Effect of energy source and energy intake on plasma glucose-dependent insulinotropic polypeptide, and its association with respiratory quotient.

Thesis

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Abstract

Two experiments were conducted to evaluate the association between energy intake and plasma glucose-dependent insulinotropic polypeptide (GIP) concentration, and the association of plasma GIP concentration with gas exchange, carcass characteristics, and growth performance in feedlot cattle. In Experiment 1, I used 60 individually fed backgrounded Angus × SimAngus-crossbred steers (n=30) in a randomized complete block design. Steers (paired by body weight and gain to feed ratio (G:F)) were randomly allocated to one of the following treatments: *ad libitum* (AI) or restricted intake (RI; the same diet fed at 85% of the AI) of a finishing diet. The diet contained 61 % cracked corn, 9 % corn silage, 15 % DDGS, 5% soyhulls, and 10 % of a protein-mineral-vitamin premix. Measurement of CO_2 emission, and consumption of O_2, were taken using the Greenfeed system (n=15/treatment) once the steers were fed for at least 140 days. Plasma and gas samples were collected 10 d before slaughter, 1 h before and 2 h after feeding. Plasma glucose, non-esterified fatty acids (NEFA), GIP, and insulin concentration and gasses (O_2 and CO_2) were analyzed using the MIXED procedure of SAS evaluating the fixed effect of treatment, time (repeated measurement) and their interaction, and the random effect of block. Final body weight and carcass characteristics were analyzed with a similar model, without the time statement and its interaction. Compared with RI, AI steers had greater ($P < 0.01$) DMI and average daily gain (ADG). Steers on AI tended to have greater final body weight (BW) ($P = 0.07$) and ribeye area ($P = 0.09$). There was no treatment effect ($P \geq 0.11$) on G:F, subcutaneous (backfat, BF) and intramuscular (IM) fat, O_2 consumption and CO_2 emission. Plasma glucose concentration of AI steers were

greater before and after feeding than RI ($P < 0.05$). In conclusion, feeding steers ad libitum increased DMI, ADG, and plasma glucose and GIP concentration, but does not affect G:F, BF, IM fat, CO_2 emission, and O_2 consumption. In Experiment 2, I used 60 individually fed backgrounded Angus × SimAngus-crossbred steer calves during the growing and finishing period in an unbalanced incomplete randomized block design, with a 2 x 2 factorial arrangement of treatments. The calves (blocked by BW and G:F) were randomly allocated to one of the following treatments, restricted-fed or offered diets *ad libitum*; with or without 4% supplemental fat for 78 d. Animal growth performance, O_2 consumption, CO_2 emissions, respiratory quotient (RQ), plasma glucose, NEFA, insulin, and GIP concentration were analyzed as Experiment 1. Growth performance was analyzed over days, and hormones and metabolite concentration were compared pre- versus post-feeding. Measurement of CO_2 emission, and consumption of O_2, were taken using the Greenfeed system and blood samples during the growing and finishing periods. Compared to restricted intake and fat supplemented animals, steers fed *ad libitum* without fat supplemented fed (AN) had greater (intake by fat interaction; $P < 0.01$) final BW, DMI, ADG, and G:F. Also, supplementation of fat increased ($P < 0.05$) plasma GIP, NEFA, and glucose concentration while *ad libitum* intake increased ($P < 0.01$) plasma insulin concentration and tended ($P = 0.08$) to decrease plasma NEFA concentration in the finishing period. In conclusion, steers AN had greater final BW, DMI and ADG, but does not affect G:F, RQ and consumption of O_2. Supplementation of fat increased plasma GIP and glucose concentration while restricting intake decrease plasma insulin concentration while increasing plasma NEFA concentration in the growing period. In

feedlot cattle, differences in plasma GIP concentration were due to an interaction between dietary fatty acids, energy intake, and time of sampling (relative to feeding).

Dedication

To my God, Jehovah:

for making this possible.

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Chapter 1 Introduction

Intramuscular (IM) fat, also known as marbling, is one of the most important traits of meat quality (Khan et al., 2019). Increasing marbling is desirable and a key aspect for improving meat quality (Khan et al., 2019). Intramuscular fat is considered by consumers as one of the most determinant factors in beef quality (Nunes et al., 2015). Moreover, IM fat percentage is highly correlated with organoleptic characteristics such as juiciness, flavor, and tenderness (Nunes et al., 2015). Among these characteristics, tenderness is the indicator that has the greatest impact on meat quality (Nunes et al., 2015). Therefore, improving IM fat deposition will improves meat quality.

Improving the quality and efficiency in meat production has been the goal to meet the global demand for meat products because global demand continues to increase as the population grows (Zhang et al., 2020). Feed costs for beef cattle account for up to 70% of total production costs (Zhang et al., 2020). Thus, improvement of feed efficiency in these animals increases profitability while reducing the environmental impact, since those efficient animals produce less fecal output and methane (Zhang et al., 2020).

The hormone glucose-dependent insulinotropic polypeptide (GIP) is an insulinotropic gut hormone, and it is associated with lipid metabolism in non-ruminants (Baldassano et al., 2019; Lee et al., 2019). However, little is known about the basic biology of GIP in ruminants, such as the stimulus for secretion and action, compared to non-ruminant animals. There is limited data on GIP. Its role in lipid metabolism and its effect on feedlot cattle (Freitas et al., 2020). Recently, it was reported that GIP had a positive linear relationship with IM fat deposition (Freitas et al., 2020). However, Freitas

et al. (2020) could not conclude that GIP increases marbling accretion and/or decreases intramuscular fat lipolysis in beef cattle. In sheep, GIP decreases lipolysis in the subcutaneous adipose tissue (Martin et al., 1993b). Because of the importance of marbling in feedlot cattle, it is important to understand the action GIP as a potential modulator of energy partitioning. This could lead to the use of GIP to manipulate, through diet changes, to improve meat quality in feedlot cattle.

Dietary fatty acids has been suggested by many to be a potent and a more important nutrient in stimulating GIP compared to glucose in ruminants (Martin et al., 1993a; Martin and Faulkner, 1993; Martin and Faulkner, 1994). There are some confounding factors in the studies that suggest that the models, ruminants vs. non-ruminants, might be different. The nutrients that increase plasma GIP concentration remain unclear in feedlot cattle. It seems that ruminants and non-ruminants have differences in mechanisms stimulating GIP secretion. Although dietary fatty acid has been reported to be a potent stimulator of plasma GIP concentration, energy content in the diet and other underlying mechanisms could be a more important factor in stimulating GIP secretion.

Chapter 2 Literature Review

This literature review provides an overview of the adipose tissue and factors involved in intramuscular fat deposition. Moreover, the literature review will examine what is currently known about the secretion and main responses of the hormone GIP as possible factor to improve and manipulate IM fat deposition.

2.1 Adipose tissue

2.1.1 Composition and lipid metabolism

Adipose tissue is composed by fat cells that store and release fatty acids (Scherer et al., 2011; Pérez-Torres et al., 2019). There are different adipose depots in mammals, such as subcutaneous, visceral, intramuscular, and intermuscular fat (Khan et al., 2019). There are two variants that are distinguished: brown and white adipose tissue (Pérez-Torres et al., 2019). Although brown adipose tissue produces heat, white adipose tissue stores energy (Pérez-Torres et al., 2019). Furthermore, white adipose tissue is composed of mature adipocytes and a stromal vascular fraction (Pérez-Torres et al., 2019). White adipose tissue has two metabolic functions: storing and releasing non-esterified fatty acids (NEFA), which are done through lipogenesis and lipolysis, respectively (Pérez-Torres et al., 2019). Both processes oppose each other. Lipogenesis synthesizes triglycerides (TG) when fatty acids derived from lipoproteins are esterified with glycerol (Pérez-Torres et al., 2019). On the other hand, lipolysis is the process by which those stored TG are catabolized releasing free fatty acids and glycerol (Scherer et al., 2011; Pérez-Torres et al., 2019). In these processes, there are several enzymes involved, such as

lipoprotein lipase (LPL), adipose triglyceride lipase (ATGL), monoacylglycerol lipase (MAGL), and hormone sensitive lipase (HSL) (Abulmeaty et al., 2019; Pérez-Torres et al., 2019).

Lipid metabolism is the process that involves the dietary intake of lipids, lipogenesis, and lipolysis (Pérez-Torres et al., 2019). In beef cattle, the adipose tissue is the principal site of de novo fatty acid (FA) synthesis (Dodson et al., 2010). The hormone insulin has a role in regulating glucose homeostasis and lipid metabolism (Petersen and Shulman, 2018). It suppresses lipolysis, increases glucose uptake by the adipocyte, and increases lipogenesis (Petersen and Shulman, 2018). Although insulin function is the most widely known, the process is regulated by multiple hormones, such as cortisol, growth hormone, glucagon, and GIP (Christiansen et al., 2007; Harrison et al., 2007; Christensen et al., 2011).

2.1.2 Intramuscular fat deposition

Adipose tissue accretion plays a major role in the determination of meat sensory quality in animals used for production (Khan et al., 2019). Among the factors that influence meat quality, IM fat is one of the most important (Nunes et al., 2015; Park et al., 2018; Khan et al., 2019). Marbling is the amount of IM fat accumulated between muscle fibers and a key factor in determining carcass value (Cesar et al., 2015; Flowers et al., 2018). Moreover, IM fat is the sum of phospholipids and triglycerides or lipid droplets between muscle fibers or within muscle cells (Cesar et al., 2015). Many factors influence and regulate IM fat deposition, such as sex, age, nutrition, and genetics (Cesar

et al., 2015; Cafferky et al., 2019). Other factors such as feed withdrawal, transport time, and stress during transport can negatively affect meat quality (Cafferky et al., 2019).

There are processes that are associated with deposition of IM fat that involve preadipocyte proliferation, differentiation, and maturation (Khan et al., 2019). Several enzymes are involved in regulating IM fat deposition in cattle such as ATGL, MAGL, and HSL, which hydrolyzes TG (Abulmeaty et al., 2019; Khan et al., 2019). Reducing the activity of ATGL and MAGL promotes IM fat deposition (Khan et al., 2019).

Furthermore, an increased in IM fat accretion is associated with tenderness, juiciness, color, flavor, water-holding capacity (WHC)-related traits and consumer satisfaction (Cesar et al., 2015; Cafferky et al., 2019). Producers are incentivized to produce cattle with great IM fat deposition because its effects in meat quality, muscle characteristics, and carcass value (Cafferky et al., 2019; Freitas et al., 2020). Also, IM fat percentage is highly correlated with tenderness, which is regarded by consumers to be one of the most determinant factors in beef quality (Nunes et al., 2015). Meat quality and carcass value or traits are important for optimizing the profitability of the beef cattle industry and have been incorporated in worldwide beef cattle breeding programs (Grigoletto et al., 2020).

2.2 Indirect calorimetry

2.2.1 Gas Exchange

In the rumen, there is a complex ecosystem of bacteria, protozoa, and fungi where nutrients that are consumed are digested in a process known as fermentation

(Castillo-González et al., 2014). Once degradation of the feed components occur and generate end-products known as short-chain VFAs, carbon dioxide (CO_2), methane, and ammonia (Hernandez-Sanabria et al., 2010). The CO_2 and methane, product of fermentation, are released through eructation (Mccann et al., 2014). The main gas released is CO_2 (65.5%) while oxygen is released at a lesser percent (0.5%). The composition of the gases depends on the fermentation rates and ecology of the rumen. When ruminants are fed simple carbohydrates which are easily fermentable, growth of bacteria that utilize lactate (Kameshwar et al., 2019). The fermentation of starch and other nonstructural carbohydrates favor propionate production (Aguerre et al., 2011). In animals, cells utilize nutrients to produce energy in a process known as cellular respiration (Li et al., 2015; Ray and Fry, 2015). Cellular respiration is an oxidative process in which an electron donor is oxidized and oxygen is reduced to produce CO_2, H_2O, and energy (Li et al., 2015; Ray and Fry, 2015). Glucose is converted into pyruvate by the glycolysis metabolic pathway to be converted into acetyl-CoA and CO_2 (Li et al., 2015). The acetyl-CoA enters the citric acid cycle where it is oxidized to CO_2 and H_2O (Li et al., 2015).

2.2.2 Function and Respiratory quotient

In open-circuit indirect calorimetry technique, while outside air circulates around the animal's head, the expired and inspired air is collected (Fernández et al., 2019). Gaseous exchange is determined by measuring the total airflow in the system and the difference in gas concentration between inspired and expired air. This method measures

the gas exchange that is associated with the oxidation of energy substrates and estimates the associated heat production (HP) (Liu et al., 2018; Fernández et al., 2019). This technique uses head chambers and facemasks to quantify gaseous exchange. Moreover, the CH_4 and CO_2 emissions are measured because they are closely related to feed intake.

Indirect calorimetry is a noninvasive method used to determine energy expenditure (EE) (Oshima et al., 2017; Liu et al., 2018; González-Haro, 2019). Based on the close association between HP and the oxidation of organic matter in which O_2 is utilized and CO_2 and CH_4 are emitted, EE is assessed as HP (Arthur et al., 2018). In addition, EE can be determined using mathematical transformations of oxygen consumption (VO_2), carbon dioxide production (VCO_2), or both (Oshima et al., 2017; Kaiyala et al., 2019). The ratio of VCO_2 to VO_2 is known respiratory quotient (RQ), which ranges between ~0.7 and ~1.0, depending largely on the proportions of carbohydrate, fat, and protein being oxidized (Kaiyala et al., 2019). This ratio allows us to determine the respiratory exchange ratio (RER) (González-Haro, 2019).

2.2.3 Greenfeed system

Automated Head-Chamber System (AHCS) is an automated gas measurement system that provides reliable and accurate estimates of gas emissions from beef and dairy cattle (Arthur et al., 2018). One AHCS is the GreenFeed emission Monitor (GEM; C-Lock Inc., Rapid City, SD). It measures CH_4 and CO_2 production (Arthur et al., 2018) and O_2 consumption (Fernández et al., 2019) from animals. This system allows us to estimate these emissions for individual animals through multiple short-term measurements within a day (Arthur et al., 2018).

2.3 Glucose-Dependent Insulinotropic Polypeptide (GIP)

2.3.1 Secretion and Stimuli

Glucose-dependent insulinotropic polypeptide is a 42 amino acid peptide hormone synthesized in, and released from, K cells (Song et al., 2007; Weaver et al., 2008; Fujii et al., 2014). These K cells are located in the proximal duodenum and jejunum of the gastrointestinal tract (Song et al., 2007; Weaver et al., 2008). Also, GIP is released post-prandially; thus, it is released upon nutrient digestion (Song et al., 2007; Fujii et al., 2014). Then, GIP exerts its function through its receptor, known as GIP receptor (GIPR) (Fujii et al., 2014). This receptor is a $G\alpha_s$-protein-coupled receptor (GPCR) that works through cAMP production (Fujii et al., 2014; Tharp et al., 2020).

In non-ruminants, GIP is secreted when carbohydrate or fat are ingested (Kim et al., 2007; Weaver et al., 2008; Tharp et al., 2020). However, many researchers agree that fed starch does not stimulate GIP in ruminants (Martin and Faulkner, 1993; Martin and Faulkner, 1994; Rose et al., 1998; Dawson et al., 1999) , but it is still unclear what nutrients increase plasma GIP concentration because there are some confounding factors in the studies. In feedlot cattle, Freitas et al. (2020) noted that corn processing had no effect on plasma GIP and glucose concentrations. In that experiment (Freitas et al., 2020), the authors noted that the lack of effect of differences of plasma glucose concentration between different treatments could be because cattle that were fed cracked corn generated a greater proportion of propionate in the rumen compared to cattle fed whole-shelled

corn. The conversion of propionate to glucose in the liver could lead to similar blood glucose concentration on both treatments. However, they did not measure the total amount of glucose reaching the small intestine. Freitas et al. (2020) suggested that the lack of differences of plasma GIP and glucose concentration between the different treatments could mean that there are different underlying mechanisms in terms of stimulating plasma GIP secretion in ruminants compared to non-ruminants.

Dietary fatty acids have been suggested by many to be a potent and a more important nutrient in stimulating GIP compared to starch in non-ruminants (Martin et al., 1993a; Martin and Faulkner, 1993; Martin and Faulkner, 1994). Moreover, Dawson et al. (1999) suggested that dietary fatty acids are considered a more important stimulus because little glucose is absorbed from the small intestine and fermentation occurs in the rumen. However, the lack of differences in plasma GIP concentration among treatments in the experiment by Dawson et al. (1999) may be due to the diet used in their experiment, which may not provide enough fat or glucose that reach the small intestine, or the amount of starch or fat and rate of absorption might not had been that different to observe changes in GIP. Also, the experimental design and statistical analysis as described by Dawson et al. (1999) are not clear; therefore, Dawson et al. (1999) may have confounded some of the results. Overall, many studies suggests that fat is a potent stimulator for GIP secretion (Martin et al., 1993a). However, those studies did not measure a potential association between energy intake and fat intake; therefore, the type of energy intake was not compared in these studies.

