EVALUATING MARBLING ACTIVITY UTILIZING METABOLOMICS AND ULTRASONOGRAPHY

A Thesis

by

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ABSTRACT

Intramuscular (i.m.) adipose tissue deposition, or marbling is one of the most important traits related to meat quality and palatability in beef, but difficult to accurately predict before slaughter. This study aimed to identify plasma metabolites and hormones associated with marbling. We hypothesized that there would be greater plasma metabolite and hormone concentrations in grain-fed heifers, which would have greater percent intramuscular fat (%IMF).

At 12 mo age, 7 heifers were fed a grain-based diet and 14 were fed a hay-based diet. Heifers were weighed every 28 d. Ultrasound and blood samples were taken before feeding at 0 d and 84 d of treatment. Plasma was analyzed for glucose, acetate, propionate, butyrate, insulin, ghrelin, adiponectin, and fatty acid composition. Data was analyzed using single-factor analysis of variance, simple regression, and split-plot design.

Rib fat thickness and plasma ghrelin concentration was greater in grain-fed heifers than in hay-fed heifers. There was a diet x time interaction for body weight (BW) and a weak interaction with %IMF. Plasma α-linolenic acid (18:3n-3) proportions tended to be elevated in hay-fed heifers than grain-fed heifers. There was a positive correlation between BW and %IMF and between BW and change in %IMF (weak). There were positive correlations between plasma palmitoleic acid (16:1n-7) proportions, oleic acid (18:1n-9) proportions, oleic:stearic acid (weak) ratio, and monounsaturated fatty acid (MUFA):SFA (weak) ratio and %IMF and weak, negative correlations between stearic acid (18:0) and saturated fatty acids (SFA) and %IMF. Plasma ghrelin concentrations were correlated with ADI (negative), feed:gain ratio (negative), rib fat thickness (positive), and ADG (positive). There were negative correlations between plasma myristic acid (14:0), palmitic acid (16:0) (weak) and palmitoleic acid (16:1n-7) proportions and

plasma ghrelin concentrations and a positive correlation between plasma linoleic acid proportions and plasma ghrelin concentrations.

In conclusion, there appears to be limitations in the sensitivity of a metabolic profile for marbling. Percent IMF was associated with body weight and plasma fatty acid concentrations. It was demonstrated that plasma ghrelin concentrations also are correlated with plasma fatty acid concentrations. Ghrelin and fatty acid composition show promise for predicting marbling.

DEDICATION

I would like to dedicate this thesis to all who have supported me over the years including family and friends. I have gone to a lot of different places throughout my educational and professional journey and I really appreciate everyone who has supported me along the way.

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NOMENCLATURE

%IMF	Percent intramuscular fat
ADG (kg/d)	Average daily gain
ADI (kg as-fed intake/d)	Average daily intake
AMPK	5’ adenosine monophosphate-activated protein kinase
BCNRM	Beef Cattle Nutrient Requirements Model
βHBA	β-hydroxybutyrate
BW (kg)	Body weight
CLA	Conjugated linoleic acid
CP	Crude Protein
CUP	Centralized ultrasound processing
CV	Coefficient of variation
DMI	dry matter intake
ELISA	Enzyme-linked immunosorbent assay
EPD	Expected progeny difference
GHS-R	Growth hormone secretagogue receptor
GLUT4	Insulin-stimulated glucose receptor
GOAT	ghrelin o-acyltransferase
H-RFI	High-residual feed intake
i.m.	Intramuscular
IML	Intramuscular lipid
JB	Japanese black cattle
LD	*m. longissimus dorsi*

L-RFI	Low-residual feed intake
LYPLA1	Lysophospholipase I
ME	Metabolizable energy
MS	Mass spectroscopy
MUFA	Monounsaturated fatty acid
NASEM	National Academies of Sciences, Engineering and Medicine
NEFA	Nonesterified fatty acid
NEg	Net energy for growth
NEm	Net energy for maintenance
PPARγ2	Peroxisome proliferator-activated receptor γ2
RIA	Radioimmunoassay
s.c.	Subcutaneous
SCD	Stearoyl-CoA desaturase
SFA	Saturated fatty acid
TAG	Triacylglycerol
USDA	United States Department of Agriculture
VFA	Volatile fatty acid

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CHAPTER I

INTRODUCTION

Intramuscular (i.m.) adipose tissue deposition, or marbling, is one of the most important traits related to meat quality and palatability in beef and contributes to tenderness, juiciness, and nutritional value (Geay et al., 2001; Hausman et al., 2009; Scollan et al., 2006). Additionally, the primary determinant of beef carcass value under the USDA beef grading system is the abundance of marbling (USDA, 1997). Thus, increases in marbling lead to increases in product quality and carcass value, resulting in increased profits per carcass. To improve beef quality and increase profits, improving the understanding of marbling deposition and the regulation of lipid metabolism is necessary (Baik et al., 2017; Mir et al., 2002, 2008; Bergen and Mersmann, 2005; Dhiman et al., 2005; Givens, 2005).

Marbling is a highly valued aspect of beef, but ultimate amount of marbling deposited can be difficult to accurately predict before slaughter. Genetic prediction of marbling potential via Expected Progeny Difference (EPD) is also difficult and its accuracy only increases with the increase in offspring from that animal; gestation lasts 283 d in Bovidae and typically produces only one offspring, which makes this approach extremely time consuming. Metabolomics has the potential to predict marbling potential from the analysis of metabolites in bovine plasma in the same manner as it was shown to predict disease states in dairy cattle (Hailemariam et al., 2014). According to Baik et al. (2017), there is no literature available on metabolomics evaluating i.m. adipose tissue deposition in cattle. Therefore, applying metabolomics to cattle may expand the knowledge we have on the mechanisms involved in i.m. adipose tissue deposition and related metabolic pathways (Baik et al., 2017). Metabolomics may facilitate the prediction of the

phenotypic expression potential for marbling in beef cattle and help increase the accuracy of herd improvement plans.

This study aimed to identify plasma metabolites and hormones predicted to be associated with marbling. To accomplish this, we compiled a preliminary metabolic profile associated with plasma samples taken early in production. To make differences in metabolites between high- and low-marbling cattle more demonstrable, two groups of heifers were fed different diets: a hay-based diet and grain-based diet. We chose to focus on metabolites associated with lipid metabolism and inflammation (metabolic syndrome). We hypothesized that we would find differences in the plasma concentrations of glucose, insulin, adiponectin, ghrelin, fatty acid composition, acetate, propionate, and butyrate. Additionally, we compared the plasma metabolite concentrations with the ultrasound imagery (used to evaluate marbling). We further hypothesized that there will be differences in metabolite concentrations between the hay- and grain-fed groups of heifers, with the heifers fed the grain-based diet showing metabolite concentrations that correlate with higher marbling. We also hypothesized that there will be a demonstrable statistical association between ultrasound marbling scores and metabolic profiles.

CHAPTER II

LITERATURE REVIEW

2.1 Metabolomics

Metabolomics is the study of the metabolome (Goodacre et al., 2004), which compromises the totality of metabolites present in the sampled tissue at a given time. The metabolome may encompass the metabolites present in an organism, tissue or cell, including blood. Metabolites measured for this study included hormones associated with carcass adiposity, substrate availability for *de novo* fatty acid biosynthesis, and fatty acids. Metabolomics has been used to assess and predict disease and evaluate disease risk through biomarkers, and metabolomics is increasing in use (Hailemariam et al., 2014). Others have used high-throughput tools such as mass spectroscopy and NMR spectroscopy to measure metabolites in biological samples (Park et al., 2015). By understanding the metabolic differences and important factors affecting marbling, we can pinpoint metabolites and/or hormones most likely to have measurable variations that can reflect differences in marbling capacity.

There have been a number of studies that have demonstrated the capabilities of metabolomics to evaluate disease states in cattle. This could transfer to the capability of metabolomics to predict marbling in beef cattle. In dairy cattle, the ketone bodies acetone, β-hydroxybutyric acid (BHBA), and acetoacetate in milk were evaluated as biomarkers for subclinical ketosis (Enjalbert et al., 2001). Saleem et al. (2012) confirmed that there were rumen metabolite alterations with increases in grain proportions in the diet. Other studies have found differences in metabolite concentrations between healthy cows and cows in a diseased state (Ospina et al., 2010; Piechotta et al., 2012). Imhasly et al. (2014) established a preliminary metabolic profile for hepatic lipidosis in dairy cattle using metabolomics. Hailemariam et al.

(2014) reported that healthy animals could be distinguished from diseased animals or soon-to-be-diseased animals through the use of quantitative mass spectroscopy (MS)-based metabolomics of bovine blood plasma, which was observable up to 4 wk before outward signs of disease were exhibited. Because metabolites provided evidence of disease up to 4 wk before clinical signs could be observed, this supports the notion that marbling ability (an altered metabolic state) could be detected at an early age by measuring plasma metabolites and/or hormones.

2.2 Metabolic differences in adipose tissue deposition sites

Understanding the metabolic distinctions between subcutaneous (s.c.) and i.m. adipose tissue is helpful for assessing the utility of metabolites associated with glycolysis and lipid metabolism in predicting marbling. It is generally understood that i.m. and s.c. adipose tissue are metabolically distinct with differences in preferred substrates and fatty acid synthesis rates (Smith et al., 1998). Adipose tissue deposition in ruminants differs from that of monogastrics as fatty acids are synthesized *de novo* mainly in adipose tissue instead of liver (Bauman and Davis, 1975). Nonesterified fatty acids (NEFA) and glycerol (derived from glucose) are required for triacylglycerol (TAG) synthesis and can be acquired either via the diet or *de novo* synthesis (Pethick et al., 2004). A positive energy balance is required for the deposition of i.m. adipose tissue; therefore the degree of deposition varies with differences in the energy content in the diet (Smith and Crouse, 1984).

Fatty acid synthesis rates for i.m. adipose tissue are generally 5 to 10% of the rates observed in s.c. adipose tissue (Hood and Allen, 1978; Rhoades et al., 2007; Smith and Crouse, 1984). Glucose provides more carbon for fatty acid synthesis in i.m. adipose tissue than s.c. adipose tissue and it was observed that i.m. adipose tissue was more sensitive to insulin than s.c.

adipose tissue (Cook and Miller, 1965; Gilbert et al., 2003; Rhoades et al., 2007; Smith and Crouse, 1984). Therefore, synthesis rates and deposition for i.m. adipose tissue should increase to a greater degree with increases in insulin concentrations than s.c. adipose tissue. Additionally, the process of TAG synthesis is depressed to a greater degree by starvation in s.c. than in i.m. adipose tissue (Smith et al., 1998). This is a further indication that i.m. and s.c. adipose tissues are metabolically distinct.

Smith et al. (2009) reported that high plasma concentrations of acetate would promote s.c. fatty acid synthesis, but glucose and propionate are considered precursors for the synthesis of fatty acids in i.m. adipose tissue (Chung et al., 2007; Cook and Miller, 1965; Gilbert et al., 2003; Hausman et al., 2009; Ladeira et al., 2016). This difference in preferred substrate provides an initial indicator of metabolic differences between s.c. and i.m. adipose tissues, and is one of the determining factors in metabolites selected to be measured for this study.

In addition to the metabolic differences in i.m. and s.c. fatty acid synthesis, i.m. adipocytes, alongside myofibers, are uniquely located within perimysial connective tissues, distinguishing it from other adipose depots (Brooks et al., 2011; Moody and Cassens, 1968). Intramuscular adipose tissue deposition increases as the result of increases in size and number of i.m. adipocytes in accordance with the two stages of fat deposition (Baik et al., 2017; Schoonmaker, 2012). These stages are hyperplasia, occurring during the early stage of animal growth with preadipocyte differentiation into adipocytes from stem cells and proliferation, and hypertrophy that occurs with TAG accumulation at the later stages of growth (Baik et al., 2017; Schoonmaker, 2012). These steps indicate that, in general, cattle have a predetermined maximum genetic potential that is limited by the number of adipocytes produced during hyperplasia.

2.3 Intramuscular adipose tissue

It is commonly concluded that i.m. adipose tissue is late developing (Vernon, 1981). According to Pethick et al. (2004), fat deposition is first deposited as internal fat (abdominal, renal-inguinal, and pelvic), then intermuscular, followed by s.c., and lastly i.m. adipose tissue indicating that various adipose tissue depots are not deposited at the same rate or under the same conditions. Pethick et al. (2004) stated that i.m. adipose tissue is not necessarily late maturing but rather the expression of i.m. adipose tissue deposition is late maturing. By adding grain to the diet, marbling activity can be stimulated. This allows for marbling to occur in younger cattle and for correlations for metabolites and/or hormones with marbling to be observed.

In the later stages of i.m. adipose tissue development, i.e., hypertrophy, there are both genetic and nongenetic factors, such as breed, carcass weight, animal age, that affect the formation of i.m. adipose tissue deposition and composition (Bong et al., 2012; Pethick et al., 2004; Wang et al., 2005; Zembayashi et al., 1995). Gotoh et al. (2009) reported that cattle reared under both typical Japanese and European beef production systems gained similar body weight (BW), but Japanese Black steers had greater intramuscular lipid (IML) in the *m. longissimus dorsi* (LD) than European breeds. Additionally, Choi et al. (2005) reported that Korean cattle had more IML in the LD than Australian Angus cattle. The rate of lipogenesis and i.m. adipose tissue deposition depends on the muscle growth rate, metabolic activities of other organs, and the energy content of the diet; i.m. deposition increases after puberty and sexual maturity due to the decrease in muscle development (Hocquette et al., 2009; Owens et al., 1995). Thus, castrating bulls increases marbling as it decreases the rate of muscle development; decreased testosterone concentrations allows more energy to be dedicated to adipose tissue deposition (Baik et al., 2014; Bong et al., 2012).

2.4 Hormonal regulation

2.4.1 Insulin

Insulin is a peptide hormone secreted by the pancreatic β-cells and is responsible for managing blood glucose concentration, which fluctuate within a narrow range in ruminants (2.6 – 124.3 μU/mL) (Gonzalez-Grajales, et al., 2019; Kaneko, 1997; Kasai et al., 2014; Matsuzaki et al., 1997; Sasaki, 2002). Overall, insulin inhibits glucose release from the liver (DeFronzo, 2004) and insulin secretion is stimulated by elevations in blood glucose concentration (Komatsu et al., 2013). Insulin is released in a pulsatile fashion because insulin release is more reactive than proactive (Nunemaker and Satin, 2014; Song et al., 2000). For ruminants, circulating blood glucose concentrations are approximately half that of monogastrics and fluctuate less in response to feed consumption (Fahey and Berger, 1988; Forbes, 1995). Lesser glucose fluctuation also is associated with lower insulin response in peripheral tissues (Bell and Bauman, 1997; Brockman and Laarveld, 1986; Kaske et al., 2001; Sasaki, 2002; Smith, 2017). This is due to the limited absorption of glucose from the intestines (Aschenbach et al., 2010). In ruminants, both glucose and propionate are strong secretagogues for insulin (Allen et al., 2009; Dänicke et al., 2014; Locher et al., 2015). Considering that a limited amount of glucose is absorbed from the gastrointestinal tract, most circulating glucose is derived from gluconeogenesis, and propionate is the primary substrate for gluconeogenesis in ruminants (Aschenbach et al., 2010). In ruminants, the inhibitory effect of insulin on glucose release stimulates a proportional increase in the utilization of propionate as a substrate for gluconeogenesis (Brockman, 1990).

Insulin also inhibits lipolysis and may stimulate fatty acid synthesis from acetate, uptake in peripheral tissues (a preferred substrate for lipogenesis in ruminants) (Alves-Nores et al., 2017; Jarrett et al., 1974; Vernon et al., 1985). It has not been possible to demonstrate a

stimulation of lipogenesis by insulin in bovine s.c. adipose tissue (reviewed by Smith, 2017). However, plasma insulin concentrations were positively correlated with carcass adiposity (Trenkle and Topel, 1978). It is possible that insulin concentrations could be positively correlated to i.m. adipose tissue deposition as well as carcass adiposity. Rather than stimulating lipogenesis, Vernon (1978, 1979) observed that insulin prevented decreases in fatty acid synthesis from acetate in tissue culture with ovine adipose tissue. Although less effective in bovine s.c. adipose tissue, similar effects were observed by Vernon (1978) and Miller et al. (1989). The effect of insulin on rates of lipogenesis was less potent in i.m. adipose tissue than in s.c. adipose tissue (Miller et al., 1989). Further, insulin infusion in steers stimulates an increased glucose incorporation into fatty acids in s.c. adipose tissue, but only if co-infused with glucose (Smith et al., 1983). Because co-infusion of both insulin and glucose is required to stimulate fatty acid synthesis in s.c. adipose tissue, it is possible that similar conditions are required for insulin-stimulated fatty acid synthesis in i.m. adipose tissue.

Glucose-induced insulin secretion increases with age, and the response of insulin secretion to glucose decreases as rumen function develops (Sano et al., 1996; Shingu et al., 2001; Stern et al., 1970). Insulin resistance is defined as a state where insulin receptors are less sensitive to the presence of insulin, leading to increased plasma concentrations of insulin required to stimulate insulin receptors (Kahn, 1978). A decline in insulin-stimulated glucose receptor (GLUT4) expression in the longissimus and quadriceps muscle has been observed in Holstein calves and bulls after birth (Abe et al., 2001). In porcine muscle, GLUT4 expression increases up to at least 6 mo of age (Liang et al., 2015). Comparatively, ruminants have lower plasma insulin concentrations than monogastrics and mature pigs have been shown to have relatively high insulin receptor and GLUT4 gene expression in adipose tissue (Halsey et al.,

2011; Kristensen et al., 2015). This limited number of insulin receptors and GLUT4 gene expression in bovine tissues is likely the basis for the decreased insulin sensitivity in ruminants (Baldwin et al., 1973; Vernon, 1977; Vernon et al., 1980; Miller et al., 1989). Increased adiposity and overfeeding leading to over-conditioning has been shown to decrease insulin sensitivity in dairy cattle (Agenas et al., 2003; McCann and Reimers, 1985). Beyond diet effects, genetic effects have been demonstrated with cows with high genetic merit, potential for producing genetically superior offspring, for milk production being less insulin sensitive than cows with low genetic merit for milk production (Chagas et al., 2009).

2.4.2 Ghrelin

Ghrelin is a peptide hormone that was discovered in the rat stomach (Howard et al., 1996; Kojima et al., 1999). It is currently the only orexigenic peptide hormone known to be produced in peripheral tissues (Nakazato et al., 2001; Tschöp et al., 2000). In monogastrics, ghrelin is produced by the stomach and gastrointestinal endocrine cells, but in cattle, ghrelin is synthesized by the abomasal section of ruminal tissues (Date et al., 2000; Hayashida et al., 2001; Gentry et al., 2003; Zigman and Elmquist, 2003). Ghrelin is known for its effects on energy homeostasis regulation (Müller et al., 2015). Ghrelin has been shown to stimulate appetite and fat accumulation (Kojima et al., 1999; Tschöp et al., 2000; Nakazato et al., 2001). Additionally, ghrelin is involved in insulin secretion, stress, and glucose metabolism (Broglio et al., 2001; Chuang et al., 2011; Delhanty and Van der Lely, 2011). Rigault et al. (1996) detected ghrelin and its receptor in liver, which in liver, ghrelin causes increased gluconeogenesis and decreased glucose uptake (Gauna et al., 2005; Rigault et al., 1996). Considering that glucose is an essential precursor for i.m. adipose tissue deposition, it is possible that plasma ghrelin concentrations can

be correlated with i.m. adipose tissue deposition due to its function of stimulating glucose output from the liver. Tschöp et al. (2000) observed that ghrelin promoted adipose tissue deposition by reducing TAG mobilization and ß-oxidation of fatty acids. Additional studies also have observed that ghrelin stimulates the expression of genes involved in lipogenesis (Perez-Tilve et al., 2011; Sangiao-Alvarellos et al., 2009; Theander-Carrillo et al., 2006). The combined effects ghrelin on gluconeogenesis and lipogenesis furthers the concept that ghrelin has the potential to be correlated with i.m. adipose tissue deposition.

Ghrelin can utilize a number of pathways to influence feed intake and metabolic function. In rodents, ghrelin stimulates feed intake via neuropeptides located in the hypothalamus (Inui, 2001; Nakazato et al., 2001; Shintani et al., 2001). Ghrelin is able to cross the blood-brain barrier in other regions and reach the cerebrospinal fluid (Cabral et al., 2017; Uriarte et al., 2018). Additionally, ghrelin can activate the vagus nerve to indirectly stimulate the hypothalamus (Date et al., 2006).

Plasma ghrelin concentrations increase when nutrient availability is low and decrease when energy supply is abundant (Al Massadi et al., 2014). There is an inverse correlation between ghrelin concentrations and body mass index; therefore, ghrelin concentrations are elevated in malnourished states and decreased in states such as obesity in monogastrics (Cummings et al., 2002; Mequinion et al., 2013; Nagaya et al., 2001; Otto et al., 2001; Tschöp et al., 2001). In contrast, plasma ghrelin concentrations were greater for Corriedale sheep with excessive body fat when compared with lean sheep, but a decreased number of ghrelin receptors in the hypothalamus were observed (Kurose et al., 2005). Zigman et al. (2016) also demonstrated that a decreased effect of ghrelin on food intake occurred in obese animal models. This condition has been termed “ghrelin resistance” (Cui et al., 2017; Zigman et al., 2016).

In addition to differences in circulating concentrations relative to energy state and body composition, circulating ghrelin concentrations increase before a meal and decrease after feeding (Cummings et al., 2001; Shiiya et al., 2002). Wertz-Lutz et al. (2006) and Bradford and Allen (2008) observed elevated blood ghrelin concentrations in cattle during restricted feed intake, fasting, and negative energy balance. This also has been observed in sheep (Sugino et al., 2002a,b). Interestingly, ghrelin likely is not essential for regulation of feed intake. Deletion of ghrelin receptors or related receptors did not have an effect on feed intake in mice (McFarlane et al., 2014; Sun et al., 2003, 2004, 2006; Wortley et al., 2004; Zhao et al., 2010).

