for 2 minutes/tube on a 2470 Automatic Gamma Counter (PerkinElmer Inc., Waltham, MA). The intraassay and interassay CV were 2.43% and 3.39, respectively.

Liver Biopsies and Gene Expression

Liver biopsies were performed on d 33 of the trial using a protocol modified from Sexten et al. (2012). Steers were restrained in a commercial squeeze chute for the duration of the procedure. The biopsy site was brushed clean and an 11 × 11 cm area was clipped with a 0.1 cm surgical blade. The clipped area was cleaned in a circular motion, once each with Povidone- and isopropyl alcohol-soaked gauze. Then 10-15 mL of Lidocaine (20 mg/mL) was administered between the 11^{th} and 12^{th} ribs, starting in the musculature, and ending in the subcutaneous tissue. The surgical area was cleaned again in circular motions, alternating between Povidone- and alcohol-soaked gauze at least 3

times. A 1-cm incision was made between the 11th and 12th ribs with a #22 scalpel blade after ensuring the area was completely blocked. A 14-gauge, 15-cm True Cut Style biopsy needle (Jorgensen Laboratories, Loveland, CO) was inserted through the peritoneum and directed cranially and ventrally toward the animal's left elbow. Once in the liver the sample was cut into the needle and the needle and sample removed. At least 3 samples were collected from the same biopsy site due to the small sampling size of the biopsy needles. Liver was rinsed with ultra-pure DI water, placed in a sterile micro centrifuge tube and frozen immediately with dry ice and stored at -80°C for later RNA extraction and gene expression. Incisions were closed with skin glue, sprayed with an adhesive bandage, and monitored for 5 days to ensure no complications.

Total RNA of the liver was isolated using the RNeasy Plus Mini Kit and QiaShredder columns (Qiagen, Hilden, Germany). About 10–30 mg of liver tissue was homogenized in 600 µL of RLT Plus lysis buffer with β-mercaptoethanol using a PowerGen 125 homogenizer (Fisher Scientific, Waltham, MA) for 40 s. The lysate was transferred to a QiaShredder column and centrifuged at 21,100 × g for 3 min at room temperature. Following the QiaShredder, the manufacturer's instructions for the RNeasy Plus Mini kit was followed and the total RNA was eluted in 50 µL of RNase-free water. The total RNA was quantified using a NanoDrop One spectrophotometer (ThermoFisher Scientific, Waltham, MA). The average sample RNA concentration was 582 ng/µL.

The isolated total RNA was used to synthesize cDNA using the iScript cDNA Synthesis Kit per the manufacturer's protocol (Bio-Rad, Hercules, CA). PrimePCR assays designed by Bio-Rad were used with the SsoAdvanced Universal SYBR Green Supermix to perform RT-qPCR. Five target genes were selected to analyze, including

solute carrier family 16 member 1 (*SLC16A1*), glucose-6-phosphatase (*G6PC*), phosphoenolpyruvate carboxykinase 1 (*PCK1*), phosphoenolpyruvate carboxykinase 2 (*PCK2*), and solute carrier family 2 member 2 (*SLC2A2*) with bovine control gene *G3PDH*. Each primer used was tested for efficiency by a serial dilution of a pooled cDNA sample and found to be most efficient at 1:10 dilution rate, with amplification greater than 92% efficiency.

Real Time qPCR was performed in triplicate for each cDNA sample using 10 µL of SsoAdvanced Universal SYBR Green Supermix, 1 µL of each PrimePCR assay primers, 7 µL of nuclease-free water, and 2 µL of diluted cDNA sample template. The reaction was performed using a Bio-Rad CFX96 real-time PCR detection instrument with the following protocol: 95°C for 30 seconds, 40 cycles of 95°C for 10 seconds and 60°C for 30 seconds, and a final melting curve from 65 to 95°C. The threshold cycle (C_p) for each sample was determined and used to calculate $2^{-\Delta\Delta C_t}$ along with the control primer and pooled cDNA sample.

Statistical Analysis

Data from the glucose tolerance tests were analyzed using GraphPad Prism 8.4.3 (San Diego, CA) to determine the area under the curve using the trapezoidal method. The glucose tolerance test data was also modeled as an exponential one-phase decay to calculate blood glucose peak, rate, and plateau. All other data were analyzed using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) with treatment, day, and their interaction as fixed effects with steer as experimental unit. Day was considered a repeated measure for dry matter intake, body weight, plasma NEFA, blood glucose, and blood lactate. Minute within day was considered a repeated measure for plasma insulin

concentrations. Covariance structure for repeated measures were chosen from autoregressive, compound symmetry, unstructured, and variance components based on the lowest Akaike Information Criterion (AIC). Normality of all data were tested using the Univariate procedure of SAS. Weekly plasma NEFA, lactate and insulin were determined to be non-normal, and were log transformed for analysis. The CORR procedure of SAS was used to analyze the Pearson correlations between variables. Differences were considered significant if $P \leq 0.05$ and were considered a tendency if $0.05 \leq P \leq 0.10$.

RESULTS

Dry matter intake decreased as the amount of propionate increased (Figure 2.1; $P < 0.0001$). Additionally, dry matter intake increased ($P < 0.0001$) from d 0 to d 32 for all treatments. There was a treatment × day interaction (Figure 2.2; $P = 0.027$) for steer body weight, where initial body weight did not differ and steers on the control treatment had a greater body weight on d 14 and d 28 than low and high propionate steers, respectively.

Pre-feeding plasma glucose tended (Table 2.2; $P = 0.09$) to be greater on d 21 than d 7 and 0, however there was no effect of treatment ($P = 0.58$). Weekly plasma NEFA concentrations were greater in high propionate steers than control steers ($P = 0.046$) and decreased from d 0 to d 7 and 21 ($P = 0.002$). Weekly plasma lactate concentrations were greater on d 7 than d 21 ($P = 0.053$) but did not differ between treatments ($P = 0.13$).

There was a treatment × day interaction (Table 2.3; $P = 0.036$) for fasting plasma insulin, where plasma insulin was greater on d 14 than d 28. Fasting plasma NEFA were greater ($P = 0.028$) on d 14 than d 28, but did not differ between treatments. There was an

effect of day ($P = 0.037$) on fasting plasma lactate, where concentrations were greater on d 28 than d 14. There was no treatment or day effect on fasting plasma glucose ($P \geq 0.12$).

As shown in Table 2.4, there was no treatment or day effect on glucose clearance peak, plateau, or rate ($P \geq 0.11$) for the glucose tolerance tests. Insulin AUC ($P = 0.08$) and glucose AUC ($P = 0.09$) tended to differ by day, but there was no treatment effect ($P > 0.31$). There was a treatment × minute interaction for plasma glucose ($P = 0.020$) during the glucose tolerance tests. A treatment × minute interaction for plasma insulin concentrations ($P = 0.001$) was present (Figure 2.4), where high propionate steers had greater insulin concentrations than both low and control steers, respectively, during the glucose tolerance tests. A treatment × day interaction ($P = 0.046$) was present for QUICKI calculations with HIGH having an increased sensitivity on d 28 compared to d 14 sensitivity and CON and LOW sensitivity on d 28. RQUICKI calculations were lower ($P = 0.044$) on d 14 than d 28, showing a potentially greater insulin sensitivity on d 28.

Abundance of *SLC16A1* expression in the liver was increased in high propionate steers compared to control steers ($P = 0.045$) and *SLC2A2* expression tended ($P = 0.07$) to be greater in high propionate steers than low propionate and control steers. Abundance of expression did not differ for *G6PC*, *PCK1*, or *PCK2* (Table 2.5; $P \geq 0.27$) among treatments. Phosphoenolpyruvate carboxykinase 2 showed a positive correlation with d 0 plasma glucose concentrations ($r = 0.58$, $P = 0.029$). Phosphoenolpyruvate carboxykinase 1 showed a negative correlation with d 0 plasma lactate concentrations ($r = -0.57$, $P = 0.034$) and a positive correlation with d 7 plasma glucose concentrations ($r = 0.63$, $P =$

0.015). Solute carrier family 2 member 2 showed a positive correlation with plasma NEFA concentrations on d 7 ($r = 0.54$, $P = 0.045$) and d 21 ($r = 0.57$, $P = 0.034$).

DISCUSSION

In the present study we found a decrease in DMI as propionate dose increased. The negative effect of propionate on feed intake has been demonstrated repeatedly (Allen, 2000; Oba and Allen, 2003c, b; Bradford and Allen, 2007a; Stocks and Allen, 2012). The hepatic oxidation theory supports the idea that propionate has a role in feed intake regulation as propionate can be oxidized in the liver as an energy source (Knapp et al., 1992). However, contrasting results were seen where calcium propionate did not affect DMI when it was fed in a pellet (DeFrain et al., 2005) or top dressed (Liu et al., 2010) to transition dairy cows. Additionally, when calcium propionate was top dressed on a finishing diet, DMI was not impacted (Zhang et al., 2015b).

The decrease in DMI could also be attributed to the decreased palatability of the calcium propionate (Littledike et al., 1981). The negative palatability of propionate has been seen in broilers where place avoidance tests results in pullets avoiding the propionate supplemented feed compared to the standard diet (Arrazola and Torrey, 2019). In sheep, Ralphs et al. (1995) saw decreased preference for a diet when they learned to associate the smell of a diet with increased ruminal propionate. Acetate has also shown to negatively impact palatability in silage when it is unproportionally added in relation to the other acids (Buchanan-Smith, 1990).

Zhang et al. (2015b) suggested that differences in nutrition level of cattle could impact the effect of increased propionate supply on DMI. The greater energy balance in the present study supports this suggestion as interaction of treatment and time on body

weight demonstrates that the steers were still growing while eating the highly fermentable feedlot diet compared to the negative energy balance that many of the dairy cattle trials involve. Inconsistent effects of propionate could also be a result of differences in dose volume and administration between trials. Many of the current trials looking at the effects of propionate on DMI and glucose metabolism use intraruminal infusions to administer a propionate solution, that has ranged from 0.5 *M* to 1.5 *M* for 1 h per day to 18 h straight (Oba and Allen, 2003c; Oba and Allen, 2003a; Stocks and Allen, 2012; Oh et al., 2015). These trials infuse the propionate solutions anywhere from 1 h/d for 5 days, to 14 h or 18 h for a single day. Similarly to the present trial, Zhang et al. (2015b), Liu et al. (2010), and DeFrain et al. (2005) provided propionate as a top-dress to the basal diet in 100 to 300 g/d for a minimum of 35 days. As seen in the next sections, the method of administration and level of nutrition seem to have an impact on the response due to increased propionate supply.

The inability of propionate to alter weekly pre-feeding insulin is in contrast to Zhang et al. (2015a) who saw a decrease in plasma insulin in propionate infused cattle over an 8-hour period. Similar to the results presented here, DeFrain et al. (2005) also saw no effect of feeding calcium propionate on weekly plasma insulin concentrations in pre- and postpartum cows. The intravenous glucose tolerance test (IVGTT) conducted in the present study has been used as a more practical method of measuring insulin sensitivity compared to the gold-standard hyperinsulinemia euglycemic clamp (HEC). Using the IVGTT we would expect more insulin resistant subjects to have a slower glucose disappearance (De Koster et al., 2016). Several indices for insulin sensitivity in humans have been developed to use a single blood sample after a 12 h fast and analyzing

for glucose, insulin, and NEFA. The homeostasis model of insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) use the fasting glucose and insulin concentrations to calculate insulin resistance. The revised quantitative insulin sensitivity check index (RQUICKI) includes fasting NEFA with glucose and insulin, which is thought to be a better predictor of insulin sensitivity in non-obese patients (Perseghin et al., 2001). However, due the larger differences between ruminant and human insulin, a single fasting measure of insulin may not accurately depict insulin sensitivity in cattle.