An current was conducted in lactating dairy cows (Relling and Reynolds, 2007) to evaluate the effect of feeding rumen-inert fats differing in their degree of saturation on plasma GIP concentration. They found that plasma GIP increased when fat was added to the diet even though the cows maintained a similar metabolizable energy intake. However, they found no difference across the fat supplements differing on their degree of saturation in terms of plasma GIP concentration. Similarly, in an current done with pre-ruminant goat kids fed milk, skimmed milk, or solutions of milk, fat, lactose, glucose, or casein plus lactose, Martin et al. (1993a) determined that carbohydrate absorption had no effect on increasing plasma GIP concentration. Therefore, there may be differences between pre-ruminant goat kids, which are considered in a sense as non-ruminants due to their underdeveloped rumen, and true non-ruminant animals in changing plasma GIP concentration. However, differing results could be due to differences in energy intake, whereas the treatment with skim milk had less energy than whole milk, and differences in energy could have been the contributing factor in differences of plasma GIP concentration between treatments (Martin et al., 1993a).

An current was conducted in lactating dairy cows, where cows were postruminally infused with either corn starch, casein, or soybean oil (Relling and Reynolds, 2008). On day one of infusion, plasma GIP concentration increased when vegetable oil or casein were infused and tended to increase when starch was infused compared to the control, which was postruminally infused with water. On day 7, starch and casein increased plasma GIP concentration. However, cows infused with oil were not different from the control group. The lack of difference between oil infusion and water

may be due to effects of oil infusion on decreasing dry matter intake (DMI) (Relling and Reynolds, 2008). Oil infusion decrease DMI but did not change metabolizable energy intake compared with the control cows (Relling and Reynolds, 2008). This finding suggests that stimulation of plasma GIP concentration was associated with the increase in total metabolizable energy supply, which was greater in casein and starch infusions compared to the control (Relling and Reynolds, 2008). In growing lambs, supplementation with lipids in the diet increased plasma GIP concentration in the first week of the experiment, but it decreased after 1 month of the animals in the diet, which might indicate also an adaptation to the fat content in the diet (Relling et al., 2010).

Given all these findings, it seems that ruminants and non-ruminants have differences in mechanisms stimulating GIP secretion. Although dietary fatty acid seems to be a potent stimulator of plasma GIP concentration, energy content in the diet and other underlying mechanisms could be a more important factor in stimulating GIP.

2.3.2 Target tissues and main responses

The receptor for GIP is expressed in many non-ruminant tissues, including the pancreas, adipose tissue, gastrointestinal tract, bone, heart, endothelium, and central nervous system (Kim et al., 2007; Tharp et al., 2020). In non-ruminants, when glucose is present in the small intestine, GIP is secreted and binds to GIPR (Kim et al., 2007; Tharp et al., 2020). In the β-cells of the pancreas, after GIP binds to the GIPR, the intracellular pathway is activated, which induces insulin secretion, increases proinsulin synthesis, and promotes proliferative and survival pathways (Kim et al., 2007; Tharp et al., 2020). A glucose-dependent mechanism such as through GIP signaling means that the pathways

related to insulin secretion depends on glucose as a nutrient to be stimulated in non-ruminants. Therefore, GIP is one of the incretin hormones. Incretins are peptide hormones that are secreted by the gastrointestinal tract and released during nutrient absorption (Kim et al., 2007; Weaver et al., 2008). Once glucose is absorbed, incretins promote insulin secretion from pancreatic β-cells (Kim et al., 2007; Weaver et al., 2008). However, there is a threshold of plasma glucose concentration required to illicit an insulinotropic response from GIP. In ruminants, such concentration is 120 mg/dL (Faulkner and Martin, 1999), which is mostly not reached in typical feedlot forage-based diets. However, even if it does not illicit that insulinotropic response, it can still act directly on the adipose or other tissues through GIPR binding.

When GIP was first discovered, it was named gastric inhibitory polypeptide because it inhibits gastric acid secretion in the gastrointestinal tract (Fujita et al., 2010). However, its name was changed to reflect its glucose-dependent potentiation of insulin secretion while still keeping its acronym (Dupre et al., 1973). Additionally, GIPR is expressed in adipose tissue (Lee et al., 2019), where GIP increases fat deposition (Lee et al., 2019). In the adipose tissue, GIP stimulates fatty acid synthesis and enhances insulin-stimulated incorporation of fatty acids into triglycerides (Bakhøj et al., 2003). Thus, it accelerates fat deposition and expansion of fat depots (Yamane et al., 2016).

In rodents and obese humans fed high fat diets, GIP stimulates fat deposition and obesity (Miyawaki et al., 2002; Fujita et al., 2010; Lee et al., 2019). When excessive fat is consumed, GIP is hypersecreted. Thus, hypersecreting GIP exacerbates obesity by increasing nutrient uptake and triglyceride accumulation in the adipocytes (Miyawaki et

al., 2002). Moreover, due to obesity, hyperinsulinemia results from insulin resistance, which increases fat storage in the adipocytes. Therefore, a vicious cycle causes adiposity. However, GIP receptor-deficient mice maintained normal weight (Miyawaki et al., 2002; Song et al., 2007). Also, GIPR-deficient mice had a lower respiratory quotient and increased oxidation of fat that was not efficiently accumulated in adipocytes compared to controls (Miyawaki et al., 2002). Thus, GIPR-deficient mice were resistant to obesity (Miyawaki et al., 2002). Hence, GIP antagonists could be a potential treatment for obesity (Fujita et al., 2010). Although eliminating GIP might reduce weight gain, it has consequences in bone formation and leads to osteopenia (Shimazu-Kuwahara et al., 2019).

In the adipose tissue, the enzyme lipoprotein lipase (LPL) hydrolyzes triglycerides and releases free fatty acids and monoglycerides that are stored in chylomicrons and very low-density lipoproteins (VLDL) (Wang and Eckel, 2009; Baldassano et al., 2019). Changes in expression and activity of LPL have major effects on lipid and glucose metabolism (Baldassano et al., 2019). Also, GIP promotes triglyceride accumulation in the adipose tissue by stimulating LPL activity. However, those authors noted that GIP works differently in the adipose tissue in humans compared to rodents. In humans, GIP increases adipose tissue blood flow and fat deposition, and a GIPR antagonist decreases adipose tissue triglycerides uptake and increases the free fatty acid/glycerol ratio. However, in rodents, GIP increases lipolysis, LPL activity and body weight, but GIP increases adipose tissue inflammation, visceral fat, and triglyceride concentration. Therefore, there may be differences in hormonal signaling and metabolism, even among

non-ruminants, in the adipose tissue. This suggests that lipid metabolism in the adipose tissue could be different between ruminants and non-ruminants.

Research conducted on GIP and lipid metabolism on ruminants has been limited. There have been considerably fewer studies that have been done on GIP in feedlot cattle. In feedlot cattle, Dawson et al. (1999) noted that plasma GIP concentration was negatively correlated to plasma insulin concentration; they suggested that GIP was not insulinotropic in beef cattle. Also, subcutaneous adipose tissue treated with GIP in the presence or absence of insulin in vitro had greater rates of lipogenesis compared to perirenal adipose tissue. However, Dawson et al. (1999) stated that it was unlikely that GIP plays a major role in promoting fat accretion. On the other hand, GIP at 0.01 to 10 nM concentration stimulated lipogenesis in lamb perirenal fat, subcutaneous back fat tissue, and popliteal fat tissue (Baba et al., 2000). Moreover, GIP had maximal effects at 1 nM. Furthermore, in the presence of insulin, a dose-dependent reduction of up to 50% on lipogenesis was seen in perirenal adipose tissue, but its net effect stabilized rates of lipogenesis in adipose tissue. The dose-dependent reduction on lipogenesis in the presence of insulin by GIP could have been because the infusion doses of plasma GIP was greater than physiological concentration, which is why it decreased insulin action. Therefore, the reduction on lipogenesis by GIP may not be seen in lambs because plasma GIP concentration were greater than normal physiological concentration of GIP. Overall, rates of lipogenesis were greater in perirenal adipose tissue compared to popliteal and subcutaneous adipose tissue, which these last two were similar between each other. Nonetheless, there are variations among depots some of which produce depot-specific

hormones and cytokines. Therefore, general conclusions cannot be made on one depot alone since there are differences in responses to GIP and variations in general among depots (Baba et al., 2000).

In an experiment conducted in sheep, Martin et al. (1993b) determined that GIP affects the metabolism of the subcutaneous adipose tissue; also, GIP had direct, insulin-like effect on ovine adipose tissue. Moreover, intravenous infusion of GIP decreased glycerol concentration in the subcutaneous adipose tissue extracellular matrix, which indicates that GIP inhibited lipolysis. The data suggest an increased uptake of plasma glucose associated with stimulation of lipogenesis. Similarly, when insulin concentration were increased in sheep, GIP enhanced peripheral insulin-mediated glucose uptake (Rose et al., 1998). Nonetheless, when insulin concentration was basal or near zero, GIP had no effect on whole-body glucose. These data may suggest that GIP may enhance insulin action with respect to glucose disposal following a meal in sheep but has no effect on glucose disposal pathways not responsive to insulin.

Freitas et al. (2020) detected a positive linear association between plasma GIP concentration and IM fat deposition in feedlot cattle. They did not conclude that GIP increases marbling accretion or decreased IM fat lipolysis as it did in the subcutaneous tissues in sheep as shown by Martin et al. (1993b). The positive linear association between plasma GIP concentration and IM fat deposition is only an association (Freitas et al., 2020). There could be other factors, such as other hormones, influencing lipid metabolism. Unless an experiment is done with a continuous infusion of GIP with the

same energy intake for all animals or using an agonist or antagonist for GIPR, we would not know definitively what the function of GIP in ruminants is.

Finally, plasma GIP concentration has been positively correlated with milk energy yield in lactating dairy cows; because of a negative correlation between plasma GIP concentration and RQ, Relling et al. (2014) concluded that cows with lower plasma GIP concentration were using more fat as an energy substrate, which indicated that GIP stimulated lipogenesis. Also, the use of lipids as an energy fuel would allow more glucose to be utilized for milk synthesis. Therefore, those authors suggested that GIP may play a role in the regulation of nutrient and energy metabolism in dairy cows.

2.4 Hypothesis and objectives

Based on the described literature, I hypothesized that animals fed greater energy intake have greater plasma GIP concentration, RQ, and IM fat deposition. The increase in IM fat deposition is positively and linearly associated with changes in plasma GIP concentration. Also, animals fed greater gross energy diets from fat would have greater plasma GIP concentration compared to the other treatments; this will happen at the beginning of the experiment, but the difference disappears over time due to an adaptation to fat.

The overall objective of this thesis was to evaluate the effect of energy and lipid intakes on plasma GIP concentration, gas exchange, growth performance, and carcass characteristic in feedlot cattle. Also, the objective was to measure plasma GIP concentration to determine its association with intramuscular fat deposition in feedlot cattle at different levels of dry matter intake.

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Chapter 3 Evaluation of the Association Between Plasma Glucose-Dependent Insulinotropic Polypeptide, Respiratory Quotient, and Intramuscular Fat Deposition in Feedlot Cattle

3.1 Introduction

Intramuscular (IM) fat is a desirable trait used to improve meat quality and highly regarded by consumers (Nunes et al., 2015; Park et al., 2018; Khan et al., 2019). Increasing IM fat deposition is favorable because it is associated with organoleptic characteristics among which is tenderness and is important for optimizing the profitability of the beef cattle industry (Nunes et al., 2015; Grigoletto et al., 2020). To meet the current market demands to improve meat quality, strategies including manipulating nutrition have been implemented in beef cattle, which also has a role in growth performance (Li et al., 2014; Park et al., 2018)

Glucose-dependent insulinotropic polypeptide (**GIP**) is a 42 amino acid peptide hormone synthesized in, and released from, K cells (Song et al., 2007; Weaver et al., 2008; Fujii et al., 2014). These K cells are located in the proximal duodenum and jejunum of the gastrointestinal tract (Song et al., 2007; Weaver et al., 2008). Also, GIP is released post-prandially; thus, it is released upon nutrient digestion (Song et al., 2007; Fujii et al., 2014). In non-ruminants, GIP is secreted when glucose or fat is ingested (Kim et al., 2007; Weaver et al., 2008; Tharp et al., 2020). However, many researchers agree that fed starch does not stimulate GIP in ruminants (Martin and Faulkner, 1993; Martin and Faulkner, 1994; Rose et al., 1998; Dawson et al., 1999), but it is still unclear what nutrients increase plasma GIP concentration because there are some conflicting results. Research conducted on GIP and lipid metabolism on ruminants, especially feedlot cattle,

has been limited. Dietary fatty acids has been suggested by many to be a potent and a more important nutrient in stimulating GIP compared to fed starch in ruminants (Martin et al., 1993a; Martin and Faulkner, 1993; Martin and Faulkner, 1994). Lactating dairy cows were postruminally infused with either corn starch, casein, or soybean oil (Relling and Reynolds, 2008). On day one of infusion, plasma GIP concentration increased when vegetable oil or casein were infused and tended to increase when starch was infused compared with control (postruminally infused with water). After 7 days of infusion, starch and casein increased plasma GIP concentration. However, cows infused with oil were not different from the control group. Oil infusion decreased DMI but did not change metabolizable energy intake compared to the control cows (Relling and Reynolds, 2008). This may suggest that stimulation of plasma GIP concentration was associated with the increase in total metabolizable energy supply, which was greater in casein and starch infusions compared to the control (Relling and Reynolds, 2008). In growing lambs, supplementation with lipids in the diet increased plasma GIP concentration in the first week of the experiment, but it decreased after 1 month of the animals in the diet, which might indicate also an adaptation to the fat content in the diet (Relling et al., 2010). Although dietary fatty acid seems to be a potent stimulator of plasma GIP concentration, energy content in the diet and other underlying mechanisms could be an important factor in stimulating GIP.

The main responses of GIP on tissues are stimulating insulin secretion and fat accumulation (Dupre et al., 1973; Martin and Faulkner, 1993; Miyawaki et al., 2002; Yamane et al., 2016). Freitas et al. (2020) detected a positive linear association between

plasma GIP concentration and intramuscular (IM) fat deposition in feedlot cattle. They did not conclude that GIP increases marbling accretion; however, Martin et al. (1993b) showed that GIP decreased lipolysis in the subcutaneous in sheep.

I hypothesized that animals fed at *ad libitum* intake (**AI**), compared to restricted intake (**RI)**, have greater IM fat deposition. This increase in IM fat deposition is associated with GIP concentration. Animals given AI have greater plasma GIP concentration and a greater respiratory quotient (**RQ**), which means they are using less fat as energy substrate and have a positive effect on lipogenesis. Therefore, the objective of this experiment was to evaluate the effect of different DMI on plasma GIP concentration and its association with intramuscular fat deposition in feedlot. A second objective was to determine the effect of different levels of DMI on O_2 consumption, CO_2 emission, growth, RQ, plasma GIP concentration, and carcass characteristic in feedlot cattle.

3.2 Materials and Methods

3.2.1 Animals, experimental design, and treatments

Animal procedures and husbandry practices were approved by the Institutional Animal Care and Use Committee (# 2019A00000112) of The Ohio State University and followed the guidelines recommended in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

The experiment used 60 individually fed backgrounded Angus × SimAngus-crossbred steers (n = 30 per treatment) in a randomized complete block design. Steers were weaned at 7 months of age, preconditioned for 45 days and move to the feedlot

facility. From the arrival to the facility until the experiment started (5 months) the steers were fed a diet containing 65% of corn silage, 10% of whole shelled corn, 15% of distillers grain with solubles (DDGS) and 10% of a mineral-vitamin premixed. The transition to the experimental (finishing) diet was a step up increase in corn while the corn silage was decreased. The transition to the experimental diet lasted 3 wk.

Steers (blocked by body weight and gain-to-feed ratio (**G:F**) during the backgrounding stage) within each block were randomly allocated to AI or RI. Restricted intake steers had the same diet offered at 85% of the AI. The diet contained 61 % cracked corn, 9 % corn silage, 15 % dry distillers grain with solubles (DDGS), 5% soyhulls, and 10 % of a protein-mineral-vitamin premix (Table 3.1). The animals were fed at 0900 h.

3.2.2 Sampling and analysis

Feed samples were collected weekly for feed analysis and DMI was recorded daily. Gas exchange was measured and blood samples were collected (see below). The amount of feed that remained in the bunk at 2 h after feeding was measured. The reason for these measurements was to evaluate the amount of feed intake at the time of plasma and gas samplings. Body weight (**BW**) was measured on days 29, 49, 75, and final BW (average between the day before and the day that the steers were sent to slaughter). These measurements were taken to determine daily DMI, growth, average daily gain (**ADG**), and G:F.

During d 7 to 28, the animals were adapted to the use of the gas sensor equipment (GreenFeed System). The Greenfeed System has been proven to provide reliable information of gas data when compared to other methods for measuring gases (Patra, 2016;

Doreau et al., 2018). Twice a week for 3 wk, each steer was walked to the chute where the GreenFeed system was located. On d 1, the animals were taken to the chute, kept there for 1-2 min and walked back to their pens. On d 2 and 3, the animals were walked to the chute and introduced a bucket with 50 g of cracked corn. Then, steers remained in the chute for 3 min. On d 4, 5 and 6, the concentrate was introduced in the GreenFeed system, and the system was moved in front of them. This adaptation was used also to select the animals that had a calm behavior during the exposure of the gas sensor equipment.