2.4.3 Adiponectin

Adiponectin is one of the most abundant adipokines secreted from white adipose tissue (Arita et al., 1999; Chandran et al., 2003; Hu et al., 1996; Jacobi et al., 2004). Adiponectin affects lipogenesis, gluconeogenesis, insulin sensitivity, and anti-inflammatory activity (Ahima and Lazar, 2008; Dall'Olio et al., 2009; Galic et al., 2010; Kadowaki and Yamauchi, 2005; Pagano et al., 2005; Pineiro et al., 2005; Scherer et al., 1995; Tilg and Wolf, 2005; Tonelli et al., 2004; Wei et al., 2013; Yamauchi et al., 2001; Yokota et al., 2002). Additionally, adiponectin is involved in yield grade and body weight (BW) in cattle (Berner et al., 2004; Kissebah et al., 2000; Morsci et al., 2006; Oshima et al., 2005; Wu et al., 2002). Receptors for adiponectin can be present in liver, macrophages, hypothalamus, white adipose tissue, and blood vessels (Tomas et al., 2002; Wu et al., 2003; Yamauchi et al., 2002, 2003, 2014).

Adiponectin is negatively correlated with adipose tissue mass, meaning that plasma adiponectin concentrations are lower in obese individuals (Arita et al., 1999; Hu et al., 1996; Kadowaki and Yamauchi, 2005; Matsubara et al., 2002; Ouchi et al., 1999; Turer et al., 2011).

The negative correlation between adiponectin and obesity, and the anti-inflammatory effects of adiponectin may be responsible for the observation that obesity leads to widespread, low-grade inflammation in adipose tissue (Illán-Gómez et al., 2012). Adiponectin has also been shown to increase insulin sensitivity (Berg et al., 2002; Combs et al., 2001; Turer and Scherer, 2012), with low plasma adiponectin concentrations being associated with decreased insulin sensitivity (i.e. isulin resistance) (Giesy et al., 2012; Singh et al., 2014).

Adiponectin also suppresses hepatic glucose output via the activation of 5' adenosine monophosphate-activated protein kinase (AMPK) (Combs et al., 2001). This was observed in mice that had lowered blood glucose for a short period of time in response to adiponectin treatment (Berg et al., 2001; Combs et al., 2001). Further, the activation of AMPK signaling by adiponectin has been shown to be hindered in obesity (Bruce et al., 2005; Chen et al., 2005). Inhibition of the activation of AMPK signaling by adiponectin led to increased hepatic glucose output and glucose intolerance (Andreelli et al., 2006; Yamauchi et al., 2002). We propose that adiponectin will be negatively correlated with i.m. adipose tissue deposition. Lower plasma adiponectin increases glucose availability to be incorporated into fatty acids and i.m. adipose tissue.

Low carbohydrate diet-based weight loss increased plasma adiponectin concentrations in humans (Salehi-Abargouei et al., 2015), and overfeeding was shown to decrease adiponectin (Ukkola et al., 2008). These observations suggest a potential difference in adiponectin concentrations will be found in cattle fed hay-based diets versus grain-based diets.

2.5 Fatty acid composition

It is well documented that fatty acid composition in s.c. adipose tissue is affected by breed, sex, age, and nutrition (Clemens et al., 1973; Eichhorn et al., 1986; Huerta-Leidenz et al., 1993; Mandell et al., 1998). The delta 9-desaturase is responsible for the conversion of saturated fatty acids (SFA) to monounsaturated fatty acids (MUFA) (St John et al., 1991; Jiang et al., 2008; Duckett et al., 2009) and several studies have indicated that a corn-based diet increases stearoyl-CoA desaturase (SCD) gene expression in cattle; SCD is the gene encoding delta 9-desaturase (Archibeque et al., 2005; Chung et al., 2007; Duckett et al., 2009).

Fatty acid composition of bovine plasma is affected by differences in diet. Plasma fatty acid composition in cattle changed, particularly with decreases over time in stearic acid (18:0), trans-vaccenic acid (18:1trans-11), and α-linolenic acid (18:3n-3), and increases in linoleic acid (18:2n-6) (Archibeque et al., 2005; Chung et al., 2006). In a study done comparing calf-feds (calves fed a concentrate diet immediately following feeding) and yearling-feds (cattle fed a concentrate diet at 12 mo of age), there were significant differences seen in the fatty acid composition of blood between calf-fed and yearling-fed steers (Brooks et al., 2011), especially for conjugated linoleic acid (CLA) isomers, stearic acid, and linoleic acid. The increase in stearic acid over time in yearling-fed steers may have been caused by a decline in hepatic SCD activity when fed the corn-based diet.

Chung et al. (2006) demonstrated a diet effect as well as a genetic effect on plasma fatty acid composition by comparing Angus and Wagyu cattle. Both myristoleic and palmitoleic acid plasma concentrations were elevated in steers fed a corn-based diet, but stearic acid concentrations were less than those fed a hay-based diet (Chung et al., 2006). Additionally, Angus steers had greater plasma palmitic and palmitoleic acid concentrations and a greater 16:1-

to-18:0 ratio than Wagyu steers (Chung et al., 2006). Along with the differences in plasma fatty acid concentrations, Chung et al. (2006) reported greater marbling scores in corn-fed steers than hay-fed steers. Further, Choi et al. (2014) observed that plasma palmitoleic acid, trans-isomers, and cis-vaccenic acid were decreased with arginine and alanine infusion. Additionally, plasma palmitic acid was higher in arginine-infused steers than in alanine-infused steers and that co-infusion with conjugated linoleic acid (CLA) decreased α-linolenic acid and increased both CLA isomers (cis9, trans11 and trans10, cis 12) in both arginine- and alanine-infused steers (Choi et al., 2014). The differences in fatty acid composition in bovine plasma due to differences in diet in these studies suggest that there will be differences in fatty acid composition in bovine plasma that reflect differences in the degree of marbling and/or diet.

CHAPTER III

MATERIALS AND METHODS

3.1 Animal handling

Twenty-one Angus heifers approximately 8 mo of age were fed hay and distiller's grain until 12 mo of age. This study is a portion of a larger study with 3 treatment groups (Hay, Hay, Grain; Hay Grain, Hay; Grain, Hay, Hay) changing dietary treatment every 3 mo with this study focusing on the first session of dietary treatment. At 12 mo of age, 14 heifers (Hay, Hay, Grain; Hay, Grain, Hay) remained on the hay/distillers grain diet. The remaining 7 heifers (Grain, Hay, Hay) were fed a standard finishing, grain-based diet. Care, handling and sampling of heifers were approved by the Texas A&M University Institutional Animal Care and Use Committee. At the beginning of the feed trial period, heifers averaged 242.24 kg and 234.98 kg for hay and grain groups, respectively. Diets were formulated by Dr. Jason Sawyer using the National Academies of Sciences, Engineering and Medicine for 2016 (NASEM 2016), the Nutrient Requirements of Beef Cattle and the Beef Cattle Nutrient Requirements Model (BCNRM). The hay/distillers grain diet was 65% alfalfa hay supplemented with 35% distiller's grain on an as-fed basis formulated to achieve an ADG of 0.9 kg/d. The grain-based diet was 25% alfalfa, 40% dry rolled corn, and 35% dried distiller's grain on an as-fed basis formulated to achieve an average daily gain (ADG) of 1.36 kg/d. Both diets are the same diets used by Lunt et al. (2005) and Chung et al. (2007). Heifers had free access to water throughout the treatment period. Weight was recorded every 28 d. Ultrasound was performed at the beginning of treatment (0 d) and after 84 d to estimate marbling amount and rib fat thickness. Ultrasound images were captured using an Aloka 500V SSD Ultrasound with a 17 cm probe and read at 3.5 mHz. Ultrasound images were analyzed by Kendrick Leblanc using the UICS program Centraliced Ultrasound Processing

(CUP) lab (The CUP Lab, LLC., Ames, IA). Change in %IMF was calculated by subtracting the %IMF at 84 d on treatment from the %IMF at 0 d on treatment. Average daily intake (amount consumed per day on an as-fed basis) was recorded using the GrowSafe System (Calgary, A.B., Canada). The GrowSafe System measures the difference in weight of feed in the bunk when an animal enters the bunk. The heifers have radio frequency identification tags placed in the ear, which is registered every time a heifer puts their head into the bunk. The system records the weight of the bunk at the time the heifer enters and the weight after the heifer leaves and the difference between the two weights was used to record individual consumption per day.

Table 1. Diet Composition

	Hay	Corn
CP (%)	20.6	18.2
Fat (%)	4.83	5.70
TDN (%)	67.3	80.1
ME (Mcal/kg)	2.40	2.89
NEm (Mcal/kg)	1.52	1.93
NEg (Mcal/kg)	0.93	1.28

3.2 Plasma analysis

Blood samples were taken in the morning before feeding from the jugular vein at 0 d and 84 d of treatment. Following the methodology by Takemoto et al. (2017), the plasma was collected in vacutainer tubes containing EDTA and chilled on ice. Samples were centrifuged for 15 min at 2,000 x g at 2°C, and plasma was stored at -80°C until analysis. Plasma glucose was measured using a commercially available kit (Glucose Enzyme Kit, CBA086, EMD Millipore Corporation, Temecula, CA) and plasma insulin was measured using a commercially available kit (Insulin Ab ELISA Kit, KA1107, Abnova, Taipei, Taiwan) with a mean inter- and intra-assay CV ranges of 2.5 to 4.0% and 4.3 to 6.0%, respectively. Plasma ghrelin and adiponectin were

measured using commercially available ELISA kits (GHRL ELISA Kit (Cattle) (OKCD02570) and ADIPOQ ELISA Kit (Bovine) (OKEH03794), respectively, Aviva Systems Biology, San Diego, CA). Mean inter- and intra-assay precision for the adiponectin assay were < 8.4% and < 5.7%, respectively. The mean inter- and intra-assay CV were ≤ 12% and ≤ 10%, respectively for the ghrelin assay. Kits were used according to protocol. All hormones were measured at 450 nm and glucose was measured at 570 nm using a Biotek Epoch Microplate Reader (Winooski, VT). Plasma acetate, propionate, and butyrate were sent to the University of Nevada in Reno to be measured as described by Remesy and Damigne (1974) with modifications (Klusmeyer et al., 1987). Additional modifications to the procedure were using 1.5 mL of plasma with 3 mL instead of 2 mL of ethanol, 150 µL instead of 100 µL of 0.2 N NaOH, 100 µL instead of 200 µL of distilled water, and 5 µL instead of 10 µL of formic acid. All chemicals were adjusted accordingly for samples with less than 1.5 mL of plasma. Some samples were thawed upon arrival in Reno. Plasma volatile fatty acids (VFA) were measured using gas chromatography (Shimadzu Nexis GC 2030, Shimadzu Co., Japan) with a split ratio of 50:1 at 300°C and 153 kpa with a 1 µL injection volume. Separations were done using a fused silica capillary column (0.25 mm x 100 m, Supelco Inc., Bellefonte, PA). % VFAs were calculated by totaling the concentrations of all VFAs (acetate, propionate, and butyrate) and calculating proportion (e.g. acetate concentration/total VFA concentration x 100). Lipids were extracted in chloroform:methanol (2:1 vol/vol) from 1 mL plasma or serum by the method of Folch et al. (1957). The samples were filtered to remove precipitated protein. Fatty acid methyl esters (FAME) were prepared as described by Morrison and Smith (1964). The FAME were analyzed using gas chromatography as described previously by Archibeque et al. (2005) with hydrogen as

the carrier gas. Fatty acid composition was measured with a Clarus 500 Gas Chromatograph from Perkin Elmer (Waltham, Massachusetts) using a 1:10 split ratio.

3.3 Statistical analysis

Data for plasma glucose, VFAs, fatty acids, and hormones were analyzed using single factor analysis of variance and simple regression (SuperAnova, Abacus Concepts, Inc., Berkeley, CA). Body weight (BW), %IMF, and VFAs were analyzed as a split-plot design with animal as the replicate, diet as the split plot, and time of collection as the whole plot. When analysis of variance indicated significant differences ($P < 0.05$), means were separated by the Fisher's Protected LSD method.

CHAPTER IV

RESULTS

4.1 Production characteristics

Initial BW was numerically lower for grain-fed heifers than hay-fed heifers (234.98 kg vs. 242.24 kg; $P = 0.55$). Final body weight (BW) tended to be greater for grain-fed heifers (360.91 ± 6.73 kg) than for hay-fed heifers (335.65 ± 6.73 kg; $P = 0.076$) (Table 1). Average daily gain (ADG) was higher in grain-fed heifers (1.49 ± 0.05 kg/d) than in hay-fed heifers (1.10 ± 0.05 kg/d; $P = 0.0001$) (Table 1). Feed:gain ratio was lower for grain-fed heifers (7.14 ± 0.38) than for hay-fed heifers (10.13 ± 0.38; $P = 0.0001$) (Table 1). Average daily intake (ADI) was not affected by dietary treatment ($P = 0.12$). Rib fat thickness was greater in grain-fed heifers (0.421 ± 0.02 cm) than in hay-fed heifers (0.342 ± 0.02 cm; $P = 0.049$). No differences were seen for %IMF between treatments ($P = 0.18$) (Table 1). Change in %IMF from January to April tended to be greater in grain-fed heifers (0.220 ± 0.14%) than in hay-fed heifers (-0.314 ± 0.14%; $P = 0.07$) (Table 1). There was a diet x time interaction for BW ($P = 0.0001$; Figure 1) and a tendency for an interaction of BW with %IMF ($P = 0.07$; Figure 2).

Table 2. Body weight, percent intramuscular fat, average daily intake, average daily gain, feed:gain ratio, rib fat thickness, and change in intramuscular fat

	Hay (n = 14)	Grain (n = 7)	SEM	P-value
Initial BW (kg)	242.24	234.98	5.53	0.55
Final BW (kg)	335.65	360.91	6.73	0.076
Average daily intake (kg/d)	11.09	10.51	0.17	0.12
Average daily gain (kg/d)	1.10	1.49	0.05	0.0001
Feed:Gain ratio	10.13	7.14	0.38	0.0001
Rib Fat (cm)	0.342	0.421	0.02	0.049
%IMF	2.6	3.0	0.20	0.18
Change in %IMF	-0.314	0.220	0.14	0.072

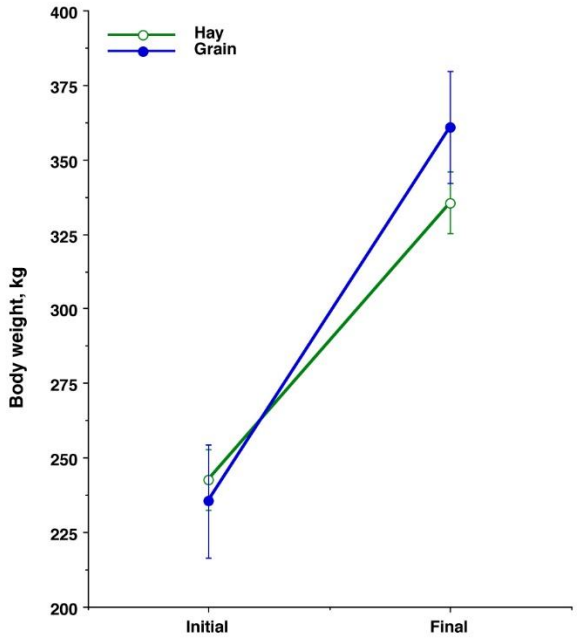

Figure 1. BW as a function of diet and time on feed (P = 0.0001).

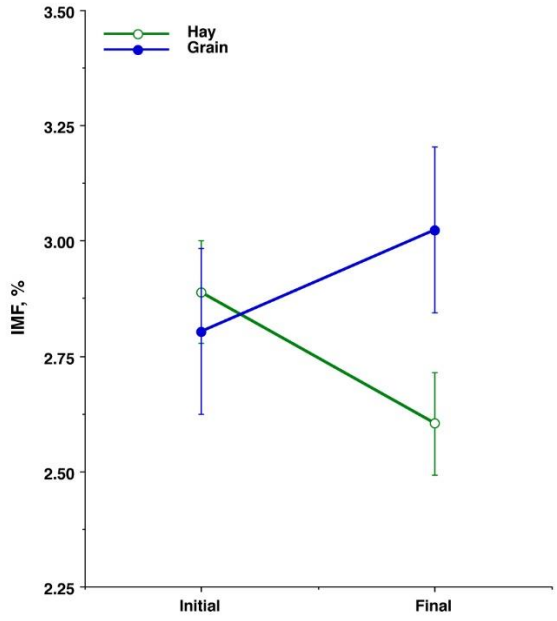

Figure 2. Percent IMF as a function of diet and time on feed (P = 0.07).

4.2 Plasma hormones and metabolites

There were no differences in plasma proportions of acetate, propionate, or butyrate between heifers fed the hay-based diet and heifers fed the grain-based diet. There also were no diet x time interactions for molar proportions of plasma acetate, propionate, or butyrate ($P > 0.05$). There was no dietary effect for plasma glucose concentrations ($P = 0.92$) (Table 2). Plasma adiponectin and insulin concentrations were not affected by dietary treatment ($P = 0.64$ and $P = 0.41$, respectively) (Table 3). Plasma ghrelin concentrations were greater in grain-fed heifers (2,877 ± 231 pg/mL) than in hay-fed heifers (1,630 ± 231 pg/mL; $P = 0.0072$) (Table 3). There were no differences among fatty acid species except for plasma α-linolenic acid (18:3n-3) proportions which tended to be greater in hay-fed heifers (0.41 ± 0.11 g/100 g fatty acids) than in grain-fed heifers (0.0 ± 0.11 g/100 g fatty acids; $P = 0.08$) (Table 4).

Table 3. Plasma acetate, propionate, butyrate, and glucose proportions and concentrations.

	Hay (n = 14)	Grain (n = 7)	SEM	P-value
Initial acetate (%)	86.08	84.63	1.98	0.74
Initial propionate (%)	10.62	11.79	2.08	0.80
Initial butyrate (%)	3.30	3.57	0.77	0.87
Final acetate (%)	86.63	81.31	2.13	0.25
Final propionate (%)	9.06	10.03	1.44	0.76
Final butyrate (%)	4.30	8.67	1.88	0.29
Glucose (mmol/L)	2.67	2.63	0.15	0.92

Table 4. Plasma insulin, ghrelin, and adiponectin concentrations.

Hormone	Hay (n = 14)	Grain (n = 7)	SEM	P-value
Insulin (U/mL)	34.5	27.4	4.0	0.41
Ghrelin (pg/mL)	1,630	2,877	231	0.007
Adiponectin (µg/mL)	4.15	5.37	1.18	0.64

Table 5. Plasma fatty acid composition, g/100 g total fatty acids.

Fatty acid	Hay (n =14)	Grain (n = 7)	SEM	P-value
Myristic (14:0)	0.37	0.06	0.13	0.26
Myristoleic (14:1)	0.04	0	0.02	0.32
Palmitic (16:0)	19.78	19.95	1.20	0.95
Palmitoleic (16:1n-7)	0.61	0.37	0.19	0.57
Stearic (18:0)	39.80	42.80	2.39	0.57
trans-Vaccenic (18:1trans-11)	4.23	2.80	0.47	0.16
Oleic (18:1n-9)	21.50	20.43	1.56	0.76
cis-Vaccenic (18:1n-7)	0.92	0.70	0.11	0.33
Linoleic (18:2n-6)	10.51	12.18	0.81	0.35
α-Linolenic (18:3n-3)	0.41	0.0	0.11	0.08
Arachidonic (20:4n-6)	0.47	0.18	0.14	0.34
Eicosapentaenoic (20:5n-3)	0.27	0.0	0.15	0.42
Docosahexaenoic (22:6n-3)	0.19	0.0	0.12	0.45
18:1:18:0	0.68	0.53	0.11	0.53
MUFA	27.53	24.30	1.93	0.44
SFA	60.05	62.81	2.06	0.54
MUFA:SFA	0.50	0.41	0.05	0.47

4.3 Correlations

There were correlations between final BW, fatty acid species and %IMF (Table 5). Rib fat thickness was negatively correlated with ADI and feed:gain ratio (Table 5). Final molar proportions of plasma acetate tended to be positively correlated with plasma insulin concentrations (Table 5). Final molar plasma propionate proportions were positively correlated with final BW (Table 5). Final molar plasma butyrate proportions tended to be negatively correlated with ADI (Table 5). Plasma ghrelin and linoleic acid (18:2n-6) concentrations were negatively correlated with feed:gain ratio (Table 5). Final BW and plasma ghrelin concentrations were positively correlated with ADG (Table 5). Plasma ghrelin concentrations were weakly, negatively correlated with ADI (Table 5).

Table 6. Correlations between final BW, ADG, ADI, F:G, %IMF and metabolites.

	Final BW	ADG	ADI	F:G	%IMF
Final BW		0.573**	0.024	-0.544***	0.518**
Rib fat	0.301	0.355	-0.379*	-0.485**	0.072
Glucose	-0.206	-0.034	0.105	0.019	-0.332
Acetate	-0.221	-0.131	0.345	0.299	-0.075
Propionate	0.482**	0.146	0.009	-0.157	0.042
Butyrate	-0.120	0.036	-0.397*	-0.218	0.051
Insulin	-0.035	-0.215	0.079	0.175	-0.242
Ghrelin	0.169	0.450**	-0.388*	-0.552***	0.301
Adiponectin	-0.08	0.242	-0.150	-0.237	0.134
Palmitic (16:0)	-0.18	-0.062	0.124	0.082	0.098
Stearic (18:0)	0.153	0.112	-0.130	-0.143	-0.407*
Oleic (18:1n-9)	-0.188	-0.089	0.151	0.183	0.478**
Linoleic (18:2n-6)	0.378*	0.316	-0.340	-0.431*	0.161
18:1:18:0	-0.232	-0.139	0.212	0.242	0.473**
SFA	0.061	0.086	-0.061	-0.106	-0.406*
MUFA	-0.249	-0.187	0.152	0.254	0.360
MUFA:SFA	-0.214	-0.158	0.165	0.231	0.419*

*$P < 0.10$; **$P < 0.05$; ***$P < 0.01$

4.3.1 Production characteristics

There was a positive correlation between final BW and final %IMF (r = 0.518; $P = 0.02$) (Table 5, Figure 3) and a weak, positive correlation between final BW and change in %IMF (r = 0.440; $P = 0.06$) (Figure 4). There was a strong, positive correlation between final BW and ADG (r = 0.573; $P = 0.007$) (Table 5, Figure 5). There also was a negative correlation between final BW and feed:gain ratio (r = -0.544; $P = 0.011$) (Table 5, Figure 6). Rib fat thickness was negatively correlated with ADI (r = -0.379; $P = 0.099$) and feed:gain ratio (r = -0.485; $P = 0.03$) (Figure 7 and Figure 8, respectively).