To validate the use of these "surrogate" indices in cattle, De Koster et al. (2016) compared the IVGTT, HOMA-IR, QUICKI, and RQUICKI to the gold standard HEC test in dry dairy cows. There was no significant correlations between the surrogate indices and insulin sensitivity indices from the HEC test (De Koster et al., 2016). It is suggested that the lack of correlation between calculated indices could be a result of the lack of variation in fasting insulin seen in the dry cows. Also, to create an equivalent fasting state to a human subject for these indices it would require much longer than 12 h in ruminants, which has been seen to alter glucose, insulin and NEFA as a method of compensating for a lack of a fed state (Bradford and Allen, 2007a; Schoenberg et al., 2012). Finally, the dry cow has a large concentration of glucose going to the gravid uterus and/or mammary tissue that is separate from insulin response. These factors not only can cause a difference between the reliability of the surrogate indices between humans and cows but could also account for differences seen between the dry cows and the steers in the current trial.

In the present study there was a positive correlation between insulin AUC and HOMA-IR and a negative correlation with QUICKI and RQUICKI indices. Similar to De

Koster et al. (2016), there was a lack of difference in fasting insulin concentrations between the treatments. However, the presence of correlations in the current trial could be due to the lack of the large glucose uptake by the mammary tissues and gravid uterus seen in the dairy cows. It has been suggested by Bradford and Allen (2007a) that fasting insulin concentrations are more indicative of the nutritional status in cattle instead of insulin sensitivity. Although the correlations present were seen with the current study suggest that the surrogate indices could be utilized in finishing steers, actual insulin response measured during the IVGTT agree with previous research showing insulin sensitivity is not accurately measured in a single fasting sample. Due to the large variation between cattle in different production stages, additional validation should be done to determine if these surrogate indices for insulin sensitivity are a reliable method for use in ruminants.

The lack of treatment effects seen on blood metabolites and hormones in the present trial could be a results of the high energy content of the basal diet not requiring the steers to depend heavily on the exogenous supply of glucose precursors through propionate (DeFrain et al., 2005). Yost et al. (1977) saw propionate production rates of 1,032 g/d in steers feed finishing ration ad libitum, and of similar body weight to steers in the current trial. When comparing steers fed ad libitum to steers fed just above maintenance in the same study, propionate production mirrored the increase in DMI, and stayed around 173 g propionate/kg feed intake (Yost et al., 1977). We can expect that the steers in the current study may have had similar propionate production due to a similar trend seen even in mature lactating cows fed a high concentrate diet and producing 2,296 g propionate/d and consuming 14.3 kg of feed/d (Bauman et al., 1971). In the same trial,

when fed a forage-based diet, mature lactating cows consuming 16.1 kg DMI/d were producing 985.3 g of propionate/d (13.3 mol/d). When steers (415 kg BW) were fed a forage-based diet, propionate production was 573.4 g/d (7.74 mol/d) when steers consumed 8.4 kg/d (Prange et al., 1978). Compared to the treatment doses in the current study, an additional 300 g propionate/d provide to the HIGH treatment steers would increase propionate concentrations in the rumen roughly 50% on a forage diet, while only 30% in concentrate fed steers. As shown in these trials, propionate production from a high concentrate diet may be large enough that an additional 300 g/d propionate may not increase the effects seen in roughage-based diets.

Recently the solute carrier family 16 member 1 gene (*SLC16A1*), which encodes the monocarboxylate transporter 1 (MCT1) protein, has been identified in the ruminant liver by Kirat et al. (2007), although functional studies in ruminants are limited. In other models, MCT1 has been shown to be a transmembrane protein that transports short chain monocarboxylates across the plasma membrane, including lactate and propionate (Müller et al., 2002). It is speculated that MCT1 in the bovine liver is at least partially responsible for the uptake of propionate into hepatocytes for gluconeogenesis or oxidation. Koho et al. (2005) found a high affinity for propionate by MCT1 transporters in reindeer hepatocytes. In the human colon, propionate was found to not have any effect on the regulation of *SLC16A1* expression (Cuff et al., 2002). Given the increased expression of *SLC16A1* observed in HIGH steers in this study, it is likely that an increase in ruminal propionate is causing an increase in propionate uptake via increased *SLC16A1* expression.

Solute carrier family 2 member 2 (*SLC2A2*) encodes for the bidirectional, facilitated transport of glucose, via glucose transporter 2 (GLUT2), in the liver of most mammals (Zhao et al., 1993). Due to the use of dietary carbohydrates for the production of VFA, little glucose is taken up by the bovine hepatocytes but GLUT2 is still required for transfer of glucose from hepatic cytoplasm to the blood stream (Thorens, 2015). With a tendency of increased *SLC2A2* expression in HIGH steers in the present study, it could be concluded that the potential increase in propionate uptake is increasing glucose production and therefore output via GLUT2. It was also seen by Gelardi et al. (1999) that as GLUT2 expression increased so did insulin resistance in lambs, which could also partly explain the decreased insulin sensitivity seen with the GTT in the present study.

Cytosolic phosphoenolpyruvate carboxykinase (*PCK1*), a protein coding gene, acts as a control point for gluconeogenesis regulation in the liver (Chakravarty and Hanson, 2008). It was reported that the flux of propionate into the gluconeogenesis pathway is mostly regulated by the activity of hepatic PEPCK (Greenfield et al., 2000; Al-Trad et al., 2010). *PCK1* expression has been seen to closely regulate PEPCK activity (Hartwell et al., 2001) and is positively regulated by propionate (Koser et al., 2008). In the current study, a lack of increased *PCK1* expression with and increased propionate supply falls in line with similar reports of mid-lactation cows in a positive energy balance (Zhang et al., 2015a). Zhang et al. (2015a) proposed that a possible explanation for this lack of change could be due to elevated plasma insulin. Insulin has been reported to quickly decrease the expression of *PCK1* in the human liver (Granner et al., 1983; Chakravarty and Hanson, 2008). However, the lack of increased weekly insulin in steers from the present study would not have an impact on the expression of *PCK1*. In early

lactation dairy cows it has been shown that increased DMI was accompanied with an increase in hepatic *PCK1* expression as a result of an expected increase in ruminal propionate (Greenfield et al., 2000).Therefore in HIGH steers, the increased supply of additional calcium propionate would in theory maintain *PCK1* expression, regardless of a decrease in DMI.

Mitochondrial phosphoenolpyruvate carboxykinase (*PCK2*) is a protein coding gene for a mitochondrial enzyme to catalyze oxaloacetate to phosphoenolpyruvate, whereas *PCK1* regulates this same conversion in the cytosol. The lack of change in *PCK2* expression is consistent with other reports that it is not heavily controlled by metabolic or hormonal changes in ruminants and has shown to have an inherent concentration, this in contrast to *PCK1* (Narkewicz et al., 1993; Velez and Donkin, 2005). Expression of PCK2 has also been found to be unaffected by feed restriction (Velez and Donkin, 2005) and resulting metabolic changes (Croniger et al., 2002). In contrast to PCK1, which has shown to have a negative relationship with elevated insulin (Zhang et al., 2015a). Glucose-6-phosphatate carboxylase works as the last step in gluconeogenesis to allow glucose to be transported out of the liver. The lack of increased *G6PC* expression in the current study could be due to the lack of increased *PCK1* and *PCK2* regulating the entry of propionate into the liver and maintaining at "normal" levels across all treatments. A similar lack of response to propionate was seen in cultured calf hepatocytes (Zhang et al., 2016).

Although a large portion of propionate is taken up by the liver for gluconeogenesis or oxidation, propionate can also be converted to other VFA in the rumen or metabolized by the rumen epithelium. Interconversions between acetate,

butyrate and propionate have been seen using isotope labeled carbon in VFA infusions. Bergman et al. (1965) however saw a greater conversion of acetate to butyrate, or vice versa, than propionate to either acetate or butyrate in sheep. In a high concentrate diet, the conversion of acetate and propionate to butyrate was much higher than in high forage diets (Sharp et al., 1982). With a ground corn concentrate diet 15.4% and 8.4% of the substrate propionate was converted to acetate and butyrate, respectively, in the rumen (Sharp et al., 1982). Epithelial metabolism of propionate to lactate or CO_2 has been reported to account for roughly 50% of the propionate produced in the rumen of sheep (Bergman, 1975). However, in cattle only 3-15% of propionate was converted to lactate (Cook et al., 1969; Weigand et al., 1972). Interconversions of propionate to other VFA in the rumen and epithelial propionate metabolism could account for a lack of increased propionate uptake by the liver.

A negative correlation between plasma NEFA and cow energy balance was found by Canfield and Butler (1991) and has been thought to act as an indicator of lowered energy status. In the current study, as the steers increase their time on the finishing ration their energy status increases, in turn decreasing their need for mobilizing NEFA in the blood from d 0 to d 21. The large decrease in intake seen in steers receiving the HIGH treatment however could have led to the need for the consistently elevated NEFA concentrations compared to the CON and LOW steers. The increase in NEFA with increasing calcium propionate is inconsistent with previous studies. Liu et al. (2010) saw a decrease in blood NEFA in lactating dairy cows fed increasing amounts of calcium propionate, with the lowest concentrations in cows receiving 300 g/d calcium propionate. DeFrain et al. (2005) saw a decrease in plasma NEFA concentrations as well, with a

greater decrease seen in cows receiving 178 g/d of propionic acid compared to cows receiving 120 g/d. Allen et al. (2009) proposed that increased NEFA in the lipolytic state could instead be causing the decrease in DMI, as it is providing an additional oxidative substrate for the liver, and NEFA concentrations are often elevated prior to a drop in DMI.

CONCLUSION

This experiment suggests that increasing calcium propionate supply for steers fed a finishing ration could alter glucose metabolism. Calcium propionate did not show an effect on basal circulating blood glucose but did seem to decrease insulin sensitivity. The hypophagic effects seen with increased propionate are supported by the hepatic oxidation theory in regulating feed intake. By providing propionate in addition to what the rumen produces an increase in blood glucose could have been expected, however the availability of oxidative fuels may have caused an increase in satiety. Additional research would need to be conducted to further identify if the palatability of calcium propionate a factor in the decreased DMI seen.