Measurements of CO_2 emission and O_2 consumption were taken using the Greenfeed system (Hristov et al., 2015) (n=15/treatment). Plasma and gas samples were collected 10 d before slaughter, 1 h before and 2 h after feeding. The ratio CO_2/O_2 was used to determine the respiratory coefficient as a marker of substrate used to supply energy. Blood samples were taken from the steers via the jugular vein to measure glucose, non-esterified fatty acids, GIP, and insulin as markers for energy metabolism. Blood samples were immediately transferred to tubes containing solutions of disodium EDTA and benzamidine HCl (1.6 and 4.7 mg/mL of blood, respectively) and placed on ice. After centrifugation for 30 min (1,800 × g and 4°C), blood was aliquoted into individual polypropylene tubes and stored at −80°C until analyzed. During d 90 to 115, animals were harvested commercially depending on the amount of back fat (BF; visual appraisal) of the control steer. Steers in the RI treatment were sent to slaughter at the same time when the AI steer (control steer) reached visually the amount of back fat targeted. Hot carcass weight was recorded on day of slaughter. A sample from the 12th to 13th rib the Longissimus muscle dorsi (**LM**) was collected at harvest for latter analysis of

LM area, BF and IM fat concentration. Intramuscular fat was measured using ether extract (AOCS, 2005) except the samples were freeze dried, instead of dried in an oven, during 24 h using a freeze drier (HarvestRight 4-shelf bench top freeze dryer, HarvestRight, North Salt Lake, UT, USA). The LM area was determined at the 12th and 13th rib interface of the LM with tracing techniques; the method for this procedure has been described in the Appendix 1.

Concentration of insulin were measured using radioimmunoassay (RIA) as described by Miqueo et al. (2019). Plasma GIP concentration was measured using a modified RIA based on the Phoenix pharmaceutical assay kit (Phoenix pharmaceutical RK-027-02) as described in the Appendix 2. Plasma glucose concentration was measured using a colorimetric assay (#1070 Glucose Trinder, Stanbio Laboratory, Boerne, TX). Plasma NEFA concentration was measured using micro-titer plates and plate reader in a two-reaction, enzyme-based assay (Wako Chemicals USA, Richmond, VA) as described by Johnson and Peters (1993).

3.2.3 Statistical Analysis

The experiment was analyzed as a complete randomized block design. Plasma glucose, NEFA, GIP, insulin concentration, and gases (O_2, CO_2, and RQ) were analyzed using the MIXED procedure of SAS (9.4) with repeated measurements. The model evaluated the fixed effect of treatment, time (repeated measurement), and their interaction. The animal was the subject and the most appropriate covariance structure was chosen as having the lowest Akaike Information Criterion. The first-order autoregressive covariance structure was used for analysis of RQ, plasma glucose, and GIP concentration.

The unstructured covariance structure was used for analysis of plasma insulin and NEFA concentration. The compound symmetry covariance structure was used for the analysis of O_2 consumption and CO_2 emission. Final BW, DMI at 2 h after feeding, and carcass characteristics were analyzed with a similar model, without the time statement and its interaction. A Pearson correlation (Proc CORR of SAS) was used to evaluate the association between plasma GIP concentration and IM fat deposition. Significance was declared at $P \leq 0.05$ and tendencies were considered at $0.05 < P \leq 0.10$.

3.3 Results and Discussions

3.3.1 Growth Performance

Steers on AI tended to have greater final BW ($P = 0.07$; Table 3.2). The tendency in greater final BW in AI animals is supported in an experiment done with growing cattle fed a grass silage diet that had increasing final BW with increasing level of intake (*ad libitum* vs. 80 and 65 % restriction; Ouellet et al., 2001). Similarly, growing cattle fed *ad libitum* a grass silage had greater final BW than restricted fed calves (80%) (Prezotto et al., 2017). The tendency for greater final BW in AI steers contradicts the results from Li et al. (2014) in which no difference was observed in final BW.

Compared to RI, AI steers had greater ($P < 0.01$) daily DMI and ADG. By design, restricted animals consumed less feed than *ad libitum* fed animals. However, the DMI after 2 hours of feed offered, on the days that gas exchange and blood samples were taken, was greater ($P < 0.01$) for the RI steers compared with the AI steers. Li et al. (2014) reported that ADG was not affected by energy levels in F1 Angus × Chinese

Xiangxi yellow Cattle, which is in contrast to the current experiment where AI steers tended to have greater ADG. One possible explanation for contrasting results is because Angus breeds have greater growth rates than Chinese Xiangxi yellow Cattle (Laborde et al., 2001; Albertí et al., 2008). Similar improvements in ADG has been reported previously (Holt et al., 2000). Steers fed *ad libitum* a high corn diet for 21 d had a greater ADG than restricted fed (70 to 80%) steers (Holt et al., 2000). At the end of a 63-d period, steers that were restricted for the first 21 d had lesser ADG than steers restricted for the first 42 d (Holt et al., 2000). Although after 63 d ADG was different between restricted steers they were similar to *ad libitum* fed steers (Holt et al., 2000). Similarly, steers fed a high-corn diet *ad libitum*, compared to restricted intake (80%), had greater ADG for the first 56 d (Hicks et al., 1990). After the first 56 d, the steers with restricted intake were fed *ad libitum* for 82 d, and there were no differences between treatments on ADG for those 82 d (Hicks et al., 1990). However, steers fed *ad libitum* for 138 d had greater ADG overall compared to steers with restricted intake (Hicks et al., 1990). An improvement of ADG was also observed in Angus, Simmental, and Shorthorn growing calves fed a forage based diet *ad libitum* compared to limit fed at 80% of the *ad libitum* intake (Prezotto et al., 2017). Similarly, growing cattle fed a grass silage diet had increasing ADG with increasing level of intake (Ouellet et al., 2001). Additionally, ADG was greater in steers fed *ad libitum* a high concentrate diet compared to restricted steers (77%) for 84 d (Holt et al., 2000). This difference remained after the restricted animals were subsequently fed *ad libitum* (Mcgregor et al., 2012).The contradictory results in growth observed in the different studies might be due to the type of diet (hay vs.

concentrate), the time that the diet was offered, the degree of feed restriction, or their interactions. Also, in the current experiment steers that had restricted intake were never switched back to *ad libitum* feeding.

There was no effect of treatment (P = 0.77) on G:F. The lack of difference in efficiency between the treatments is similar to other experiments (Hicks et al., 1990; Hayden et al., 1993; Holt et al., 2000). No differences in G:F was also reported in steers fed *ad libitum* a high concentrate diet and restricted steers (77%) for 84 d (Mcgregor et al., 2012). But feed-to-gain ratio (F:G) was lesser for steers fed a 90% haylage diet compared to steers fed a high concentrate diet (Mcgregor et al., 2012). After 84 d steers that were limit fed, had *ad libitum* access to the high concentrate diet; only British steers, but not Continental steers, had improved F:G (Mcgregor et al., 2012). The authors (Mcgregor et al., 2012) discussed that the improvement in efficiency in limit-fed and then fed *ad libitum* steers was because they reduce maintenance costs and improve diet digestibility compared to *ad libitum* fed steers. However, greater efficiency (lesser F:G) were seen in crossbred steers fed 70 to 80% of *ad libitum* DMI that transitioned to *ad libitum* DMI for an additional 20 days (Holt et al., 2000). Holt et al. (2000) suggested that the contrasting results in efficiency was because of differences in diet digestibility and that restricted fed cattle have previously shown greater efficiency of metabolizable energy use for body energy gain. Murphy et al. (1994) reported that mild restrictions in DMI resulted in improvements in digestibility in steers fed a corn silage based diet. The improvements could be results of increased hindgut fermentation of fiber components (Murphy et al., 1994). The observed results in G:F differ from previous research limiting

feed intake of a forage based diet. Improvement in G:F when restricting intake was reported in Angus, Simmental, and Shorthorn growing calves fed a forage based diet for 84 d *ad libitum* (Prezotto et al., 2017). Prezotto et al. (2017) reported that *ad libitum* DMI steers had greater G:F than those limit-fed at 80% of the *ad libitum* DMI. Prezotto et al. (2017) discussed that the results of limit-feeding may differ depending on the level of restriction, type of diet (forage compared with concentrate), and energy density of the diet. Those factors are possible explanations for when Ouellet et al. (2001) noted that F:G decreased linearly with increasing level of DMI in growing cattle fed a grass silage diet, which meant *ad libitum* animals were more efficient; however, animals had a different diet and level of restriction (80 and 65%) compared to the present experiment.

3.3.2 Plasma Hormone and Metabolite Concentration

Plasma glucose concentration of AI steers was than RI steers ($P = 0.02$; Table 3.3). There was an effect of time on plasma glucose concentration ($P = 0.01$). There was a significant effect of time ($P < 0.05$) on plasma insulin concentration. There was no effect of treatment ($P \geq 0.45$) on plasma insulin and NEFA concentration. However, steers tended to have a time effect on plasma NEFA concentration ($P = 0.06$). There was an effect of time and treatment on GIP ($P \leq 0.04$). Plasma GIP concentration was greater after eating and greater in AI animals. There was no effect of treatment ($P \geq 0.11$) for any other plasma hormone and metabolite concentration measured in the present experiment.

The plasma glucose concentration of AI steers is similar to the one reported by Becú-Villalobos et al. (2007) in Angus and Angus–Hereford feedlot steers fed a high

concentrate diet (40.6% corn grain) for 84 d. The time effect on plasma glucose and insulin concentration are consistent with established data (Brockman and Laarveld, 1986; Becú-Villalobos et al., 2007). Blood glucose regulation is integrated with regulation of other metabolic processes through common hormones such as insulin (Brockman and Laarveld, 1986). Plasma insulin concentration was greater and plasma glucose concentration was lesser 2 h after feeding in both treatments and may be because plasma insulin concentration is positively correlated with feed intake and facilitates glucose and amino acid uptake by peripheral tissues (Brockman and Laarveld, 1986). Plasma glucose concentration was lesser 2 h after feeding, perhaps because insulin increases uptake of glucose into peripheral tissues such as muscle and fat (Brockman, 1978; De Koster and Opsomer, 2013). Ouellet et al. (2001) reported using a grass silage diet that pre-prandial plasma glucose concentration was lesser in *ad libitum* fed cattle compared to limit fed cattle (80 and 65 % restrictions) after 79 d; also plasma glucose concentration decreased with feeding and was greater in restricted animals. In the present experiment, plasma glucose concentration did decrease with feeding, but was greater in *ad libitum* animals.

Ouellet et al. (2001) reported that plasma insulin concentration before feeding was greater in *ad libitum* animals compared to feed restricted steers fed a grass silage (timothy or bromegrass) diet after 78 d; which contradicts the results of the present experiment from which there was no treatment effect. However, Ouellet et al. (2001) also reported that, on d 79, plasma insulin concentration 2 h after feeding were greater in *ad libitum* fed animals compared to restricted fed animals which contrasts with this experiment.

The time effect for plasma NEFA concentration was because of a greater concentration of NEFA pre-feeding compared to post-feeding. Greater concentration of plasma NEFA could be explain based on the need for energy; NEFA are released to circulation, from the adipose tissue, to meet the metabolic needs of the animals (Bowden, 1971; De Koster and Opsomer, 2013). Plasma concentration of NEFA is also associated with lesser insulin concentration (Bowden, 1971; De Koster and Opsomer, 2013). This is similar to previous research reporting that in ruminants plasma NEFA concentration is related primarily to time of feeding (Bowden, 1971). These observed changes in concentration of plasma NEFA could also be related to the increase in plasma insulin concentration after feeding because it has been reported that insulin could inhibit the release of NEFA in ruminants (Bowden, 1971). Also, in growing lambs, a negative association was reported between plasma concentration of GIP and NEFA (Relling et al., 2010). The authors discussed that the negative association between plasma GIP and NEFA concentration could possibly be due to a role reported in GIP in decreasing lipolysis in ovine adipose tissue (Martin et al., 1993b). Greater NEFA concentration indicate lipid mobilization and fatty acid oxidation (Wathes et al., 2009). The observed results of plasma NEFA concentration differ from growing cattle fed a grass silage diet, in which there was an effect of time and intake on plasma NEFA concentration (Ouellet et al., 2001). Restricted fed cattle (80 and 65 %) had greater plasma NEFA concentration before and after feeding compared to the *ad libitum* fed cattle (Ouellet et al., 2001). Also, in that experiment plasma NEFA concentration on all treatment decreased after feeding (Ouellet et al., 2001). The authors discussed that animals with greater intakes had lesser

plasma NEFA concentration because they received more silage, and therefore a greater nutrient supply (Ouellet et al., 2001).

I had hypothesized that steers fed AI would have greater plasma GIP than the RI steers concentration, as seen in the present experiment . Plasma GIP concentration was greater after feeding time, which is in agreement with established data that GIP is released upon nutrient digestion (Song et al., 2007; Fujii et al., 2014). In steers fed at AI, plasma GIP concentration was greater compared to RI animals, despite that DMI of RI steers was greater than AI steers 2 hours after feeding. Similar results were reported in lactating dairy cows that were postruminally infused with either corn starch, casein, or soybean oil (Relling and Reynolds, 2008). On d 7, starch, and casein increased plasma GIP concentration. This suggest that stimulation of plasma GIP was associated with the increase in total metabolizable energy supply, which was greater in casein and starch infusions compared to the control (Relling and Reynolds, 2008). The observed results are similar to those reported in non-ruminants because GIP was secreted when glucose was ingested (Kim et al., 2007; Weaver et al., 2008; Tharp et al., 2020). Previously, fat was thought to be an important nutrient in stimulating GIP compared to glucose in ruminants (Martin et al., 1993a; Martin and Faulkner, 1993; Martin and Faulkner, 1994). Results in the present experiment could indicate that energy content in the diet is still a factor in stimulating plasma GIP concentration in ruminants since there was no fat supplemented in the diet and the animals were fed similar diets.

3.3.3 Gas exchange

I did not observe a treatment or treatment x time interaction effect ($P \geq 0.11$; Table 3.3) on O_2 consumption, RQ, and CO_2 and CH_4 emission. After feed ingestion the oxidation of glucose through cellular respiration the animal produces more CO_2, which is what was expected to occur 2 h after the feed was offered in the present experiment. However, the decrease of post-prandial CO_2 emissions cannot be explained or supported with existing literature.

Main effect of time ($P < 0.01$) was observed on CH_4 emission (Table 3.3). Post-feeding CH_4 emission were greater than pre-feeding for both treatments. A possible explanation for the time effect on CH_4 emission could be because it is by-product of microbial fermentation of carbohydrates (Hristov et al., 2013).

I had hypothesized that animals fed AI would have greater plasma GIP concentration and greater respiratory quotient (**RQ**), which means they would be using less fat as substrate and increase lipogenesis. The main responses of GIP on tissues are stimulating insulin and fat accumulation (Dupre et al., 1973; Martin and Faulkner, 1993; Miyawaki et al., 2002; Yamane et al., 2016). The steers in both groups had lesser RQ before feeding and approximately 0.7 which could indicate that fat was used as an energy substrate (Rogobete et al., 2019). Nonetheless, RQ results before and after feeding were approximately 0.7 which mean they were still using mainly fat as a substrate. Therefore, my hypothesis was rejected because feed intake or plasma GIP concentration could not be associated to RQ. However, it was proposed that in ruminants a glucose threshold of at

least 4.5 mM (81.1mg/dl) is needed for GIP to act as insulinotropic that was possibly not met in the current experiment (Faulkner and Martin, 1997; Holst, 1997).

3.3.4 Carcass Characteristics

I had hypothesized that feeding AI to beef steers would increase IM fat deposition; however, there was no treatment effect ($P \geq 0.11$; Table 3.4) on HCW, BF and IM fat. There was no correlation ($P = 0.82$) between plasma GIP concentration and IM fat deposition. Steers on AI tended to have greater LM area ($P = 0.09$) compare with steers on RI. A lack of difference in LM area, IM fat, and HCW was observed when comparing different DMI in continental and British steers fed a high moisture corn diet *ad libitum* or limit-fed (77%) for 84 d (Mcgregor et al., 2012). Similarly, steers fed a high-wheat diet *ad libitum* or restricted for 149 d had no differences in HCW, LM area or BF (Hicks et al., 1990). Also, Hereford steers fed a high-corn diet *ad libitum* for 138 d tended to have greater HCW but no differences in LM area or BF compared with restricted-fed animals (restricted during 56 d followed by a period of *ad libitum* feeding for 82 d; Hicks et al., 1990). No differences in HCW, BF, and LM area was observed also in previous research on F1 Angus × Chinese Xiangxi yellow cattle; when cattle was fed under two levels of dietary energy and protein (Li et al., 2014). Additionally, Li et al. (2014), reported that cattle fed diets with greater energy had greater IM fat. The data from Li et al. (2014) differs from the results of the present experiment; where there was no effect of level of DMI on IM fat and a tendency for greater LM area. However, data from different breeds such as Angus × Chinese Xiangxi yellow cattle are not always comparable with studies using Angus and Angus crossbred animals (Li et al., 2014).

Animals in the present experiment had greater BW and HCW in general compared to the experiment by Li et al. (2014); because Angus breeds have greater growth rate, which results in greater carcass weight at the finishing phase (Laborde et al., 2001; Albertí et al., 2008). Results in the present experiment differ from growing cattle that had greater carcass weight with increasing feeding level (Ouellet et al., 2001). However, the experiment was during the growing period with a silage-based feed which contrasts from the present experiment that fed steers high-concentrate diet during the finishing period (Ouellet et al., 2001).

3.4 Conclusions

In conclusion, feeding steers at *ad libitum* increased ADG and plasma glucose and GIP concentration but did not affect G:F, BF, IM fat, CO_2 emission, RQ, and consumption of O_2. Results in the present experiment could indicate that plasma GIP concentrations in ruminants is not stimulated only by fat content in the diet. Also, metabolizable energy content in the diet could still be an important factor leading to differences plasma GIP concentration in ruminants. Although there was a significant effect of treatment on plasma GIP concentration, there were no differences in RQ. These results do not confirm that GIP is positively associated with fat deposition and greater plasma insulin concentration as it did in feedlot cattle.