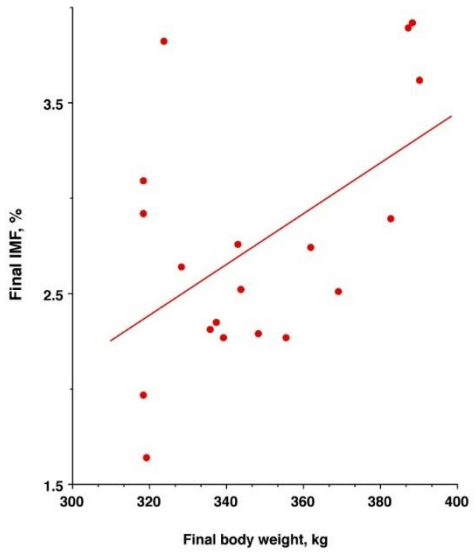

Figure 3. Final %IMF as a function of final BW (r = 0.518; *P* = 0.02) (n = 19).

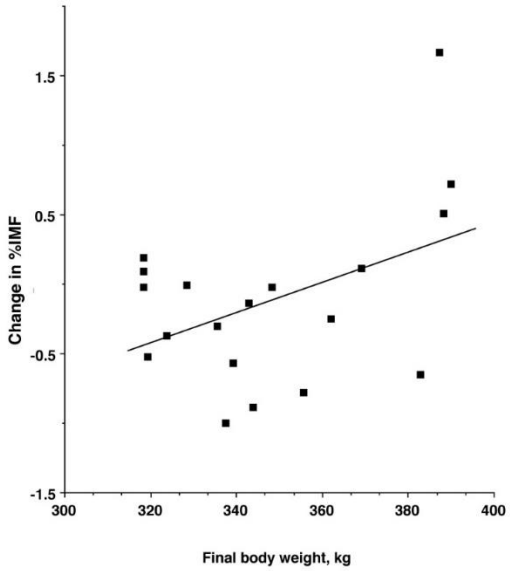

Figure 4. Change in %IMF as a function of final BW (r = 0.440; *P* = 0.06) (n = 19).

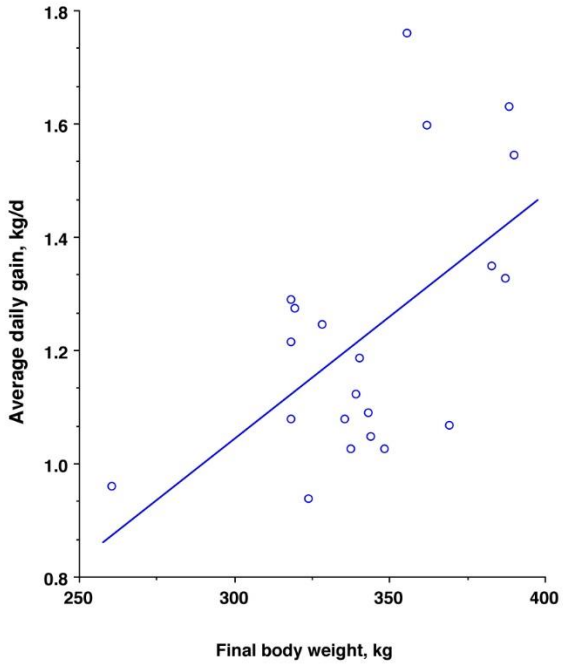

Figure 5. ADG as a function of final BW (r = 0.573; *P* = 0.007

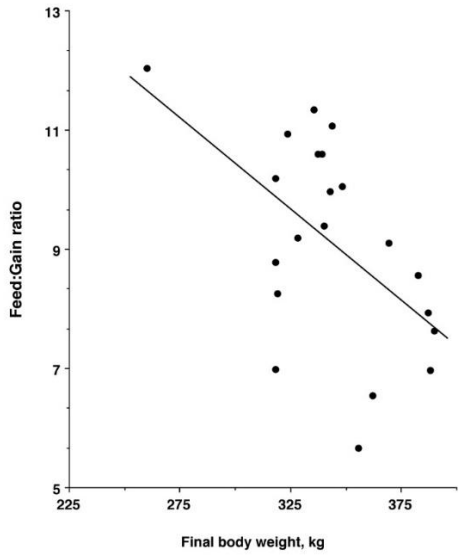

Figure 6. Feed:Gain ratio as a function of final BW (r = 0.544; *P* = 0.011).

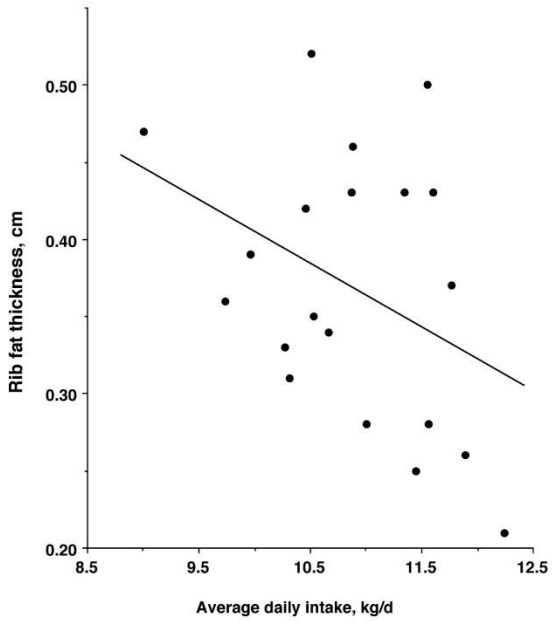

Figure 7. ADI as a function of rib fat thickness (r = -0.379; *P* = 0.099).

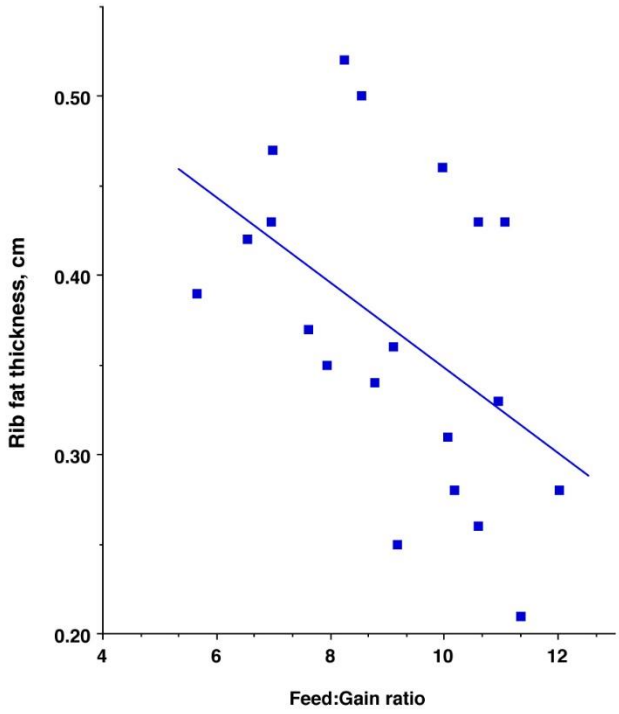

Figure 8. Feed:gain ratio as a function of rib fat thickness (r = -0.485; *P* = 0.03).

4.3.2 Plasma metabolites

Final molar proportions of plasma acetate (r = 0.386; *P* = 0.08) tended to be positively correlated with plasma insulin concentrations (Figure 9). Final molar plasma propionate proportions were positively correlated with final BW (r = 0.482; *P* = 0.03) (Figure 10). Final molar plasma butyrate proportions tended to be negatively correlated with ADI (r = -0.397; *P* = 0.07) (Figure 11). There was a positive correlation between plasma glucose concentrations and plasma trans-vaccenic acid (18:1trans) proportions (r = 0.470; *P* = 0.03) (Figure 12). There were no correlations between glucose and %IMF, BW, or any of the hormones (Table 5).

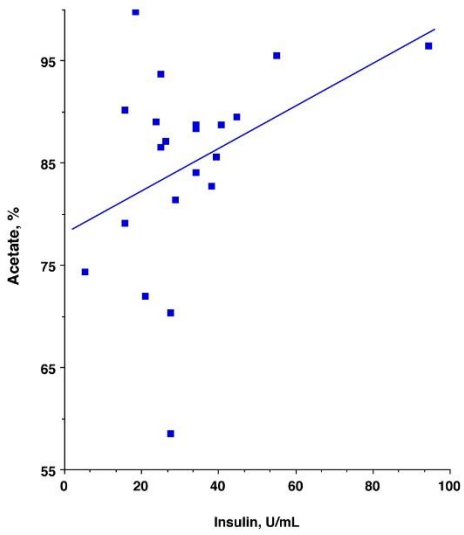

Figure 9. Final plasma proportions of acetate as a function of plasma insulin concentrations (r = 0.386; *P* = 0.08).

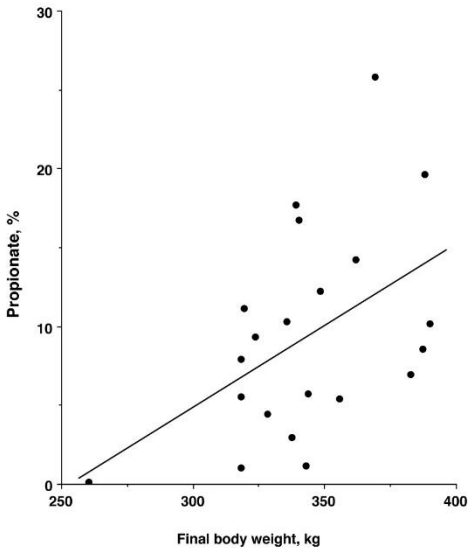

Figure 10. Final plasma proportions of propionate as a function of final BW (r = 0.482; *P* = 0.03).

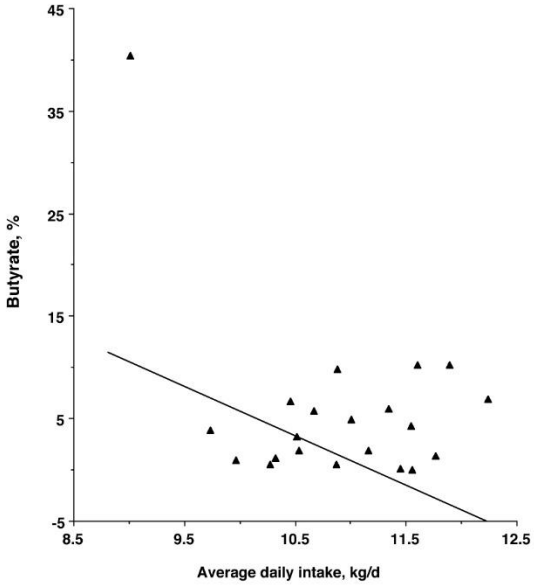

Figure 11. Final plasma proportions of butyrate as a function of ADI (r = -0.397; *P* = 0.07).

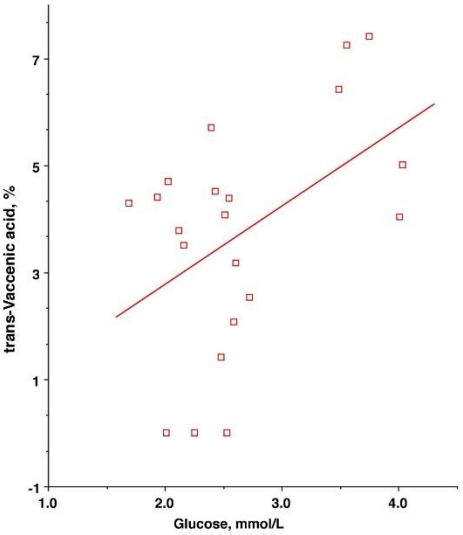

Figure 12. Plasma trans-vaccenic acid proportions as a function of plasma glucose concentrations (r = 0.470; *P* = 0.0315).

Proportions of plasma linoleic acid (18:2n-6) tended to be positively correlated with final BW (r = 0.378; *P* = 0.09) (Figure 13). Plasma trans-vaccenic acid proportions tended to be negatively correlated with ADG (r = -0.396; *P* = 0.08) (Figure 14). Plasma linoleic acid proportions tended to be negatively correlated with feed:gain ratio (r = -0.431; *P* = 0.05) (Figure 15). There were positive correlations observed for plasma proportions of palmitoleic acid (16:1n-7) (r = 0.469; *P* = 0.043) and oleic acid (18:1n-9) (r = 0.478; *P* = 0.04) and %IMF (Figure 16). Additionally, plasma proportions of stearic acid (18:0) (r = -0.407; *P* = 0.08) and SFA (r = -0.406; *P* = 0.09) tended to be negatively correlated with final %IMF (Figure 16). The oleic:stearic acid ratio was positively correlated to final %IMF (r = 0.473; *P* = 0.04) and the MUFA:SFA ratio tended to be positively correlated to final %IMF (r = 0.419; *P* = 0.07) (Figure 17).

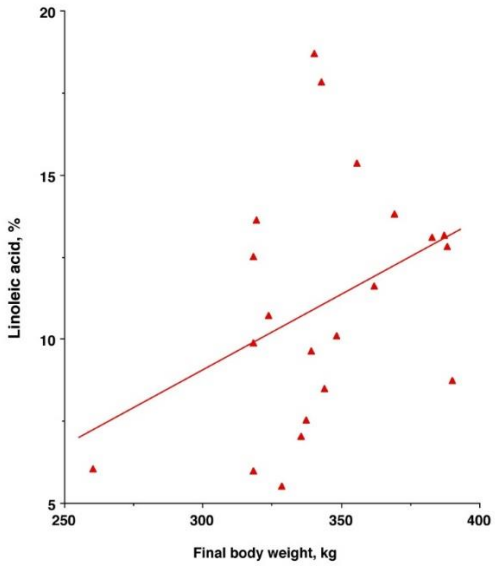

Figure 13. Plasma linoleic acid proportions as a function of final BW (kg) (r = 0.378; *P* = 0.09).

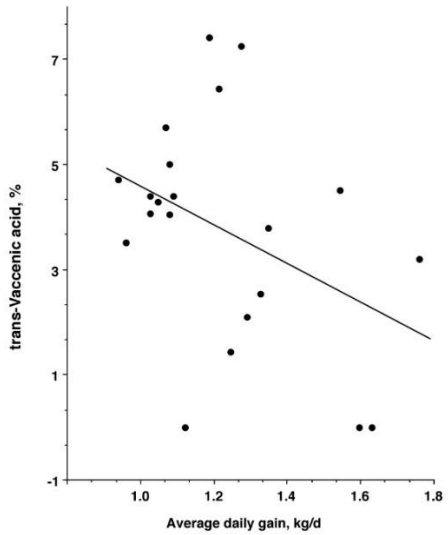

Figure 14. Plasma trans-vaccenic acid proportions as a function of ADG (r = -0.396; *P* = 0.08).

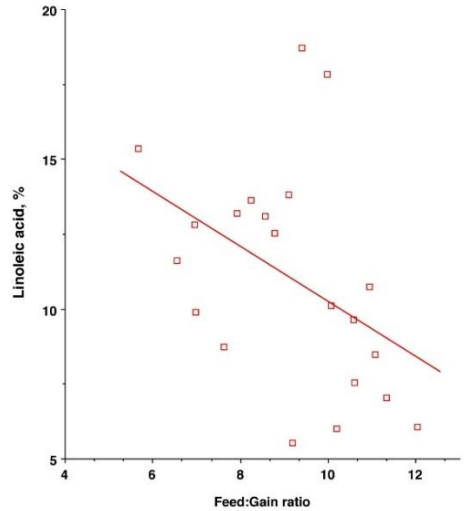

Figure 15. Plasma linoleic acid proportions as a function of feed:gain ratio (r = -0.431; P = 0.051).

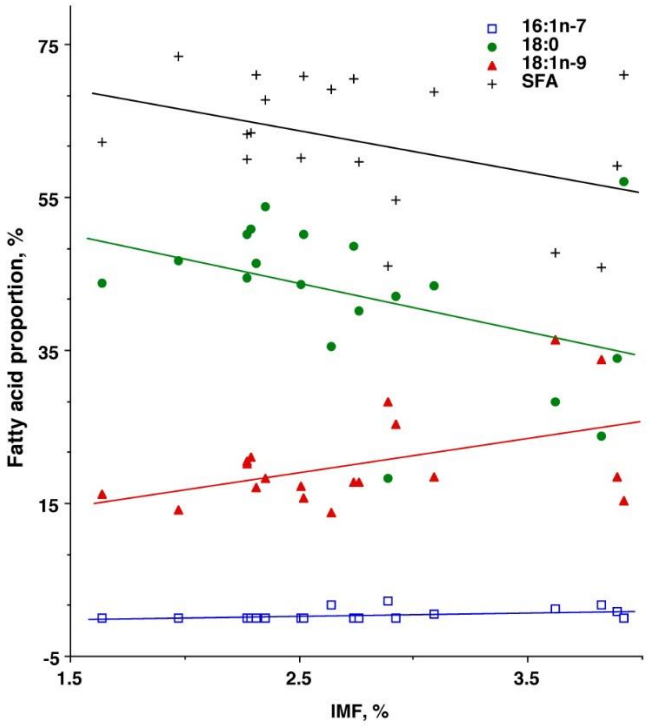

Figure 16. Plasma proportions of 16:1n-7(r = 0.469; *P* = 0.043), 18:0 (r = -0.407; *P* = 0.08), 18:1n-9 (r = 0.478; *P* = 0.04), and SFA (r = -0.406; *P* = 0.09) as functions of final %IMF.

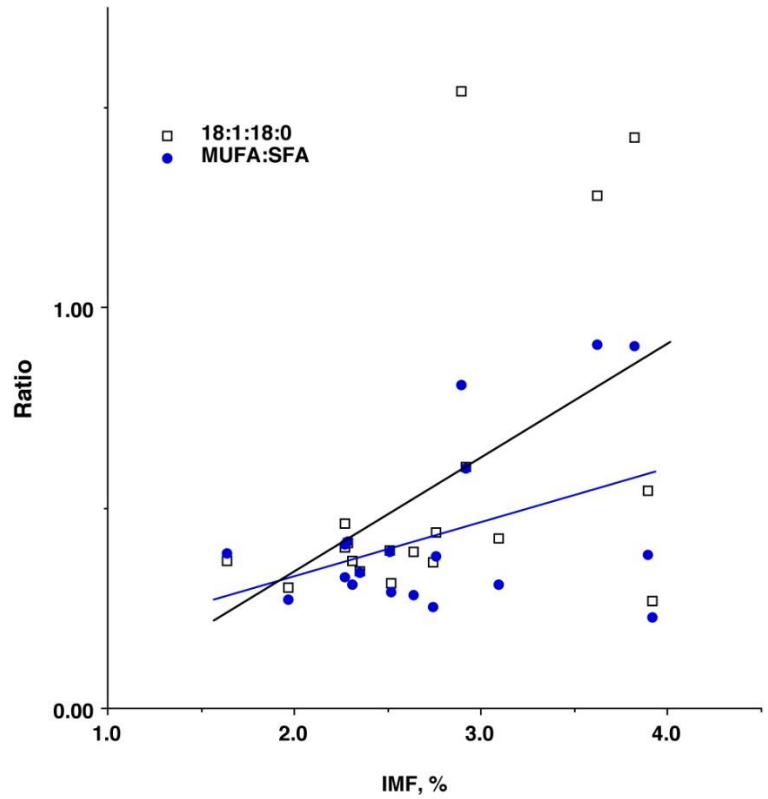

Figure 17. Oleic:stearic acid (r = 0.473; *P* = 0.04) and MUFA:SFA (r = 0.419; *P* = 0.07) ratios as functions of final %IMF.

4.3.3 Hormones

There were no significant correlations observed between plasma concentrations of insulin, adiponectin, or ghrelin and %IMF. Plasma ghrelin concentrations were negatively correlated with ADI (r = -0.388; *P* = 0.08) (Figure 18) and feed:gain ratio (r = -0.552; *P* = 0.0095) (Figure 19). Plasma ghrelin concentrations also were positively correlated with ADG (r = 0.450; *P* = 0.04) (Figure 18). Plasma ghrelin concentrations were positively correlated with rib fat thickness (r = 0.459; *P* = 0.04) (Figure 20).

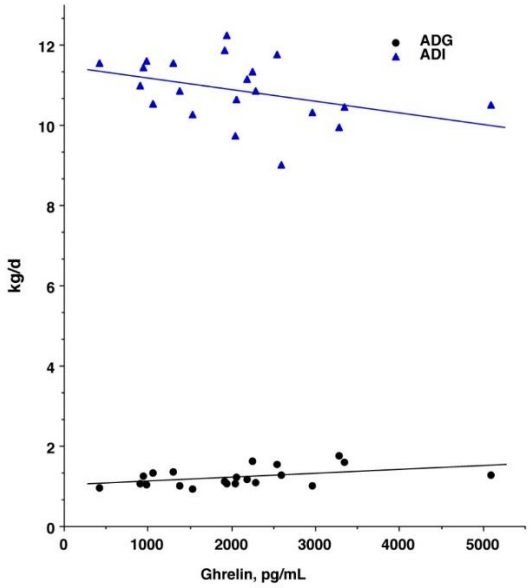

Figure 18. ADG (r = 0.450; *P* = 0.04) and ADI (r = -0.388; *P* = 0.08) as functions of plasma ghrelin concentrations.

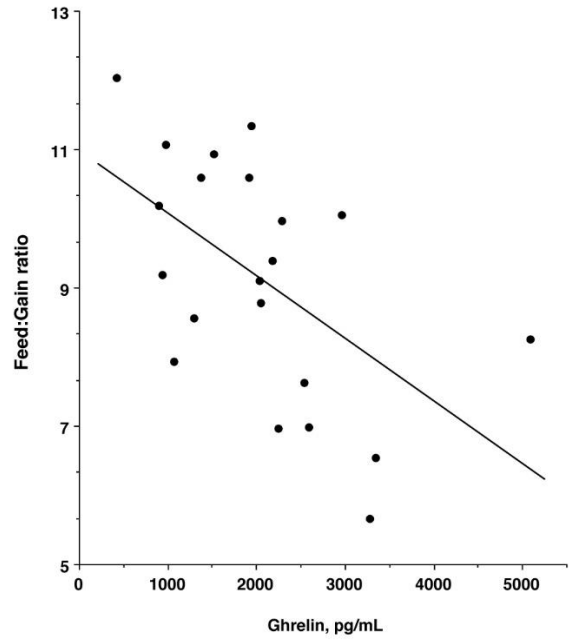

Figure 19. Feed:gain ratio as a function of plasma ghrelin concentrations (r = -0.552; *P* = 0.0095).