Table 2.1 Ingredient and nutrient composition of diet

Ingredient, % of DM	
Rolled corn	60.0
SweetBran[1]	20.0
Prairie hay	6.0
Alfalfa hay	4.0
Liquid Supplement[2]	5.0
Dry Supplement[3]	5.0
Nutrient Composition, DM basis	
Dry Matter, %	80.54
Crude Protein, %	12.79
Neutral Detergent Fiber, %	20.30
Acid Detergent Fiber, %	8.11
Ether Extract, %	3.28
Ash, %	5.45
NE_m, Mcal/kg	1.73
NE_g, Mcal/kg	1.11

[1]SweetBran (Cargill Inc., Dalhart, TX)

[2] Liquid supplement formulated to contain (% DM basis) 45.86% corn steep, 36.17% cane molasses, 6% hydrolyzed vegetable oil, 5.46% 80/20 vegetable oil blend, 5.2% water, 1.23% urea (55% solution), and 0.10 xanthan gum

[3] Dry supplement formulated to contain (% DM basis) 40.0% ground corn, 29.6% limestone, 20.0% wheat middlings, 7.0% urea, 1.0% salt, 0.53% magnesium oxide, 0.51% zinc sulfate, 0.17% manganese oxide, 0.13% copper sulfate, 0.08% selenium premix (0.6%), 0.0037% cobalt carbonate, 0.32% Vitamin A (30,000 IU/g), 0.10% vitamin E (500 IU/g), 0.009% vitamin D (30,000 IU/g), 0.20% tylosin (Tylan-40; Elanco Animal Health, Greenfield, IN), and 0.33% monensin (Rumensin-90; Elanco Animal Health)

Table 2.2 Effect of propionate treatment on weekly, pre-feeding metabolites and insulin

	Treatments[1]				Day				*P*-value		
Variable	Control	Low	High	SEM[2]	0	7	21	SEM[2]	Trt	Day	Trt × day
Blood Glucose, mg/dL	69.9	67.2	67.3	2.02	67.6	66.7	70.1	1.49	0.58	0.09	0.28
Plasma logNEFA	1.90[b]	1.99[ab]	2.08[a]	0.051	2.15[x]	1.96[y]	1.87[y]	0.051	0.051	0.002	0.64
Plasma NEFA, µEq/L	89.7	107.8	144.5	-	152.5	112.4	77.1	-	-	-	-
Blood logLactate	0.857	0.936	0.787	0.0478	0.858[ab]	0.913[a]	0.808[b]	0.0423	0.13	0.053	0.63
Blood Lactate, mg/dL	7.49	9.58	6.23	-	7.83	8.94	6.54	-	-	-	-
Plasma logInsulin	-0.044	-0.236	-0.304	0.0823	-0.075	-0.230	-0.280	0.066	0.11	0.04	0.74
Plasma Insulin, ng/mL	1.002	0.700	0.622	-	1.012	0.718	0.594	-	-	-	-

[1] Treatments included: Control, 0 g/d calcium propionate; Low, 100 g/d calcium propionate; High, 300 g/d calcium propionate

[2] Standard error of the mean (n = 5)

[a, b, c] Values within row with differing superscripts differ ($P < 0.05$)

Table 2.3 Effect of propionate treatment on fasting metabolites and insulin

	Treatments[1]				Day			*P*-value		
Variable	Control	Low	High	SEM[2]	14	28	SEM[2]	Trt	Day	Trt × day
Blood Glucose, mg/dL	74.8	73.1	71.5	2.21	71.8	74.5	1.51	0.58	0.12	0.43
Plasma logNEFA	2.65	2.61	2.67	0.046	2.70[a]	2.58[b]	0.037	0.58	0.028	0.32
Plasma NEFA, µEq/L	460.2	431.9	496.9	-	525.2	400.8	-	-	-	-
Blood logLactate	0.822	0.984	0.853	0.0816	0.794[b]	0.979[a]	0.0702	0.30	0.037	0.88
Blood Lactate, mg/dL	6.97	7.74	10.13	-	6.72	9.85	-	-	-	-
Plasma logInsulin	-0.635	-0.588	-0.639	0.0936	-0.580	-0.662	0.0756	0.91	0.22	0.036
Plasma Insulin, ng/mL	0.253	0.310	0.260	-	0.285	0.264	-	-	-	-

[1] Treatments included: Control, 0 g/d calcium propionate; Low, 100 g/d calcium propionate; High, 300 g/d calcium propionate

[2] Standard error of the mean (n = 5)

[a, b, c] Values within row with differing superscripts differ ($P < 0.05$)

Table 2.4 Effect of propionate treatment on insulin and glucose area under the curve (AUC) and glucose clearance parameters

	Treatments[1]				Day			*P*-value		
Variable	Control	Low	High	SEM[2]	14	28	SEM[2]	Trt	Day	Trt × day
Insulin AUC	145.3	155.8	212.3	40.25	193.1	149.2	31.21	0.46	0.08	0.18
Glucose AUC	16873	15675	15906	567.1	15771	16532	382.4	0.31	0.09	0.18
Glucose Peak, mg/dL	390.1	309.7	370.3	33.35	356.8	356.6	26.72	0.19	1.00	0.16
Glucose Plateau, mg/dL	105.2	90.7	99.6	5.87	95.5	101.5	4.70	0.20	0.36	0.20
Glucose Clearance Rate	0.153	0.081	0.136	0.0282	0.135	0.111	0.0226	0.16	0.46	0.15
HOMA-IR[3]	26.1	31.9	26.0	8.22	28.6	27.4	7.04	0.83	0.86	0.08
QUICKI	0.381	0.377	0.386	0.0137	0.376	0.387	0.0109	0.89	0.24	0.046
RQUICKI	0.440	0.442	0.441	0.0180	0.423[b]	0.459[a]	0.0157	0.99	0.044	0.46

[1] Treatments included: Control, 0 g/d calcium propionate; Low, 100 g/d calcium propionate; High, 300 g/d calcium propionate

[2] Standard error of the mean (n = 5)

[3] Calculated surrogate insulin sensitivity indices: homeostasis model of insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), revised quantitative insulin sensitivity check index (RQUICKI)

Table 2.5 Effect of propionate treatment on the expression of genes required for gluconeogenesis

	Treatments[1]				
Gene	Control	Low	High	SEM[2]	*P*-value
SLC16A1	-0.1112[b]	-0.0199[ab]	0.0088[a]	0.0321	0.045
G6PC	0.0125	-0.0604	0.0240	0.0390	0.29
PCK1	0.1116	-0.1986	-0.0885	0.1361	0.27
PCK2	0.0460	-0.0057	0.0286	0.1045	0.93
SLC2A2	-0.0425	0.0277	0.1800	0.0625	0.07

[1] Treatments included: Control, 0 g/d calcium propionate; Low, 100 g/d calcium propionate; High, 300 g/d calcium propionate

[2] Standard error of the mean ($n = 5$)

[a, b]Within row, values with unlike superscripts are different ($P < 0.05$)

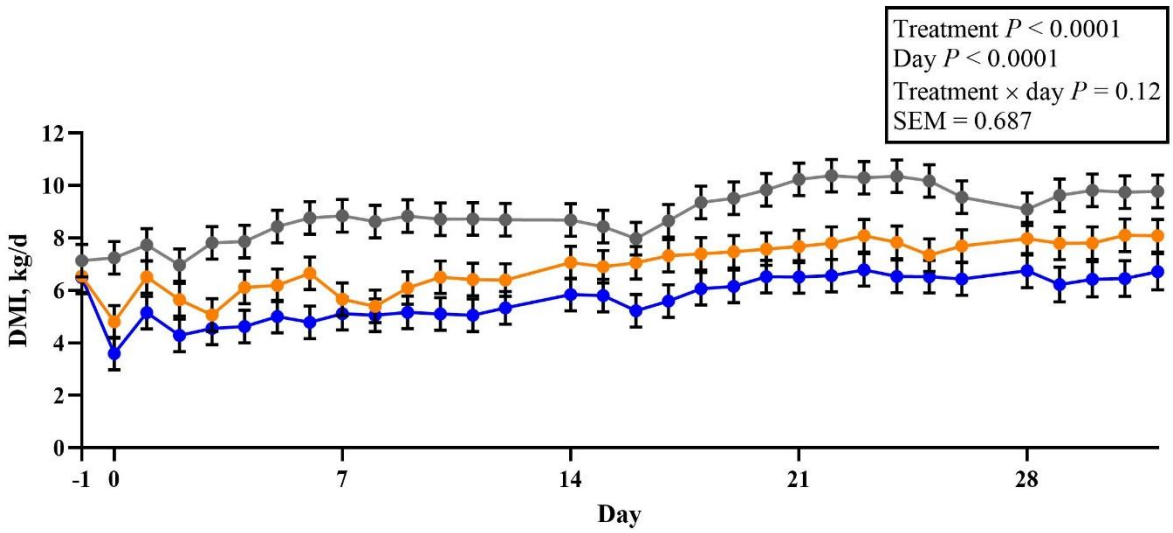

Figure 2.1 Dry matter intake of steers receiving control (0 g/d, ●), low (100 g/d, ●), or high (300 g/d, ●) calcium propionate treatments.

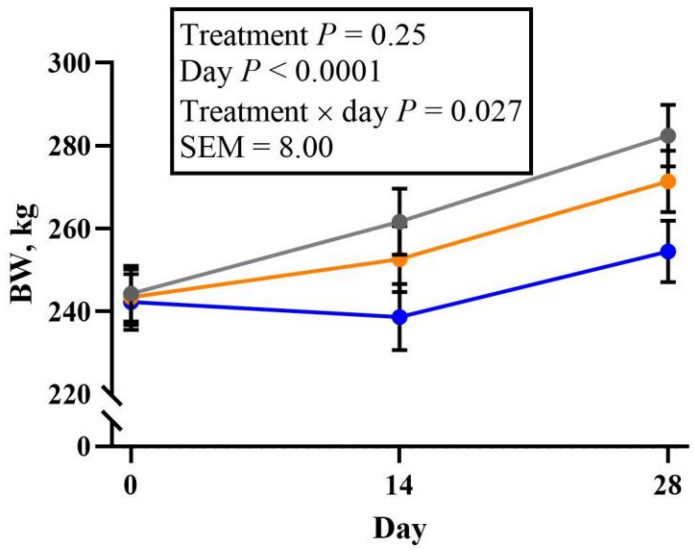

Figure 2.2 Body weight of steers receiving control (0 g/d, ●), low (100 g/d, ●), or high (300 g/d, ●) calcium propionate treatments.

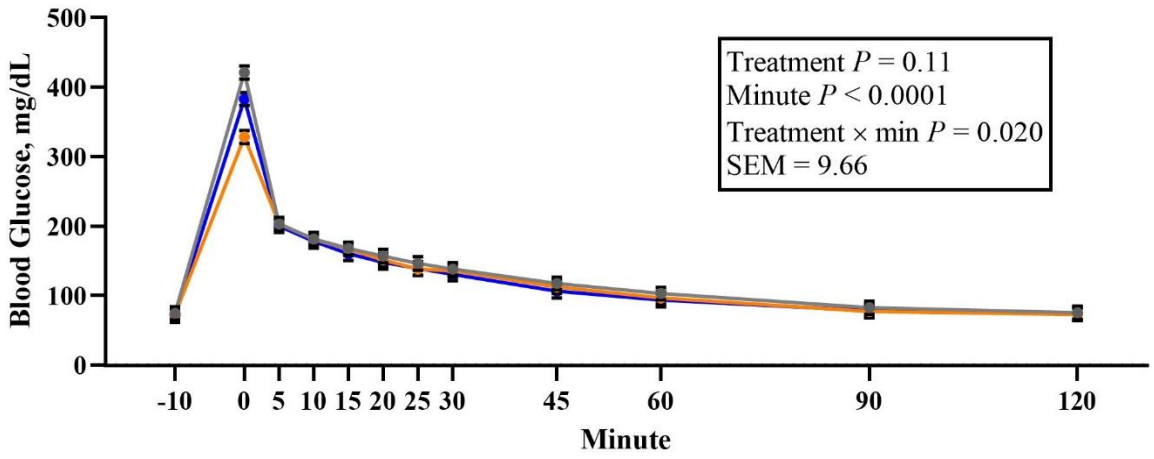

Figure 2.3 Blood glucose concentrations during a glucose tolerance test of steers receiving control (0 g/d, ●), low (100 g/d, ●), or high (300 g/d, ●) calcium propionate treatments.