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3.6 Tables

Table 3.1. Dietary and chemical composition (% DM basis) of the control diet.

Item	Amount
Ingredient	
Cracked corn	67.46
DDGS	15
Corn silage	9
Soy hulls	5
Urea	0.37
Limestone	1.66
Minerals/vitamins[1]	1.51
Composition	
CP, %	11.75
NDF, %	17.58
EE, %	3.62
Ash	5.57

[1]Mineral and vitamin mix, contained 4.615% of Sodium Chloride, 0.068% of Vitamin A, 30,000 IU/g, 0.068% of Vitamin D, 3,000 IU/g, 0.205% of Vitamin E, 44 IU/g, 6.461% of Ca Sulfate, 0.351% of Selenium, 0.203%, Rumensin 90 (Elanco Animal Health, Greenfield, IN), 2.769% of Potassium Chloride, 0.06% of Copper Sulfate, 0.185% of Zinc Sulfate, 0.111% of Manganese Sulfate, and 0.001% of Cobalt Carbonate.

Table 3.2. Mean ± SEM for body weight (BW), dry matter intake (DMI), average daily gain (ADG), gain to feed ratio of backgrounded beef cattle fed a finishing diet *ad libitum* and a similar diet from the control group, but at 85% of the ad libitum intake during the finishing phase in a feedlot[a]

Items	*Ad libitum*	Restricted	SEM	P-value
Animals	30	30	-	-
Initial BW, kg	398.7	399.4	11.88	0.93
Final BW, kg	541.8	524.4	8.45	0.07
Daily DMI, kg/d	7.66	6.39	0.297	< 0.01
2 h, DMI, kg[b]	5.13	6.64	0.321	< 0.01
ADG, kg	1.32	1.13	0.056	0.01
Gain:feed ratio	0.178	0.180	0.0135	0.77

[a] Backgrounded cattle. Diets contained 9 % corn silage, 61 % cracked corn, 15 % DDGS, 5% soyhulls, and 10 % of a protein-mineral-vitamin premix on a DM basis.

[b] 2 h, DMI is the amount of DMI at the 2 hours after the feed was offered (n = 15) during the days that gas was sampled.

Table 3.3. Mean ± SEM plasma glucose, insulin, glucose-dependent insulinotropic polypeptide (GIP), and non-esterified fatty acids (NEFA) concentration, O_2 consumption, CO_2 emission, CH_4 emission, and RQ from beef cattle fed *ad libitum* or restricted diets during the finishing phase in feedlot.

	Ad libitum		Restricted		SEM	P-values		
Items[1]	Pre[2]	Post[3]	Pre[2]	Post[3]		Treatment	Time	T x I[4]
Animals, n	15	15	15	15	-	-	-	-
Glucose, mM	4.9	4.4	4.3	3.9	0.26	0.02	0.01	0.54
Insulin, pmol/mL	79	93	65	97	11.6	0.66	< 0.01	0.13
GIP, pmol/mL	65	76	46	52	7.2	0.01	0.04	0.47
NEFA, μM	136.7	132.1	133.6	123.4	6.61	0.45	0.06	0.46
CO_2, g/d	13178	11954	11858	11502	498.47	0.11	0.04	0.24
O_2, g/d	9072.90	8742.00	8223.70	8548.13	320.10	0.16	0.99	0.12
CH_4, g/d	15.67	38.93	14.30	39.15	4.62	0.88	< 0.01	0.79
RQ	0.689	0.738	0.696	0.745	0.0189	0.66	< 0.01	0.98

[1]GIP = glucose-dependent insulinotropic polypeptide; NEFA = non-esterified fatty acids; RQ = respiratory quotient.
[2]Pre = 1 h before feeding.
[3]Post = 2 h after feeding time.
[4]T x I = interaction of treatment and time main effects.

Table 3.4. Mean ± SEM hot carcass weight (HCW), Back fat, Intramuscular (IM) fat, and Longissimus dorsi muscle (LM) area for *ad libitum* and restricted fed steers given a concentrate diet[a]

Items[b]	*Ad libitum*	Restricted	SEM	P-value
Animals	15	15	-	-
HCW[d], kg	344	335	6.5	0.15
Back fat, cm	1.81	1.56	0.167	0.11
IM fat[b], %	6.28	5.96	0.488	0.53
LM[c] area, cm^2	79.1	74.1	2.66	0.09

[a]Backgrounded cattle. Diets contained 9 % corn silage, 61 % cracked corn, 15 % DDGS, 5% soyhulls, and 10 % of a protein-mineral-vitamin premix on a DM basis.
[b]IM fat= ether-extractable intramuscular fat; LM= Longissimus dorsi muscle; HCW = hot carcass weight.

Chapter 4 Effect of Amount and Source of Energy on Growth Performance, Gas Exchange, and Glucose-Dependent Insulinotropic Polypeptide Concentration in Feedlot Cattle

4.1 Introduction

Glucose-dependent insulinotropic polypeptide (GIP) is a 42 amino acid peptide hormone synthesized in, and released from, K cells located in the small intestine (Song et al., 2007; Weaver et al., 2008; Fujii et al., 2014). In non-ruminants, GIP is secreted when glucose or fat are ingested (Kim et al., 2007; Weaver et al., 2008; Tharp et al., 2020). However, in ruminants, research conducted on feedlot cattle examining GIP concentration. In ruminants, dietary fatty acids have been suggested to be a potent and a more important nutrient in stimulating GIP secretion compared to glucose (Martin et al., 1993a; Martin and Faulkner, 1993; Martin and Faulkner, 1994). Although dietary fatty acid seems to be a potent stimulator of GIP in ruminants, energy content in the diet and other underlying mechanisms could be a more important factor in stimulating GIP (Relling and Reynolds, 2008). In feedlot cattle, differences in metabolizable energy content in the diet led to differences in plasma GIP concentration (Chapter 3). However, in feedlot cattle it is still unknown if dietary fatty acids lead to differences in plasma GIP concentration.

Dietary glucose does not increase plasma GIP concentration in ruminants (Martin and Faulkner, 1993; Martin and Faulkner, 1994; Rose et al., 1998; Dawson et al., 1999). Most of the fed starch is converted to VFA in the rumen and less glucose is absorbed from the small intestine. In feedlot cattle, Freitas et al. (2020) noted that corn processing

had no effect on plasma GIP and glucose concentrations. However, they did not measure the total amount of glucose reaching the small intestine. Freitas et al. (2020) suggested that the lack of differences of plasma GIP and glucose concentration between the different treatments could mean that there are different underlying mechanisms in terms of stimulating plasma GIP secretion in ruminants compared to non-ruminants that remain unknown. In lactating dairy cows, postruminally infused (corn starch, casein, or soybean oil), plasma GIP concentration increased on day 1 when vegetable oil or casein were infused and tended to increase when starch was infused compared to control. On day 7, starch and casein increased plasma GIP concentration compared to the control and the oil infused cows. This may suggest that stimulation of plasma GIP concentration is associated with the increase in total metabolizable energy supply, which was greater in casein and starch infusions compared to the control and the vegetable oil infused cows (Relling and Reynolds, 2008). In growing lambs, supplementation with lipids in the diet increased plasma GIP concentration in the first week of the experiment, but it decreased after 1 month of the animals consuming the diet, which might indicate also an adaptation to the fat content in the diet (Relling et al., 2010).

The main responses of GIP on tissues are stimulating insulin secretion by the pancreas and fat accumulation (Dupre et al., 1973; Martin and Faulkner, 1993; Miyawaki et al., 2002; Yamane et al., 2016). Freitas et al. (2020) detected a positive linear association between plasma GIP concentration and intramuscular (IM) fat deposition in feedlot cattle. They did not conclude that GIP increases marbling accretion but GIP decreased fat lipolysis as it did in the subcutaneous adipose tissue in sheep Martin et al.

(1993b). In studies with GIP receptor knockout mice with the same energy intake, knockout mice were less efficient because they used more fatty acids as an energy substrate (lower respiratory quotient (RQ)) compared to the control (Miyawaki et al., 2002). This was because of a lack of action of GIP in the GIP receptor knockout mice (Miyawaki et al., 2002). However, in dairy cattle there was an inverse correlation between RQ and plasma GIP concentration (Relling et al., 2014). Results in dairy cattle indicate that GIP increased lipogenesis because cows with lesser plasma GIP concentration used more fatty acids as an energy substrate (Relling et al., 2014). However, if dairy cattle are using lipids as fuel, more glucose would be allowed for milk synthesis, which means they are more efficient (Relling et al., 2014).

I hypothesized that animals fed greater gross energy intake would have greater plasma GIP concentration and RQ compared with the lesser gross energy intake. Also, animals fed greater gross energy diets from fat would have greater plasma GIP concentration compared to the other treatments at the beginning of the experiment, but this difference would disappear over time due to an adaptation to dietary fat. Thus, the objective of this experiment was to evaluate the effect of energy and lipid intake on plasma GIP concentration, gas exchange, and growth.

4.2 Materials and Methods

4.2.1 Animals, experimental design, and treatments

Animal procedures and husbandry practices were approved by the Institutional Animal Care and Use Committee (#2019A00000112) of The Ohio State University and followed the guidelines recommended in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

The experiment used 60 individually fed Angus × SimAngus-crossbred steer calves (n=15 / treatment). The steers had 9 months of age and were adapted to eat in an individual pen for one month before the experiment started. The steers (paired blocked by body weight and gain to feed ratio (**G:F**) during the adaptation to the diet) were randomly allocated to one of the following treatments (Table 4.1): greater energy intake diet with fat supplemented (fed *ad libitum;* **AW**), greater energy intake diet with no fat supplemented (fed *ad libitum;* **AN**), lesser energy intake diet with fat supplemented (fed at 85% of the *ad libitum* AW diet; **RW**), and lesser energy intake diet with no fat supplemented (fed at 85% of the *ad libitum* AN diet; **RN**). The control feeding for the treatments RW and RN was the DMI of the AN. The feed that was offered to the feed intake restricted steers (RN and RW) was divided in 2 feedings to have a more homogeneous feed intake during the day (60% in the morning feeding at approx. 0900 h and 40 % at approx. 1300 h; this proportion was based on the DMI of the ad libitum steers DMI of Chapter 3). Steers that were fed *ad libitum* were fed at 0900 h. The growing diet was fed for 30 days. The steers were transitioned to a finishing diet during 7

d. The transition diet for the steers in the AN consisted of 35% corn silage, 38% cracked corn, 28% DDGS, and 7% supplement. The transition diet for the steers in the AW consisted of 35% corn silage, 34% cracked corn, 20% DDGS, 4% Ca salts of fatty acids and 7% supplement. The supplement used was EnerGII (Virtus Nutrition, LLC, Corcoran, CA) and was used because it has a blend of inert source of fatty acids and we are not evaluating the fatty acids profile as part of the objectives for this experiment. Fatty acid profile of the Ca salts is presented in Table 4.2. The magnitude of supplementation was chosen at 4% to maximize inclusion in the diet to see results but reduce possible problems associated with interference in digestibility of fiber. The supplement during the transition was 5% of the growing diet and 2% of the finishing diet. The finishing diet was fed for 40 d. A timeline description of sampling times and diet changes is outlined in Figure 4.1.

4.2.2 Sampling and analysis

Feed samples were collected weekly for feed analysis and dry matter intake (**DM**I) was recorded daily. These measurements were taken to determine daily DMI and gain to feed ratio. Steers were weighed on d 78.

Steers were adapted to the use of the gas sensor equipment (GreenFeed System) from d -26 to -4 (relative to the start of the experiment) 3 to 5 times a week until all steers had 6 visits (Figure 4.1). The Greenfeed System has been proven to provide reliable information of gas data when compared to other methods for measuring gases (Patra, 2016; Doreau et al., 2018). All 60 steers in the experiment were adapted except one steer that after 3 visits to the GreenFeed area was eliminated because of dangerous behavior. On

visits 1 and 2, the steers were taken to the chute, kept there for 5 min and walked back to their pens. On visits 3 and 4, the steers were walked to the chute and introduced to a bucket with 50 g of cracked corn. Then, the steer remained there for 5 min. On visits 5 and 6, the concentrate was introduced in the GreenFeed system, the steers were moved into the chute and the system was moved in front of them, where the steer remained for 5 min. This adaptation was used also to select the steers that had a calm behavior during the exposure of the gas sensor equipment.

The days that the treatments were imposed were considered d 1 (Figure 4.1). On d 8, 9, and 10 (period 1) measurements of CO_2 emission and consumption of O_2 were taken using the Greenfeed system (9 per treatment) (Hristov et al., 2015). The steers were divided into 2 groups (18 per group). The steers in the second group started 7 days after the first group started. This allowed to have 2 homogeneous groups and be able to do the measurements at the same day relatively to the starting day of the experiment. All measurements were the same for both groups. The O_2 consumption and CO_2 emission data was used to calculate the ratio CO_2/O_2 (RQ; as a marker of nutrient used to supply energy). Each animal had 8 gas measurements which were taken every 9 h during 3 consecutive days. The steers were divided into 3 more groups (9 steers per group) and each group visited the GreenFeed at alternating times to allow for visits every 9 h. Therefore, the gas measurements were taken at 0200, 0500, 0800, 1100, 1400, 1700, 2000, and 2300 h. The reason for the sampling sequence is based on the results that 8 samples per day are enough for gas data (Hristov et al., 2015; Hristov et al., 2016; Islam et al., 2021). After the gas measurement, the steers were moved to a different chute and blood samples were taken

from the jugular vein to measure glucose, non-esterified fatty acids, GIP, and insulin concentration. The blood samples were taken during the gas samplings at 0800, 1100, 1400, and 1700 h. The reason for this sampling time are based on the daily changes on plasma hormone concentration observed previously taken at 30-min intervals from 0700 to 1400 h in which there was greater variation after feeding (Relling and Reynolds, 2007). There was a gradual increase in plasma hormones from pre-feeding to post-feeding in which plasma hormones reach a plateau and steady-state after 4 h post-feeding (Relling and Reynolds, 2007; Bradford et al., 2008). Blood samples were immediately transferred to tubes containing solutions of disodium EDTA and benzamidine HCl (1.6 and 4.7 mg/mL of blood, respectively) and placed on ice. After centrifugation for 30 min (1,800 × g and 4°C), the blood was aliquoted into individual polypropylene tubes and stored at −80°C until analyzed. The gas and blood measurements were repeated on d 28, 29, and 30 (period 2) and on d 63, 64, and 65 (period 3). On d 31, the steers started to transition to a finishing diet. On d 38, the steers completely transitioned to the finishing diet. After gas exchange was measured the feeding time was segregated to start feeding with a 15-min window between the steers. The time between steers allowed to have 15 min for each steer for gas collection. The average time between the entrance of 2 consecutive animals for gas collection time was 12 min. Therefore, all the gas samplings were always at the same time relative to feeding time.

Plasma concentration of insulin, GIP, glucose, and NEFA were measured as described in Chapter 3. Plasma GIP concentration was analyzed from blood samples taken at 0800 and 1400 h during all 3 periods.

4.2.3 Statistical Analysis

The experimental design was an unbalanced incomplete randomized block design, with a 2×2 factorial arrangement of treatments. Growth performance, O_2 consumption, RQ, plasma glucose, NEFA, insulin, and GIP concentration were analyzed using the repeated statement of time (time of sampling) within period with the MIXED procedure of SAS (9.4, SAS Inst. Inc., Cary, NC), with animal as the subject, to test the random effects of block and the fixed effects of intake, fat, time, period, and their interactions. The most appropriate covariance structure was chosen as having the lowest Akaike Information Criterion. The first-order autoregressive covariance structure was used for the analysis of RQ, O_2 consumption, plasma glucose, insulin, NEFA, and GIP concentration. The GROUP statement was included in the model for the intake main effect. A similar model without the GROUP statement was used to analyze CO_2 emissions because convergence criteria was not met. The compound symmetry covariance structure was used for the analysis of CO_2 emissions. The LS means were separated using the PDIFF and SLICE option of SAS. The SLICE option was used when an interaction with time or period was found. Significance was declared at $P \leq 0.05$ and tendencies were considered at $0.05 < P \leq 0.10$.

4.3 Results and Discussions

4.3.1 Growth Performance

There was an energy intake × fat interaction for final BW ($P = 0.01$; Table 4.3) Steers on AN had greater final BW compared to AW, RW, and RN. Steers on RN had

lesser final BW compared to all treatments. The observed results of final BW differ from previous research in which feedlot steers supplemented Ca salts of fatty acids had greater final BW than the control group (Becú-Villalobos et al., 2007). However, the diet used in the experiment by Becú-Villalobos et al. (2007) was composed of a corn grain rich diet with lipid supplementation that was adjusted every 15 d to 0.13% of the mean BW. Although Becú-Villalobos et al. (2007) did not discuss how much fat was supplemented every 15 d, the quantity could have been greater compared to the diet in the current experiment, which remained constant. Greater final BW with increasing intake is supported by similar findings from an experiment with growing cattle fed a grass silage diet (Ouellet et al., 2001). In an experiment on growing lambs, lambs fed at *ad libitum* had greater BW over time compared to restricted lambs (Relling et al., 2010). Also, supplementing dietary fat in the experiment by Relling et al. (2010) did not affect BW change over time in the restricted fed lambs, which is in contrast to the present experiment in which the intake × fat interaction was observed on final BW. However, and based on the results of DMI, presented later, the difference on BW might be associated with changes in net energy intake.