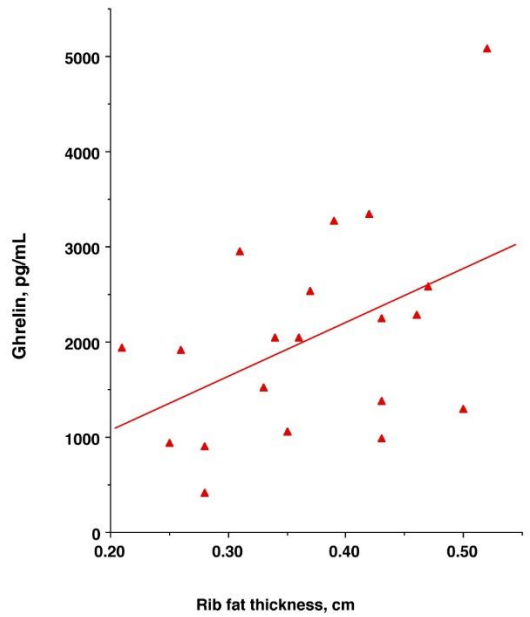

Figure 20. Plasma ghrelin concentrations as a function of rib fat thickness (r = 0.459; *P* = 0.04).

4.3.4 Hormones and metabolites

There were negative correlations between proportions of plasma myristic acid (14:0) (r = -0.464; *P* = 0.03) and palmitoleic acid (r = -0.474; *P* = 0.03) and plasma ghrelin concentrations (Figure 21). There also was a positive correlation between plasma concentrations of linoleic acid and plasma ghrelin concentrations (r = 0.462; *P* = 0.04) (Figure 21). Additionally, plasma proportions of palmitic acid tended to be negatively correlated with plasma ghrelin concentrations (r = -0.406; *P* = 0.07) (Figure 21). Plasma proportions of palmitic acid also tended to be negatively correlated with plasma adiponectin concentrations (r = -0.375; *P* = 0.09) (Figure 22).

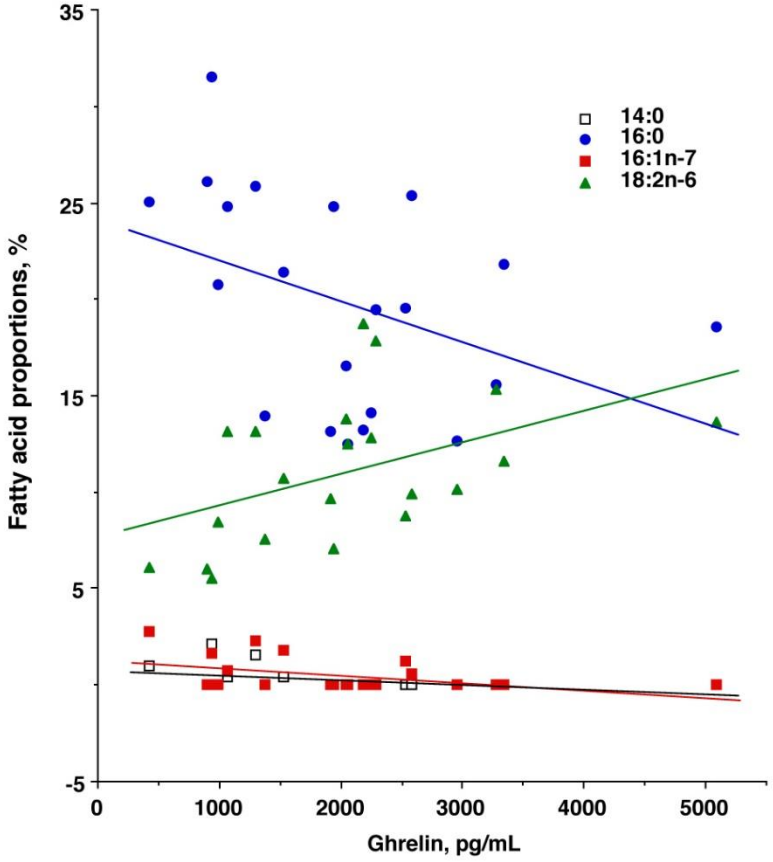

Figure 21. Plasma proportions of 14:0 (r = -0.464; *P* = 0.03), 16:0 (r = -0.406; *P* = 0.07), 16:1n-7 (r = -0.474; *P* = 0.03), and 18:2n-6 (r = 0.462; *P* = 0.04) as functions of plasma ghrelin concentrations.

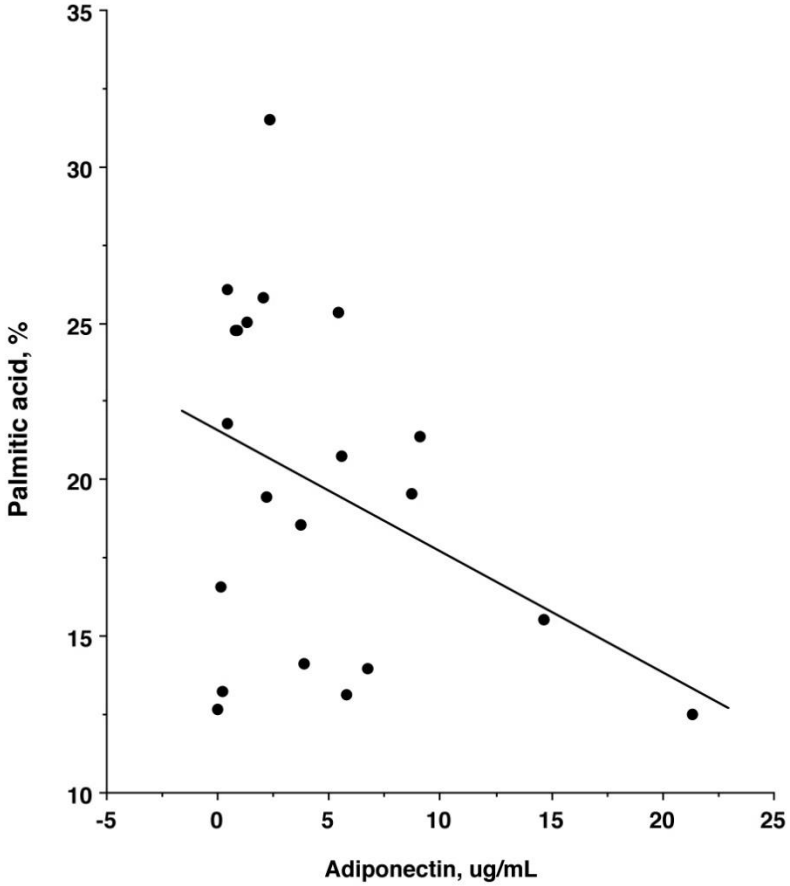

Figure 22. Plasma proportions of palmitic acid as a function of plasma adiponectin concentrations (r = -0.375; *P* = 0.09).

CHAPTER V

DISCUSSION

5.1 Production characteristics

Smith and Crouse (1984) reported greater backfat thickness and kidney, pelvic, and heart fat in cattle fed a corn-based than in cattle fed a hay-based diet that likely accounted for the greater BW of cattle fed the corn-based diet. Jennings et al. (2011) also reported that carcass fat mass was greater for steers fed a high-energy, grain-based, diet for both growing and finishing phases. As a proportion of shrunk BW (BW without final meal prior to slaughter; BW*0.96), cumulative fat accumulation was greater for steers fed the high-energy diet for both phases than for steers fed a low-energy diet during the growing phase (Jennings et al., 2011). This agrees with the results of this study where heifers fed grain had greater rib fat thickness than heifers fed hay. As per the design of the study, ADG was greater for heifers on the grain-based than for heifers on the hay-based diet. Brooks at al. (2011) also reported higher ADG for calf-fed steers (steer calves fed a concentrate diet immediately following feeding) on a corn-based diet than steers fed hay/pasture. Similarly, the feed:gain ratio was less for heifers on the grain-based diet indicating greater feed efficiency. These correlations coincide with the negative correlation between rib fat thickness and feed:gain ratio and the weak, negative correlation between rib fat thickness and ADI. There was a negative correlation between BW and feed:gain ratio, which agrees with the differences seen between both BW and feed:gain ratio with dietary treatment. Along with the differences in ADG and feed:gain ratio, there was a diet x time interaction for BW with heifers on the grain-based diet increasing in BW at a greater rate than heifers on the hay-based diet.

In the present study, diet was used to stimulate marbling deposition to establish a difference in marbling between dietary groups. No difference was observed with %IMF between diet groups, which did not support our hypothesis that grain-fed heifers would have a greater %IMF than hay-fed heifers. This lack of difference for %IMF with diet and the positive correlation between %IMF and BW is consistent with results observed by Nour et al. (1983), who found that carcass weight had a greater positive effect on marbling than diet. Previous studies showed that overall carcass fatness and s.c. fat thickness were associated with marbling (Pearson 1966; Crouse and Smith 1978; Harrison et al. 1978; Tatum et al. 1980; Dolezal et al. 1982; Huffman et al. 1990; May et al., 1992). In contrast, there was no correlation between rib fat thickness and %IMF in the present study. Previous reports of correlations for carcass fatness and s.c. fat thickness were in mature or harvested cattle, whereas the heifers in the present study ended treatment at 15 mo of age. Also, the current study fed 40% corn on an as-fed basis and did not step up the proportion of concentrate throughout the feeding period, as would have been done in a commercial feedlot operation.

Heifers fed the hay-based diet tended to have a decrease in %IMF with time on feed whereas heifers fed the grain-based diet tended to have an increase %IMF over 3 mo of treatment. We previously reported that marbling score increases for steers on corn-based diets at a greater rate than for steers on a hay-based diet (Chung et al., 2007; Lunt et al., 2005). Further, there was a significant, positive correlation between BW and %IMF. The results of the present study coincide with Jennings et al. (2011) who reported that marbling score increased at a greater rate for commercial Angus steers that started on a high energy diet for the growth phase (0 to 111 d) and stayed on a high-energy diet for a finishing phase (112 to 209 d) than cattle that started on a low-energy diet for the growth phase and which were transitioned onto the high energy diet for

the finishing phase. Further, the %IMF expressed as a percent of LD increased at a greater rate for steers on the high-energy diet for both phases than steers started on the low-energy diet (Jennings et al., 2011). Change in %IMF per day also was increased throughout the experiment for steers on high-energy diets for both phases (Jennings et al., 2011), which confirms the observations in the present study of a positive diet x time interaction for %IMF with heifers on the grain-based diet.

5.2 Volatile fatty acids

Microbial activity and diversity is influenced by diet (Bevans et al., 2005; Turnbaugh et al., 2006, Hernandez-Sanabria et al., 2012). Cook and Miller (1965) demonstrated that plasma propionate concentrations in ewes and goats were higher when fed a grain-based diet. When fed alfalfa hay, plasma acetate and propionate averaged at 187.67 and 9 µmol/100 mL, respectively; when fed grain, plasma acetate and propionate averaged at 133 and 24 µmol/100 mL, respectively. Butyrate was undetectable in ewes fed either diet (Cook and Miller, 1965). Orskov et al. (1991) reported that cattle fed high-concentrate diets produce a greater proportion of propionate than when fed forage diets. Further, increased i.m. adipose tissue deposition was reported in steers fed a concentrate diet that generated 39.3% more propionate (Choat et al., 2003). There were no differences in molar proportions of plasma acetate, propionate, or butyrate between hay- and grain-based diet groups in the present study, but plasma molar proportions of propionate were positively correlated with BW. Although BW only tended to be greater in the grain-fed heifers than in hay-fed heifers, ADG was significantly greater in the grain-fed heifers than in hay-fed heifers. Therefore, there is a distant relationship between propionate and diet in the present study. Differences in bacterial population also are reported among individuals (Brulc

et al., 2009; Hernandez-Sanabria et al., 2010). Hernandez-Sanabria et al. (2012) reported that rumen propionate and butyrate were not different between high- and low-energy diets in the low-residual feed intake (L-RFI) (the difference between the animal's expected intake and actual intake) group, but the difference in rumen propionate proportion approached significance in the high-RFI (H-RFI) group ($P = 0.10$) than in the L-RFI group ($P = 0.71$). Rumen acetate differed more in the L-RFI group than in H-RFI the group (Hernandez-Sanabria et al., 2012).

In the present study, plasma molar proportions of butyrate was weakly, negatively correlated with ADI. Hernandez-Sanabria et al. (2012) reported that in L-RFI steers, rumen propionate and total VFA were negatively correlated with RFI. Also, Hernandez-Sanabria et al. (2012) reported different phylotypes of bacteria were associated with L-RFI and H-RFI steers suggesting different populations of microflora within the rumen influencing individual feed efficiency. Further, there were different phylotypes reported for high and low DMI steers (Hernandez-Sanabria et al., 2012). This is likely one factor influencing the weak, negative correlation between plasma butyrate proportions and ADI.

Quigley et al. (1991) reported total plasma VFA concentrations of 0.1 to 0.95 mmol/L whereas rumen VFA concentrations range from 100 to 140 mmol/L (Bauman et al., 1971). There is little available research on plasma VFA concentrations that demonstrate dietary effect, but rumen VFA concentrations are well-known to be responsive to diet as was reported by Bauman et al. (1971) in Holstein cows. Therefore, although there were no correlations between acetate, propionate, or butyrate and %IMF or change in %IMF, rumen VFA concentrations may be correlated and reflective of marbling status.

There was a weak, positive correlation between molar proportions of plasma acetate and plasma insulin concentrations. This could be an indicator of insulin activity stimulating

lipogenesis, but none of the VFAs were correlated with rib fat thickness in the present study. Previous studies investigated changes in plasma VFA concentration in response to diet composition (Cook and Miller, 1965) and the difference in substrate preference for fatty acid biosynthesis between s.c. and i.m. adipose tissue (Chung et al., 2007; Cook and Miller, 1965; Gilbert et al., 2003; Hausman et al., 2009; Ladeira et al., 2016). Subcutaneous adipose tissue incorporates acetate into fatty acids at a much greater rate than i.m. adipose tissue. Rhoades et al. (2007) and Smith and Crouse (1984) reported a greater rate of incorporation of acetate into fatty acids in s.c. than in i.m. adipose tissue; the relative rate of incorporation was not influenced by diet. Also, the highest incorporation of acetate into fatty acids was observed in the s.c. adipose tissue of steers fed a high energy diet (Smith and Crouse, 1984). Proportionally, acetate provided 70-80% of the acetyl units to fatty acid synthesis in s.c. adipose tissue and 10 to 26% for i.m. adipose tissue (Smith and Crouse, 1984). Insulin inhibits lipolysis and may stimulate fatty acid synthesis from acetate, a preferred substrate for lipogenesis in ruminants, uptake in peripheral tissues although it has not been possible to demonstrate a stimulation of lipogenesis by insulin in bovine s.c. adipose tissue (Alves-Nores et al., 2017; Jarrett et al., 1974; reviewed by Smith, 2017; Vernon et al., 1985). However, plasma insulin concentrations were positively correlated with carcass adiposity (Trenkle and Topel, 1978). Rather than stimulating lipogenesis, Vernon (1978, 1979) observed that insulin prevented decreases in fatty acid synthesis from acetate in tissue culture with ovine adipose tissue. Vernon (1978) and Miller et al. (1989) reported similar, but less effective effects in bovine s.c. adipose tissue. This effect of insulin on lipogenesis rates was less apparent in i.m. adipose tissue than s.c. adipose tissue (Miller et al., 1989). To the author's knowledge, there are no reports on the direct relationship between plasma proportions of

acetate and plasma insulin concentrations or the correlation between plasma VFA proportions and marbling deposition or adiposity.

5.3 Glucose

González-Grajales et al. (2019) observed basal glucose concentrations ranging from 4.39 to 5.25 mmol/L and maximal glucose concentrations ranging from 13.1 to 16.1 mmol/L in Holstein cattle. Alves-Nores et al. (2017) reported that basal serum glucose concentrations ranged from 2.65 to 4.03 mmol/L and peak serum glucose concentrations varied from 19.54 to 28.15 mmol/L in cattle. Vanhatalo et al. (2003) infused casein and glucose into the abomasum, but did not see significant differences in plasma glucose concentration. In contrast, Locher et al. (2015) conditioned dairy cattle on an energy-dense diet for 15 wk and observed an increase in plasma glucose concentrations with time on treatment (3.06 mmol/L at 0 wk and 4.72 mmol/L at 15 wk conditioning). Although this would indicate that increases in plasma glucose concentrations are associated with time and adiposity, it was not stated whether there was a direct connection between glucose concentrations and body condition score or any other direct measure of adiposity. The results of the current study do not coincide with the results seen by Locher et al. (2015) because cattle on the grain-based diet were "conditioned" for 12 wk, but there were no differences in plasma glucose concentrations between grain-fed heifers and hay-fed heifers. Glucose concentrations ranged from 1.68 to 4.03 mmol/L in the present study, which are at the low end of concentrations observed in previous studies (Alves-Nores et al., 2017; González-Grajales et al., 2019). The lack of difference with plasma glucose concentrations also coincides with the lack of difference in plasma insulin concentrations in the present study.

Plasma glucose concentrations are higher in male than in female cattle (Hayhurst et al., 2009). Plasma glucose concentrations in dairy calves ranged from 4.26 to 4.73 mmol/L in females and 5.10 mmol/L in males. In addition to sex effects, breed effects also have been related to plasma glucose concentrations. Glucose concentrations were greater in Holstein steers than in Japanese Black (JB) steers ranging from 4.44 to 5.56 mmol/L for Holstein steers and 3.61 to 4.17 mmol/L for JB steers (Matsuzaki et al., 1997).

The lack of difference in glucose concentrations in the present study could be responsible for the lack of difference in %IMF between diets groups. The cattle in the present study were genetically similar (Angus heifers), so glucose and insulin concentrations would not have been affected by breed type or sex. This is in agreement with previous reports that plasma glucose and insulin concentrations fluctuate within a narrow range in ruminants (González-Grajales, et al., 2019; Kaneko, 1997; Kasai et al., 2014; Sasaki, 2002), but contradicts a previous study that reported increases in plasma glucose concentrations in cattle fed a high-energy diet (Locher et al., 2015).

Plasma trans-vaccenic acid concentrations were positively correlated to plasma glucose concentrations. This perhaps was due to dietary effects on ruminal microflora. Increasing grain in the diet decreases ruminal fatty acid biohydrogenation (Fukuda et al., 2006; Nagaraja and Titgemeyer, 2007; van de Vossenberg and Joblin, 2003; Wallace et al., 2006). As the microbial populations responsible for fatty acid isomerization and biohydrogenation decrease, there would be a resulting decrease in reaction products (stearic and trans-vaccenic acids) (Brooks et al., 2011). In the present study plasma trans-vaccenic acid concentrations were numerically, but not significantly, lower in grain-fed heifers (2.80 vs. 4.23 g/100 g fatty acid for grain- and hay-fed groups, respectively).

5.4 Hormones

5.4.1 Insulin

In the present study, plasma concentrations of insulin did not differ between diet groups, nor were plasma insulin concentrations correlated with marbling although Jennings et al. (2011) reported plasma insulin concentrations were lower in steers fed a low-energy diet than in steers fed a high-energy diet. The results of the present study do not support our hypothesis that insulin concentrations would be higher in grain-fed heifers than in hay-fed heifers and that plasma insulin concentrations would be correlated with marbling. This finding could be attributed in part to the limited range of %IMF of the heifers in this study.

Differences in plasma insulin concentrations have been observed between different breeds of cattle. Satou et al. (1998a) reported JB heifers had higher basal plasma insulin concentrations than Holstein heifers. Shingu et al. (2001) further observed greater plasma insulin concentrations in mature JB cattle than Holstein cattle fed the same diet. Plasma insulin concentrations were 13.94 and 9.85 μU/mL at 12 mo of age and 29.17 and 12.75 μU/mL at 18 mo of age for JB and Holstein heifers, respectively. Shingu et al. (2001) also observed that during the suckling period and at 18 mo of age, JB heifers had significantly higher maximum insulin concentrations after glucose injection than Holstein heifers indicating a greater insulin response in JB heifers. Satou et al (1998a,b) observed similar results in JB heifers. Similar results with steers also have been observed (Matsuzaki et al., 1997). Shingu et al. (2001) speculated that this difference in insulin level and secretion in response to glucose treatment could be due to beef vs. dairy cattle (muscle accumulation and marbling in beef cattle versus milk production in dairy cattle) that favor anabolic processes (JB) or catabolic processes (Holstein). Matsuzaki et al. (1997) observed that plasma insulin concentrations increased with increasing BW from 3.3 to

124.3 µU/mL, from 10.8 to 45.2 µU/mL, and from 2.6 to 73.9 µU/mL for the JB, Japanese Brown, and Holstein cattle, respectively. At 400 kg BW, JB steers had higher plasma insulin concentrations than either Japanese Brown or Holstein cattle, but only were higher than Japanese Brown at 600 kg BW (Matsuzaki et al., 1997). Similar results were observed by Grigsby and Trenkle (1986), who reported higher plasma insulin concentrations in smaller-framed Angus cattle than in larger-framed Limousin and Simmental cattle. Matsuzaki et al. (1997) found that the different plasma insulin concentrations between JB and Japanese Brown were greater for JB steers, but carcass fatness was similar to Japanese Brown steers. JB had greater plasma insulin concentrations as well as carcass fat than Holstein steers even with different degrees of carcass fatness. Carcass fatness differed between Holstein and Japanese Brown cattle, but plasma insulin concentrations were similar. This could be due to the closer genetic similarity between JB and Japanese Brown cattle. Matsuzaki et al. (1997) determined that the differences in plasma insulin concentrations are more likely due to genetic differences rather than differences in feed intake.

The above studies investigated plasma insulin concentrations in breeds of cattle that varied genetically in both BW, frame-size, and marbling potential. All these factors, either combined or independently, have shown to be associated with differences in plasma insulin concentrations. The present study utilized genetically similar cattle (Angus) and there were no differences in plasma insulin concentrations. What remains to be determined is whether greater differences in marbling status than was seen in this study is correlated with plasma insulin concentrations.

5.4.2 Ghrelin

Ghrelin has two, major circulating isomers, acyl-ghrelin and des-acyl ghrelin. Des-acyl ghrelin circulates at much higher concentrations than acyl-ghrelin (active ghrelin) (Hosoda et al., 2000; ThidarMyint et al., 2006). The functions of des-acyl ghrelin in cattle are largely unknown. Foote et al. (2014) reported that plasma active ghrelin concentrations differed among breeds of cattle, but plasma total ghrelin concentrations did not. Foote et al. (2014), therefore, speculated that the activation of ghrelin (acylation and deacylation) could be more important for regulating ghrelin activity and signaling than the removal of the ghrelin peptide from circulation. Ghrelin o-actlytransferase (GOAT), the enzyme that catalyzes the addition of the octanoyl group onto ghrelin (Yang et al., 2008), increases expression in the stomach, hypothalamus, and pituitary with fasting in mice (Gahete et al., 2010). Des-acyl ghrelin has been suggested to induce a negative energy balance (Tschöp et al. 2000, Nakazato et al. 2001) and possesses peripheral effects (Gauna et al., 2005; Thompson et al., 2004), indicating it may be a natural antagonist to active ghrelin. Gauna et al. (2004) reported that injection of acyl-ghrelin induced a rapid rise in plasma insulin and glucose concentrations, but caused decreased insulin sensitivity in human models. The same authors also reported that in an in vitro study, acyl-ghrelin induced while des-acyl ghrelin inhibited glucose output from primary porcine hepatocytes (Gauna et al., 2005). Although the function of des-acyl ghrelin is still unclear in ruminants and only total ghrelin was quantified in the present study, this antagonistic relationship between the ghrelin isomers and glucose and insulin metabolism could be why differences were not observed for plasma glucose and insulin concentrations, but total ghrelin was elevated in grain-fed heifers.