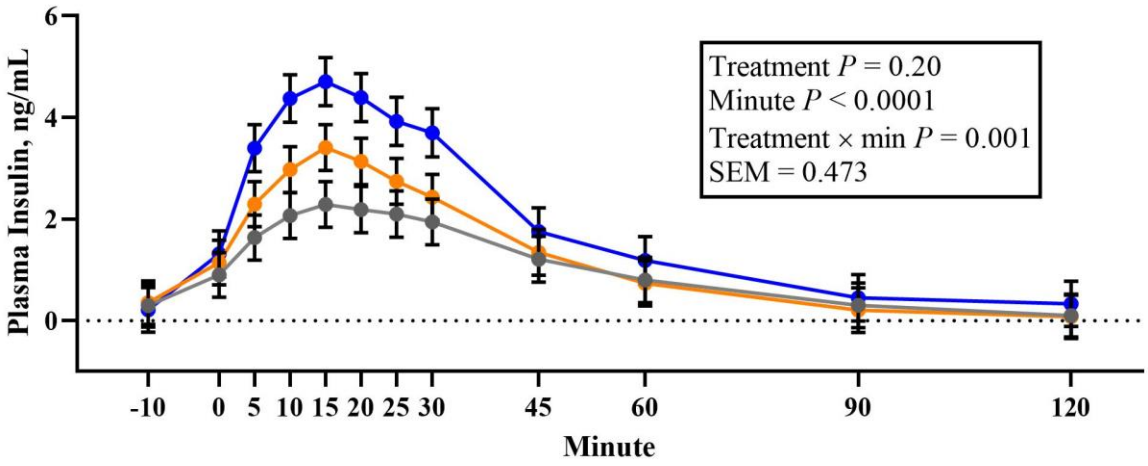

Figure 2.4 Plasma insulin concentrations during a glucose tolerance test of steers receiving control (0 g/d, ●), low (100 g/d, ●), or high (300 g/d, ●) calcium propionate treatments.

CHAPTER III

EFFECTS OF INCREASING RUMINAL PROPIONATE ON FEEDING BEHAVIOR AND GLUCOSE METABOLISM IN STEERS FED A FINISHING RATION

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ABSTRACT: The objective of this experiment was to determine if increasing propionate alters DMI, feeding behavior, glucose clearance rate, blood metabolite, insulin concentrations and rumen fluid metabolites in steers fed a finishing diet. Ruminally cannulated Holstein steers (n = 6) were fed a finishing diet ad-libitum. Steers were randomly assigned to one of three treatments in a 3 × 6 Latin rectangle design. Treatments of no Ca propionate (CON), 100 g/d (LOW), or 300 g/d (HIGH) were ruminally dosed daily. Individual intake was measured using an Insentec feeding system. Weekly blood samples and body weight were collected on d 0, 7, and 14. A glucose tolerance test was conducted on d 14 of each period and liver biopsies on d 15. Weekly

plasma samples were analyzed for glucose, lactate, NEFA, and insulin. Liver samples were analyzed for expression of genes involved in gluconeogenesis. Data were analyzed using a mixed model with period, treatment, day and their interaction included, with day and minute within period as a repeated measure and steer as a random effect. Dry matter intake, meal size, and number of meals per day was decreased ($P < 0.049$) in HIGH steers. Body weight was greater ($P < 0.0001$) for steers on d 7 and 14 than d 0, but was not effected by treatment ($P = 0.65$). Weekly plasma glucose tended ($P = 0.07$) to be greater on d 7 than d 0. There was an effect of hour ($P < 0.0001$) on rumen fluid pH, with an increase from 0 h to 6 h and then a decrease until 12 h. There was no treatment effect ($P \geq 0.13$) on weekly body weight, plasma glucose, NEFA, lactate, or insulin, rumen fluid lactate or pH, or glucose clearance peak, plateau and rate. There was no day effect ($P \geq 0.77$) on weekly plasma NEFA, lactate or insulin concentrations. There was no hour effect ($P = 0.12$) on rumen fluid lactate concentrations. There was no effect of treatment ($P \geq 0.57$) on hepatic gene expression. These data indicate that increased propionate may decrease DMI and alter feeding behavior.

Key words: cattle, finishing diet, glucose metabolism, glucose tolerance test, propionate

INTRODUCTION

Microbial metabolism of dietary carbohydrates in the rumen limits the availability of glucose to be absorbed. The volatile fatty acids (VFA) produced from microbial fermentation are used as precursors for gluconeogenesis in the ruminant liver, with propionate providing 60-74% (Aschenbach et al., 2010). Current research investigating

the relationship between increasing propionate supply involves prepartum or lactating dairy cattle on a high forage, lactating diet. It has been shown that diet has a large impact on VFA production, altering the ratio of acetate:propionate (Bauman et al., 1971; Wang et al., 2020). On a forage-based diet, acetate is produced in greater proportions than propionate (65:25:10, acetate:propionate:butyrate) and this balance shifts as the inclusion rate of concentrates increased, leading to greater quantities of propionate produced (50:40:10) (Owens and Goetsch, 1993). Additionally, on a forage based diet Sutton et al. (2003) saw quantities of 57, 17, 7 mol/d of acetate, propionate, and butyrate, respectively, in dairy cows. In the same study, cows fed a high concentrate diet had production rates of 49, 36, and 5 mol/d of acetate, propionate, and butyrate, respectively. The shift in production rates seen by Sutton et al. (2003) support the proportions ratio discussed previously.

Alterations in dry matter intake (DMI) has shown variable results when propionate is infused or fed. Depending on the stage of production cows are in, propionate has decreased DMI in late lactation (Oba and Allen, 2003c) and no change has been seen in early lactating cows (DeFrain et al., 2005). Variation in feed intake caused by increased propionate could be impacted by energy requirements of ruminant animals at different production stages. Oba and Allen (2003c) saw a decrease in metabolizable energy intake with increasing propionate infusion which negates the idea that ruminants are eating to a certain energy requirement.

The hepatic oxidation theory (HOT) describes the role of the ruminant liver in controlling feed intake with hepatic oxidation of NEFA, lactate, and propionate (Allen, 2014). The relationship between propionate as an oxidative fuel in the liver was seen by

Anil and Forbes (1988) when denervation of the liver prevented the hypophagic effects of propionate. Since feed intake is measured as a function of both meal size and meal frequency, a collection of research has looked at how propionate infusions alter these feeding behaviors (Oba and Allen, 2003c, b; Bradford and Allen, 2007c; Bradford and Allen, 2007b). The rapid metabolism of propionate has shown that it may have a larger impact on meal size, with other oxidative fuels altering meal frequency such as NEFA and lactate (Allen, 2014). Since much of this research has utilized lactating dairy cows eating a forage-based diet, little is known how these factors would alter behavior with a highly fermentable concentrate-based diet.

In addition to DMI varying between studies, the impact of increasing ruminal propionate on the metabolism of the oxidative fuels has been inconsistent. Some have seen no impact of propionate treatments on plasma NEFA or glucose (DeFrain et al., 2005; McNamara and Valdez, 2005; Ferreira and Bittar, 2011). Some others have seen an increase of plasma glucose and insulin with increased ruminal propionate (DiCostanzo et al., 1999; Oba and Allen, 2003a; Liu et al., 2010). An increase in plasma NEFA is often seen in early lactating dairy cows due to the dramatic increase in energy requirements with a decrease or maintained intake (Bell, 1995). Therefore the decrease in plasma NEFA seen by DiCostanzo et al. (1999) would be plausible if propionate is providing the additional energy.

Little research has looked at increased propionate supply in a feedlot finishing setting and how it may impact the performance and metabolism of steers. What has been done has shown no change in dry matter intake when steers are fed calcium propionate; however, the effects of propionate feeding on alterations in metabolic and endocrine

factors have not been investigated (Zhang et al., 2015b). The current experiment was performed to follow up on the previous study conducted (Chapter 2) and further investigate the impacts of increased ruminal propionate in finishing steers. Therefore, the objective of this experiment was to determine if increasing propionate alters DMI, feeding behavior, glucose clearance rate, basal blood metabolite, insulin response, rumen fluid lactate and hepatic gene expression in steers fed a finishing diet. It was hypothesized that an increase in ruminal propionate would decrease DMI by decreasing meal size and potentially decreasing meal frequency, along with decreasing insulin sensitivity.

MATERIALS AND METHODS

All animal procedures were approved by the Oklahoma State University Institutional Animal Care and Use Committee (Protocol #19-77).

Animal Management

Six ruminally cannulated Holstein steers (average initial BW = 418 ± 17.74 [SEM] kg) were group housed at the Oklahoma State University Willard Sparks Beef Research Center (Stillwater, OK) for the duration of the trial with automatic waterers. Steers were fed the basal finishing diet (Table 3.1), ad libitum, for 14 d prior to initiating the experiment. The basal finishing diet was mixed at the Oklahoma State University Willard Sparks Beef Research Center (Stillwater, OK) and fed once daily. Steers were fed daily with three Insentec Roughage Intake Control system (Hokofarm Group, Marknesse, Netherlands) with adjustments made to insure ad libitum intake with constant access to water. Steers were given one week to adapt to the Insentec feeders before transitioning to the basal finishing diet. Daily feed intake, number of meals, and meal size

were collected by the Insentec system. Meal size was based on weight change of the feed present in each bunk and the timestamp of each visit tracked by each steers individual radio-frequency identification tag (RFID). Bunk visits within a 10-minute period were considered a single meal event.

Dietary Treatments

Steers were randomly assigned to one of three treatments in a 3 × 6 Latin rectangle: Control (CON) receiving no supplemental propionate; Low Propionate (LOW) receiving 100 g propionate/d; or High Propionate (HIGH) receiving 300 g/d of Calcium Propionate (CaP; Niacet CrystalPro Calcium Propionate; Ingredi, Wilkes-Barre, PA). Steers were dosed with half of the treatment amount directly through the rumen cannula at 0600h and 1800h, daily. A 100 g daily feed subsample was collected each day and composited weekly for nutrient analysis. Body weights were collected on d 0, 7 and 14 of each period of the experiment and the day 14 BW was used to calculate dosing volumes for glucose tolerance tests on d 14 of each period. A five-day washout period was included between periods, where steers did not receive any treatment.

Blood Sample Collection and Analysis

Jugular blood samples were collected on d 0 and 7 prior to the morning feeding via jugular venipuncture (9 mL neutral Sarstedt Monovette, Sarstedt AG & Co. KG, Nümbrecht, Germany) with K_2EDTA added at 1.5 mg/mL, inverted and immediately placed on ice. Blood samples were centrifuged for 20 minutes at 3,000 × g at 4°C. Plasma was collected and stored at -20°C in 2 mL aliquots until further analysis.

A glucose tolerance test (GTT) was conducted on d 14 of each period following a 12 hour fast using the methods of Joy et al. (2017). A temporary indwelling jugular

catheter (16-gauge x 13 cm; Jorgensen Labs, Loveland, CO) was placed in each steer about 1 h prior to sampling with a 76.2-86.4 cm catheter extension set (Oasis, Mettawa, IL). A 2.78 *M* glucose solution was infused at 7.57 *mmol*/kg $BW^{0.75}$ via the jugular catheter at a continuous rate over 2 minutes. Blood samples were collected at -10, 0, 5, 10, 15, 20, 25, 30, 45, 60, 90, and 120 minutes after the glucose infusion and immediately placed on ice. Catheters were flushed with 10 mL of heparinized physiological saline (10 IU/mL; Thermo Fisher Scientific Chemicals, Inc., Ward Hill, MA) immediately after each blood collection. Blood samples were immediately set on ice and plasma was collected and stored as described previously. After completion of each GTT, steers were dosed and monitored for digestive upset after giving access to feed again.