Similarly, steers on AN had greater ADG compared to the other treatments (intake × fat; P = 0.01; Table 4.3). The ADG improved in Angus, Simmental, and Shorthorn growing calves fed a forage based diet *ad libitum* compared to limit-fed at 80% of the *ad libitum* DMI (Prezotto et al., 2017). The observation of improved ADG in *ad libitum* fed steers is similar to results reported by Hicks et al. (1990), when Hereford cattle were fed a high-corn diet *ad libitum*, compared to 80% of *ad libitum*, had greater

ADG for the first 56 d. Similar improvements in ADG were reported in crossbred steers that were fed *ad libitum* a corn gluten feed or dry rolled corn based ration compared with steers fed a corn gluten feed based ration based restricted 70 to 80% of the *ad libitum* intake for 21 days (Holt et al., 2000). Improvement in ADG with intake is similar to findings in growing cattle fed a grass silage diet had increasing ADG with increasing level of intake (Ouellet et al., 2001). Additionally, ADG was greater in continental and British steers fed *ad libitum* a high moisture corn diet compared to 77% limit-fed steers of the corn diet for 84 d; and it remained like that even after the restricted steers were subsequently fed *ad libitum* (Mcgregor et al., 2012). Warner et al. (2015) reported similar decreases in ADG when supplementing Ca salts of fatty acids in high-corn *ad libitum* diets. The authors discussed that ADG were lesser in fat supplemented steers because they had lesser DMI (Warner et al., 2015). Similar results in ADG were seen in steers and heifers supplemented Ca salts of fatty acids that resulted in lesser DMI and a tendency for lesser ADG (Hill and West, 1991). The authors also determined that ADG was not improved in supplemented steers because of depressed DMI (Hill and West, 1991). The interaction observed in the present experiment may be because the restricted fed animals had similar DMI and was set by treatment and not due to the maximum DMI set point of the animals, as it is described latter in the text. Therefore, no effects of DMI depression was observed in the RW steers compared with the RN steers.

There was an effect of treatment on DMI (intake × fat; $P < 0.01$; Table 4.3). Steers on AN had greater DMI compared to steers on AW, RW, and LNW, which had lesser DMI ($P < 0.01$). By design, restricted steers consumed less feed than *ad libitum* fed

steers. Supplemental fat can increase energy intake and density of diets, which leads to a decrease in voluntary DMI (Allen, 2000; Bradford et al., 2008). Also, a linear increase in Ca salts of fatty acids in the diet has linearly decreased DMI (Palmquist, 1994; Allen, 2000). Fatty acids have been reported to increase the gut peptides cholecystokinin (CCK) and glucagon-like peptide 1 (GLP-1), which are hormones that control feed intake in dairy cattle (Bradford et al., 2008; Relling and Reynolds, 2008). Also, unsaturated fatty acids may stimulate satiety through hepatic oxidation which explains that feed intake is control by oxidation of fuels in the liver from diet and tissues (Allen, 2020). In high-starch diets, hepatic oxidation likely controls feed intake to a greater extent than physical distention in high-forage diets. Also, Conrad et al. (1964) proposed that physical and physiological factors regulating feed intake change in ruminants. Ruminants eat to meet their energy requirements unless limited by gut fill (Conrad et al., 1964). Release of satiety-inducing gut peptides and energy satiety are possible explanations as to why AW steers had lesser DMI than AN. Also, Warner et al. (2015) discussed that steers supplemented Ca salts of fatty acids could have had decreased DMI because the smell and taste could influence palatability of the whole diet.

There was a main effect of fat on G:F ($P < 0.05$; Table 4.3); steers that were fed diets with the addition of 4% Ca salts of fatty acids had greater G:F without an interaction. The observed results in G:F differ from previous research with growing calves fed *ad libitum* that had greater G:F than limit-fed calves for 84 d (Prezotto et al., 2017). However, the type of diet used by Prezotto et al. (2017) was high in forage compared to the present experiment; the current experiment had a feeding period of high-

forage and then high-concentrate diet. Also, Prezotto et al. (2017) restricted the calves by 80% compared to 85% in the present experiment. The authors Prezotto et al. (2017), discussed that the effects of limit-feeding on efficiency depend on the level of restriction, type of diet (forage compared to concentrate) and energy density of the diet. The lack of effect of level of intake on G:F is similar to other studies measuring efficiency as feed:gain ratio where no differences where observed in cattle fed *ad libitum* or restrictively (Hicks et al., 1990; Hayden et al., 1993; Holt et al., 2000). Ouellet et al. (2001) reported that feed:gain decreased linearly with increasing level of DMI in growing cattle fed a grass silage diet, which meant *ad libitum* steers were more efficient than feed restricted steers. Those results (Ouellet et al., 2001) differs from the current experiment where fat was the only effect on efficiency. However, steers in the experiment by Ouellet et al. (2001) had a different diet and larger level of restriction (80 and 65%) compared to the present experiment. Additionally, no differences in G:F due to different intakes reported in crossbred Continental and British steers between *ad libitum* feeding or restricting intake (77%) a high moisture corn diet for 84 d (Mcgregor et al., 2012); G:F was similar between breeds. After 84 d steers that were limit fed had *ad libitum* access to the 77% corn diet only British steers had improved G:F compared to continental steers. The AW steers had greater G:F compared to AN possibly because AW steers consumed less feed and had similar ADG. Similar results were seen in RW steers that consumed less feed than AW steers and had similar gains in weight. Also, the fat effect on G:F could be because Ca salts of fatty acids have 2.25 more energy than carbohydrates (Palmquist, 1994; Allen, 2000; Stelmach-Mardas et al., 2016). In the diets with fat, 4% of

cracked corn was replaced with 4% of Ca salts of fatty acids which increased the energy density of the diet 2.25 more that could have resulted in greater G:F. In ruminants, restricting intake can improve diet utilization, but the magnitude of change depends on diet energy density (Trubenbach et al., 2019) which could explain differences in efficiency between RW and RN.

4.3.2 Plasma Hormone and Metabolite Concentration

Plasma glucose concentration was affected by the time × fat interaction ($P \leq 0.01$) and period main effect ($P < 0.01$; Fig 4.2); there was no effect ($P \geq 0.11$) of intake and the rest of the interactions. The mean ± SEM plasma glucose concentration was 5.29, 5.14, and 5.48 mM for periods 1, 2, and 3 (SEM = 0.129), respectively. Plasma glucose concentration at 0800 h was very similar between treatments. However, post-prandial plasma glucose concentration (1100 and 1400) decreased over time; and the decrease was larger in steers on treatments with no supplemental Ca salts of fatty acids. At 1700h plasma glucose concentration increased again and was similar between treatments. There was no effect ($P \geq 0.11$) of intake, fat × intake, time × intake, time × fat × intake, period × intake, period × fat, period × fat × intake, period × time, period × time × intake, period × time × fat, nor period × time × fat × intake. The time effect on plasma glucose concentration is consistent with established data suggesting regulation of blood glucose is integrated with regulation of other metabolic processes through common hormones such as insulin (Brockman and Laarveld, 1986; Qaid and Abdelrahman, 2016). Becú-Villalobos et al. (2007) reported that plasma glucose concentration was not different when comparing a control group and supplemented Ca salts of fatty acids group, which it

is in contrast to the present experiment in which the interaction of time and fat had an effect. However, they took blood samples before feeding which means that basal plasma glucose concentration are similar in both groups (Becú-Villalobos et al., 2007) as in the present experiment. The observed results of post-prandial plasma glucose concentration in steers supplemented fat (W) compared to no supplementation (N) was also reported in ewes supplemented Ca soaps of palm oil fatty acids compared to a control group (El-Nour et al., 2012). In the present experiment, a possible explanation for lesser post-prandial plasma glucose concentration in N steers could be that, after a meal, there is absorption of glucose and glucose-precursors that stimulate secretion of incretins that facilitates glucose uptake by peripheral tissues (Brockman and Laarveld, 1986; Qaid and Abdelrahman, 2016). The treatments N may have stimulated greater plasma insulin concentration because they had more soluble carbohydrates in the diet compared to W steers that led to lesser plasma insulin concentration after eating. Ouellet et al. (2001) reported that before feeding plasma glucose concentration was similar in all treatments on d 78. On d 79, feeding decreased plasma glucose concentration and was lesser in ad libitum animals (Ouellet et al., 2001). Relling et al. (2010) reported that plasma glucose concentration was greater in growing lambs fed the control diet compare with those supplemented fat, which contrasts with the present experiment. Pre-prandial plasma glucose concentration was less than post-prandial concentration in the restricted lambs, which could be because of differing patterns of intake in the restricted steers (Relling et al., 2010). Relling et al. (2010) also reported that plasma glucose concentration decreased with the dietary supplementation of fat, which differs from the present

experiment. However, Relling et al. (2010) supplemented Ca salts of fatty acids at 6% in the diet, which is more than the present experiment (4%). Differences in plasma glucose concentration between the present experiment and previous studies discussed could be explained by differences in animal models (steers vs. lambs), diets (forage vs. concentrate), supplementation of fat, magnitude of supplementation of fat, and their interactions. A possible explanation for greater plasma glucose concentration on period 3, considering that concentration was lesser during period 2 (compared to period 1), could be because steers were fed a high-concentrate diet during period 3 or the steers were increasing adipose tissue in their carcass.

There were effects on the interactions of period × intake ($P < 0.01$) and period × time ($P = 0.05$) on plasma insulin concentration (Fig 4.3). During period 1, plasma insulin concentration was very similar, regardless of intake treatment. During period 2, plasma insulin concentration was similar for both intakes on all the times except 1700 h, when steers with *ad libitum* intake had greater plasma insulin concentration. During period 3, plasma insulin concentration was greater in steers fed *ad libitum* in all sampling times. Also, there was a tendency for the fat × intake interaction ($P = 0.08$) on plasma insulin concentration. The mean ± SEM plasma insulin concentration was 45.58, 28.36, 45.97, and 39.28 pmol/mL for the AN, RN, AW, and RW, respectively (± 3.894). Plasma insulin concentration of the RN tended be lesser compared to all of the other treatments. There was no effect ($P \geq 0.11$) of time, time × intake, time × fat, time × fat × intake, period × fat, period × fat × intake, period × time × intake, period × time × fat, and period × time × fat × intake on plasma insulin concentration. Previous experiment where

supplementation of Ca salts of fatty acids in finishing feedlot steers fed a high-concentrate diet had no effect on plasma insulin concentration (Becú-Villalobos et al., 2007). However, in that experiment, the steers were not feed restricted (Becú-Villalobos et al., 2007); therefore, the effect of the lipid supplementation was only observed when animals were feed-restricted. Ouellet et al. (2001) reported that beef cattle fed *ad libitum* had a greater plasma insulin concentration than restricted fed cattle. As mentioned previously, the diets used by Ouellet et al. (2001) had a greater concentration of fiber and the restriction used was greater 80 and 65% compared to the *ad libitum* intake. Ouellet et al. (2001) discussed that the increments of plasma insulin concentration after the grass silage meal were lesser overall compared to cattle fed high concentrate diets in other experiments (Jenny et al., 1974; Vasilatos and Wangsness, 1980), which is in accordance with this experiment from which plasma insulin concentration were greater after a meal on period 3 when animals were fed high concentrate diets. A possible explanation for greater plasma insulin concentration in high-starch diets is because the high energy diet yield more propionate from rumen fermentation, which can be converted to glucose (Espinoza et al., 1997; Qaid and Abdelrahman, 2016). The increase in glucose avialability causes rapid secretion of insulin (Espinoza et al., 1997; Qaid and Abdelrahman, 2016). Also, greater plasma insulin concentration was observed in *ad libitum* lambs compared to restricted animals which was similar to results seen in the present experiment. The post prandial increase in plasma insulin secretagogues could be because of an increase in the absorption of propionate of glucose (Relling et al., 2010) . In addition, an interaction intake × fat supplementation × time relative to feeding was

detected for plasma insulin concentration (Relling et al., 2010). The observed tendency in the present experiment for the lesser plasma insulin concentration of the RN compared with the other treatments may be because they consumed less energy or starch. That additional energy of the RW compared to RN steers may be have been enough to affect plasma insulin concentration. Studies in non-ruminants show that lipids can regulate insulin secretion by binding to a free fatty acid receptor in the pancreas (Itoh et al., 2003). Although the authors (Itoh et al., 2003) discussed that the mechanism is not clearly understood free fatty acids in humans may act as signaling molecules in insulin secretion. Therefore, the differences in the restricted animals could be because of a regulation of plasma insulin concentration by lipids in the RW animals that were not present in RN. The tendency in the present experiment of the effect of fat × intake interaction on plasma insulin concentration could be because of a similar mechanism. However, I do not have enough supporting literature to confirm this assumption in ruminants. Also, the period × time effect on plasma insulin concentration are consistent with established theories that metabolism is regulated by maintaining constant concentration of glucose in the blood (Brockman and Laarveld, 1986).

Plasma NEFA concentration was affected by the interaction of period × time ($P < 0.01$). In addition, there was a main effect demonstrated for fat ($P < 0.01$). The mean ± SEM plasma NEFA concentration during period 1 was 171, 110, 100, and 103 μM for the 0800, 1100, 1400, and 1700 h, respectively (± 8.4; data not shown); period 2, 158, 120, 118, and 139 μM; period 3, 162, 115, 138, and 131 μM. There was a tendency for a period × intake interaction on plasma NEFA concentration ($P = 0.08$; Figure 4.4). Plasma

NEFA concentration was 147 and 114 for steers supplemented fat (W) and for steers not supplemented fat (N), respectively. There was no effect ($P \geq 0.11$) of intake, fat × intake, time × intake, time × fat, time × fat × intake, period × fat, period × fat × intake, period × time × intake, period × time × fat, and period × time × fat × intake on plasma NEFA concentration. A possible explanation for the tendency is that plasma NEFA concentration is determined by the balance between lipolysis and lipogenesis (Allen, 2020). Based on the insulin response, I expected them to have lesser plasma NEFA concentration; during period 3, the biggest differences in insulin were also seen between treatments. However, the plasma NEFA concentration was not associated with plasma insulin or GIP concentration. Therefore, in finishing animals, there may be another mechanism associated with plasma NEFA concentration. The metabolism of fatty acids may be more complex in finishing steers and cannot be explained with the data I collected for the present experiment. Only insulin and GIP were measured in the current experiment; there could be other hormones regulating lipid metabolism that we did not measure e.g., cortisol, glucagon, and growth hormone (Brockman, 1978; Brockman and Laarveld, 1986; Allen, 2020). The observed results of plasma NEFA concentration are similar from growing cattle that had an effect of time and intake on plasma NEFA concentration and had greater plasma NEFA concentration before and after feeding compared to *ad libitum* fed animals (Ouellet et al., 2001); the author discussed that animals with greater intakes had lesser plasma NEFA concentration. The greater concentration of plasma NEFA before eating in all treatments and periods could be explain because when there is lesser plasma insulin concentration NEFA are released in

the blood plasma from the adipose tissue to meet the metabolic needs of the animals (Bowden, 1971; De Koster and Opsomer, 2013). This is similar to previous research reporting that, in ruminants, plasma NEFA concentration are related primarily to time of feeding (Bowden, 1971). Also, in an experiment by Bowden (1971) plasma NEFA concentration decreased after feeding for all treatment. The observed results of greater plasma NEFA concentration in animals supplemented fat in the present experiment are similar to studies done in lactating dairy cows that were infused vegetable oil into the abomasum (Gagliostro and Chilliard, 1991; Benson et al., 2002; Relling and Reynolds, 2008). Additionally, a greater plasma NEFA concentration due to supplementation with 6% of Ca salts of fatty acids and level of intake was also observed in growing lambs (Relling et al., 2010). A possible explanation for greater plasma NEFA concentration after infusion of oil in the abomasum might be due to a liking of the fatty acids from plasma lipoproteins (Gagliostro and Chilliard, 1991).

There was a time × fat × intake interaction ($P = 0.04$) for plasma GIP concentration (Figure 4.5). There was also period effect ($P < 0.01$) on plasma GIP concentration. The means ± SEM plasma GIP concentration were 87.2, 120.3, and 105.7 pmol/mL (± 7.38) for periods 1, 2, and 3, respectively (averaged over treatments; data not shown). There was no effect ($P \geq 0.13$) of intake, fat × intake, time × fat, period × intake, period × fat, period × fat × intake, period × time, period × time × intake, period × time × fat, and period × time × fat × intake on plasma GIP concentration. At the 0800 h sampling, RN steers had lesser plasma GIP concentration compared to the other 3 treatments. At the 1400 h sampling, steers AN and RN had similar plasma GIP

concentration between them and lesser compared with steers supplemented with fat. This is in partial agreement with previous research in ruminants that dietary fatty acids are a more potent and a more important nutrient in stimulating GIP compared to fed starch in non-ruminants (Martin et al., 1993a; Martin and Faulkner, 1993; Martin and Faulkner, 1994). Although dietary fatty acids are a more potent nutrient in stimulating GIP, diets without supplementation of fat but high in energy could increase pre-feeding plasma GIP concentration. Also, the differences in plasma GIP concentration can be observed depending on the time of sampling, relative to the feeding time. I had hypothesized that animals fed greater gross energy intake and animals fed high gross energy diets from fat would have greater plasma GIP concentrations compared to the other treatments at the beginning of the experiment. This hypothesis was partially accepted, because during the 0800 h sampling, lipid content of the diet and *ad libitum* feed intake increased plasma GIP compared to RN; this difference disappeared later in the day. This change in the daily pattern of GIP may be due to an adaptation to energy during the day in diets without supplementation of fat. However, in the present experiment, there was no adaptation to fat observed for plasma GIP concentration. Growing lambs fed at *ad libitum* with 6% Ca salts of palm oil had greater plasma GIP concentration compared to those fed restricted or without fat during the first week of the experiment, but the difference diminished after 1 month of the experiment (Relling et al., 2010), which differs from the present experiment. Relling et al. (2010) also reported that the addition of fat in the diet did not change plasma GIP concentration in restricted-fed lambs, but fat increased plasma GIP concentration in those fed *ad libitum*, which does not agree with the results from the

current experiment. Regardless of restriction, the addition of fat increased plasma GIP concentration (Figure 4.5). The reduction over time in plasma GIP concentration and adaptation to fat/high-energy diets in growing lambs (Relling et al., 2010) and the lack of difference in the current experiment with beef steers suggests that there could be differences among ruminants on the stimulus and regulation of plasma GIP concentration. In period 3, there was lesser overall plasma GIP concentration compared to period 2 that could be associated with the change of diet (high-concentrate) and an adaptation of stimulation of GIP to high energy diets (data not shown). Therefore, there may be differences in plasma GIP concentration depending on the type of diet or phase (growing vs. finishing).