Plasma ghrelin concentrations were negatively correlated with feed:gain ratio, which coincides with the difference seen in feed:gain ratio and dietary treatment with heifers fed the

grain-based diet being lower than those fed the hay-based diet. Also, plasma ghrelin concentrations tended to be negatively correlated with ADI. These results partially supported our hypothesis that ghrelin concentrations would be greater in heifers fed the grain-based diet than heifers fed the hay-based diet, but did not support a correlation between ghrelin and marbling. Foote et al. (2014) reported that plasma active ghrelin concentrations were not correlated with dry matter intake (DMI) (intake on a dry matter-basis), but the active:total ghrelin ratio was positively correlated with DMI, and plasma total ghrelin concentrations were negatively correlated with DMI. The negative relationship between total ghrelin and DMI reported by Foote et al. (2014) supports the results of the present study. Foote et al. (2014) argues that the active:total ghrelin ratio is a better indicator and/or predictor of DMI than plasma active or total ghrelin concentrations individually because the active:total ghrelin ratio accounted for more variation explained by the model than either the plasma active or total ghrelin concentrations did. Foote et al. (2014) also reported that ADG was negatively correlated to plasma total ghrelin concentrations, but in the present study, plasma ghrelin concentrations were positively correlated with ADG. This could be due to the diet effect in the present study, as there was no dietary treatment in the study by Foote et al. (2014). The results of the present and previous studies suggest that ghrelin is a potential indicator of production performance, including feed efficiency and ADG. In future studies, measurement of the ratio of active:total ghrelin would be beneficial for exploring the relationship between ghrelin and dietary treatment, marbling, and growth.

A polymorphism near the gene LYPLA1 was found to be significantly associated with DMI in cattle (Lindholm-Perry et al., 2012). LYPLA1 encodes for lysophospholipase I, which cleaves the Octanoyl group from serine 3 on ghrelin (Shanado et al., 2004). Octanoyl group cleavage is thought to prevent ghrelin from activating the growth hormone secretagogue receptor

(GHS-R), which is important for stimulating appetite (Al Massadi et al., 2011; Kojima and Kangawa, 2002). Foote et al. (2014) speculated that the LYPLA1 polymorphism could affect LYPLA1 expression and alter acyl-ghrelin concentrations, but ghrelin likely is not essential for regulation of feed intake. Deletion of ghrelin receptors or related receptors in mice did not affect feed intake (McFarlane et al., 2014; Sun et al., 2003, 2004, 2006; Wortley et al., 2004; Zhao et al., 2010). Wertz-Lutz et al. (2006) reported that ghrelin infusion of satiated steers did not increase DMI, therefore, ghrelin likely is not able to overcome satiety factors and/or signals. Therefore, the LYPLA1 polymorphism probably is not a driving force influencing DMI or ghrelin signaling to stimulate feed intake.

Plasma ghrelin concentrations in the present study, which used an ELISA assay, were 4 to 10 times that reported in previous studies that used radioimmunoassay (RIA) to quantify plasma ghrelin concentrations (1,630 pg/mL and 2,877 pg/mL vs 123 to 690 pg/mL) (Wertz-Lutz et al., 2006). ThidarMyint et al. (2006) reported Holstein heifers at 6 mo age had basal total plasma ghrelin concentrations averaging at 2,800 pg/mL, which was approximately 16 times higher than the basal active plasma ghrelin concentrations they measured. The total plasma ghrelin concentrations observed by ThidarMyint et al. (2006) are closer to the concentrations observed in the present study. This indicates that previous reports are likely quantifying active plasma ghrelin concentrations, although this is not specified. In the present study, blood was taken before feeding, so the elevated concentrations were consistent with previous studies that reported that plasma ghrelin concentrations are elevated prior to feeding (Miura et al., 2004; Wertz-Lutz et al., 2006). Miura et al. (2004) observed greater pre-feeding ghrelin concentrations for mature Holstein cows fed a solely forage diet at 1.3% BW. Wertz-Lutz et al. (2006) observed greater plasma ghrelin concentrations prior to feeding in Simmental x Angus crossbred steers fed

a diet composed of 80% grain at 2.3% BW. In monogastrics, ghrelin concentrations were suppressed post-feeding proportionally to ingested caloric load, and this was dependent on the macronutrient composition of the meal (Callahan et al., 2004).

Jennings et al. (2011) reported a significant diet effect on plasma ghrelin concentrations. Plasma ghrelin concentrations were similar during the growing phase between dietary treatment, which was started at 8 mo of age, but were greater during the finishing phase in steers that were fed a high-energy diet for both growing and finishing phases than steers fed a low-energy diet for the growing phase (Jennings et al., 2011). This partially agrees with the results seen in the present study, where heifers fed the grain-based diet had greater plasma ghrelin concentrations than heifers fed the hay-based diet. The cattle in the present study began treatment at 12 mo of age whereas the cattle in Jennings et al. (2011) were started on treatment at 8 mo of age. Miura et al. (2004) demonstrated that 3-mo-old calves had lower plasma ghrelin concentrations and less fluctuation relative to feeding time than mature cows, demonstrating an age effect. Therefore, age may have played a role in the differences in results seen between the present study and Jennings et al. (2011). In contrast, Jennings et al. (2011) reported that the abundance of GHS-R, a receptor for ghrelin signaling, in s.c. adipose tissue, liver, and muscle tissue did not differ with dietary treatment although plasma ghrelin concentrations differed. GHS-R differed with time and body composition; the greatest abundance of GHS-R in s.c. adipose tissue was at the final slaughter, and liver had the greatest abundance of GHS-R at the 1.0 cm rib fat compositional endpoint (Jennings et al., 2011). This indicates that the GHS-R is influenced by body composition (perhaps adiposity) rather than diet, which affects plasma ghrelin concentrations.

Heifers tended to have greater plasma active ghrelin concentrations but lower DMI than steers (Foote et al., 2014). Also, heifers had greater total plasma ghrelin concentrations, but

similar active:total ghrelin ratios than steers (Foote et al., 2014). There also were breed effects observed by Foote et al. (2014); Gelbveih-sired cattle had the greatest plasma active ghrelin concentrations and Charolais-sired cattle had the least, and Angus-sired cattle were intermediate. The active:total ghrelin ratios mirrored the plasma active ghrelin concentration results because there were no differences with plasma total ghrelin concentrations among sire breeds (Foote et al., 2014). These results suggest that plasma ghrelin concentrations are influenced by both genetics and diet.

In the present study, plasma ghrelin concentrations were greater in heifers fed the grain-based diet than heifers maintained on hay, but there was no correlation observed between plasma concentrations of ghrelin and %IMF. Choi et al. (2003) reported that ghrelin stimulates adipogenesis in 3T3 cells through the stimulation of the expression of peroxisome proliferator-activated receptor γ2 (PPARγ2), a transcription factor involved in adipocyte differentiation. Jennings et al. (2011) speculated that increased ghrelin in steers fed the high-energy diet for both phases may be signaling increased differentiation of preadipocytes to lipid-filled adipocytes in cattle consuming energy-dense diets. Infusion with ghrelin in rat adipose tissue increased glucose transporter and lipogenic precursor expression (Davies et al., 2009). There also was an additive effect of ghrelin and insulin on glucose uptake in rodent adipocytes (Patel et al., 2006). Therefore, considering glucose is the preferred precursor for fatty acid synthesis in i.m. adipose tissue, Jennings et al. (2011) speculated ghrelin could promote i.m. adipose tissue deposition. This is supported by the greater rate of increase in %IMF in the present study and Jennings et al. (2011) for heifers and steers fed high-energy diets.

Jennings et al. (2011) alternatively speculated that increased ghrelin in cattle fed high-energy diets may be the result of lower receptor abundance in target tissues. However, Jennings

et al. (2011) observed no differences in GHS-R in s.c. adipose tissue, liver, or muscle tissue between dietary treatments, further supporting that the abundance of GHS-R is influenced by body composition rather than diet. There was a decrease in the abundance of receptors observed in the hypothalamus in obese sheep by Kurose et al. (2005). Zigman et al. (2016) demonstrated a decreased effect of ghrelin on food intake in obese animal models, suggesting ghrelin resistance with obesity (Cui et al., 2017).

GHS-R abundance was not measured in the present study, but the present study confirmed that dietary treatment does affect plasma ghrelin concentrations. Also, body composition (especially adiposity) is a possible factor affecting plasma ghrelin concentrations as seen by the greater %IMF and rib fat thickness and plasma ghrelin concentrations with high-energy diets in the present study and in the studies done by Jennings et al. (2011) and Kurose et al. (2005). In the present study, rib fat thickness was positively correlated with plasma ghrelin concentrations, which also agrees with the greater rib fat thickness measured in grain-fed heifers than in hay-fed heifers. Future studies investigating the influence of ghrelin on lipid metabolism and adiposity are warranted. The active:total ghrelin ratio also needs to be considered when looking at the relationship between ghrelin and feed intake, adiposity, and marbling in the future.

5.4.3 Adiponectin

Plasma adiponectin concentrations did not differ between diets, nor was there a significant correlation between plasma adiponectin concentrations and %IMF. These results did not support our hypotheses that plasma adiponectin concentrations would be lower in heifers fed the grain-based diet than in heifers fed the hay-based diet and that there would be a correlation of adiponectin with %IMF. Adiponectin concentrations are reportedly associated with sex in cattle

and obesity in humans. Serum adiponectin concentrations were less for bulls than heifers of the same age (25.1 vs 31.0 µg/mL, respectively) (Heinz et al., 2015). In humans, adiponectin concentrations were greater in non-obese subjects (8.9 mg/mL) than in obese subjects (3.7 mg/mL) (Arita et al., 1999). There was a strong, negative correlation observed between plasma adiponectin concentrations and body mass indices in humans (Arita et al., 1999). Additionally, circulating adiponectin concentrations are negatively correlated with plasma glucose, insulin, and TAG, in healthy human subjects (Hirose et al., 2010). There were heifers with undetectable concentrations of adiponectin in both diet groups in the current study, but there was no correlation between plasma adiponectin concentrations and rib fat thickness. This contradicts previous reports on the relationship between adiponectin and white adipose tissue mass.

A lack of significant difference and/or correlation between plasma adiponectin concentrations and %IMF and/or rib fat thickness could be due to the cattle being the same breed and sex with genetically similar marbling potential. Also, the range for %IMF and rib fat thickness of the heifers in the present study was narrow. Although the goal of this study was to establish a preliminary model that could provide information on marbling status and/or potential regardless of breed, more differences in %IMF and back fat thickness due to diet may be required. Further, it would be interesting to examine differences between breeds of cattle with known differences in genetic marbling potential. Most studies that have established the relationships between obesity, white adipose tissue mass, and adiponectin concentrations have been done in humans and rodents, and there are limited data examining adiposity and adiponectin concentrations in ruminants. The differences in metabolic function between monogastrics and ruminants may be a significant factor as to why significant differences were not seen in this study. Adiponectin concentrations in white adipose tissue were significantly

decreased with increased BW, body condition, and fat cell size in cattle (Locher et al., 2015). Locher et al. (2015) also reported that blood adiponectin concentrations decreased with weight gain, but the significance was less than with white adipose tissue adiponectin concentrations suggesting that fluctuations in adiponectin concentrations are more apparent in white adipose tissue than in plasma. Therefore, there may be a significant difference seen in adiponectin concentrations in white adipose tissue with diet, rib fat thickness, and/or %IMF in contrast to what was observed in this study, which used plasma adiponectin concentrations.

5.5 Fatty acid composition

Although previous studies reported that diet and time on feed influence fatty acid composition (Sturdivant et al., 1992; May et al., 1993; Huerta-Leidenz et al., 1996; Chung et al., 2006), there were no differences seen among fatty acid species due to diet except for plasma α-linolenic acid proportions, which were greater in heifers fed the hay-based diet than heifers fed the grain-based diet. Brooks et al. (2011) compared calf-fed and yearling-fed angus steers, and reported greater proportions of plasma linoleic acid in yearling-fed (pasture-fed) steers than in calf-fed steers. Plasma linoleic acid proportions tended to be negatively correlated to feed:gain ratio and tended to be negatively correlated with final BW in the present study, which agrees with the results seen by Brooks et al. (2011), when considering the higher feed:gain ratio and final BW observed in heifers fed the hay-based diet than in heifers fed the grain-based diet.

Increasing grain in the diet changes the microflora population in the rumen, leading to decreases in ruminal fatty acid biohydrogenation (Fukuda et al., 2006; Nagaraja and Titgemeyer, 2007; van de Vossenberg and Joblin, 2003; Wallace et al., 2006). Therefore, as the microbial populations responsible for fatty acid isomerization and biohydrogenation decrease, the

substrates for their reactions (unsaturated fatty acids) would accumulate, resulting in a decrease in the reaction products (stearic and trans-vaccenic acids) (Brooks et al., 2011). Brooks et al. (2011) reported increased proportions of linoleic acid and 18:2*cis*-9,*trans*-11 conjugated linoleic acid (CLA) in duodenal digesta of calf-fed steers indicating a decrease in SFA exiting the rumen, therefore, reduced substrate for the SCD reaction. In the present study, plasma trans-vaccenic acid proportions tended to be negatively correlated with ADG, suggesting less ruminal bacterial isomerization in grain-fed heifers (which had higher ADG) than hay-fed heifers. Brooks et al. (2011) fed diets composed largely of Bermuda hay whereas, the bulk of the diets in the present study consisted of alfalfa hay and distiller's grains. According to Dal Bosco et al. (2014), alfalfa contains 0.375 g α-linolenic acid per 100 g of fatty acids compared to the limited amounts of α-linolenic acid in corn (Archibeque et al., 2005; Gilbert et al., 2003). In the current study, there were only low concentrations of plasma α-linolenic acid in heifers fed hay and α-linolenic acid was undetectable in the plasma of heifers fed grain.

There were positive correlations observed between plasma proportions of palmitoleic and oleic acid and %IMF. Additionally, plasma proportions of stearic acid and SFA tended to be negatively correlated with %IMF. The 18:1:18:0 ratio was significantly, positively correlated to %IMF, and the MUFA:SFA ratio tended to be positively correlated to %IMF. Delta 9-desaturase is responsible for converting SFA to MUFA (St John et al., 1991; Jiang et al., 2008; Duckett et al., 2009) and studies have indicated that corn-based diets increase mRNA levels of SCD, the gene responsible for delta 9-desaturase, gene expression (Archibeque et al., 2005; Chung et al., 2007; Duckett et al., 2009; Jiang et al., 2008; Martin et al., 1999). Although Archibeque et al. (2005) argues against using fatty acid ratios to predict SCD activity due to the presence of oleic acid from the diet, Chung et al. (2007) argued that palmitoleic acid is a strong

indicator of SCD activity because it can only be produced endogenously. In the present study, palmitoleic acid was positively correlated to %IMF and stearic acid had a weak, negative correlation with %IMF. This suggests increased SCD activity in the adipose tissue and/or liver of heifers with higher marbling. Brooks et al. (2011) reported a higher SCD gene expression and SCD index in i.m. adipose tissue of calf-fed steers than in yearling-fed steers. Further, Chung et al. (2007) reported a greater increase in SCD activity in corn-fed steers than in forage-fed steers and differences with marbling score were reported being higher in steers fed corn than steers fed forage (Lunt et al., 2005). This agrees with the correlations observed in the present study between 18:1:18:0 and MUFA:SFA ratios and %IMF. Although there were no differences found with MUFA nor %IMF between diet groups, the correlations in the present study may suggest that the mechanisms responsible for stimulating SCD gene expression are closely related to i.m. adipose tissue deposition regardless of diet. These observations, along with the greater marbling in steers fed corn than steers fed forage (Brooks et al., 2011; Chung et al., 2007; Lunt et al., 2005), suggest that SCD gene expression in i.m. adipose tissue is influenced by diet and that SCD gene expression could differ between cattle with differences in genetic marbling potential regardless of diet. Cattle with higher genetic marbling potential may have higher base level SCD gene expression than cattle with lower genetic marbling potential. Any increase in i.m. lipid (or marbling scores) generally is positively correlated with an accumulation of MUFA when feeding corn to finishing cattle (Chung et al., 2006; reviewed in Smith et al., 2006). Further research is warranted to establish relationships between SCD gene expression in i.m. adipose tissue with marbling in cattle.

Previous studies reported that JB accumulate more MUFA and fewer SFA in s.c. and i.m. adipose tissue than Angus steers (Sturdivant et al., 1992; May et al., 1993; Chung et al., 2006). A

greater MUFA:SFA ratio also was demonstrated in Wagyu compared to Angus steer s.c. adipose tissue on the same, high-roughage diet and at the same maturity (May et al., 1993). This effect between breeds with known genetic differences agrees with the results of the present study that demonstrated a positive correlation with 18:1:18:0, MUFA:SFA and %IMF. Further, there were negative correlations for stearic acid and SFA with %IMF. This suggests that the correlations and differences observed in the current study and previous studies could be due differences in marbling activity rather than breed differences alone. In contrast, Cameron et al. (1994) reported no differences in SCD enzyme activity or SCD gene expression in s.c. adipose tissues of Wagyu and Angus steers fed the same diet. Chung et al. (2007) also found that there was no difference in SCD enzyme activity in s.c. adipose tissue between Wagyu and Angus steers. Considering the differences in substrate preference for s.c. and i.m. adipose tissue, it is possible that SCD enzyme expression and activity only differs in i.m. adipose tissue, and not s.c. adipose tissue.

5.5.1 Fatty acid composition and hormones

There were further results not considered in the original hypotheses of this study. There was a positive correlation between plasma proportions of linoleic acid and plasma ghrelin concentrations. This correlation with linoleic acid is consistent considering the higher plasma ghrelin concentrations associated with the grain diet. Corn contains an average fatty acid proportion of 52.7% linoleic acid (Carrillo et al., 2017) whereas alfalfa contains approximately 6.4% linoleic acid as a proportion of total fatty acids (calculated from Dal Bosco et al., 2014). Linoleic acid also tended to be positively correlated with BW. This coincides with the greater plasma ghrelin concentrations in the grain-fed heifers than in the hay-fed heifers.

Plasma palmitic acid proportions tended to be negatively correlated with both plasma ghrelin and adiponectin concentrations and plasma palmitoleic acid proportions were negatively correlated with plasma ghrelin concentrations. Alfalfa does have approximately 2 times the proportion of palmitic acid (25%) in its fatty acid composition than corn does (12.6%) (Carrillo et al., 2017; Toral et al., 2016), which could explain the negative correlation between plasma ghrelin concentrations and plasma palmitic acid proportions. These correlations possibly are due to increased SCD activity with grain-based diets (Archibeque et al., 2005; Chung et al., 2007; Duckett et al., 2009; Jiang et al., 2008; Martin et al., 1999). Either SCD expression is upregulated in cattle with higher ghrelin concentrations or the fatty acid composition of the diet affects plasma ghrelin and adiponectin concentrations. Chung et al. (2007) argued that palmitoleic acid is a strong indicator of SCD gene activity because it can only be produced endogenously. Long-chain fatty acids suppressed ghrelin release from monogastric gastric mucosal cells in vitro (Lu et al., 2012; Sakata et al., 2012), but, no research has been reported that relates plasma ghrelin or plasma adiponectin concentrations to plasma fatty acid concentrations. Additionally, there was an additive effect of ghrelin and insulin on glucose use for fatty acid synthesis in rodent adipocytes (Davies et al., 2009; Patel et al., 2006), which needs to be investigated in ruminants.

CHAPTER VI

SUMMARY

The heifers in this study did not differ in %IMF, but %IMF decreased in hay-fed heifers and increased in grain-fed heifers over time. There may be a minimal level of difference in %IMF required to further develop a metabolic profile that could evaluate marbling status and/or future marbling potential.

Plasma ghrelin concentrations have been confirmed to be greater in grain-fed cattle than in hay-fed cattle and have demonstrated correlations with production characteristics (ADI and ADG). Ghrelin also has demonstrated a positive correlation with adiposity (rib fat thickness). Ghrelin also was positively correlated with %IMF, although not significantly. Subcutaneous and i.m. adipose tissue depots are metabolically distinct, thus the difference in significance with correlations between s.c. and i.m. adipose tissue depots and plasma ghrelin concentrations. With this, ghrelin shows promise in predicting carcass adiposity and marbling. Fatty acid composition also shows promise in predicting marbling due to its correlation with %IMF in the present study.

Future investigation into active ghrelin and the active:total ghrelin ratio and their potential influence on fatty acid composition and vice versa is needed. Novel data correlating ghrelin to fatty acid composition suggests it's involvement in lipid metabolism, especially concerning preadipocyte differentiation and SCD expression. Active ghrelin and the active:total ghrelin ratio need further investigation related to their effect on lipid metabolism. Further research also is needed to explore whether ghrelin is a true indicator of production performance. It is not clear whether ghrelin affects fatty acid composition or fatty acid composition affects ghrelin activity, so the effect of dietary fatty acids on plasma ghrelin concentrations need to be further explored. The effect of diet and adiposity on the abundance of GHS-R in both central

nervous tissue and peripheral tissue needs further examination to clarify whether the driving force of ghrelin activity is the abundance of ghrelin (total or active) or GHS-R or a combination of both.

REFERENCES

Abe, H., Y. Kawakita, K. Hodate, and S. Masayuki. 2001. Postnatal development of glucose transporter proteins in bovine skeletal muscle and adipose tissue. J. Vet. Med. Sci. 63: 1071-1075. doi: doi:10.1292/jvms.63.1071

Agenas, S., E. Burstedt, and K. Holtenius. 2003. Effects of feeding intensity during the dry period. 1. feed intake, body weight, and milk production. J. Dairy Sci. 86(3): 870-882.

Ahima, R. S., and M. A. Lazar. 2008. Adipokines and the peripheral and neural control of energy balance. Mol. Endocrinol. 22: 1023-1031.