Plasma glucose and L-lactate were analyzed using the YSI Biochemistry Analyzer 2900 (YSI Inc., Yellow Springs, OH). Plasma Nonesterified fatty acids (NEFA) were analyzed using a modified protocol of the NEFA-HR (2) kit (Wako Pure Chemical Corporation, Osaka, Japan) based on the acyl-CoA synthetase-acyl-CoA oxidase method. Samples were analyzed in duplicate in 96-well polystyrene plates (brand info) on a microplate reader (Biotek EPOCH, Biotek Instruments Inc., Winooski, VT) at 550 nm. The intraassay and interassay CV were 6.04% and 4.85%, respectively. Plasma insulin was analyzed using a commercially available porcine insulin radioimmunoassay (RIA) kit (Millipore Corporation, Billerica, MA) with insulin from bovine pancreas (Sigma-Aldrich Inc., St. Louis, MO) used to construct a standard curve. The RIA kit had a sensitivity of 0.080 ng/mL with a sample size of 100 µL, and 90% specificity to bovine insulin. Samples were prepared for analysis in 12 x 75 mm glass culture tubes and counted in duplicate for 2 minutes/tube on a 2470 Automatic Gamma Counter

(PerkinElmer Inc., Waltham, MA). The intraassay and interassay CV were 2.22% and 2.16%, respectively.

Rumen Fluid Collection and Analysis

Rumen fluid was serially collected at 0, 2, 4, 6, 8, 10, and 12 hours after dosing on d 13 of each period. Samples were collected through a 0.297 mm screen (Rumen Fluid Sampler Tube, Bar Diamond, Parma, ID) attached to 101 cm extension set and a 60 mL syringe. Samples were taken from the cranial and ventral sacs of the rumen. The 0 h and 12 h samples were collected prior to each treatment dosing. A total of 50 mL of rumen fluid was collected at each time point with three 2 mL aliquots frozen at -20°C for later analysis of L-lactate concentrations and VFA in the future. Rumen fluid pH was immediately measured after collection using an Oakton pH 6+ Handheld pH meter (Cole-Parmer, Vernon Hills, IL). L-lactate were analyzed using the YSI Biochemistry Analyzer 2900 (YSI Inc., Yellow Springs, OH).

Liver Biopsies and Gene Expression

Liver biopsies were performed on d 15 of each period using a protocol modified from Sexten et al. (2012). Steers were restrained in a commercial squeeze chute for the duration of the procedure. The biopsy site was brushed clean and an 11x11 cm area was clipped with a 0.1 cm surgical blade. The clipped area was cleaned in a circular motion, once each with Povidone and isopropyl alcohol-soaked gauze. Then 10-15 mL of Lidocaine (20 mg/mL) was administered between the 11^{th} and 12^{th} ribs, starting in the musculature and ending in the subcutaneous tissue. The surgical area was cleaned again in circular motions, alternating Povidone and alcohol-soaked gauze at least 3 times. A 1-cm incision was made between the 11^{th} and 12^{th} ribs with a #22 scalpel blade after

ensuring the area was completely blocked. An 11-gauge, 15-cm Jamshidi™ biopsy needle (CareFusion, Vernon Hills, IL) was inserted through the peritoneum and directed cranially and ventrally toward the animal's left elbow. Once in the liver the sample was cut into the needle and the needle and sample removed. At least 3 samples were collected from the same biopsy site due to the small sampling size of the biopsy needles. Liver was rinsed with ultra-pure DI water, placed in a sterile micro centrifuge tube and frozen immediately with dry ice and stored at -80°C for later RNA extraction and gene expression. Incisions were closed with skin glue, sprayed with an adhesive bandage and monitored for 5 days to ensure no complications.

Total RNA of the liver was isolated using the RNeasy Plus Mini Kit and QiaShredder columns (Qiagen, Hilden, Germany). About 10 – 30 mg of liver tissue was homogenized in 600 µL of RLT Plus lysis buffer with β-mercaptoethanol using a PowerGen 125 homogenizer (Fisher Scientific, Waltham, MA) for 40 s. The lysate was transferred to a QiaShredder column and centrifuged at 21,100 × g for 3 min at room temperature. Following the QiaShredder, the manufacturer's instructions for the RNeasy Plus Mini kit was followed and the total RNA was eluted in 50 µL of RNase-free water. The total RNA was quantified using a NanoDrop One spectrophotometer (ThermoFisher Scientific, Waltham, MA).

The previously isolated total RNA was used to synthesize cDNA using the iScript cDNA Synthesis Kit per the manufacturer's protocol (Bio-Rad, Hercules, CA). PrimePCR assays designed by Bio-Rad were used with the SsoAdvanced Universal SYBR Green Supermix to perform RT-qPCR. Five target genes were selected to analyze, including solute carrier family 16 member 1 (*SLC16A1*), glucose-6-phosphatase (*G6PC*),

phosphoenolpyruvate carboxykinase 1 (*PCK1*), phosphoenolpyruvate carboxykinase 2 (*PCK2*), and solute carrier family 2 member 2 (*SLC2A2*) with bovine control gene *G3PDH*. Each primer used was tested for efficiency by a serial dilution of a pooled cDNA sample and found to be most efficient at 1:10 dilution rate.

Real Time qPCR was performed in triplicate for each cDNA sample using 10 µL of SsoAdvanced Universal SYBR Green Supermix, 1 µL of each PrimePCR assay, 7 µL of nuclease-free water, and 2 µL of diluted cDNA sample template. The reaction was performed using a Bio-Rad CFX96 real-time PCR detection instrument with the following protocol: 95°C for 30 seconds, 40 cycles of 95°C for 10 seconds and 60°C for 30 seconds, and a final melting curve from 65 to 95°C for 5 seconds. The threshold cycle (C_p) for each sample was determined and used to calculate $2^{-\Delta\Delta C_t}$ along with the control primer and pooled cDNA sample.

Statistical Analysis

Data from the glucose tolerance tests were analyzed using GraphPad Prism 8.4.3 (San Diego, CA) to determine the area under the curve for glucose and insulin. This data was also modeled as an exponential one-phase decay to calculate blood glucose peak, rate, and plateau. All other data were analyzed using the MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) with period, treatment, and day as fixed effects and steer as a random effect. Day was considered a repeated measure for dry matter intake, number of meals, meal size, body weight, weekly plasma NEFA, glucose, and lactate. Hour within day was considered a repeated measure for rumen fluid lactate and pH. Minute within day was considered a repeated measure for plasma insulin and glucose concentrations. Covariance structure for repeated measures were chosen from

autoregressive, compound symmetry, unstructured, and variance components based on the lowest Akaike Information Criterion (AIC). Weekly plasma NEFA, lactate, insulin, and rumen fluid lactate were log transformed to test normality using UNIVARIATE procedure of SAS. The CORR procedure of SAS was used to analyze the Pearson correlations between hepatic gene expression and other variables. Means were considered significantly different if $P \leq 0.05$ and were considered tendencies if $0.05 \leq P \leq 0.10$.

RESULTS

Dry matter intake decreased as the amount of propionate increased (Figure 3.1b; $P < 0.0001$) with control and low steers eating more than the high treatment steers. Control and low steers also had a greater (Figure 3.2b; $P = 0.049$) average meal size and a greater (Figure 3.3b; $P = 0.046$) number of meals during the day compared to high steers. There was no effect of treatment on steer body weight (Figure 3.4; $P = 0.65$) but steers did increase in body weight ($P < 0.0001$) from d 0 to d 14.

Weekly pre-feeding metabolites and insulin are shown in Table 3.2. Weekly plasma glucose tended ($P = 0.06$) to increase from d 0 to d 7, but there was no treatment effect. There was no treatment or day effect on plasma NEFA or lactate ($P \geq 0.33$). Plasma insulin was greater ($P = 0.019$) in control steers compared to low and high treatment steers. There was no effect of treatment on fasting plasma glucose, lactate, or insulin (Table 3.3; $P \geq 0.44$).

There was an effect of treatment on rumen fluid lactate (Figure 3.5; $P = 0.034$) where low steers had a greater concentration than high steers. Hour tended to effect lactate concentrations as well, with the 0 h samples having the lowest lactate concentrations and 10 h post dosing having the highest. Treatment did not affect (Figure

3.6; $P = 0.65$) rumen fluid pH but there was an effect of hour ($P < 0.0001$), where pH was lowest at 0 h and highest between 4 and 10 h post dosing.

As shown in Table 3.4, there was no treatment effect on glucose peak, plateau, or clearance rate, and plasma insulin or glucose AUC ($P \geq 0.50$). There was no treatment effect (Figure 3.7 and 3.8; $P \geq 0.41$) on plasma glucose or insulin during the glucose tolerance tests. There was no treatment effect ($P \geq 0.34$) on HOMA-IR or QUICKI surrogate insulin indices.

Propionate treatment did not affect liver gene expression (Table 3.5; $P \geq 0.57$). *SLC16A1* showed a negative correlation with d 7 plasma lactate ($r = -0.84$, $P < 0.0001$) and fasting plasma lactate ($r = -0.55$, $P = 0.028$). *SLC2A2* tended to have a positive correlation with fasting glucose ($r = 0.44$, $P = 0.09$), fasting lactate ($r = 0.43$, $P = 0.09$), and glucose AUC ($r = 0.46$, $P = 0.07$). There were no correlations present between *G6PC*, *PCK1*, or *PCK2* and any other variables measured ($P \geq 0.15$).

DISCUSSION

The effects on propionate on DMI and feeding behavior are often inconsistent due to the variation in dose amount, mode of administering the dose, and the production period targeted. In contrast to the current study, DMI was not effected by increasing propionate supplementation in transition and early lactation dairy cows (DeFrain et al., 2005; Liu et al., 2010) or in finishing cattle (Zhang et al., 2015b). Feed intake was also not effected by an intrajugular administration of propionate to crossbred wether sheep fed a concentrate based diet (Deetz and Wangsness, 1981). McNamara and Valdez (2005) reported an increase in DMI when early lactation cows were supplied 125 g/d calcium propionate in a pelleted form. Calcium propionate was also found to increase intake of

low quality straw in lambs when supplemented at 8.3 g/d, but then decreased dry matter intake when that does was doubled to 16.6 g/d calcium propionate (Villalba and Provenza, 1996). Dry matter intake over a 18 h period linearly decreased as the propionate fraction of total VFA ruminally infused increased, 0 to 1 (Oba and Allen, 2003c). When compared to an 1 mol/L acetate infusion, 1 mol/L propionate decreased DMI 20% over the 18 h infusion (Stocks and Allen, 2012). Sheperd and Combs (1998) saw a similar effect in lactating cows with a continuous ruminally infused propionate dose on a high forage diet. Oba and Allen (2003c) attribute this depressed dry matter intake to the hypophagic effects of propionate, altering satiety and hunger signals. Directly dosing steers in the current study removes the potentially for decreased palatability of the calcium propionate which was thought to be present in the preceding study (Chapter II).