4.3.3 Gas exchange

Main effects of time ($P < 0.01$) and period ($P < 0.01$) were observed for O2 consumption. There was no effect ($P \geq 0.12$) of intake, fat, fat × intake, time × intake, time × fat, time × fat × intake, period × intake, period × fat, period × fat × intake, period × time, period × time × intake, period × time × fat, and period × time × fat × intake on O2 consumption. The observed results for O2 consumption followed a normal pattern in which O2 consumption increased whenever we gave feed to the steers (Osellame et al., 2012). There was a period × intake ($P = 0.01$; Fig 4.6) and a time × fat × intake interaction ($P < 0.01$; Fig 4.7) for CO_2 emissions. There was no effect ($P \geq 0.11$) of intake, fat, fat × intake, time × intake, time × fat, period × fat, period × fat × intake, period × time, period × time × intake, period × time × fat, and period × time × fat × intake for CO_2 emissions. The results and highly complex effects of the interactions on

CO_2 could be related with to body weight of the animal as they age and change in diets, which could have increased the oxidation of molecules that led to a greater production of CO_2.

Main effects of time (P = 0.04) and period (P = 0.05) were observed on RQ. The mean ± SEM RQ demonstrated a tendency for a fat × intake interaction (P = 0.07; Figure 4.8). For this tendency, the mean (± SEM) RQ of the treatments [0.69 and 0.70 (± 0.012) for the N and W, respectively] were similar. Also, the mean ± SEM RQ of the treatments RN and AW [0.70 and 0.71 (± 0.017)] were similar. The mean ± SEM RQ was 0.68, 0.63, 0.72, 0.67, 0.73, 0.69, 0.70, and 0.73 (± 0.025) for 0200, 0500, 0800, 1100, 1400, 1700, 2000, and 2300 h, respectively (data not shown). The mean ± SEM RQ for period 1 was 0.71, 0.67 for period 2, and 0.70 (± 0.015) for period 3 (data not shown). There was no effect ($P \geq 0.28$) of intake, fat, time × intake, time × fat, time × fat × intake, period × intake, period × fat, period × fat × intake, period × time, period × time × intake, period × time × fat, and period × time × fat × intake on RQ. I had hypothesized that animals fed greater gross energy intake would have greater RQ compared to animals fed a lesser gross energy intake. Based on the results, this hypothesis is rejected. In finishing steers, the substrate for energy is mainly lipids based on RQ results that were approximately 0.7 in the treatments. There is not enough literature in beef cattle to explain the results of the current experiment, in particular why treatments AN and RW have similar RQ. Relling et al. (2014) reported that cows with lesser plasma GIP concentration had a lesser RQ compared to cows with greater plasm GIP concentration. Therefore, cows with the lesser plasma GIP concentration cows were using more fat as an energy substrate, which may

indicate that GIP stimulated lipogenesis. The results in dairy cows (Relling et al., 2014) differ from the results of the current experiment. Also, Relling et al. (2014) discussed that use of lipids as fuel would allow more glucose to be utilized for milk synthesis. Those authors suggested that GIP may play a role in the regulation of nutrient and energy metabolism in dairy cows. However, in the current experiment there was variability in RQ between cattle with greater plasma GIP concentration. Therefore, plasma GIP concentration provides no evidence for association with RQ in growing feedlot steers in this experiment.

There was a period × fat ($P = 0.02$; Fig 4.9) and a period × time interaction ($P = 0.04$; data not shown) for CH_4 emissions. Steers supplemented Ca salts of fatty acids had greater CH_4 emissions during period 1 but lesser CH_4 emissions during period 3 (Fig. 4.9). Throughout the 3 periods emission of CH_4 of W steers was similar ($P = 0.3$). However, emission of CH_4 of N steers increased from period 1 to 2 ($P < 0.01$) and tended to increase from period 2 to 3 ($P = 0.08$). Main effects of time ($P < 0.01$) and period ($P < 0.01$) were observed on CH_4 emissions (data not shown). The mean ± SEM CH_4 emissions was 0.08, 0.09, 0.05, 0.10, 0.11, 0.10, 0.09, and 0.08 kg/d (± 0.008) for 0200, 0500, 0800, 1100, 1400, 1700, 2000, and 2300 h, respectively (data not shown). The mean ± SEM CH_4 emissions for period 1 was 0.07 kg/d, 0.09 kg/d for period 2, and 0.10 kg/d (± 0.006) for period 3 (data not shown). There was no effect ($P \geq 0.26$) of intake, fat, fat × intake, time × intake, time × fat, time × fat × intake, period × intake, period × fat × intake, period × time × intake, period × time × fat, and period × time × fat × intake on CH_4 emissions. During period 1, the W steers had greater CH_4 emissions compared to N

steers. However, this difference did not remain during period 2 which was when the steers could have been better adapted to the diet. During period 3 the W steers had lesser CH_4 emissions compared to N steers. The effect of dietary fatty acids seen in period 3 is consistent with evidence that lipids suppress CH_4 emissions (Hristov et al., 2013). Dietary lipids have an overall suppressive effect on bacteria and protozoa. This suppressive effect could explain why emission of CH_4 remained similar on all 3 periods for W steers and increased in N steers. Also, the reduction in DMI and increased feed efficiency could have led to a reduction in CH_4 emissions compared to N steers.

4.4 Conclusions

In conclusion, supplementation of 4% of Ca salts of fatty acids and restriction of DMI affected plasma hormones and metabolites differently Fatty acids in the diet increased plasma glucose concentration after feeding and plasma NEFA concentration. However, differences in plasma insulin concentration were because of differing intakes during the finishing period. In finishing steers, the substrate for energy is mainly lipids based on RQ; that is not associated with plasma GIP concentration. Supplementation of fat and *ad libitum* feed intake increased pre-prandial plasma GIP concentration. This difference in plasma GIP concentration disappeared after the animals were fed; post-prandial lipid supplementation increased plasma GIP concentration. There could be differences among ruminants on the stimulus and regulation of plasma GIP concentration because there was no adaptation to fat in the diet. Results confirm previous findings that, in ruminants, dietary fatty acids are a more potent and a more important nutrient in stimulating plasma GIP concentration. I observed that the plasma concentration of GIP

not only depends of fatty acids in the diet or energy intake, but it depends on the time of sampling. This differences in plasma GIP are not associated with RQ, which differs from results in dairy cows. Therefore, the type of diet and the selection of cattle for meat or milk production may alter nutrient utilization.

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4.6 Tables

Table 4.1. Dietary and chemical composition of growing and finishing phase diets (% DM basis)

	Growing diet		Finishing diet	
Item	AN[1]	AW[1]	AN[1]	AW[1]
Cracked corn	24	20.26	58.6	53.58
Corn silage	50	50	20	20
DDGS	20	20	20	21
Calcium salts of FA[2]	0	4.0	0	4.0
Urea	0.2	0.5	0	0.15
Soybean meal	2.12	2.74	0	0.26
Calcium carbonate	2.13	0	0.8	0
Calcium sulfate	0.7	0.65	0.26	0.26
Limestone	0	1.0	0	0.4
Minerals/Vitamins[3]	0.86	0.86	0.35	0.35
Composition				
CP, %	13.17	13.92	12.13	12.50
NDF, %	25.30	25.26	17.65	17.59
EE, %	3.58	6.71	3.76	6.95
Ash	7.79	7.22	4.09	4.39
NEm, Mcal/kg	1.78	2.08	2.03	2.31

[1]AN= *ad libitum* diet without supplemental fat; AW= *ad libitum* diet with 4% of Ca salts of fatty acids.
[2]EnerGII, Virtus Nutrition LLC, Corcoran, CA.
[3]Mineral and vitamin mix, contained 4.609% of Sodium Chloride, 0.068% of Vitamin A, 30,000 IU/g, 0.068% of Vitamin D, 3,000 IU/g, 0.205% of Vitamin E, 44 IU/g, 0.35% of Selenium, 0.157% of Rumensin 90 (Elanco Animal Health, Greenfield, IN), 2.765% of Potassium Chloride, 0.055% of Copper Sulfate, 0.184% of Zinc Sulfate, 0.088% of Manganese Sulfate, and 0.001% of Cobalt Carbonate for the growing phase, and 4.615% of Sodium Chloride, 0.068% of Vitamin A, 30,000 IU/g, 0.068% of Vitamin D, 3,000 IU/g, 0.205% of Vitamin E, 44 IU/g, 0.351% of Selenium, 0.203% of Rumensin 90 (Elanco Animal Health, Greenfield, IN), 2.769% of Potassium Chloride, 0.06% of Copper Sulfate, 0.185% of Zinc Sulfate, 0.111% of Manganese Sulfate, and 0.001% of Cobalt Carbonate for the finishing phase.

Table 4.2. Fatty acid profile of the Calcium salts of fatty acids (EnerGII; Virtus Nutrition, LLC, Corcoran, CA).

Fatty acid	Ca Salts of FA
C8:0 + C10:0 + C12:0	0.62
C14:0	1.17
C16:0	45.87
C16:1	0.20
C18:0	5.14
C18:1 c9	36.27
C18:1 other	1.10
C18:2	8.03
C20:0	0.37
C20:1	0.09
C18:3	0.20
C20:5	0.13
Other	0.80

Table 4.3. Mean ± SEM body weight (BW), dry matter intake (DMI) average daily gain (ADG) and gain:feed ratio (G:F) of beef steers given one of four treatments; 1. *ad libitum* access to a high energy diet without supplemented fat (AN), 2. *ad libitum* access to a high energy diet supplemented with 4% Ca salts of fatty acids (AW), 3. Restricted access to a low energy diet without supplemented fat (RN), or 4. Restricted access to a low energy diet supplemented with 4% Ca salts of fatty acids (RW). Treatments were administered over 65 days during the growing and finishing phase in a feedlot[1]

	Ad libitum		Restricted		SEM[3]		P-value[2]		
Treatments	AN	AW	RN	RW	Ad-lib	Res	Intake	Fat	I × F
Animals	15	15	15	15	-	-	-	-	-
Initial BW, kg	281	279	277	277	4.3	4.6	0.27	0.74	0.67
Final BW, kg	402[a]	396[ab]	376[c]	387[b]	4.6	4.7	< 0.01	0.45	0.01
ADG, kg/d	1.58[a]	1.51[ab]	1.25[c]	1.39[b]	0.057	0.059	< 0.01	0.45	0.01
DMI, kg/d	13.3[a]	12.4[b]	11.1[c]	11.4[c]	0.31	0.27	< 0.01	0.07	< 0.01
G:F ratio	0.119	0.122	0.112	0.122	0.0036	0.0038	0.22	0.02	0.23

[1]Backgrounded cattle. AN=high energy intake diet without fat supplemented fed *ad libitum*; AW= high energy intake diet with 4% of Ca salts of fatty acids supplemented fed *ad libitum*; RN= low energy intake diet without fat supplemented fed at 85% of the *ad libitum* AN diet; RW=low energy intake diet with 4% Ca salts of fatty acids supplemented fed at 85% of the *ad libitum* AW diet.

[2]Intake = main effect of the intake treatment; Fat = main effect of the addition of fat in the diet; I × F = interaction of intake and fat main effects.

[3]Reported SEM is greatest of all treatments within intake (*Ad libitum* vs Restricted).

[a,b,c,]Within rows, means without a common superscript differ ($P \leq 0.05$).

4.7 Figures

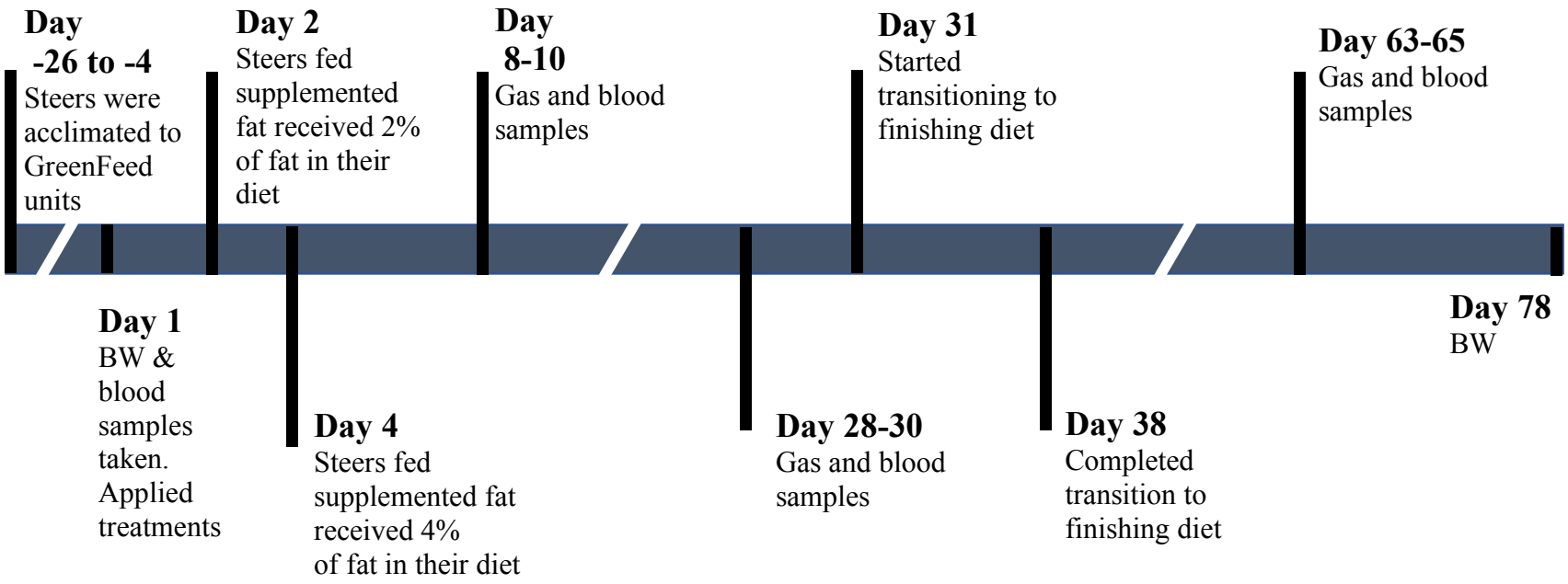

Figure 4.1. A schematic timeline of experiment two including diet changes, oxygen, carbon dioxide gas and blood measurements. During the gas sampling periods, the animals were sampled every 9 h for a duration of 3 d.

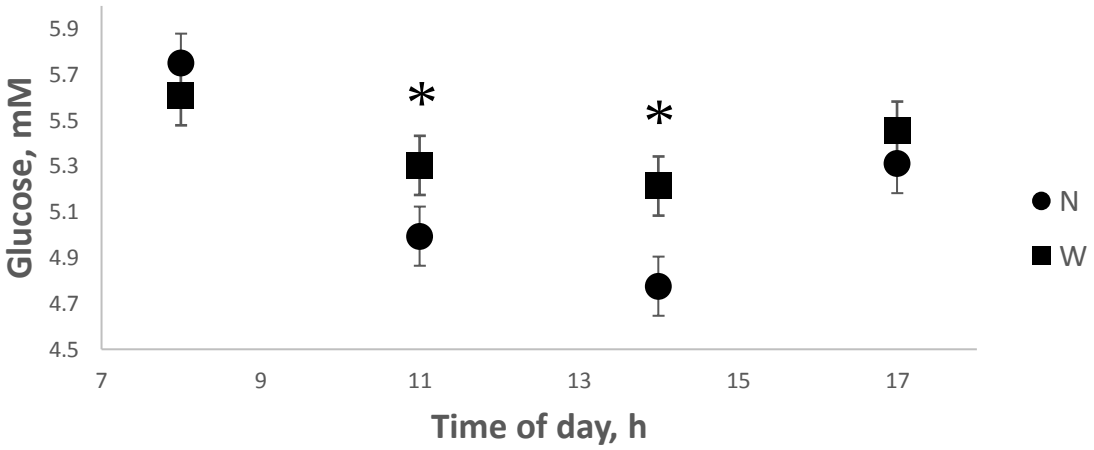

Figure 4.2. Mean ± SEM plasma glucose concentration of beef cattle for 12 hours treated with 4% Ca salts of fatty acids in the diet (■) or no fatty acids in the diet (●) from three sampling days during a 65-d feeding period. A time × fat interaction was demonstrated ($P = 0.01$). There was no effect ($P \geq 0.11$) intake, fat × intake, time × intake, time × fat × intake, period × intake, period × fat, period × fat × intake, period × time, period × time × intake, period × time × fat, period × time × fat × intake. * $P \leq 0.05$ for means separated using the SLICE and PDIFF option (SAS Inst. Inc., Cary NC).