Al Massadi, O., P. V. Lear, T. D. Muller, M. Lopez, C. Dieguez, M. H. Tschöp, and R. Nogueiras. 2014. Review of novel aspects of the regulation of ghrelin secretion. Curr. Drug Metabol. 15: 398-413.

Al Massadi, O., M. H. Tschöp, and J. Tong. 2011. Ghrelin acylation and metabolic control. Peptides. 32: 2301-2308.

Allen, M. S., B. J. Bradford, and M. Oba. 2009. Board-invited review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. J. Anim. Sci. 87: 3317-3334.

Alves-Nores, V., C. Castillo, J. Hernandez, and A. Abuelo. 2017. Comparison of surrogate indices for insulin sensitivity with parameters of the intravenous glucose tolerance test in early lactation dairy cattle. Domest. Anim. Endocrinol. 61: 48-53. doi: http://dx.doi.org/10.1016/j.domaniend.2017.06.003

Andreelli, F., M. Foretz, C. Knauf, P. D. Cani, C. Perrin, M. A. Iglesias, B. Pillot, A. Bado, F. Tronche, G. Mithieux, S. Vaulont, R. Burcelin, and B. Viollet. 2006. Liver adenosine monophosphate-activated kinase-alpha2 catalytic subunit is a key target for the control of hepatic glucose production by adiponectin and leptin but not insulin. Endocrinology. 147: 2432-2441.

Archibeque, S. L., D. K. Lunt, C. D. Gilbert, R. K. Tume, and S. B. Smith. 2005. Fatty acid indices of stearoyl-CoA desaturase do not reflect actual stearoyl-CoA desaturase enzyme activities in adipose tissues of beef steers finished with corn-, flaxseed-, or sorghum-based diets. J. Anim. Sci. 83: 1153-1166.

Arita, Y., S. Kihara, N. Ouchi, M. Takahashi, K. Maeda, J. Miyagawa, K. Hotta, I. Shimomura, T. Nakamura, K. Miyaoka, H. Kuriyama, M. Nishida, S. Yamashita, K. Okubo, K. Matsubara, M. Muraguchi, Y. Ohmoto, T. Funahashi, and Y. Matsuzawa. 1999.

Paradoxical decrease of an adipose-Specific protein, adiponectin, in obesity. Biochem. Biophys. Res. Commun. 257: 79-83.

Aschenbach, J. R., N. B. Kristensen, S. S. Donkin, H. M. Hammon, and G. B. Penner. 2010. Gluconeogenesis in dairy cows: The secret of making sweet milk from sour dough. IUBMB Life. 62(12): 869-877. doi: 10.1002/iub.400

Baik, M., J. Y. Jeong, T. T. T. Vu, M. Y. Piao, and H. J. Kang. 2014. Effects of castration on the adiposity and expression of lipid metabolism genes in various fat depots of Korean cattle. Livestock Science. 168: 168-176. doi: https://doi.org/10.1016/j.livsci.2014.08.013

Baik, M., H. J. Kang, S. J. Park, S. W. Na, M. Piao, S. Y. Kim, D. M. Fassah, and Y. S. Moon. 2017. TRIENNIAL GROWTH AND DEVELOPMENT SYMPOSIUM: Molecular mechanisms related to bovine intramuscular fat deposition in the longissimus muscle. J. Anim. Sci. 95(5): 2284-2303. doi: https://doi.org/10.2527/jas.2016.1160

Baldwin, R. L., J. R. Reichl, S. Louis, N. E. Smith, Y. T. Yang, and E. Osborne. 1973. Effects of age, pregnancy, and lactation on rat, guinea pig, and cow adipose enzyme activities and cow adipose tissue metabolism. J. Dairy Sci. 56: 340-349. doi: 10.3168/jds. S0022-0302(73)85177-X

Bauman, D. E., and C. L. Davis. 1975. Regulation of Lipid Metabolism. Pages 496-509 in Digestion and Metabolism in the Ruminant. McDonald, I. W., and A. C. I. Warner, eds. Univ. New England Publ. Unit, Armidale.

Bauman, D. E., C. L. Davis, and H. F. Bucholtz. 1971. Propionate production in the rumen of cows fed either a control or high-grain, low-fiber diet. J. Dairy Sci. 54(9): 1282-1287. doi: https://doi.org/10.3168/jds.S0022-0302(71)86021-6

Bell, A. W., and D. E. Bauman. 1997. Adaptations of glucose metabolism during pregnancy and lactation. J. Mammary Gland Biol. Neoplasia. 2(3): 265-278.

Berg, A. H., T. P. Combs, X. Du, M. Brownlee, and P. E. Scherer. 2001. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat. Med. 7: 947-953.

Berg, A. H., T. P. Combs, and P. E. Scherer. 2002. Arcp30/adiponectin: An adipokine regulating glucose and lipid metabolism. Trends Endocrinol. Metab. 13: 84-89.

Bergen, W. G., and H. J. Mersmann. 2005. Comparative aspects of lipid metabolism: Impact on contemporary research and use of animal models. J. Nutr. 135(11): 2499-2502. doi: https://doi.org/10.1093/jn/135.11.2499

Berner, H. S., S. P. Lyngstadaas, A. Spahr, M. Monjo, L. Thommesen, C. A. Drevon, U. Syversen, and J. E. Reseland. 2004. Adiponectin and its receptors are expressed in bone-forming cells. Bone. 35: 842-849.

Bevans, D. W., K. A. Beauchemin, K. S. Schwartzkopf-Genswein, J. J. McKinnon, and T. A. McAllister. 2005. Effect of rapid or gradual grain adaptation on subacute acidosis and feed intake by feedlot cattle. J. Anim. Sci. 83: 1116-1132.

Bong, J. J., J. Y. Jeong, P. Rajasekar, Y. M. Cho, E. G. Kwon, H. C. Kim, B. H. Paek, and M. Baik. 2012. Differential expression of genes associated with lipid metabolism in longissimus dorsi of Korean bulls and steers. Meat Sci. 91(3): 284-293. doi: https://doi.org/10.1016/j.meatsci.2012.02.004

Bradford, B. J., and M. S. Allen. 2008. Negative energy balance increases periprandial ghrelin and growth hormone concentrations in lactating dairy cows. Domest. Anim. Endocrinol. 34: 196-203.

Brockman, R. P. 1990. Effect of insulin on the utilization of propionate in gluconeogenesis in sheep. Br. J. Nutr. 64(1): 95-101.

Brockman, R. P., and B. Laarveld. 1986. Hormonal-regulation of metabolism in ruminants—a review. Livest. Prod. Sci. 14(4): 313-334.

Broglio, F., E. Arvat, A. Benso, C. Gottero, G. Muccioli, M. Papotti, J. van der Lely, R. Deghenghi, and E. Ghigo. 2001. Ghrelin, a natural GH secretagogue produced by the stomach, induces hyperglycemia and reduces insulin secretion in humans. J. Clin. Endocrinol. Metab. 86(10): 5083-5086. doi: https://doi.org/10.1210/jcem.86.10.8098

Brooks, M. A., C. W. Choi, D. K. Lunt, H. Kawachi, and S. B. Smith. 2011. Subcutaneous and intramuscular adipose tissue stearoyl-coenzyme A desaturase gene expression and fatty acid composition in calf- and yearling-fed angus steers. J. Anim. Sci. 89: 2556-2570. doi: 10.2527/jas.2010-3369

Bruce, C. R., V. A. Mertz, G. J. Heigenhauser, and D. J. Dyck. 2005. The stimulatory effect of globular adiponectin on insulin-stimulated glucose uptake and fatty acid oxidation is impaired in skeletal muscle from obese subjects. Diabetes. 54: 3154-3160.

Brulc, J. M., D. A. Antonopoulos, M. E. Miller, M. K. Wilson, A. C. Yannarell, E. A. Dinsdale, R. E. Edwards, E. D. Frank, J. B. Emerson, P. Wacklin, P. M. Coutinho, B. Henrissat, K. E. Nelson, and B. A. White. 2009. Gene-centric metagenomis of the fiber-adherent bovine rumen microbiome reveals forage specific glycoside hydrolases. Proc. Natl. Acad. Sci. U. S. A. 106: 1948-1953.

Cabral, A., E. J. Lopez Soto, J. Epelbaum, and M. Perello. 2017. Is ghrelin synthesized in the central nervous system? Int. J. Mol. Sci. 18

Callahan, H. S., D. E. Cummings, M. S. Pepe, P. A. Breen, C. C. Matthys, and D. S. Weigle. 2004. Postprandial suppression of plasma ghrelin level is proportional to ingested caloric load but does not predict intermeal interval in humans. J. Clin. Endocrinol. Metab. 89: 1319-1324.

Cameron, P. J., M. Rogers, J. Oman, S. G. May, D. K. Lunt, and S. B. Smith. 1994. Stearoyl coenzyme A desaturase enzyme activity and mRNA levels are not different in subcutaneous adipose tissue from angus and american wagyu steers. J. Anim. Sci. 72: 2624-2628.

Carrillo, W., C. Carpio, D. Morales, E. Vilcacundo, M. Álvarez, and M. Silva. 2017. Content of fatty acids in corn (*zea mays l.*) oil from Ecuador. Asian J. Pharm. Clin. Res. 10(8): 150-153. doi: 10.22159/ajpcr.2017.v10i8.18786

Chagas, L. M., M. C. Lucy, P. J. Back, D. Blache, J. M. Lee, P. J. S. Gore, A. J. Sheahan, and J. R. Roche. 2009. Insulin resistance in divergent strains of Holstein-friesian dairy cows offered fresh pasture and increasing amounts of concentrate in early lactation. J. Dairy Sci. 92(1): 216-222. . doi: https://doi.org/10.3168/jds.2008-1329

Chandran, M., S. A. Phillips, T. Ciaraldi, and R. R. Henry. 2003. Adiponectin, more than just another fat cell hormone? Diabetes Care. 26: 2442-2450.

Chen, M. B., A. J. McAinch, S. L. Macaulay, L. A. Castelli, P. E. O'Brien, J. B. Dixon, D. Cameron-Smith, B. E. Kemp, and G. R. Steinberg. 2005. Impaired activation of AMP-kinase and fatty acid oxidation by globular adiponectin in cultured human skeletal muscle of obese type 2 diabetics. J. Clin. Endocrinol. Metab. 90: 3665-3672.

Choat, W. T., C. R. Krehbiel, G. C. Duff, R. E. Kirksey, L. M. Lauriault, J. D. Rivera, B. M. Capitan, D. A. Walker, G. B. Donart, and C. L. Goad. 2003. Influence of grazing dormant native range or winter wheat pasture on subsequent finishing cattle performance, carcass characteristics, and ruminal metabolism. J. Anim. Sci. 81: 3191-3201.

Choi, S. H., B. Y. Park, J. H. Kim, I. H. Hwang, J. H. Kim, and J. M. Lee. 2005. Fatty acid profiles and sensory properties of longissimus dorsi, triceps brachii, and semimembranosus muscles from Korean Hanwoo and Australian Angus beef. Asian-Australasian J. Anim. Sci. 18(12): 1786-1793. doi: https://doi.org/10.5713/ajas.2005.1786

Choi, K., S. G. Roh, Y. H. Hong, Y. B. Shrestha, D. Hishikawa, C. Chen, M. Kojima, K. Kangawa, and S. I. Sasaki. 2003. The role of ghrelin and growth hormone secretagogues receptor on rat adipogenesis. Endocrinology. 144: 754-759.

Choi, S. H., T. A. Wickersham, G. Wu, L. A. Gilmore, H. D. Edwards, S. K. Park, K. H. Kim, and S. B. Smith. 2014. Abomasal infusion of arginine stimulates SCD and C/EBPß gene expression, and decreases CPT1ß gene expression in bovine adipose tissue independent of conjugated linoleic acid. Amino Acids. 46: 353-366. doi: 10.1007/s00726-013-1622-x

Chuang, J. C., M. Perello, I. Sakata, S. Osborne-Lawrence, J. M. Savitt, M. Lutter, and J. M. Zigman. 2011. Ghrelin mediates stress-induced food-reward behavior in mice. J. Clin. Invest. 2011(121): 2684-2692. doi: 10.1172/JCI57660

Chung, K. Y., D. K. Lunt, C. B. Choi, S. H. Chae, R. D. Rhoades, T. H. Adams, B. Booren, and S. B. Smith. 2006. Lipid characteristics of subcutaneous adipose tissue and *M. longissimus thoracis* of Angus and Wagyu steers fed to US and Japanese endpoints. Meat Sci. 73: 432-441. doi: 10.1016/j.meatsci.2006.01.002

Chung, K. Y., D. K. Lunt, H. Kawachi, H. Yano, and S. B. Smith. 2007. Lipogenesis and stearoyl-CoA desaturase gene expression and enzyme activity in adipose tissue of short- and long-fed Angus and Wagyu steers fed corn- or hay-based diets. J. Anim. Sci. 85(2): 380-387. doi: https://doi.org/10.2527/jas.2006-087

Clemens, E., V. Arthaud, R. Mandigo, and W. Woods. 1973. Fatty acid composition of bulls and steers as influenced by age and dietary energy level. J. Anim. Sci. 37(6): 1326-1331.

Combs, T. P., A. H. Berg, S. Obici, P. E. Scherer, and L. Rossetti. 2001. Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. J. Clin. Invest. 108: 1875-1881.

Cook, R. M., and L. D. Miller. 1965. Utilization of volatile fatty acids in ruminants. I. removal of them from portal blood by the liver. J. Dairy Sci. 48(10): 1339-1345. doi: https://doi.org/10.3168/jds.S0022-0302(65)88460-0

Crouse, J. D., and G. M. Smith. 1978. Relationship of selected beef carcass traits with meat palatability. J. Food Sci. 43: 152-157.

Cui, H., M. Lopez, and K. Rahmouni. 2017. The cellular and molecular bases of leptin and ghrelin resistance in obesity. Nat. Rev. Endocrinol. 13: 338-351.

Cummings, D. E., J. Q. Purnell, R. S. Frayo, K. Schmidova, B. E. Wisse, and D. S. Weigle. 2001. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes. 50: 1714-1719.

Cummings, D. E., D. S. Weigle, R. S. Frayo, P. A. Breen, M. K. Ma, E. P. Dellinger, and J. Q. Purnell. 2002. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. N. Engl. J. Med. 346: 1623-1630.

Dal Bosco, A., C. Mugnai, V. Roscini, S. Mattioli, S. Ruggeri, and C. Castellini. 2014. Effect of dietary alfalfa on the fatty acid composition and indexes of lipid metabolism of rabbit meat. Meat Sci. 96(1): 606-609. doi: https://doi.org/10.1016/j.meatsci.2013.08.027

Dall'Olio, S., D. Roberta, L. Buttazzoni, P. Zambonelli, and R. Vincenzo. 2009. Study of porcine adiponectin (ADIPOQ) gene and association of a missense mutation with EBVs for production and carcass traits in italian duroc heavy pigs. Livestock Sci. 125: 101-104.

Dänicke, S., U. Meyer, J. Winkler, K. Schulz, S. Ulrich, J. Frahm, S. Kersten, J. Rehage, G. Breves, S. Häußler, H. Sauerwein, and L. Locher. 2014. Description of a bovine model for studying digestive and metabolic effects of a positive energy balance not biased by lactation or gravidity. Arch. Anim. Nutr. 68(6): 460-477. doi: 10.1080/1745039X.2014.973243

Date, Y., M. Kojima, H. Hosoda, A. Sawaguchi, M. S. Mondal, T. Suganuma, S. Matsukura, K. Kangawa, and M. Nakazato. 2000. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. Endocrinology. 141(4255): 4261.

Date, Y., T. Shimbara, S. Koda, K. Toshinai, T. Ida, N. Murakami, M. Miyazato, K. Kokame, Y. Ishizuka, Y. Ishida, H. Kageyama, S. Shioda, K. Kangawa, and M. Nakazato. 2006. Peripheral ghrelin transmits orexigenic signals through the noradrenergic pathway from the hindbrain to the hypothalamus. Cell Metabol. 4(323): 331.

Davies, J. S., P. Kotokorpi, S. R. Eccles, S. K. Barnes, P. F. Tokarczuk, S. K. Allen, H. S. Whitworth, I. A. Guschina, B. A. J. Evans, A. Mode, J. M. Zigman, and T. Wells. 2009. Ghrelin induces abdominal obesity via GHS-R-dependent lipid retention. Mol. Endocrinol. 23: 914-924.

DeFronzo, R. A. 2004. Pathogenesis of type 2 diabetes mellitus. Med. Clin. North Am. 88(4): 787-835.

Delhanty, P. J. D., and A. J. Van der Lely. 2011. Ghrelin and glucose homeostasis. Peptides. 32: 2309-2318.

Dhiman, T. R., S. H. Nam, and A. L. Ure. 2005. Factors affecting conjugated linoleic acid content in milk and meat. Crit. Rev. Food Sci. Nutr. 45(6): 463-482. doi: https://doi.org/10.1080/10408390591034463

Dolezal, H. G., G. C. Smith, J. W. Savell, and Z. L. Carpenter. 1982. Comparison of subcutaneous fat thickness, marbling and quality grade for predicting palatability of beef. J. Food Sci. 47: 397-401.

Duckett, S. K., S. L. Pratt, and E. Pavan. 2009. Corn oil or corn grain supplementation to steers grazing endophyte-free tall fescue. II. effects on subcutaneous fatty acid content and lipogenic gene expression. J. Anim. Sci. 87(1120): 1128.

Eichhorn, J. M., L. J. Coleman, E. J. Wakayama, G. J. Blomquist, C. M. Bailey, and T. G. Jenkins. 1986. Effects of breed type and restricted versus ad libitum feeding on fatty acid composition and cholesterol content of muscle and adipose tissue from mature bovine females. J. Anim. Sci. 63(3): 781-794.

Enjalbert, F., M. C. Nicot, C. Bayourthe, and R. Moncoulon. 2001. Ketone bodies in milk and blood of dairy cows: Relationship between concentrations and utilization for detection of subclinical ketosis. J. Dairy Sci. 84(3): 583-589. doi: https://doi.org/10.3168/jds.S0022-0302(01)74511-0

Fahey Jr., G. C., and L. L. Berger. 1988. Carbohydrate Nutrition of Ruminants. Pages 269-297 in The Ruminant Animal—Digestive Physiology and Nutrition. D.C. Church ed. Waveland Press Inc., Prospect Heights, IL.

Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226(1): 497-509.

Foote, A. P., K. E. Hales, C. A. Lents, and H. C. Freetly. 2014. Association of circulating active and total ghrelin concentrations with dry matter intake, growth, and carcass characteristics of finishing beef cattle. J. Anim. Sci. 92: 5651-5658. doi: 10.2527/jas2014-8291

Forbes, J. M. 1995. Metabolites and Hormones in Voluntary Food Intake and Diet Selection in Farm Animals. 2nd ed. CAB Int., Wallingford, UK.

Fukuda, S., Y. Y. Suzuki, M. Murai, N. Asanuma, and T. Hino. 2006. Augmentation of vaccenate production and suppression of vaccenate biohydrogenation in cultures of mixed ruminal microbes. J. Dairy Sci. 89: 1043-1051.

Gahete, M. D., J. Córdoba-Chacón, R. Salvatori, and J. P. Castaño. 2010. Metabolic regulation of ghrelin O-acyl transferase (GOAT) expression in the mouse hypothalamus, pituitary, and stomach. Mol. Cell. Endocrinol. 317: 154-160.

Galic, S., J. S. Oakhill, and G. R. Steinberg. 2010. Adipose tissue as an endocrine organ. Mol. Cell Endocrinol. 316: 129-139.

Gauna, C., P. J. Delhanty, L. J. Hofland, J. A. Janssen, F. Broglio, R. J. Ross, E. Ghigo, and A. J. van der Lely. 2005. Ghrelin stimulates, whereas des-octanoyl ghrelin inhibits, glucose output by primary hepatocytes. J. Clin. Endocrinol. Metab. 90: 1055-1060.

Gauna, C., F. M. Meyler, J. A. Janssen, P. J. Delhanty, T. Abribat, P. van Koetsveld, L. J. Hofland, F. Broglio, E. Ghigo, and A. J. van der Lely. 2004. Administration of acylated ghrelin reduces insulin sensitivity, whereas the combination of acylated plus unacylated ghrelin strongly improves insulin sensitivity. J. Clin. Endocrinol. Metab. 89: 5035-5042.

Geay, Y., D. Bauchart, J. F. Hocquette, and J. Culioli. 2001. Effect of nutritional factors on biochemical, structural and metabolic characteristics of muscles in ruminants, consequences on dietetic value and sensorial qualities of meat. Reprod. Nutr. Dev. 41(1): 1-26.

Gentry, P. C., J. P. Willey, and R. J. Collier. 2003. Ghrelin, a growth hormone secretagogue, is expressed by bovine rumen. J. Anim. Sci. 81: 123.

Giesy, S. L., B. Yoon, W. B. Currie, J. W. Kim, and Y. R. Boisclair. 2012. Adiponectin deficit during the precarious glucose economy of early lactation in dairy cows. Endocrinology. 153: 5834-5844.

Gilbert, C. D., D. K. Lunt, R. K. Miller, and S. B. Smith. 2003. Carcass, sensory, and adipose tissue traits of Brangus steers fed casein-formaldehyde-protected starch and/or canola lipid. J. Anim. Sci. 81(10): 2457-2468. doi: https://doi.org/10.2527/2003.81102457x

Givens, D. I. 2005. The role of animal nutrition in improving the nutritive value of animal-derived foods in relation to chronic disease. Proc. Nutr. Soc. 64(3): 395-402. doi: 10.1079/PNS2005448

González-Grajales, L. A., L. Pieper, S. Görner, P. Görner, and R. Staufenbiel. 2019. Short communication: Repeatability of intravenous glucose tolerance test traits in young Holstein-friesian cattle. J. Dairy Sci. 102(4): 3609-3614. doi: 10.3168/jds.2018-15617

Goodacre, R., S. Vaidyanathan, W. B. Dunn, G. G. Harrigan, and D. B. Kell. 2004. Metabolomics by numbers: Acquiring and understanding global metabolite data. Trends Biotech. 22(5): 245-252. doi: https://doi.org/10.1016/j.tibtech.2004.03.007

Gotoh, T., E. Albrecht, F. Teuscher, K. Kawabata, K. Sakashita, H. Iwamoto, and J. Wegner. 2009. Differences in muscle and fat accretion in Japanese Black and European cattle. Meat Sci. 82(3): 300-308. doi: https://doi.org/10.1016/j.meatsci.2009.01.026

Grigsby, M. E., and A. Trenkle. 1986. Plasma growth hormone, insulin, glucocorticoids and thyroid hormones in large, medium and small breeds of steers with and without an estradiol implant. Domest Anim Endocrinol. 3: 261-267.