Feed intake is an influenced by both meal size and meal frequency. The decrease in meal size in steers dosed with the HIGH CaP treatment in the current study was previously seen in lactating dairy cows by Oba and Allen (2003c) where meal size tended to decrease with increasing propionate infusion over 12 h. Stocks and Allen (2012) did not see an effect of increased propionate infused on meal size over 18 h. The increased fermentability of traditional, high starch, feedlot diets have shown to increase propionate production in the rumen. Additionally, propionate, once in the blood stream, is easily taken up by the liver and stimulates hepatic oxidation of acetyl CoA through the tricarboxylic acid (TCA) cycle (Allen et al., 2009). The oxidation of acetyl CoA is thought to decrease the rate of firing of the hepatic vagus nerve signaling satiety during a meal (Anil and Forbes, 1988). The decrease in meal size with high starch diets implies

that propionate taken up by the liver within the timeframe of a meal is the major fuel causing satiety.

If satiety was being primarily caused by other oxidative fuels (lactate, glycerol, or amino acids) that are absorbed post-ruminally, then a decreased meal frequency or increased time between meals would show a greater effect on decreased daily DMI (Allen, 2014). Unlike the current study, propionate infusion did not decrease number of meals (meal frequency) over the infusion period in lactating dairy cows (Oba and Allen, 2003c; Stocks and Allen, 2012). Although both a decrease in meal size and frequency was seen in the current study, the variability in meal frequency day to day in all treatments demonstrates that propionate had a more consistent effect on decreased DMI through decreased meal size. Between meals, lipolysis may increase the supply of NEFA to the liver to be oxidized, further increasing the time between meals. Although post-meal NEFA concentrations were not measured in the current study, slightly elevated basal plasma NEFA concentrations in HIGH CaP steers could help explain the decreased meal frequency. Much of the current research looking at the effects of propionate on feeding behavior has concentrated on behavior over a 24 h period or less, unlike the current study which monitored behavior for 14 days each period. Due to the daily changes in feed intake seen on high starch feedlot diets, including the current study, creating a long term, consistently elevated propionate pool in the rumen would give a better indication of persistent changes in feeding behavior in response to increased propionate.

The difference of hypophagic effects between propionate and acetate could also be expected to be due to the increased energy concentration of propionate. However, Oba and Allen (2003c) observed a decrease in metabolizable energy (ME) intake in lactating

cows as propionate concentration increased in relation to acetate. When isocaloric solutions of propionate and acetate were infused by Sheperd and Combs (1998) a decrease in DMI was seen again in lactating cows.

Weekly plasma insulin increased as calcium propionate supplementation increased in early lactating dairy cows (Liu et al., 2010). However, in transitioning dairy cows, pre-weaning dairy calves, and goats plasma insulin was not altered by increased propionate supply (Stern et al., 1970; Bunting et al., 2000; DeFrain et al., 2005; Stocks and Allen, 2012). The depressed weekly pre-feeding plasma insulin with increased propionate supply seen in the current study contrasts the idea that increased propionate causes satiety through increased insulin signaling. While propionate has been shown to act as an insulin secretagogue and insulin then acting as a satiety hormones and decrease DMI (Allen et al., 2009), it has also been shown that propionate can decrease DMI without altering insulin concentrations (Frobish and Davis, 1977; Farningham and Whyte, 1993). Allen et al. (2005) proposed that insulin has an indirect effect on DMI by potentially speeding up the clearance of oxidative fuels from the blood, resulting in increased hepatic oxidation.

Stern et al. (1970) investigated the insulin response to propionate infusions at different concentrations and methods of administrations (0.1 vs. 0.25 vs. 0.5 *M* intraruminally or 1m*M*/min vs. 4 m*M*/min intravenously) in mature goats. Plasma insulin concentrations were not increased by 0.1 or 0.25 *M* propionate solutions infused into the rumen, but 0.5 *M* propionate infused into the rumen significantly increased plasma insulin concentrations. When intravenously infused propionate had a greater effect on plasma insulin at 4 m*M*/min infusion into the ruminal vein. The 0.5 *M* infusion was

estimated to be ten times the normal hourly propionate production in the goats, therefore Stern et al. (1970) concluded that propionate was not a significant regulator of insulin secretion in ruminants. In another study, propionate did not consistently increase plasma insulin when infused at 1.2 or 2.5 mM/min so it was determined again that insulin was not causing the satiety seen with propionate infusion (Farningham and Whyte, 1993).

As discussed in the previous chapter, surrogate indices have been developed to measure insulin sensitivity based on fasting plasma glucose and insulin concentrations. The homeostasis model of insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) calculate insulin resistance using these single samples. Similar to the previous study discussed in Chapter 2, there was a positive correlation between insulin AUC and HOMA-IR and a negative correlation between insulin AUC and QUICKI. A lack of significant difference in fasting insulin concentrations between steers reinforces that the single fasting blood samples are not an accurate indicator of insulin sensitivity in ruminant animals compared to results of the hyperinsulinemia euglycemic clamp (HEC) or IVGTT.

The lack of treatment effect on pre-feeding plasma glucose and NEFA was also seen in dairy calves and lactating dairy cow ration (Bunting et al., 2000; DeFrain et al., 2005; Ferreira and Bittar, 2011). A linear increase in plasma glucose and decrease in plasma NEFA was seen by Liu et al. (2010) in early lactating dairy cows. The lack of treatment effects seen on blood metabolites in the current study could be a results of the high energy content seen in finishing rations not requiring the steers to depend heavily on the exogenous supply of glucose precursors through propionate (DeFrain et al., 2005). The findings in Chapter 2 indicate that pre-feeding plasma NEFA concentrations

increased with an increase in propionate supply. Due to the contrast between the results of the current study and those in Chapter 2, it can be inferred that the elevated NEFA concentrations in the previous study are due to more than just a decrease in DMI. The differences observed between these two studies could suggest that the steers were in more of a lipolytic state in the previous study than the current, and therefore the mobilized NEFA were providing an additional oxidative fuel to the liver (Allen et al., 2009).

Lower rumen fluid lactate concentrations in high propionate steers compared to low steers could indicate that the increase in propionate supply is increasing the metabolisms of lactate to other VFA, but excess propionate is likely not converted to lactate. Further analysis of the VFA concentrations in the rumen fluid is required to better understand how the lactate and excess propionate are being metabolized. In contrast to the current experiment, Liu et al. (2009) found a linear decrease in rumen fluid pH in steers feed increasing amounts of calcium propionate. Calves fed 5% calcium propionate (DM) did not experience a change in rumen fluid pH compared to their control counterparts (Cao et al., 2020). Similarly, pH did not differ after lambs were infused with varying propionate concentrations (Villalba and Provenza, 1996).

As discussed in Chapter 2, the solute carrier family 16 member 1 gene (*SLC16A1*) was identified in the ruminant liver to regulate monocarboxylate transporter 1 (MCT1) for the transportation of lactate and propionate across the plasma membrane (Müller et al., 2002; Kirat et al., 2007). On the other end of gluconeogenesis, solute carrier family 2 member 2 (*SLC2A2*) encodes glucose transporter 2 (GLUT2) for the facilitated transportation of glucose (Zhao et al., 1993). The lack of a significant difference in the expression of either of these genes in the current study implies that there was not an

increased flux of propionate into the gluconeogenic pathways and therefore not an increase of glucose out of the liver with increased rumen propionate.

In vitro studies of propionate uptake by the liver have determined that the saturation point is between 2-5 m*M* (Armentano, 1992) but still higher than the estimated blood propionate concentrations (Looney et al., 1987). It is possible that on a concentrate-based feedlot diet, the hepatic saturation point was reached, and increased uptake of propionate was not attainable. Further investigation into plasma propionate concentrations in the current study could explain where the increased propionate was, if not in the liver.

Cytosolic phosphoenolpyruvate carboxykinase (*PCK1*) expression regulates PEPCK protein activity (Hartwell et al., 2001) which has been noted to regulate propionate flux into the gluconeogenesis pathway in the liver (Greenfield et al., 2000; Al-Trad et al., 2010). The lack of increased *PCK1* expression with increasing ruminal propionate follows what was seen in the previous study (Chapter 2) and by Zhang et al. (2015a) in mid lactation dairy cows. The maintenance of *PCK1* expression could be explained by the increase in ruminal propionate supply making up for the decrease in DMI seen in both the previous and current study. Greenfield et al. (2000) saw that increased ruminal propionate production due to an increase in feed intake resulted in increased hepatic *PCK1* expression.

Mitochondrial phosphoenolpyruvate carboxykinase (*PCK2*), unlike *PCK1*, simply codes for a mitochondrial enzyme that catalyzes oxaloacetate to phosphoenolpyruvate in the TCA cycle. It has been reported that *PCK2* is not greatly influenced by metabolic and hormonal changes in the ruminant liver (Narkewicz et al., 1993) which is supported by

the lack of change increased ruminal propionate had on expression in the current study. Glucose-6-phosphate carboxylase (*G6PC*) regulates the final export of glucose from the gluconeogenesis pathway. The lack of significant change in *G6PC* expression with propionate treatment in the current study follows that of the previous study (Chapter 2) and Zhang et al. (2016). This consistent expression of *G6PC* is likely due to the lack of increased expression of genes involved in propionate uptake like *SLC16A1* and *PCK1*. The overall lack of change in hepatic gene expression in the current study suggests that gluconeogenesis in the liver is not influenced by an increase in propionate as a precursor.

CONCLUSION

This experiment suggests that increasing ruminal propionate supply for steers fed a finishing ration could decrease dry matter intake by altering feeding behavior even in the absence of potential palatability issues. The decrease of both meal size and meal frequency support previous research that rapid uptake of propionate to the liver causes satiety and potentially the excess propionate available for oxidation can increase meal intervals. The lack of effect of increased propionate supply on circulating glucose and insulin suggest that at a higher energy balance glucose metabolism is less likely to be impacted.

Table 3.1 Ingredient and nutrient composition of diet

Ingredient, % of DM	
Rolled corn	62.0
SweetBran[1]	20.0
Prairie hay	8.0
Liquid Supplement[2]	5.0
Dry Supplement[3]	5.0
Nutrient Composition, DM basis	
Dry Matter, %	79.51
Crude Protein, %	13.50
Neutral Detergent Fiber, %	23.23
Acid Detergent Fiber, %	8.60
Ether Extract, %	3.91
Ash, %	5.22
NE_m, Mcal/kg	1.73
NE_g, Mcal/kg	1.11

[1]SweetBran (Cargill Inc., Dalhart, TX)

[2] Liquid supplement formulated to contain (% DM basis) 45.86% corn steep, 36.17% cane molasses, 6% hydrolyzed vegetable oil, 5.46% 80/20 vegetable oil blend, 5.2% water, 1.23% urea (55% solution), and 0.10 xanthan gum

[3] Dry supplement formulated to contain (% DM basis) 40.0% ground corn, 29.6% limestone, 20.0% wheat middlings, 7.0% urea, 1.0% salt, 0.53% magnesium oxide, 0.51% zinc sulfate, 0.17% manganese oxide, 0.13% copper sulfate, 0.08% selenium premix (0.6%), 0.0037% cobalt carbonate, 0.32% Vitamin A (30,000 IU/g), 0.10% vitamin E (500 IU/g), 0.009% vitamin D (30,000 IU/g), 0.20% tylosin (Tylan-40; Elanco Animal Health, Greenfield, IN), and 0.33% monensin (Rumensin-90; Elanco Animal Health)

Table 3.2 Effect of propionate treatment on weekly, pre-feeding plasma metabolites and insulin