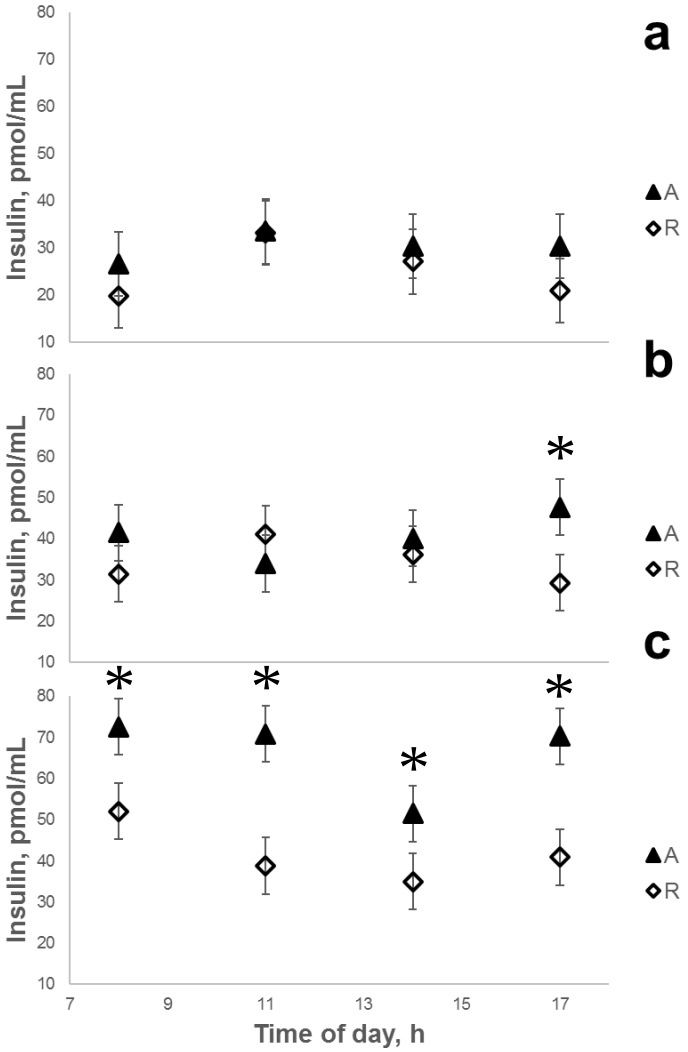

Figure 4.3. Mean ± SEM plasma insulin concentration in beef steers given *ad libitum* access to their diet (▲) or restricted access to their diet (◊) during three sampling periods; a, 8-10 d, b, 28-30 d, and c, 63-65 d. A period × time and period × intake interaction was demonstrated ($P \leq 0.05$). There was no effect ($P \geq 0.11$) of time, time × intake, time × fat, time × fat × intake, period × fat, period × fat × intake, period × time × intake, period × time × fat, and period × time × fat × intake on plasma insulin concentration. * $P \leq 0.05$ for means separated using the SLICE and PDIFF option (SAS Inst. Inc., Cary NC).

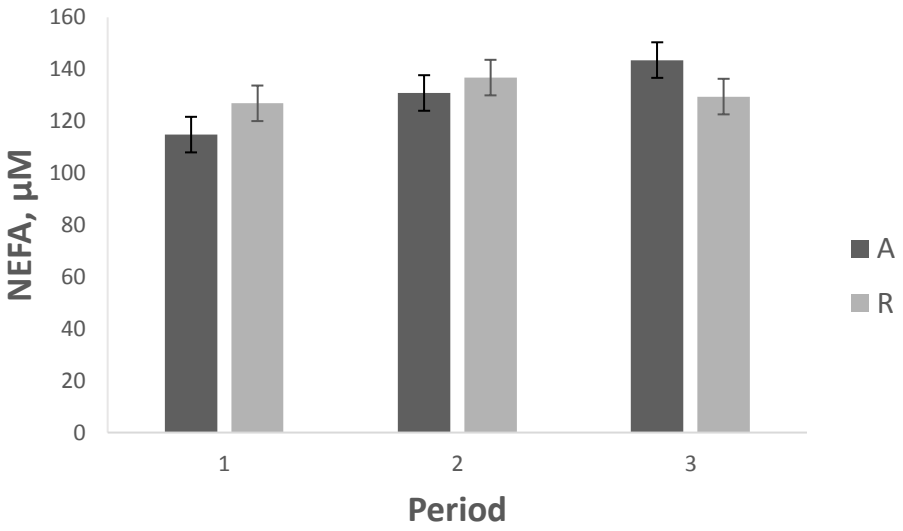

Figure 4.4. Mean ± SEM plasma non-esterified fatty acids (NEFA) concentration of beef cattle given *ad libitum* access to their diet (■) or restricted access to their diet (□) during 3 sampling periods; a, 8-10 d, b, 28-30 d, and c, 63-65 d. A tendency for a period × intake interaction was demonstrated ($P = 0.08$). There was no effect ($P \geq 0.11$) intake, fat × intake, time × intake, time × fat, time × fat × intake, period × fat, period × fat × intake, period × time × intake, period × time × fat, and period × time × fat × intake on plasma NEFA concentration. Means were separated using the SLICE and PDIFF option (SAS Inst. Inc., Cary NC; $P > 0.11$).

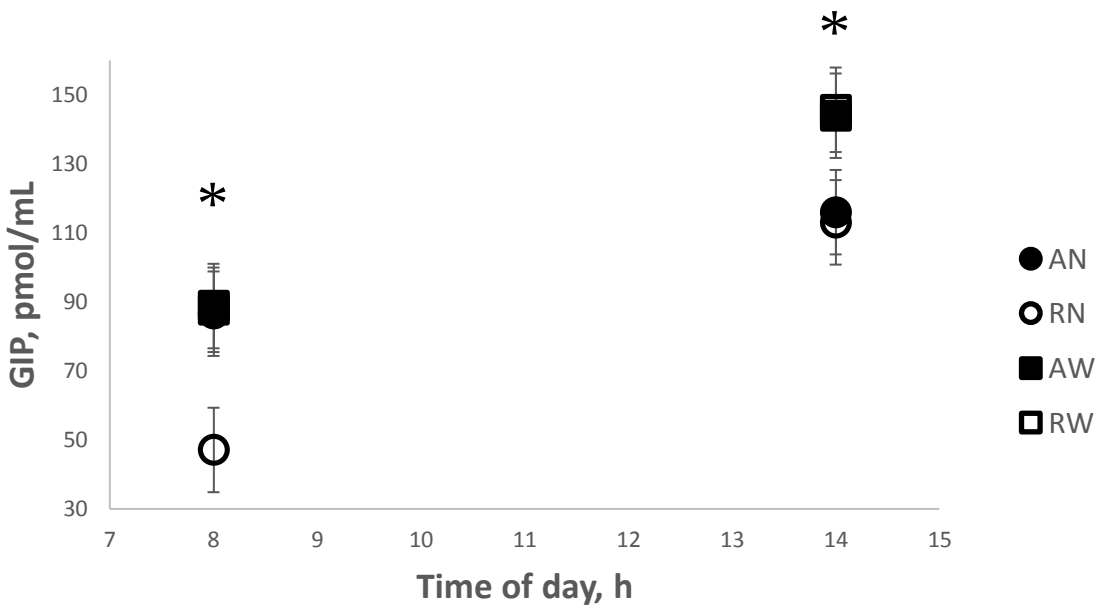

Figure 4.5. Mean ± SEM plasma glucose-dependent insulinotropic polypeptide (GIP) concentration in beef steers given one of four treatments; 1. *ad libitum* access to a high energy diet without supplemented fat (AN; ●), 2. *ad libitum* access to a high energy diet supplemented with 4% Ca salts of fatty acids (AW; ■), 3. Restricted access to a low energy diet without supplemented fat (RN; ○), or 4. Restricted access to a low energy diet supplemented with 4% Ca salts of fatty acids (RW; □). All steers were sampled at 0800 and 1400 h during the growing and finishing phase. A time × fat × intake interaction occurred ($P = 0.04$). The results are averaged over periods because there was no interaction with period main effect. There was no effect ($P \geq 0.13$) of intake, fat × intake, time × fat, period × intake, period × fat, period × fat × intake, period × time, period × time × intake, period × time × fat, and period × time × fat × intake on plasma GIP concentration. * $P \leq 0.05$ for means separated using the SLICE and PDIFF option (SAS Inst. Inc., Cary NC).

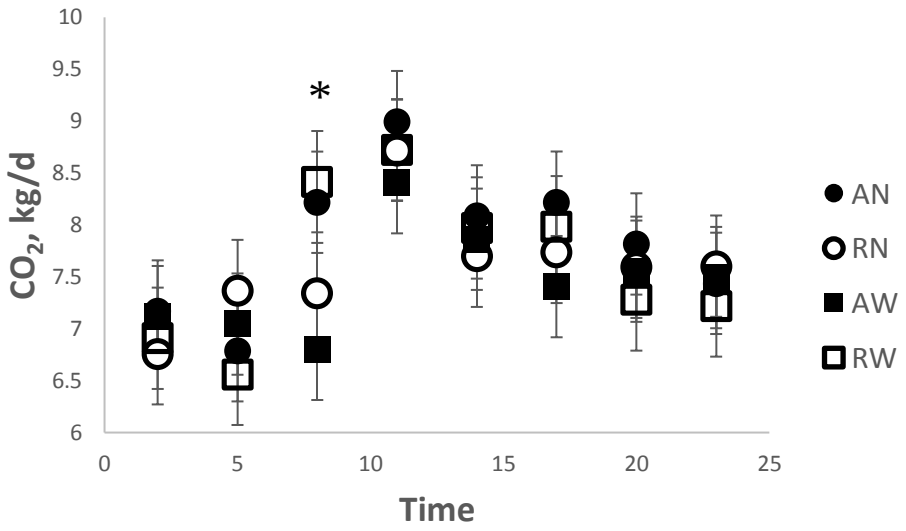

Figure 4.6. Mean ± SEM **c**arbon dioxide **(**CO_2) emissions in beef steers given one of four treatments; 1. *ad libitum* access to a high energy diet without supplemented fat (AN; ●), 2. *ad libitum* access to a high energy diet supplemented with 4% Ca salts of fatty acids (AW; ■), 3. Restricted access to a low energy diet without supplemented fat (RN; ○), or 4. Restricted access to a low energy diet supplemented with 4% Ca salts of fatty acids (RW; □). Gas emission sampling was undertaken at 0200, 0500, 0800, 1100, 1400, 1700, 2000, and 2300 h during 3 sampling periods within 65 days. A time × fat × intake interaction was demonstrated ($P < 0.01$). There was no effect ($P \geq 0.11$) of intake, fat, fat × intake, time × intake, time × fat, period × fat, period × fat × intake, period × time, period × time × intake, period × time × fat, and period × time × fat × intake for CO_2 emissions. * $P \leq 0.05$ for means separated using the SLICE and PDIFF option (SAS Inst. Inc., Cary NC).

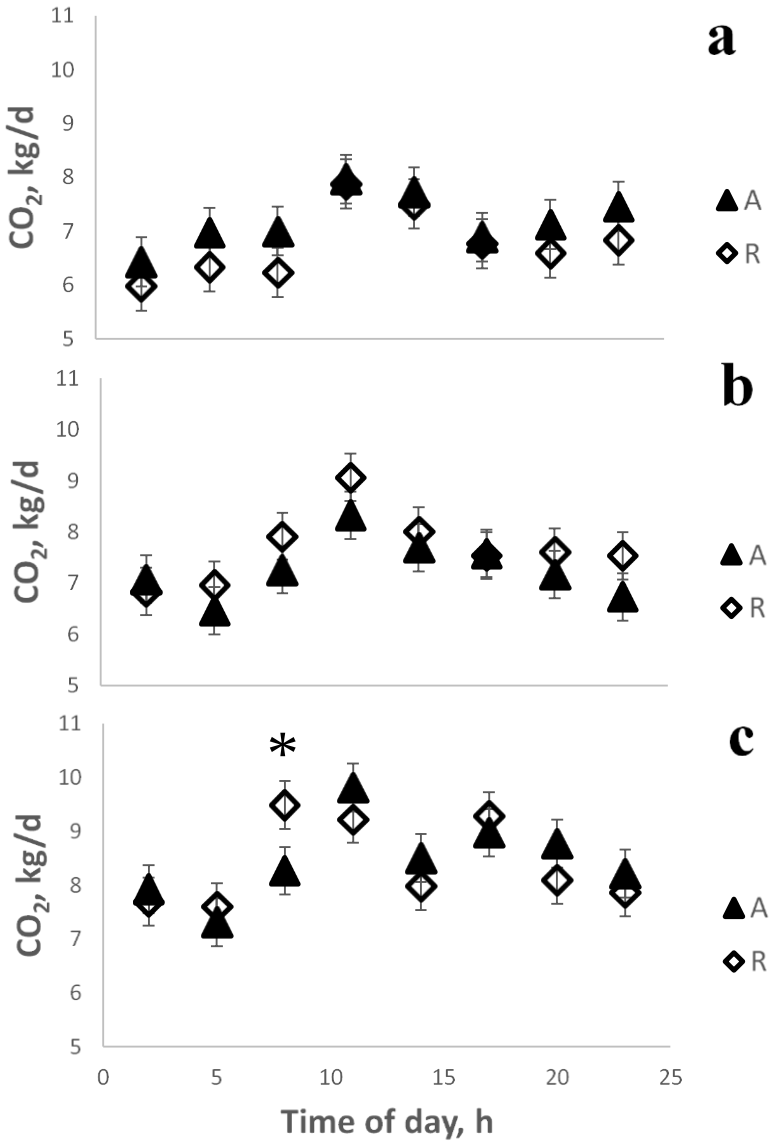

Figure 4.7. Mean ± SEM carbon dioxide (CO_2) emissions in beef steers given *ad libitum* access to their diet (▲) or restricted access to their diet (◊) during three sampling periods; Period a, 8-10 d, Period b, 28-30 d, and Period c, 63-65 d. A period × intake interaction was demonstrated ($P < 0.01$). There was no effect ($P \geq 0.11$) of intake, fat, fat × intake, time × intake, time × fat, period × fat, period × fat × intake, period × time, period × time × intake, period × time × fat, and period × time × fat × intake for CO_2 emissions. * $P \leq 0.05$ for means separated using the SLICE and PDIFF option (SAS Inst. Inc., Cary NC).

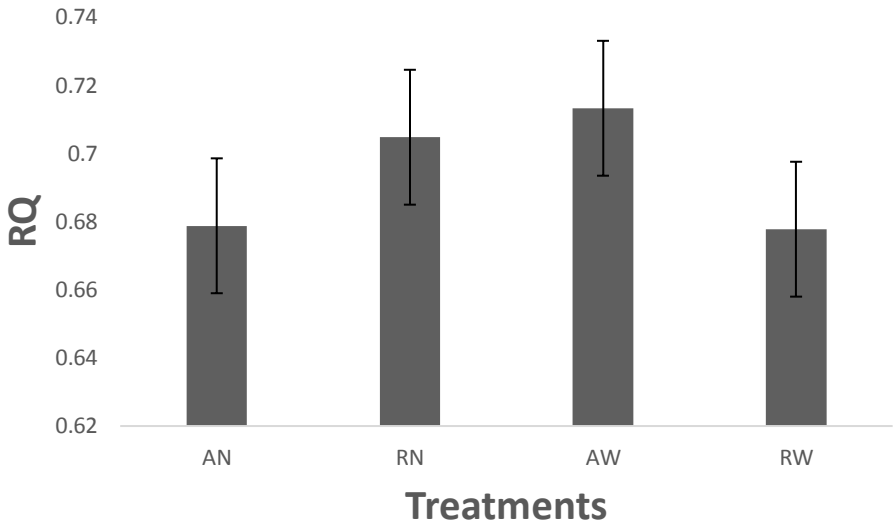

Figure 4.8. Mean ± SEM respiratory quotient (RQ) from beef steers given one of four treatments; 1. *ad libitum* access to a high energy diet without supplemented fat (AN), 2. *ad libitum* access to a high energy diet supplemented with 4% Ca salts of fatty acids (AW), 3. Restricted access to a low energy diet without supplemented fat (RN), or 4. Restricted access to a low energy diet supplemented with 4% Ca salts of fatty acids (RW). Respiratory quotient was calculated from gas emission sampling taken at 0200, 0500, 0800, 1100, 1400, 1700, 2000, and 2300 h during 3 sampling periods within 65 days. A fat × intake interaction was demonstrated ($P = 0.07$). There was no effect ($P \geq 0.28$) of intake, fat, time × intake, time × fat, time × fat × intake, period × intake, period × fat, period × fat × intake, period × time, period × time × intake, period × time × fat, and period × time × fat × intake on RQ. Means were separated using the SLICE and PDIFF option (SAS Inst. Inc., Cary NC; $P > 0.10$).