Hailemariam, D., R. Mandal, F. Saleem, S. M. Dunn, D. S. Wishart, and B. N. Ametaj. 2014. Identification of predictive biomarkers of disease state in transition dairy cows. J. Dairy Sci. 97(5): 2680-2693. doi: https://doi.org/10.3168/jds.2013-6803

Halsey, C. H. C., P. S. Weber, S. S. Reiter, B. N. Stronach, J. L. Bartosh, and W. G. Bergen. 2011. The effect of ractopamine hydrochloride on gene expression in adipose tissues of finishing pigs. J. Anim. Sci. 89: 1011-1019. doi: 10.2527/jas.2010-3269

Harrison, A. R., M. E. Smith, D. M. Allen, M. C. Hunt, C. L. Kastner, and D. H. Kropf. 1978. Nutritional regime effects on quality and yield characteristics of beef. J. Anim. Sci. 47: 383-388.

Hausman, G. J., M. V. Dodson, K. Ajuwon, M. Azain, K. M. Barnes, L. L. Guan, Z. Jiang, S. P. Poulos, R. D. Sainz, S. Smith, M. Spurlock, J. Novakofski, M. E. Fernyhough, and W. G. Bergen. 2009. BOARD-INVITED REVIEW: The biology and regulation of preadipocytes and adipocytes in meat animals, J. Anim. Sci. 87(4): 1218-1246. doi: https://doi.org/10.2527/jas.2008-1427

Hayashida, T., K. Murakami, K. Mogi, M. Nishihara, M. Nakazato, S. Mondal, Y. Horii, M. Kojima, K. Kangawa, and N. Murakami. 2001. Ghrelin in domestic animals: Distribution in the stomach and its possible role. Domest. Anim. Endocrinol. 21: 17-24.

Hayhurst, C., A. P. F. Flint, P. Løvendahl, J. A. Woolliams, and M. D. Royal. 2009. Genetic variation of metabolite and hormone concentration in UK Holstein-friesian calves and the genetic relationship with economically important traits. J. Dairy Sci. 92: 4001-4007.

Heinz, J. F. L., S. P. Singh, U. Janowitz, M. Hoelker, D. Tesfaye, K. Schellander, and H. Sauerwein. 2015. Characterization of adiponectin concentrations and molecular weight forms in serum, seminal plasma, and ovarian follicular fluid from cattle. Theriogenology. 83: 326-333. doi: http://dx.doi.org/10.1016/j.theriogenology.2014.06.030

Hernandez-Sanabria, E., L. A. Goonewardene, Z. Wang, O. N. Durunna, S. S. Moore, and L. L. Guan. 2012. Impact of feed efficiency and diet on adaptive variations in the bacterial community in the rumen fluid of cattle. Appl. Environ. Microbiol. 78(4): 1203-1214. doi: 10.1128/AEM.05114-11

Hernandez-Sanabria, E., L. L. Guan, L. A. Goonewardene, M. Li, D. F. Mujibi, P. Stothard, S. S. Moore, and M. C. Leon-Quintero. 2010. Linkage of particular bacterial PCRDGGE patterns to bovine ruminal fermentation parameters and feed efficiency traits. Appl. Environ. Microbiol. 76(19): 6338-6350. doi: 10.1128/AEM.01052-10

Hirose, H., Y. Yamamoto, Y. Seino-Yoshihara, H. Kawabe, and I. Saito. 2010. Serum high-molecular-weight adiponectin as a marker for the evaluation and care of subjects with metabolic syndrome and related disorders. J. Atheroschler. Thromb. 17: 1201-1211.

Hocquette, J. F., I. Cassar-Malek, A. Scalbert, and F. Guillou. 2009. Contribution of genomics to the understanding of physiological functions. J. Physiol. Pharmacol. 60(3): 5-16.

Hood, R. L., and C. E. Allen. 1978. Lipogenesis in isolated intramuscular adipose tissue from four bovine muscles. J. Anim. Sci. 46(6): 1626-1633.

Hosoda, H., M. Kojima, H. Matsuo, and K. Kangawa. 2000. Ghrelin and des-acyl ghrelin: Two major forms of rat ghrelin peptide in gastrointestinal tissue. Biochem. Biophys. Res. Commun. 279: 909-913.

Howard, A. D., S. D. Feighner, D. F. Cully, J. P. Arena, P. A. Liberator, C. I. Rosenblum, M. Hamelin, D. L. Hreniuk, O. C. Palyha, J. Anderson, P. S. Paress, C. Diaz, M. Chou, K. K. Liu, K. K. McKee, S. S. Pong, L. Y. Chaung, A. Elbrecht, M. Dashkevicz, R. Heavens, M. Rigby, D. J. Sirinathsinghji, D. C. Dean, D. G. Melillo, A. A. Patchett, R. Nargund, P. R. Griffin, J. A. DeMartino, S. K. Gupta, J. M. Schaeffer, R. G. Smith, and L. H. Van der Ploeg. 1996. A receptor in pituitary and hypothalamus that functions in growth hormone release. Science. 273: 974-977.

Hu, E., P. Liang, and B. M. Spiegelman. 1996. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J. Boil. Chem. 271: 10697-10703.

Huerta-Leidenz, N. O., H. R. Cross, J. W. Savell, D. K. Lunt, J. F. Baker, and L. S. Pelton. 1993. Comparison of the fatty acid composition of subcutaneous adipose tissue from mature Brahman and Hereford cows. J. Anim. Sci. 71(3): 625-630.

Huerta-Leidenz, N. O., H. R. Cross, J. W. Savell, D. K. Lunt, J. F. Baker, and S. B. Smith. 1996. Fatty acid composition of subcutaneous adipose tissue from male calves at different stages of growth. J. Anim. Sci. 74: 1256-1264.

Huffman, R. D., S. E. Williams, D. D. Hargrove, D. D. Johnson, and T. T. Marshall. 1990. Effects of percentage brahman and angus breeding, age, season of feeding and slaughter end point on feedlot performance and carcass characteristics. J. Anim. Sci. 68: 2243-2252.

Illán-Gómez, F., M. Gonzálvez-Ortega, I. Orea-Soler, M. S. Alcaraz-Tafalla, A. Aragón-Alonso, M. Pascual-Díaz, M. Pérez-Paredes, and M. L. Lozano-Almela. 2012. Obesity and inflammation: Change in adiponectin, C-reactive protein, tumour necrosis factor-alpha and interleukin-6 after bariatric surgery. Obes. Surg. 22(6): 950-955. doi: 10.1007/s11695-012-0643-y

Imhasly, S., H. Naegeli, S. Baumann, M. von Bergen, A. Luch, H. Jungnickel, S. Potratz, and C. Gerspach. 2014. Metabolomic biomarkers correlating with hepatic lipidosis in dairy cows. BMC Vet. Res. 10: 122. doi: 10.1186/1746-6148-10-122

Inui, A. 2001. Ghrelin: An orexigenic and somatotrophic signal from the stomach. Nature Rev. Neurosci. 2: 551-560.

Jacobi, S. K., K. M. Ajunwon, T. E. Weber, J. L. Kuske, and C. J. Dyer. 2004. Cloning and expression of porcine adiponectin, and its relationship to adiposity, lipogenesis and the acute phase response. J. Endocrinology. 182: 133-144.

Jarrett, I. G., O. H. Filsell, and F. J. Ballard. 1974. Metabolic and endocrine interrelationships in normal and diabetic sheep. Horm. Metab. Res. Suppl. Ser. 4: 111-116.

Jennings, J. S., A. E. Wertz-Lutz, R. H. Pritchard, A. D. Weaver, D. H. Keisler, and K. Bruns. 2011. Circulating ghrelin and leptin concentrations and growth hormone secretagogue receptor abundance in liver, muscle, and adipose tissue of beef cattle exhibiting differences in composition of gain. J. Anim. Sci. 89: 3954-3972.

Jiang, Z., J. J. Michal, D. J. Tobey, T. F. Daniels, D. C. Rule, and M. D. MacNeil. 2008. Significant associations of stearoyl-CoA desaturase (SCD1) gene with fat deposition and composition in skeletal muscle. Int. J. Biol. Sci. 4: 345-351.

JMGA. 1988. New beef carcass grading standards. Japan Meat Grading Association. Tokyo, Japan.

Kadowaki, T., and T. Yamauchi. 2005. Adiponectin and adiponectin receptors. Endocrine Reviews. 26: 439-451.

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

Kaneko, J. J. 1997. Carbohydrate Metabolism and its Diseases. Pages 45-81 in Clinical Biochemistry of Domestic Animals. Kaneko, J. J., W. J. Harvey, and L. M. Bruss, eds. 6th ed. Academic Press, San Diego, CA.

Kasai, H., H. Hatakeyama, M. Ohno, and N. Takahashi. 2014. Exocytosis in Islet β-Cells. Pages 476-503 in Islets of Langerhans. 2nd ed.

Kaske, M., B. Elmahdi, W. von Engelhardt, and H. P. Sallmann. 2001. Insulin responsiveness of sheep, ponies, miniature pigs and camels: Results of hyperinsulinemic clamps using porcine insulin. J. Comp. Physiol. B. 171(7): 549-556.

Kissebah, A. H., G. E. Sonnenberg, J. Myklebust, M. Goldstein, K. Broman, R. G. James, J. A. Marks, G. R. Krakower, H. J. Jacob, J. Weber, L. Martin, J. Blangero, and A. G. Comuzzie. 2000. Quantitative trait loci on chromosomes 3 and 17 influence phenotypes

of the metabolic syndrome. Proceedings of the National Academy of Sciences of the United States of America. 97: 14478-14483.

Klusmeyer, T. H., J. H. Clark, J. L. Vicini, M. R. Murphy, and G. C. Fahey Jr. 1987. Effects of feeding or infusing ammonium salts of volatile fatty acids on ruminal fermentation, plasma characteristics, and milk production of cows. J. Dairy Sci. 70: 50-63.

Kojima, M., H. Hosoda, Y. Date, M. Nakazato, H. Matsuo, and K. Kangawa. 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature. 402: 656-660.

Kojima, M., and K. Kangawa. 2002. Ghrelin, an orexigenic signaling molecule from the gastrointestinal tract. Curr. Opin. Pharmacol. 2: 665-668.

Komatsu, M., M. Takei, H. Ishii, and Y. Sato. 2013. Glucose-stimulated insulin secretion: A newer perspective. J. Diabetes Investig. 4(6): 511-516.

Kristensen, T., M. Fredholm, and S. Cirera. 2015. Expression study of GLUT4 translocation-related genes in a porcine prediabetic model. Mamm. Genome. 26: 650-657. doi: 10.1007/ s00335-015-9601-z

Kurose, Y., J. Iqbal, A. Rao, Y. Murata, Y. Hasegawa, Y. Terashima, M. Kojima, K. Kangawa, and I. J. Clarke. 2005. Changes in expression of the genes for the leptin receptor and the growth hormone-releasing peptide/ghrelin receptor in the hypothalamic arcuate nucleus with long-term manipulation of adiposity by dietary means. Neuroendocrinology. 17: 331-340.

Ladeira, M. M., J. P. Schoonmaker, M. P. Gionbelli, J. C. O. Dias, T. R. S. Gionbelli, J. R. R. Carvalho, and P. D. Teixeira. 2016. Nutrigenomics and beef quality: A review about lipogenesis. J. Molecular Sci. 17(6): 918. doi: 10.3390/ijms17060918

Liang, Y., X. M. Yang, Y. R. Gu, X. Tao, Z. Z. Zhong, J. J. Gong, X. H. Chen , and X. B. Lu. 2015. Developmental changes in the expression of the GLUT2 and GLUT4 genes in the longissimus muscle of Yorkshire and Tibetan pigs. Genet. Mol. Res. 14: 1287-1292. doi: 10.4238/2015.February.13.7

Lindholm-Perry, A. K., L. A. Kuehn, T. P. L. Smith, C. L. Ferrell, T. G. Jenkins, H. C. Freetly, and W. M. Snelling. 2012. A region on BTA14 that includes the positional candidate genes LYPLA1, XKR4 and TMEM68 is associated with feed intake and growth phenotypes in cattle. Anim. Genet. 43: 216-219.

Locher, L., S. Häussler, L. Laubenthal, S. P. Singh, J. Winkler, A. Kinoshita, A. Kenéz, J. Rehage, K. Huber, H. Sauerwein, and S. Dänicke. 2015. Effect of increasing body condition on key regulators of fat metabolism in subcutaneous adipose tissue depot and

circulation of nonlactating dairy cows. J. Dairy Sci. 98: 1057-1068. doi: http://dx.doi.org/10.3168/jds.2014-8710

Lu, X., X. Zhao, J. Feng, A. P. Liou, S. Anthony, S. Pechhold, Y. Sun, H. Lu, and S. Wank. 2012. Postprandial inhibition of gastric ghrelin secretion by long-chain fatty acid through GPR120 in isolated gastric ghrelin cells and mice. Am. J. Physiol. Gastrointest. Liver Physiol. 303: G367-G376.

Lunt, D. K., K. Y. Chung, C. B. Choi, and S. B. Smith. 2005. Production characteristics and carcass quality of angus and wagyu steers fed to US and japanese endpoints. J. Anim. Vet. Adv. 4(11): 949-953.

Mandell, I. B., J. G. Buchanan-Smith, and C. P. Campbell. 1998. Effects of forage vs. grain feeding on carcass characteristics, fatty acid composition, and beef quality in Limousin-cross steers when time on feed is controlled. J. Anim. Sci. 76(10): 2619-2630.

Martin, G. S., D. K. Lunt, K. G. Britain, and S. B. Smith. 1999. Postnatal development of stearoyl coenzyme A desaturase gene expression and adiposity in bovine subcutaneous adipose tissue. J. Anim. Sci. 77: 630-636.

Matsubara, M., S. Maruoka, and S. Katayose. 2002. Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. Eur. J. Endocrinol. 147: 173-180.

Matsuzaki, M., S. Takizawa, and M. Ogawa. 1997. Plasma insulin, metabolite concentrations, and carcass characteristics of Japanese Black, Japanese Brown, and Holstein steers. J. Anim. Sci. 75: 3287-3293.

May, S. G., H. G. Dolezal, D. R. Gill, F. K. Ray, and D. S. Buchanan. 1992. Effects of days fed, carcass grade traits and subcutaneous fat removal on post mortem muscle characteristics and beef palatability. J. Anim. Sci. 70: 444-453.

May, S. G., C. A. Sturdivant, D. K. Lunt, R. K. Miller, and S. B. Smith. 1993. Comparison of sensory characteristics and fatty acid composition between wagyu crossbred and angus steers. Meat Sci. 35: 289-298.

McCann, J. P., and T. J. Reimers. 1985. Glucose response to exogenous insulin and kinetics of insulin metabolism in obese and lean heifers. J. Anim. Sci. 61(3): 612-618.

McFarlane, M. R., M. S. Brown, J. L. Goldstein, and T. J. Zhao. 2014. Induced ablation of ghrelin cells in adult mice does not decrease food intake, body weight, or response to high-fat diet. Cell Metab. 20(54): 60.

Mequinion, M., F. Langlet, S. Zgheib, S. Dickson, B. Dehouck, C. Chauveau, and O. Viltart. 2013. Ghrelin: Central and peripheral implications in anorexia nervosa. Front. Endocrinol. 4: 15.

Miao, Z. G., L. P. Zhang, X. Fu, Q. Y. Yang, M. J. Zhu, M. V. Dodson, and M. Du. 2016. Invited review: Mesenchymal progenitor cells in intramuscular connective tissue development. Animal. 10(1): 75-81. doi: 10.1017/S1751731115001834

Miller, M. F., H. R. Cross, J. J. Wilson, and S. B. Smith. 1989. Acute and long-term lipogenic response to insulin and clenbuterol in bovine intramuscular and subcutaneous adipose tissues. J. Anim. Sci. 67: 928-933. doi: 10.2527/jas1989.674928x

Mir, P. S., Z. Mir, P. S. Kubert, C. T. Gaskins, E. L. Martin, M. V. Dodson, J. A. Calles, K. A. Johnson, J. R. Busboom, A. J. Wood, G. J. Pittenger, and J. J. Reeves. 2002. Growth, carcass characteristics, muscle conjugated linoleic acid (CLA) content, and response to intravenous glucose challenge in high percentage Wagyu, Wagyu x Limousin, and Limousin steers fed sunflower oil-containing diet. J. Anim. Sci. 80(11): 2996-3004.

Mir, P. S., K. S. Schwartzkopf-Genswein, T. Entz, K. K. Klein, E. Okine, and M. V. Dodson. 2008. Effect of a short duration feed withdrawal followed by full feeding on marbling fat in beef carcasses. Livestock Sci. 116(1): 22-29. doi: https://doi.org/10.1016/j.livsci.2007.08.015

Miura, H., N. Tsuchiya, I. Sasaki, M. Kikuchi, M. Kojima, K. Kangawa, Y. Hasegawa, and Y. Ohnami. 2004. Changes in plasma ghrelin and growth hormone concentrations in mature Holstein cows and three-month-old calves. J. Anim. Sci. 82: 1329-1333.

Moody, W. G., and R. G. Cassens. 1968. A quantitative and morphological study of bovine longissimus fat cells. J. Food Sci. 33(1): 47-55. doi: 10.1111/j.1365-2621.1968.tb00882.x https://doi.org/10.1111/j.1365-2621.1968.tb00882.x

Morrison, W. R., and L. M. Smith. 1964. Preparation of fatty acid methyl esters and demethyl acetals from lipids with boron fluoride-methanol. J. Lipid Research. 5(10): 600-608.

Morsci, N. S., E. M. Sellner, R. D. Schnabel, and J. F. Taylor. 2006. Association analysis of adiponectin and somatostatin polymorphisms on BTA1 with growth and carcass traits in angus cattle. Animal Genetics. 37: 554-562.

Müller, T. D., R. Nogueiras, M. L. Andermann, Z. B. Andrews, S. D. Anker, J. Argente, R. L. Batterham, S. C. Benoit, C. Y. Bowers, F. Broglio, F. F. Casanueva, D. D'Alessio, I. Depoortere, A. Geliebter, E. Ghigo, P. A. Cole, M. Cowley, D. E. Cummings, A. Dagher, S. Diano, S. L. Dickson, C. Diéguez, R. Granata, H. J. Grill, K. Grove, K. M. Habegger, K. Heppner, M. L. Heiman, L. Holsen, B. Holst, A. Inui, J. O. Jansson, H. Kirchner, M.

Korbonits, B. Laferrère, C. W. LeRoux, M. Lopez, S. Morin, M. Nakazato, R. Nass, D. Perez-Tilve, P. T. Pfluger, T. W. Schwartz, R. J. Seeley, M. Sleeman, Y. Sun, L. Sussel, J. Tong, M. O. Thorner, A. J. van der Lely, L. H. T. van der Ploeg, J. M. Zigman, M. Kojima, K. Kangawa, R. G. Smith, T. Horvath, and M. H. Tschöp. 2015. Ghrelin. Mol Metab. 4(6): 437-460. doi: https://doi.org/10.1016/j.molmet.2015.03.005

Nagaraja, T. G., and E. C. Titgemeyer. 2007. Ruminal acidosis in beef cattle: The current microbiological and nutritional outlook. J. Dairy Sci. 90: E17-E38.

Nagaya, N., M. Uematsu, M. Kojima, Y. Ikeda, F. Yoshihara, W. Shimizu, H. Hosoda, Y. Hirota, H. Ishida, H. Mori, and K. Kangawa. 2001. Chronic administration of ghrelin improves left ventricular dysfunction and attenuates development of cardiac cachexia in rats with heart failure. Circulation. 104: 1430-1435.

Nakazato, M., N. Murakami, Y. Date, M. Kojima, H. Matsuo, K. Kangawa, and S. Matsukura. 2001. A role for ghrelin in the central regulation of feeding. Nature. 409(6817): 194-198. doi: 10.1038/35051587

Nour, A. Y. M., M. L. Thonney, J. R. Stouffer, and W. R. C. White Jr. 1983. Changes in carcass weight and characteristics with increasing weight of large and small cattle. J. Anim. Sci. 57: 1154-1165.

Nunemaker, C. S., and L. S. Satin. 2014. Episodic hormone secretion: A comparison of the basis of pulsatile secretion of insulin and GnRH. Endocrine. 47(1): 49-63.

Orskov, E. R., N. A. MacLeod, and Y. Nakashima. 1991. Effect of different volatile fatty acids mixtures on energy metabolism. J. Anim. Sci. 69: 3389-3397.

Oshima, K., A. Nampei, M. Matsuda, M. Iwaki, A. Fukuhara, J. Hashimoto, H. Yoshikawa, and I. Shimomura. 2005. Adiponectin increases bone mass by suppressing osteoclast and activating osteoblast. Biochem. Biophys. Res. Commun. 331: 520-526.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010. Evaluation of nonesterified fatty acids and β-hydroxybutyrate in transition dairy cattle in the northeastern united states: Critical thresholds for prediction of clinical diseases. J. Dairy Sci. 93(2): 546-554. doi: https://doi.org/10.3168/jds.2009-2277

Otto, B., U. Cuntz, E. Fruehauf, R. Wawarta, C. Folwaczny, R. L. Riepl, M. L. Heiman, P. Lehnert, M. Fichter, and M. Tschöp. 2001. Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa. Eur. J. Endocrinol. 145: 669-673.

Ouchi, N., S. Kihara, Y. Arita, K. Maeda, H. Kuriyama, Y. Okamoto, K. Hotta, M. Nishida, M. Takahashi, T. Nakamura, S. Yamashita, T. Funahashi, and Y. Matsuzawa. 1999. Novel modulator for endothelial adhesion molecules: Adipocyte-derived plasma protein adiponectin. Circulation. 100: 2473-2476.

Owens, F. N., D. R. Gill, D. S. Secrist, and S. W. Coleman. 1995. Review of some aspects of growth and development of feedlot cattle. J. Anim. Sci. 73(10): 3152-3172.

Pagano, C., G. Soardo, W. Esposito, F. Fallo, L. Basan, D. Donnini, G. Federspil, L. A. Sechi, and R. Vettor. 2005. Plasma adiponectin is decreased in nonalcoholic fatty liver disease. Eur. J. Endocrinol. 152: 113-118.

Park, S., K. C. Sadanala, and E. K. Kim. 2015. A metabolomic approach to understanding the metabolic link between obesity and diabetes. Molecules and Cells. 38(7): 587-596. doi: 10.14348/molcells.2015.0126

Patel, A. D., S. A. Stanley, K. G. Murphy, G. S. Frost, J. V. Gardiner, A. S. Kent, N. E. White, M. A. Ghatei, and S. R. Bloom. 2006. Ghrelin stimulates insulin-induced glucose uptake in adipocytes. Regul. Pept. 134: 17-22.