	Treatments[1]				Day			*P*-value		
Variable	Control	Low	High	SEM[2]	0	7	SEM[2]	Trt	Day	Trt × day
Plasma Glucose, mg/dL	77.7	75.8	76.0	1.32	75.6	77.3	1.25	0.16	0.06	0.68
Plasma logNEFA	1.89	1.94	1.98	0.039	1.93	1.94	0.032	0.33	0.92	0.73
Plasma NEFA, µEq/L	81.4	91.7	97.6	-	92.2	88.3	-	-	-	-
Plasma logLactate	1.000	0.978	0.970	0.0239	0.980	0.986	0.0221	0.40	0.75	0.35
Plasma Lactate, mg/dL	10.15	9.61	9.43	-	9.67	9.79	-	-	-	-
Plasma logInsulin	0.050[a]	-0.098[b]	-0.098[b]	0.0504	-0.066	-0.032	0.0450	0.019	0.46	0.46
Plasma Insulin, ng/mL	1.673	0.848	0.886	-	0.945	0.986	-	-	-	-

[1] Treatments included: Control, 0 g/d calcium propionate; Low, 100 g/d calcium propionate; High, 300 g/d calcium propionate

[2] Standard error of the mean (n = 6)

Table 3.3 Effect of propionate treatment fasting plasma metabolites and insulin

	Treatments[1]				
Variable	Control	Low	High	SEM[2]	*P*-value
Plasma Glucose, mg/dL	80.5	79.8	79.7	1.76	0.93
Plasma logLactate	0.947	0.991	0.924	0.0414	0.45
Plasma Lactate, mg/dL	9.27	8.52	9.85	-	-
Plasma logInsulin	-0.203	-0.277	-0.360	0.0821	0.44
Plasma Insulin, ng/mL	0.717	0.563	0.462	-	-

[1] Treatments included: Control, 0 g/d calcium propionate; Low, 100 g/d calcium propionate; High, 300 g/d calcium propionate

[2] Standard error of the mean (n = 6)

Table 3.4 Effect of propionate treatment on insulin and glucose area under the curve (AUC) and glucose clearance parameters

	Treatments[1]				
Variable	Control	Low	High	SEM[2]	*P*-value
Insulin AUC	266.1	258.8	223.7	29.13	0.50
Glucose AUC	15703.0	14984.0	15572.0	486.3	0.56
Glucose Peak, mg/dL	466.2	429.3	510.9	70.54	0.72
Glucose Plateau, mg/dL	95.4	89.2	90.2	5.96	0.72
Glucose Clearance Rate	0.164	0.147	0.180	0.0383	0.83
HOMA-IR[3]	81.5	63.2	51.7	13.48	0.34
QUICKI	0.325	0.332	0.341	0.0098	0.53

[1] Treatments included: Control, 0 g/d calcium propionate; Low, 100 g/d calcium propionate; High, 300 g/d calcium propionate

[2] Standard error of the mean (n = 6)

[3] Calculated surrogate insulin sensitivity indices: homeostasis model of insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI)

Table 3.5 Effect of propionate treatment on the expression of genes required for gluconeogenesis

	Treatments[1]				
Gene	Control	Low	High	SEM[2]	*P*-value
SLC16A1	0.0377	0.0840	0.0508	0.07406	0.87
G6PC	-0.0192	-0.0290	0.0084	0.06683	0.92
PCK1	-0.1392	-0.0670	-0.0186	0.08490	0.63
PCK2	-0.1012	-0.0486	-0.0810	0.07808	0.89
SLC2A2	-0.3568	-0.2976	-0.2674	0.05734	0.57

[1] Treatments included: Control, 0 g/d calcium propionate; Low, 100 g/d calcium propionate; High, 300 g/d calcium propionate
[2] Standard error of the mean (n = 6)

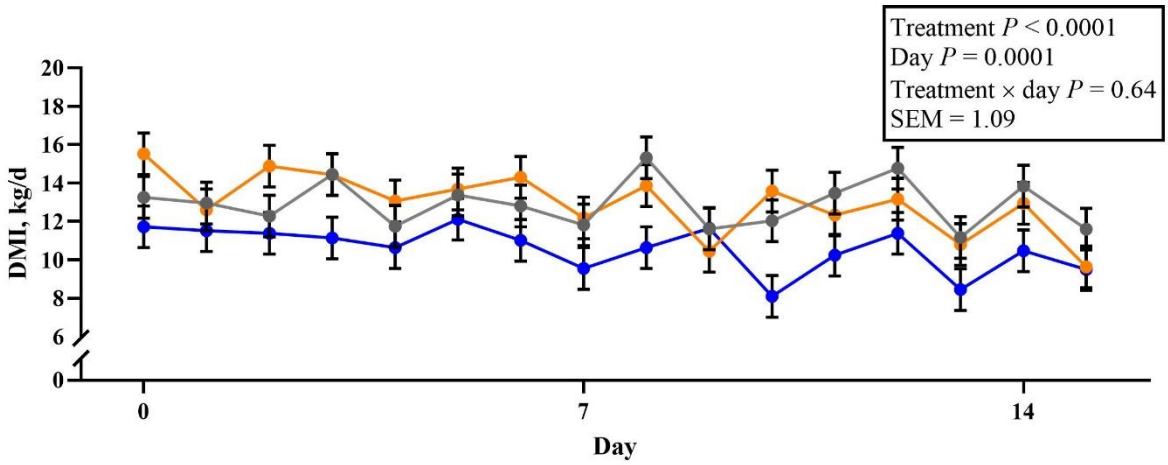

Figure 3.1a Dry matter intake of steers receiving control (0 g/d, ●), low (100 g/d, ●), or high (300 g/d, ●) calcium propionate treatments.

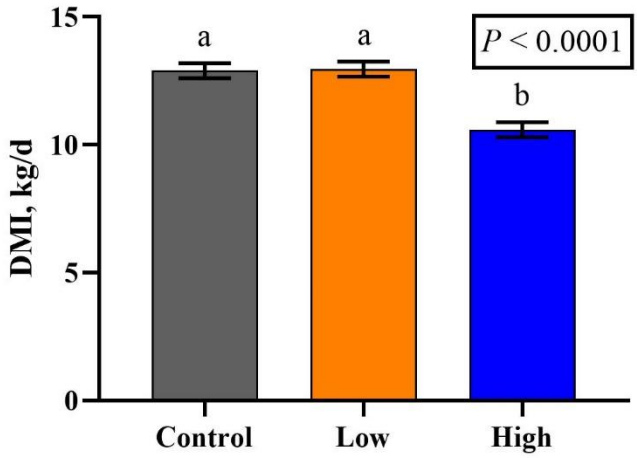

Figure 3.1b Dry matter intake of steers receiving control (0 g/d), low (100 g/d), or high (300 g/d) calcium propionate treatments.

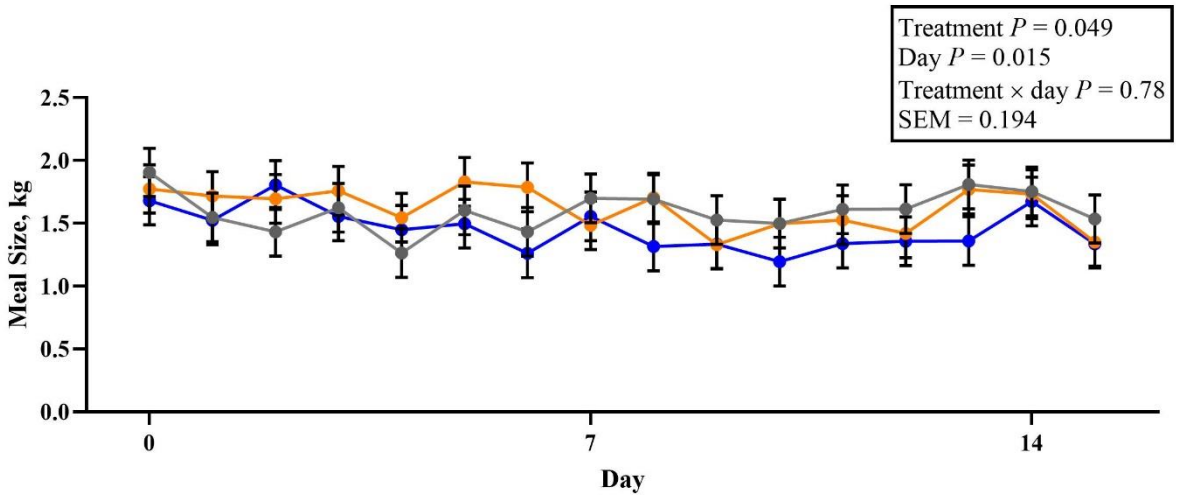

Figure 3.2a Meal size (on a DM basis) of steers receiving control (0 g/d, ●), low (100 g/d, ●), or high (300 g/d, ●) calcium propionate treatments.

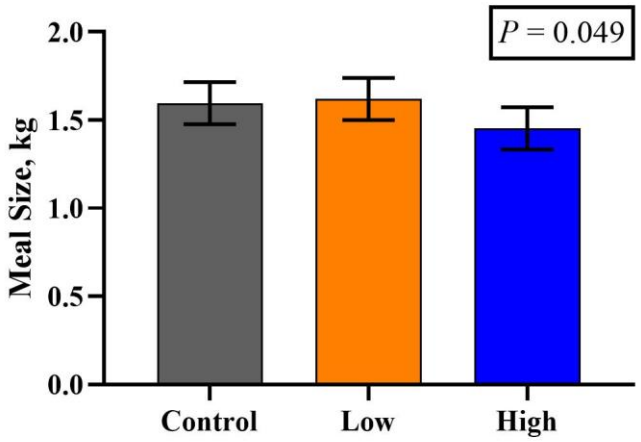

Figure 3.2b Meal size (on a DM basis) of steers receiving control (0 g/d), low (100 g/d), or high (300 g/d) calcium propionate treatments.

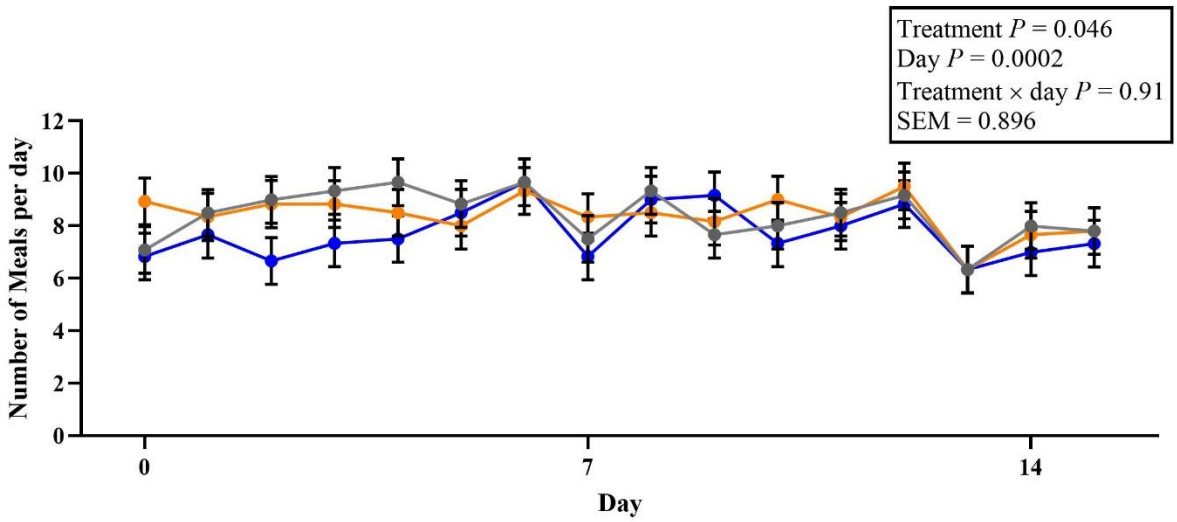

Figure 3.3a Number of meals per day of steers receiving control (0 g/d, ●), low (100 g/d, ●), or high (300 g/d, ●) calcium propionate treatments.