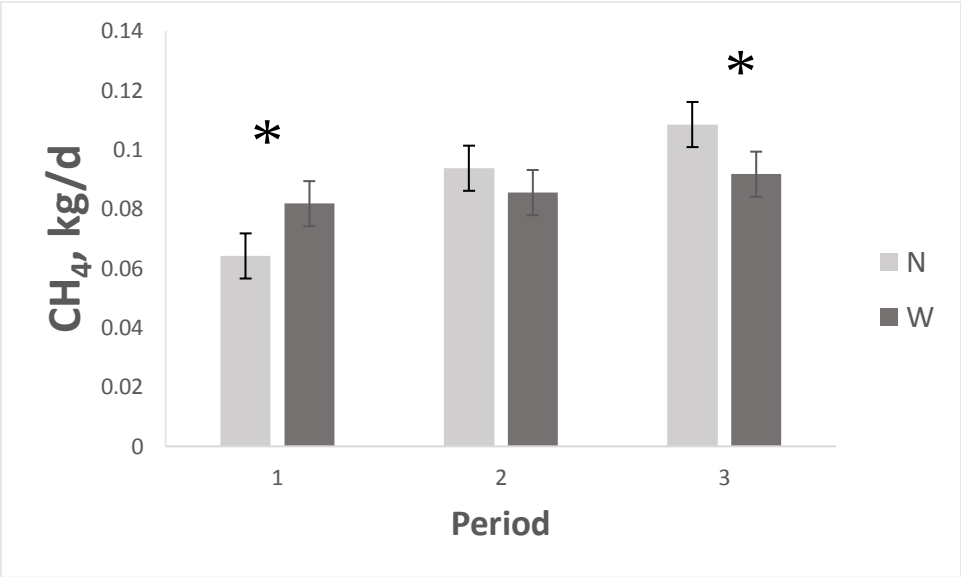

Figure 4.9. Mean ± SEM methane (CH_4) emissions of beef cattle treated with 4% Ca salts of fatty acids in the diet (■) or no fatty acids in the diet (□) during 3 sampling periods; a, 8-10 d, b, 28-30 d, and c, 63-65 d. A period × fat interaction was demonstrated ($P = 0.02$). There was no effect ($P \geq 0.26$) of intake, fat, fat × intake, time × intake, time × fat, time × fat × intake, period × intake, period × fat × intake, period × time × intake, period × time × fat, and period × time × fat × intake. * $P \leq 0.05$ for means separated using the SLICE and PDIFF option (SAS Inst. Inc., Cary NC).

Chapter 5

5.1 General Conclusions

These two experiments were conducted to evaluate the association between energy intake and plasma GIP concentration, and the association of plasma GIP concentration with RQ, carcass characteristics, and growth performance in feedlot cattle. The main hypothesis is partially rejected because animals fed greater energy intakes did not have greater RQ or IM fat deposition. Plasma GIP concentration could not be positively associated with RQ or IM fat deposition as it did in feedlot cattle. Also, in experiment 2, supplementation of fat and *ad libitum* feed intake increased plasma GIP concentration. However, this difference in plasma GIP concentration disappeared later in the day, and supplementation of fat had greater effects on stimulation. In experiment 1, there was no treatment x time interaction on plasma GIP concentration. However, similar treatments in experiment 2 (AN and RN) had similar plasma GIP concentration after feeding. A possible explanation could have been that the intake for limit fed animals in experiment 2 was divided in two daily feedings that could have change the pattern of energy intake. In feedlot cattle, it seems that there is no adaptation to fat supplementation in terms of plasma GIP concentration. Results confirm previous findings that in ruminants dietary fatty acids led to greater differences in plasma GIP concentration after feeding. Differences in plasma GIP concentration were due to an interaction between fat, energy, and time of sampling (relative to feeding). The results do not confirm that GIP is positively associated with IM fat deposition or greater plasma insulin concentration.

However, it is possible that the glucose threshold needed for GIP to act as insulinotropic was not met.

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Appendix A Muscle Tracing with Photo Scanner and Adobe Photoshop

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Title of Procedure: Muscle Tracing with Photo Scanner and Adobe Photoshop
Effective Date: October 29**,** 2020 **Version:** 1.0
Prepared by: Mairim Ortiz and Ben Bohrer

Approved by: _____ Date: _____

References:

Similar protocol:
Richardson E., B.M. Bohrer, E.K. Arkfeld, D.D. Boler, and A.C. Dilger. 2017. A comparison of intact and degraded desmin in cooked and uncooked pork longissimus thoracis and their relationship to pork quality. Meat Science. 129(7): 93-101. doi.org/10.1016/j.meatsci.2017.02.024

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Summary of Method

Ribeye area (REA) was determined for the longissimus dorsi muscle at the 12th and 13th rib interface with tracing techniques. A single individual outlined the ribeye area with a fine point marker using transparent vellum paper. The tracings were scanned using an Epson Perfection V500 photo scanner (Epson America, Inc., Long Beach, California, USA). A 5.08 cm × 5.08 cm square outline was included in each scan for calibration purposes. The tracings were quantified in Adobe photoshop (Adobe Photoshop 2020, Adobe Creative Cloud and Acrobat, San Jose, California, USA).

Equipment Needed:

- Transparent water-proof vellum or acetate paper (can be purchased at Staples).
- Computer.
- High clarity photo scanner (Epson Perfection V500 photo scanner, Epson America, Inc., Long Beach, California, USA).

- Adobe photoshop (Adobe Photoshop 2020, Adobe Creative Cloud and Acrobat, San Jose, California, USA).
- Calibration square with dark color (high contrast) and known dimensions (generated in Microsoft Word and then printed).

Guidelines and Considerations

Set-up

1. Trace muscle (e.g. ribeye area or loin eye area) using a fine point permanent marker on transparent water-proof vellum or acetate paper.
2. Prepare scanner and Adobe photoshop by setting up these programs on a computer.

Methods

1. Turn on scanner and turn on computer.
2. Plug scanner into computer.
3. Scan muscle tracings and calibration square using scanner.
4. Open images with Adobe Photoshop.
5. Measure tracing in Adobe Photoshop.
    a. Image → Analysis → Set Measurement Scale → Custom
    b. Measure one side of the calibration square and set Pixel Length, Logical Length, and Units.
    c. Confirm area of calibration square [Use magic wand tool to click on calibration square and then Image → Analysis → Record Measurements].
        i. This value should be accurate to 2 decimal places (go back to step 4a if this is not the case).
    d. Use magic wand tool to outline muscle tracing. If this does not work properly (i.e. more than just the muscle tracing is outlined) use the quick selection tool or magnetic lasso tool.
    e. Measure/Quantify Area by Image → Analysis → Record Measurements.

Appendix B Glucose-dependent insulinotropic polypeptide radioimmunoassay method

GIP RIA (Phoenix pharmaceutical RK-027-02)

This assay was modified based on the Phoenix pharmaceutical assay. The original assay is for 125 tubes. The assay yields 250 tubes. Also, all the volumes in the following protocol are for one assay, if two or more assays are going to be run, multiply all the volumes by the number of kits/tubes needed. This assay was modified using half the volumes required of reagents and two extra serial dilution of the standard was added. The volume of the serial dilutions used are double than the recommended by the protocol. The volume of plasma serum concentration and standards used was 200µl instead of the 100µl recommended by the protocol. On day 1, the volume of RIA buffer remained the same as the recommended by the protocol for the NSB tube, but changed to 150ul for the TB tubes instead of the 100ul recommended. On day 3, the volume of the RIA buffer used was 150ul for the NSB and TB tubes and 100ul for the standards, QC and samples instead of the 500ul recommended by the protocol.

INTRODUCTION

This kit is designed to measure a specific peptide and its related peptides by a competitive radioimmunoassay method. It is intended for in vitro study only. The antibody used for this assay was raised against a synthetic form of the peptide.

General Procedure:

1. Dilute in a 250 mL glassware the RIA buffer (4X concentrate buffer) (large bottle, **silver cap**) with 150 mL of distilled water. This buffer will be used to reconstitute all the other compounds in this kit and should be used for dilution of samples if needed.

   To be sure that all the concentrate buffer is used, add the 4x concentrate buffer liquid in the glassware and then add 50 mL of the distilled water in the original large bottle. Then add (mixed) the 50 mL of water with the 50 mL of the 4x concentrate buffer. Repeat this step twice.

2. Reconstitute the **standard peptide-STOCK (purple cap)** with 1 mL of RIA buffer.

   The standard is a lyophilized powder inside a microcentrifuge tube into the purple cap bottle. Vortex at least two minutes until ALL the peptide powder is completely dissolved in the microcentrifuge tube.

Note: Before adding buffer, carefully examine the microcentrifuge tube containing the standard. During shipping, part or all of the lyophilized standard may have come loose from the bottom of the tube causing it to stick to the cap or walls of the tube. Gently tap or centrifuge the tube to dislodge powder from the cap or walls. Carefully open the tube and add buffer.

3. Reconstitute the **antibody (blue cap)** with 13 mL of RIA buffer and vortex.

4. Reconstitute the **Positive Control** (small bottle, **silver cap**) with 1mL of RIA buffer and vortex the microcentrifuge tube.

5. Prepare dilutions of the standard as shown in the following figure using the dilutions of table
    - The volumes should be double of the ones in table 1. for one assay.

    a. Vortex each tube and switch each tip between dilutions

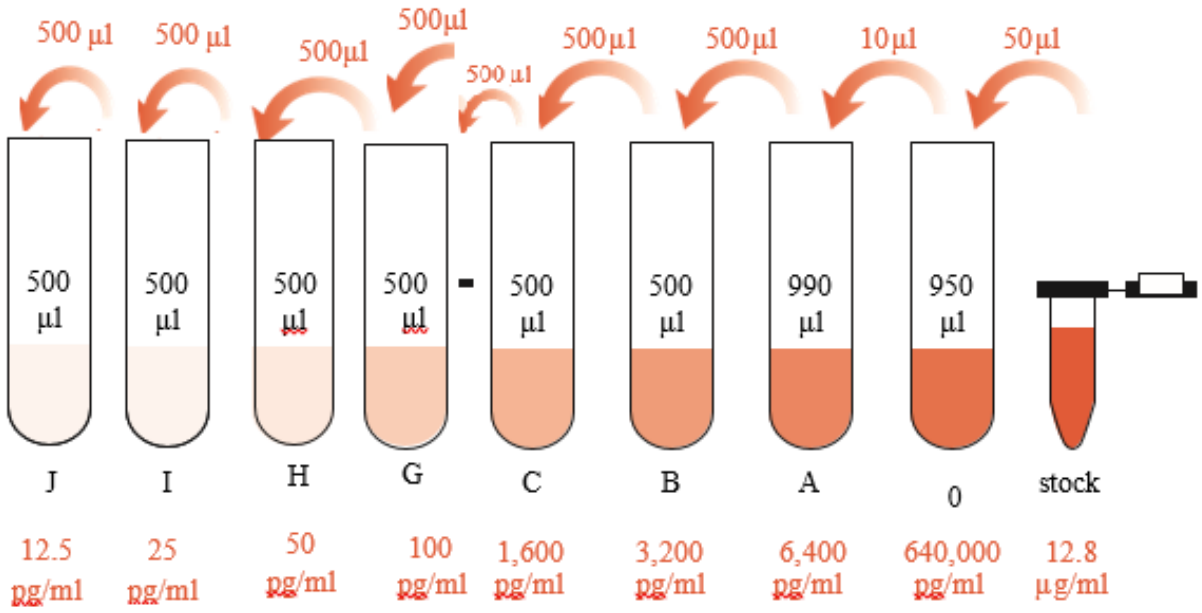

Table 1: Standard Dilutions

Tube	RIA Buffer	Standard	Std. Conc.
Stock	1 ml	Powder	12.8 µg/ml
0	950 µl	50 µl of Stock	640,000 pg/ml
A	990 µl	10 µl of 0	6,400 pg/ml
B	500 µl	500 µl of A	3,200 pg/ml
C	500 µl	500 µl of B	1,600 pg/ml
D	500 µl	500 µl of C	800 pg/ml
E	500 µl	500 µl of D	400 pg/ml
F	500 µl	500 µl of E	200 pg/ml
G	500 µl	500 µl of F	100 pg/ml
H	500 µl	500 µl of G	50 pg/ml
I	500 µl	500 µl of H	25 pg/ml
J	500 µl	500 µl of I	12.5 pg/ml

Day 1 RIA set up

6. Set up RIA reactions (see Table 2) in up to 125 12 mm x 75 mm polystyrene tubes. (DO NOT USE GLASS TUBES)

a) Number tubes (in triplicated) TC, NSB, TB, and the standards.
b) Number tubes for the positive controls (in duplicates).
c) Number tubes #30 up to end for the unknown samples (in duplicates).
d) Pipette 200 µl of RIA buffer into each NSB tube.
e) Pipette 150 µl of RIA buffer into each TB tube.
f) Pipette 200 µl of standards J (12.5 pg/mL) through E (400 pg/mL) into triplicate.

Note: The tubes should be prepared in reverse order of serial dilution so that the concentration increases as the number of the tube increases. For example: Begin by pipetting 50 µl of standard H into tubes the respective tubes, then proceed to standard G into the following three tubes,

g) Pipette 200 µl of positive control into respective tubes.
h) Pipette 200 µl of unknown sample into duplicate tubes.

i) Pipette 50 µl of antibody into all tubes (except NSB y TC) EXCEPT TC AND NSB TUBES.
j) Vortex the contents of each tube.

k) Cover and incubate all tubes at 4°C for 16-24 hours.

Day 2

7. a) Add 1 mL RIA buffer into the **^{125}I-peptide** in the eppendorf tube (red cap) and vortex. This is the Stock Tracer Solution (**STS**). Take 10 µL of STS and check its concentration (CPM/µL) using a γ-counter.

b) Prepare 15 mL RIA buffer in a polystyrene container. Add an adequate amount of STS into this container so that the concentration is 8,000-12,000 cpm/100µl. Confirm the concentration with a γ-counter. This is the Working Tracer Solution (**WTS**).

To achieve the correct volumes of STS to use and dilute, use the equation:

$$\text{Concentration 1} \times \text{Volume 1} = \text{Concentration 2} \times \text{Volume 2}$$

The concentration 1 will be the concentration obtain from the γ-counter (remember that the count is in 10 µL; therefore, you have to multiply the results from the γ-counter x 10). Volume 2 will be the volume that you need to add of the STS to the 15 mL of the buffer. Volume 2 will be 15 mL Concentration 2 will be 10,000 to 12,000 (always target to the highest CPM needed). Then

$$\frac{\text{15mL} \times \text{10,000-12,000 CPM}}{\text{γ-counter CPM read} \times 10}$$

c) Add 50 µl of the WTS to each tube.

8. Vortex the contents in each tube.

9. Cover and incubate all tubes for another 16-24 hours at 4°C.

Day 3

10. Reconstitute the Goat Anti-Rabbit IgG serum (**GAR**; gold cap) with 13 ml of RIA buffer.

11. Reconstitute the Normal Rabbit Serum (**NRS**; green cap) with 13 ml of RIA buffer.

Note: The Total Count Tubes (TC) are not involved in the following reactions.

12. Add 50 µl of GAR to each tube except the TC tubes.

13. Add 50 µl of NRS to each tube except the TC tubes.

14. Vortex the contents of each tube. Incubate all tubes at room temperature for at least 90 minutes

15. Add 150 µl of RIA buffer to NSB and TB(except the TC tubes) and vortex.

16. Add 100 µl of RIA buffer to the rest of the tubes and vortex.

17. Centrifuge all tubes (except the TC tubes) at 3,000 rpm (approx. 1700 x g) for at least 20 minutes at 4°C.

18. Carefully aspirate **ALL** the supernatant (without touching the pellet) **immediately** following centrifugation (do not decant as the pellet might be lost or excess liquid could be left). **DO NOT ASPIRATE THE TC TUBES.**

Note: For best results, the supernatant should be **immediately** aspirated after centrifugation. If the pellet sits for more than 15-30 minutes, it may become detached and make aspiration difficult. Do not aspirate any solids.

19. Use a γ-counter to count the cpm of the pellet.

Table 2: Contents in Each Tube for Incubation

Day 1						Day 2	Day 3		
ID	**Tube**	**Contents**	**RIA Buffer**	**Std or Samples**	**Primary Antibody**	**Working Tracer Solution**	**Secondary Antibody (GAR)**	**NRS**	**RIA Buffer**
TC	1 - 3	Total Counts				50 µl			
NSB	4 - 6	Non-specific binding	200 µl			50 µl	50 µl	50 µl	150 µl
TB	7 - 9	Total binding	150 µl		50 µl	50 µl	50 µl	50 µl	150 µl
STDJ	10 - 12	12.5 pg/ml		200 uL	50 µl	50 µl	50 µl	50 µl	100 µl
STDI	13 - 15	25 pg/ml		200 uL	50 µl	50 µl	50 µl	50 µl	100 µl
STDH	16 - 18	50 pg/mL		200 uL	50 µl	50 µl	50 µl	50 µl	100 µl
STDG	19 - 21	100 pg/mL		200 uL	50 µl	50 µl	50 µl	50 µl	100 µl
STDF	22 - 24	200 pg/mL		200 uL	50 µl	50 µl	50 µl	50 µl	100 µl
STDE	25 - 27	400 pg/mL		200 uL	50 µl	50 µl	50 µl	50 µl	100 µl
	28 - 29	Positive Control		200 uL	50 µl	50 µl	50 µl	50 µl	100 µl
	30 - 31	Samples		200 uL	50 µl	50 µl	50 µl	50 µl	100 µl