Pearson, A. M. 1966. Desirability of beef - its characteristics and their measurement. J. Anim. Sci. 25: 843-854.

Perez-Tilve, D., K. Heppner, H. Kirchner, S. H. Lockie, S. C. Woods, D. L. Smiley, M. Tschöp, and P. Pfluger. 2011. Ghrelin-induced adiposity is independent of orexigenic effects. FASEB J. 25: 2814-2822.

Pethick, D. W., G. S. Harper, and H. Oddy. 2004. Growth, development and nutritional manipulation of marbling in cattle: A review. Australian J. Exp. Agric. 44(7)

Piechotta, M., A. K. Sander, J. P. Kastelic, R. Wilde, M. Heppelmann, B. Rudolphi, H. J. Schuberth, H. Bollwein, and M. Kaske. 2012. Short communication: Prepartum plasma insulin-like growth factor-I concentrations based on day of insemination are lower in cows developing postpartum diseases. J. Dairy Sci. 95(3): 1367-1370. doi: 10.3168/jds.2011-4622.

Pineiro, R., M. J. Iglesias, R. Gallego, K. Raghay, S. Eiras, J. Rubio, C. Dieguez, O. Gualillo, J. R. Gonzalez-Juanatey, and F. Lago. 2005. Adiponectin is synthesized and secreted by human and murine cardiomyocytes. FEBS Letters. 579(5163): 5169.

Quigley III, J. D., Z. P. Smith, and R. N. Heitmann. 1991. Changes in plasma volatile fatty acids in response to weaning and feed intake in young calves. J. Dairy Sci. 74(1): 258-263. doi: https://doi.org/10.3168/jds.S0022-0302(91)78168-X

Remesy, C., and C. Demigne. 1974. Determination of volatile fatty acids in plasma after ethanolic extraction. Biochem. J. 141: 85-91.

Rhoades, R. D., J. E. Sawyer, K. Y. Chung, M. L. Schell, D. K. Lunt, and S. B. Smith. 2007. Effect of dietary energy source on in vitro substrate utilization and insulin sensitivity of muscle and adipose tissues of angus and wagyu steers. J. Anim. Sci. 85: 1719-1726. doi: 10.2527/jas.2006-498

Rigault, C., F. Le Borgne, B. Georges, and J. Demarquoy. 1996. Ghrelin reduces hepatic mitochondrial fatty acid beta oxidation. J. Endocrinol. Invest. 30: RC4-RC8.

Sakata, I., W. M. Park, A. K. Walker, P. K. Piper, J. C. Chuang, S. Osborne-Lawrence, and J. M. Zigman. 2012. Glucose-mediated control of ghrelin release from primary cultures of gastric mucosal cells. Am. J. Physiol. Endocrinol. Metab. 302: E1300-E1310.

Saleem, F., B. N. Ametaj, S. Bouatra, R. Mandal, Q. Zebeli, S. M. Dunn, and D. S. Wishart. 2012. A metabolomics approach to uncover the effects of grain diets on rumen health in dairy cows. J. Dairy Sci. 95(11): 6606-6623. doi: 10.3168/jds.2012-5403

Salehi-Abargouei, A., V. Izadi, and L. Azadbakht. 2015. The effect of low calorie diet on adiponectin concentration: A systematic review and meta-analysis. Horm. Metab. Res. 47(549): 555.

Sangiao-Alvarellos, S., M. J. Vazquez, L. Varela, R. Nogueiras, A. K. Saha, F. Cordido, M. Lopez, and C. Dieguez. 2009. Central ghrelin regulates peripheral lipid metabolism in a growth hormone-independent fashion. Endocrinology. 150: 4562-4574.

Sano, H., K. Asano, Y. Noguchi, K. Yoshimura, T. Senshu, and Y. Terashima. 1996. Insulin responsiveness, action and sensitivity in growing lambs and mature rams. Can. J. Anim. Sci. 76: 203-208.

Sasaki, S. 2002. Mechanism of insulin action on glucose metabolism in ruminants. Anim. Sci. J. 73: 423-433.

Satou, H., K. Chiba, M. Takeda, and A. Hagino. 1998a. Comparison of plasma metabolic hormones concentrations in Japanese Black cattle and Holstein cattle. Anim. Sci. Technol. 69(483): 488.

Satou, H., K. Chiba, M. Takeda, and A. Hagino. 1998b. Hormone responses to glucose injection in Japanese Black cattle and Holstein cattle. Anim. Sci. Technol. 69: 475-482.

Scherer, P. E., S. Williams, M. Fogliano, G. Baldini, and H. F. Lodish. 1995. A novel serum protein similar to C1q, produced exclusively in adipocytes. J. Biol. Chem. 270: 26746-26749.

Schoonmaker, J. P. 2012. Effects of lifetime nutrition on beef quality. The III international symposium of beef cattle, Saskatoon, SK, Canada.

Scollan, N., J. Hocquette, K. Nuernberg, D. Dannenberger, I. Richardson, and A. Moloney. 2006. Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. Meat Sci. 74(1): 17-33. doi: https://doi.org/10.1016/j.meatsci.2006.05.002

Shanado, Y., M. Kometani, H. Uchiyama, S. Koizumi, and N. Teno. 2004. Lysophospholipase I identified as a ghrelin deacylation enzyme in rat stomach. Biochem. Biophys. Res. Commun. 325: 1487-1494.

Shiiya, T., M. Nakazato, M. Mizuta, Y. Date, M. S. Mondal, M. Tanaka, S. Nozoe, H. Hosoda, K. Kangawa, and S. Matsukura. 2002. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. J. Clin. Endocrinol. Metab. 87: 240-244.

Shingu, H., K. Hodate, S. Kushibiki, Y. Ueda, A. Watanabe, M. Shinoda, and M. Matsumoto. 2001. Profiles of growth hormone and insulin secretion, and glucose response to insulin in growing Japanese Black heifers (beef type) : Comparison with Holstein heifers (dairy type). Comp. Biochem. Physiol. C. 130: 259-270.

Shintani, M., Y. Ogawa, K. Ebihara, M. Aizawa-Abe, F. Miyanaga, T. T. Hayashi, G. Inoue, K. Hosoda, M. Kojima, K. Kangawa, and K. Nakao. 2001. Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. Diabetes. 50: 227-232.

Singh, S. P., S. Häussler, J. F. L. Heinz, B. Saremi, B. Mielenz, J. Rehage, S. Dänicke, M. Mielenz, and H. Sauerwein. 2014. Supplementation with conjugated linoleic acids extends the adiponectin deficit during early lactation in dairy cows. Gen. Comp. Endocrinol. 198: 13-21.

Smith, S. B., K. C. Lin, J. J. Wilson, D. K. Lunt, and H. R. Cross. 1998. Starvation depresses acylglycerol biosynthesis in bovine subcutaneous but not intramuscular adipose tissue homogenates. Comp. Biochem. Pysiol. 120B: 165-174.

Smith, S. B., R. L. Prior, and H. J. Mersmann. 1983. Interrelationships between insulin and lipid metabolism in normal and alloxan-diabetic cattle. J. Nutr. 113: 1002-1015.

Smith, S. B. 2017. CELL BIOLOGY SYMPOSIUM: Practical application of the basic aspects of GLUT4 membrane trafficking and insulin signaling on issues related to animal agriculture. J. Anim. Sci. 95(5): 2185-2197. doi: 10.2527/jas.2016.0984

Smith, S. B., and J. D. Crouse. 1984. Relative contributions of acetate, lactate and glucose to lipogenesis in bovine intramuscular and subcutaneous adipose tissue. J. Nutr. 114(4): 792-800.

Smith, S. B., H. Kawachi, C. B. Choi, C. W. Choi, G. Wu, and J. E. Sawyer. 2009. Cellular regulation of bovine intramuscular adipose tissue development and composition. J. Anim. Sci. 87(14): 72-82. doi: https://doi.org/10.2527/jas.2008-1340

Smith, S. B., D. K. Lunt, K. Y. Chung, C. B. Choi, R. K. Tume, and M. Zembayashi. 2006. Review article: Adiposity, fatty acid composition, and delta-9 desaturase activity during growth in beef cattle. Anim. Sci. J. 77: 478-486. doi: 10.1111/j.1740-0929.2006.00375.x

Song, S. H., S. S. McIntyre, H. Shah, J. D. Veldhuis, P. C. Hayes, and P. C. Butler. 2000. Direct measurement of pulsatile insulin secretion from the portal vein in human subjects. J. Clin. Endocrinol. Metab. 85(12): 4491-4499.

St John, L. C., D. K. Lunt, and S. B. Smith. 1991. Fatty acid elongation and desaturation enzyme activities of bovine liver and subcutaneous adipose tissue microsomes. J. Anim. Sci. 69: 1064-1073.

Stern, J. S., C. A. Baile, and J. Mayer. 1970. Growth hormone, insulin, and glucose in suckling, weanling, and mature ruminants. J. Dairy Sci. 54: 1052-1059.

Sturdivant, C. A., D. K. Lunt, G. C. Smith, and S. B. Smith. 1992. Fatty acid composition of subcutaneous and intramuscular adipose tissues and M. longissimus dorsi of wagyu cattle. Meat Sci. 32: 449-458.

Sugino, T., Y. Hasegawa, Y. Kikkawa, J. Yamaura, M. Yamagishi, Y. Kurose, M. Kojima, K. Kangawa, and Y. Terashima. 2002a. A transient ghrelin surge occurs just before feeding in a scheduled meal-fed sheep. Biochem. Biophys. Res. Commun. 295: 255-260.

Sugino, T., J. Yamaura, M. Yamagishi, A. Ogura, R. Hayashi, Y. Kurose, M. Kojima, K. Kangawa, Y. Hasegawa, and Y. Terashima. 2002b. A transient ghrelin surge of ghrelin secretion is modified by feeding regimens in sheep. Biochem. Biophys. Res. Commun. 298: 785-788.

Sun, Y., S. Ahmed, and R. G. Smith. 2003. Deletion of ghrelin impairs neither growth nor appetite. Mol. Cell. Biol. 23: 7973-7981.

Sun, Y., M. Asnicar, P. K. Saha, L. Chan, and R. G. Smith. 2006. Ablation of ghrelin improves the diabetic but not obese phenotype of ob/ob mice. Cell. Metab. 3: 379-386.

Sun, Y., P. Wang, H. Zheng, and R. G. Smith. 2004. Ghrelin stimulation of growth hormone release and appetite is mediated through the growth hormone secretagogue receptor. Proc. Natl. Acad. Sci. USA. 101: 4679-4684.

Takemoto, S., S. Tomonaga, M. Funaba, and T. Matsui. 2017. Effect of long-distance transportation on serum metabolic profiles of steer calves. Animal Science Journal. 88(12): 1970-1978. doi: 10.1111/asj.12870

Tatum, J. D., G. C. Smith, B. W. Berry, C. E. Murphey, F. L. Williams, and Z. L. Carpenter. 1980. Carcass characteristics, time on feed and cooked beef palatability attributes. J. Anim. Sci. 50: 833-840.

Theander-Carrillo, C., P. Wiedmer, P. Cettour-Rose, R. Nogueiras, D. Perez-Tilve, P. Pfluger, T. R. Castaneda, P. Muzzin, A. Schürmann, I. Szanto, M. H. Tschöp, and F. Rohner-Jeanrenaud. 2006. Ghrelin action in the brain controls adipocyte metabolism. J. Clin. Invest. 116: 1983-1993.

ThidarMyint, H., H. Yoshida, T. Ito, and H. Kuwayama. 2006. Dosedependent response of plasma ghrelin and growth hormone concentrations to bovine ghrelin in holstein heifers. J. Endocrinol. 189: 655-664.

Thompson, N. M., D. A. Gill, R. Davies, N. Loveridge, P. A. Houston, I. C. Robinson, and T. Wells. 2004. Ghrelin and des-octanoyl ghrelin promote adipogenesis directly in vivo by a mechanism independent of the type 1a growth hormone secretagogue receptor. Endocrinology. 145: 234-242.

Tilg, H., and A. M. Wolf. 2005. Adiponectin: A key fat-derived molecule regulating inflammation. Expert Opin. Ther. Targets. 9(245): 251.

Tomas, E., T. S. Tsao, A. K. Saha, H. E. Murrey, C. C. Zhang, S. I. Itani, H. F. Lodish, and N. B. Ruderman. 2002. Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain :Acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. Proc. Natl. Acad. Sci. USA. 99: 16309-16313.

Tonelli, J., W. Li, P. Kishore, U. B. Pajvani, E. Kwon, C. Weaver, P. E. Scherer, and M. Hawkins. 2004. Mechanisms of early insulin-sensitizing effects of thiazolidinediones in type 2 diabetes. Diabetes. 53(1621): 1629.

Toral, P. G., G. Hervás, H. Missaoui, S. Andrés, F. J. Giráldez, S. Jellali, and P. Frutos. 2016. Effects of a tannin-rich legume (onobrychis viciifolia) on in vitro ruminal

biohydrogenation and fermentation. Span. J. Agric. Res. 14(1): 1-9. doi: 10.5424/sjar/2016141-8989

Trenkle, A., and D. G. Topel. 1978. Relationships of some endocrine measurements to growth and carcass composition of cattle. J. Anim. Sci. 46: 1604-1609.

Tschöp, M., D. L. Smiley, and M. L. Heiman. 2000. Ghrelin induces adiposity in rodents. Nature. 407: 908-913.

Tschöp, M., C. Weyer, P. A. Tataranni, V. Devanarayan, E. Ravussin, and M. L. Heiman. 2001. Circulating ghrelin levels are decreased in human obesity. Diabetes. 50: 707-709.

Turer, A. T., A. Khera, C. R. Ayers, C. B. Turer, S. M. Grundy, G. L. Vega, and P. E. Scherer. 2011. Adipose tissue mass and location affect circulating adiponectin levels. Diabetologia. 54: 2515-2524. doi: 10.1007/s00125-011-2252-z

Turer, A. T., and P. E. Scherer. 2012. Adiponectin: Mechanistic insights and clinical implications. Diabetologia. 55: 2319-2326.

Turnbaugh, P. J., R. E. Ley, M. A. Mahowald, V. Magrini, E. R. Mardis, and J. I. Gordon. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 444: 1027-1031. doi: 10.1038/nature05414

Ukkola, O., M. Terán-García, A. Tremblay, J. Després, and C. Bouchard. 2008. Adiponectin concentration and insulin indicators following overfeeding in identical twins. J. Endocrinol. Invest. 31: 132-137.

Uriarte, M., P. N. DeFrancesco, G. Fernandez, A. Cabral, D. Castrogiovanni, T. Lalonde, L. G. Luyt, S. Trejo, and M. Perello. 2018. Evidence supporting a role for the blood cerebrospinal fluid barrier transporting circulating ghrelin into the brain. Mol. Neurobiol.

USDA. 1997. Official United States Standards for Grades of Carcass Beefin USDA, Agric. Market. Serv. Livest. Seed Div., Washington, DC.

van de Vossenberg, J. L. C. M., and K. N. Joblin. 2003. Biohydrogenation of C18 unsaturated fatty acids to stearic acid by a strain of *Butyrivibrio hungatei* from the bovine rumen. Lett. Appl. Microbiol. 37: 424-428.

Vanhatalo, A., T. Varvikko, and P. Huhtanen. 2003. Effects of casein and glucose on responses of cows fed diets based on restrictively fermented grass silage. J. Dairy Sci. 86(10): 3260-3270.

Vernon, R. G. 1979. Lipogenesis in ovine adipose tissue in culture. Ann Rech Vet. 10: 399-400.

Vernon, R. G. 1978. Lipogenesis in sheep adipose tissue maintained in tissue culture: Effects of insulin and growth hormone. Biochem. Soc. Trans. 6: 988-990. doi: 10.1042/bst0060988

Vernon, R. G. 1977. Effect of different fatty acids on lipogenesis in rat and sheep adipose tissue in vitro. Int. J. Biochem. 8: 517-523. doi: 10.1016/0020-711X(77)90114-8

Vernon, R. G., R. Clegg, and F. J. Flint. 1980. Insulin receptors and metabolic activity of sheep adipose tissue during pregnancy and lactation. Biochem. Soc. Trans. 8: 370-371. doi: 10.1042/ bst0080370

Vernon, R. G., E. Finley, E. Taylor, and D. J. Flint. 1985. Insulin binding and action on bovine adipocytes. Endocrinology. 116: 1195-1199.

Vernon, R. G. 1981. Lipid metabolism in the adipose tissue of ruminant animals. Pages 279-362 in Lipid metabolism in ruminant animals. Christie, W.W. ed. Oxford, Pergamon Press, Inc. doi: https://doi.org/10.1016/0163-7827(80)90007-7

Wallace, R. J., L. C. Chaudhary, N. McKain, N. R. McEwan, A. J. Richardson, P. E. Vercoe, N. D. Walker, and D. Paillard. 2006. *Clostridium proteoclasticum*: A ruminal bacterium that forms stearic acid from linoleic acid. FEMS Microbiol. Lett. 265: 195-201.

Wang, Y. H., K. A. Byrne, A. Reverter, G. S. Harper, M. Taniguchi, S. M. McWilliam, H. Mannen, K. Oyama, and S. A. Lehnert. 2005. Transcriptional profiling of skeletal muscle tissue from two breeds of cattle. Mamm. Genome. 16(3): 201-210. doi: 10.1007/s00335-004-2419-8

Wei, S., L. S. Zan, H. B. Wang, G. Cheng, M. Du, Z. Jiang, G. J. Hausman, D. C. McFarland, and M. V. Dodson. 2013. Adenovirus-mediated interference of FABP4 regulates mRNA expression of ADIPOQ, LEP and LEPR in bovine adipocytes. Gen. Mol. Res. 27: 494-505.

Wertz-Lutz, A. E., T. J. Knight, R. H. Pritchard, J. A. Daniel, J. A. Clapper, A. J. Smart, A. Trenkle, and D. C. Beitz. 2006. Circulating ghrelin concentrations fluctuate relative to nutritional status and influence feeding behavior in cattle. J. Anim. Sci. 84: 3285-3300.

Wortley, K. E., K. D. Anderson, K. Garcia, J. D. Murray, L. Malinova, R. Liu, M. Moncrieffe, K. Thabet, H. J. Cox, G. D. Yancopoulos, S. J. Wiegand, and M. W. Sleeman. 2004. Genetic deletion of ghrelin does not decrease food intake but influences metabolic fuel preference. Proc. Natl. Acad. Sci. USA. 101(8227): 8232.

Wu, X., R. S. Cooper, I. Borecki, C. Hanis, M. Bray, C. E. Lewis, X. Zhu, D. Kan, A. Luke, and D. Curb. 2002. A combined analysis of genome-wide linkage scans for body mass index

from the national heart, lung and blood institute family blood pressure program. Am. J. Human Genetics. 70: 1247-1256.

Wu, X., H. Motoshima, K. Mahadev, T. J. Stalker, R. Scalia, and B. J. Goldstein. 2003. Involvement of AMP-activated protein kinase in glucose uptake stimulated by the globular domain of adiponectin in primary rat adipocytes. Diabetes. 52: 1355-1363.

Yamauchi, T., M. Iwabu, M. Okada-Iwabu, and T. Kadowaki. 2014. Adiponectin receptors: A review of their structure, function and how they work. Best Pract. Res. Clin. Endocrinol. Metab. 28: 15-23.

Yamauchi, T., J. Kamon, Y. Ito, A. Tsuchida, T. Yokomizo, S. Kita, T. Sugiyama, M. Miyagishi, K. Hara, M. Tsunoda, K. Murakami, T. Ohteki, S. Uchida, S. Takekawa, H. Waki, N. H. Tsuno, Y. Shibata, Y. Terauchi, P. Froguel, K. Tobe, S. Koyasu, K. Taira, T. Kitamura, T. Shimizu, R. Nagai, and T. Kadowaki. 2003. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature. 423(6941): 762-769.

Yamauchi, T., J. Kamon, Y. Minokoshi, Y. Ito, H. Waki, S. Uchida, S. Yamashita, M. Noda, S. Kita, K. Ueki, K. Eto, Y. Akanuma, P. Froguel, F. Foufelle, P. Ferre, D. Carling, S. Kimura, R. Nagai, B. B. Kahn, and T. Kadowaki. 2002. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat. Med. 8: 1288-1295.

Yamauchi, T., J. Kamon, H. Waki, Y. Terauchi, N. Kubota, K. Hara, Y. Mori, T. Ide, K. Murakami, N. Tsuboyama-Kasaoka, O. Ezaki, Y. Akanuma, O. Gavrilova, C. Vinson, M. L. Reitman, H. Kagechika, K. Shudo, M. Yoda, Y. Nakano, K. Tobe, R. Nagai, S. Kimura, M. Tomita, P. Froguel, and T. Kadowaki. 2001. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat. Med. 7: 941-946.

Yang, J., M. S. Brown, G. Liang, N. V. Grishin, and J. L. Goldstein. 2008. Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. Cell. 132: 387-396.

Yokota, T., C. S. Meka, K. L. Medina, H. Igarashi, P. C. Comp, M. Takahashi, M. Nishida, K. Oritani, J. Miyagawa, T. Funahashi, Y. Tomiyama, Y. Matsuzawa, and P. W. Kincade. 2002. Paracrine regulation of fat cell formation in bone marrow cultures via adiponectin and prostaglandins. J. Clin. Invest. 109: 1303-1310.

Zembayashi, M., K. Nishimura, D. K. Lunt, and S. B. Smith. 1995. Effect of breed type and sex on the fatty acid composition of subcutaneous and intramuscular lipids of finishing steers and heifers. J. Anim. Sci. 73(11): 3325-3332.

Zhao, T. J., G. Liang, R. L. Li, X. Xie, M. W. Sleeman, A. J. Murphy, D. M. Valenzuela, G. D. Yancopoulos, J. L. Goldstein, and M. S. Brown. 2010. Ghrelin O-acyltransferase (GOAT) is essential for growth hormone-mediated survival of calorie-restricted mice. Proc. Natl. Acad. Sci USA. 107: 7467-7472.

Zigman, J. M., S. G. Bouret, and Z. B. Andrews. 2016. Obesity impairs the action of the neuroendocrine ghrelin system. Trends Endocrinol. Metab. 27: 54-63.

Zigman, J. M., and J. K. Elmquist. 2003. Minireview: From anorexia to obesity-the yin and yang of body weight control. Endocrinology. 144: 3749-3756.