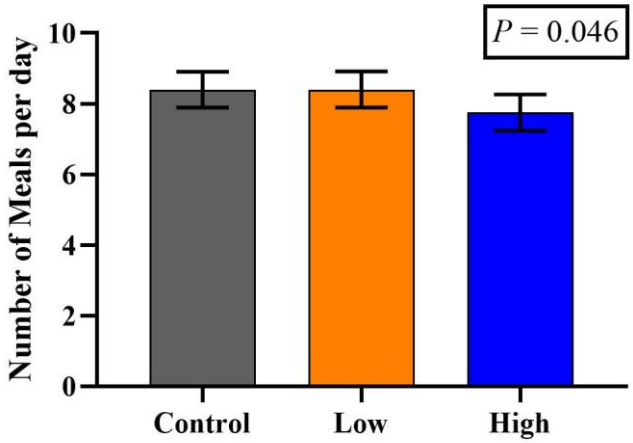

Figure 3.3b Number of meals per day of steers receiving control (0 g/d), low (100 g/d), or high (300 g/d) calcium propionate treatments.

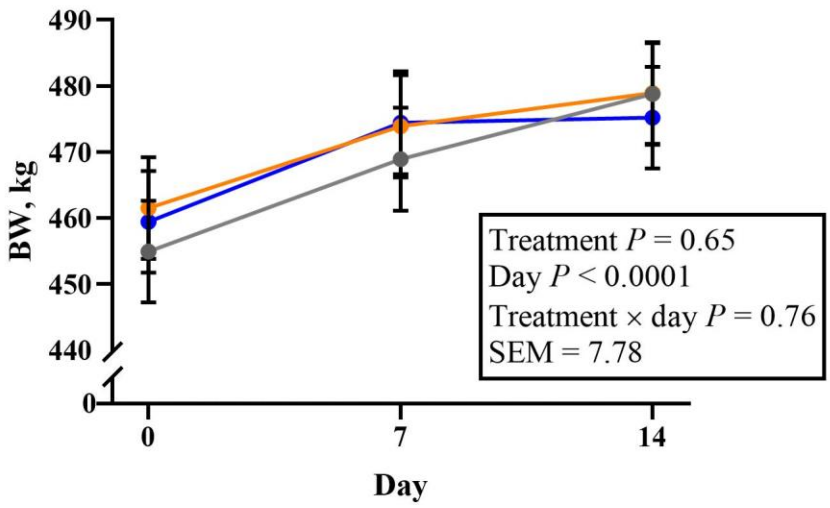

Figure 3.4 Body weight of steers receiving control (0 g/d, ●), low (100 g/d, ●), or high (300 g/d, ●) calcium propionate treatments.

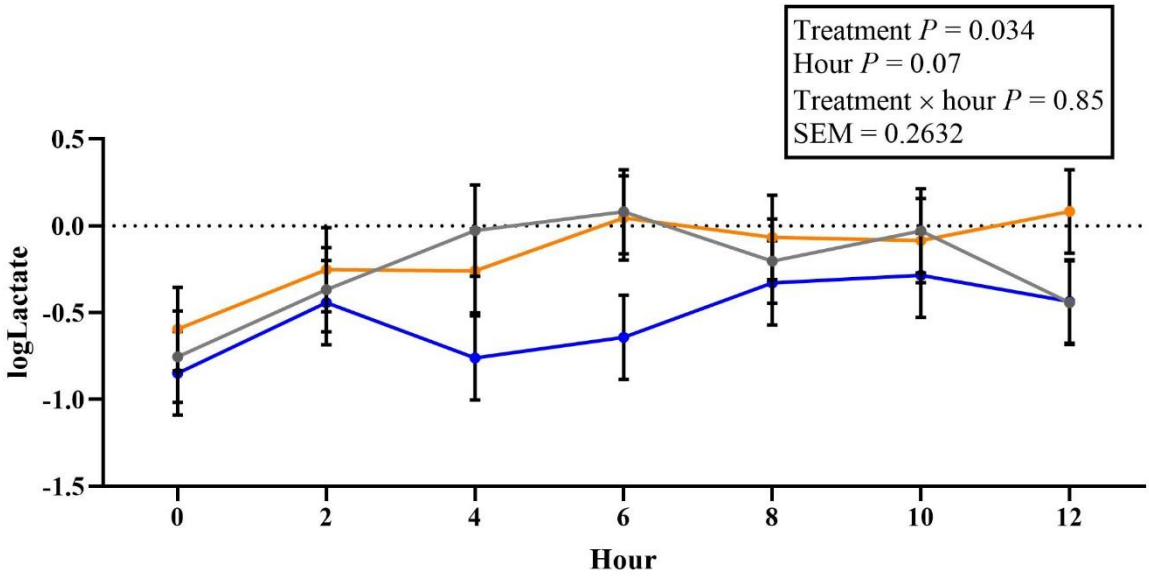

Figure 3.5 Rumen fluid logLactate concentrations of steers receiving control (0 g/d, ●), low (100 g/d, ●), or high (300 g/d, ●) calcium propionate treatments during a 12 h serially rumen fluid collection.

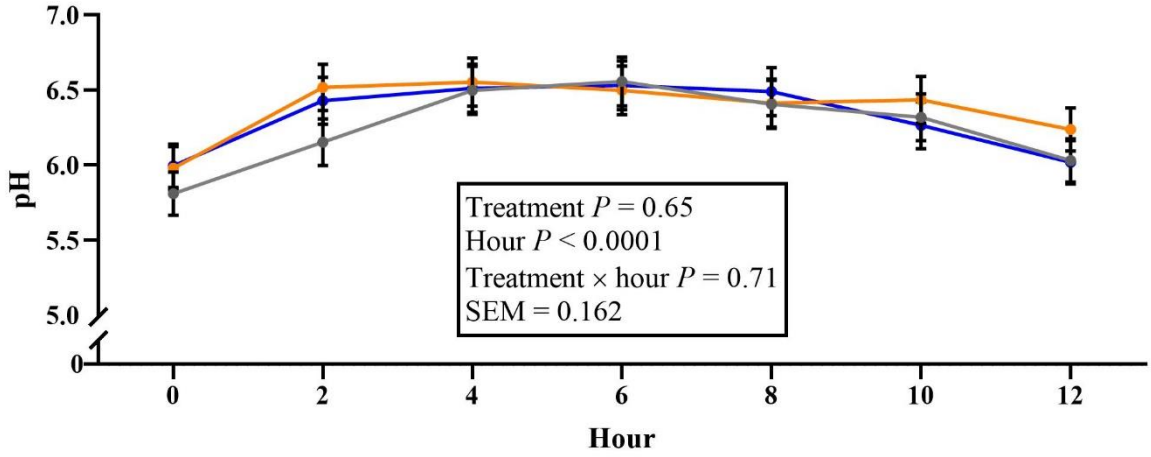

Figure 3.6 Rumen fluid pH of steers receiving control (0 g/d, ●), low (100 g/d, ●), or high (300 g/d, ●) calcium propionate treatments during a 12 h serially rumen fluid collection.

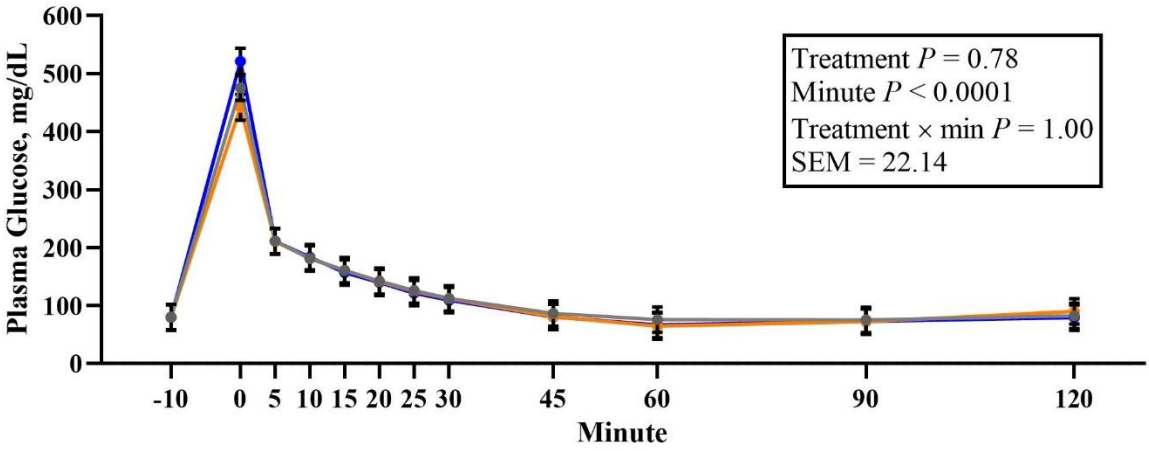

Figure 3.7 Plasma glucose concentrations during a glucose tolerance test of steers receiving control (0 g/d, ●), low (100 g/d, ●), or high (300 g/d, ●) calcium propionate treatments.

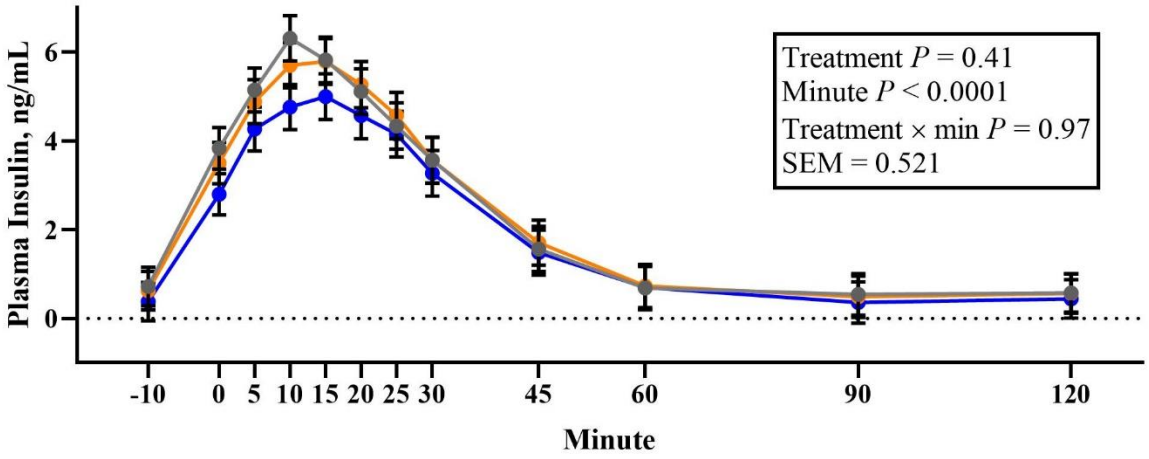

Figure 3.8 Plasma insulin concentrations during a glucose tolerance test of steers receiving control (0 g/d, ●), low (100 g/d, ●), or high (300 g/d, ●) calcium propionate treatments.

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