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# The Effect of Supplementing Rumen Undegradable Unsaturated Fatty Acids on Marbling in Early-Weaned Steers

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# THE EFFECT OF SUPPLEMENTING RUMEN UNDEGRADABLE UNSATURATED FATTY ACIDS ON MARBLING IN EARLY-WEANED STEERS

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ABSTRACT

The objective of this study was to determine if supplementation of a rumen undegradable unsaturated fatty acid (FA) source would improve marbling in early-weaned steers. All steers (Angus, n = 23 and Angus × Hereford, n = 24) were weaned at 150 ± 5 d of age. Steers were blocked by BW and breed then randomly assigned to either control (CON; 1.5 kg of corn gluten feed (CGF), n = 23) or a rumen undegradable fat isocaloric supplementation (RUF; 200 g of Megalac-R in 1.06 kg of CGF, n = 24) for 110 d (fed 5 d/wk). All steers had *ad libitum* access to pastures throughout treatment. Steer BW and blood samples were collected at 0, 55, and 110 d of supplementation, and real-time carcass ultrasound measurements were collected at d 110. Following treatment, steers were transported to Oklahoma State University for finishing and subsequent harvesting at a commercial plant. All data were analyzed using PROC MIXED procedure of SAS either as a repeated measures or ANOVA, depending on response variables. There were no significant changes in BW from beginning of treatment to harvest due to treatment. Ultrasound data showed that RUF steers tended to have more intramuscular fat ($P = 0.08$) than CON steers at d 110. Serum FA concentration showed a treatment × day interaction ($P < 0.02$) on 16:0, 18:0, 18:1 *c*-9, 18:2, 20:4, and total FA. These specific FA concentrations increased throughout treatment in CON steers, but there was a greater increase in the concentration of RUF steers. Serum triglyceride and cholesterol concentrations were increased ($P < 0.01$) on d 55 and 110 in RUF steers compared to CON steers. Serum leptin concentration in RUF steers was greater ($P < 0.01$) than CON steers at d 110. Marbling scores of the RUF carcasses tended ($P = 0.09$)

to be higher than CON carcasses. There was a tendency ($P$ = 0.09) for increased percent total lipids in RUF steaks compared to CON steaks. There was also a tendency ($P$ = 0.06) for RUF steers to have an increased percentage of 20 - 30 μm adipocytes in their intramuscular depots compared to CON steers. The results of this study indicate that supplementation of rumen undegradable unsaturated FA may positively impact marbling deposition in early-weaned steers.

Keywords: early-weaning, marbling, rumen undegradable fat, steers

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# CHAPTER I

## INTRODUCTION

Over the past four decades, beef production has become more efficient by using only 69.9 % of the animals to produce 1 billion kg of beef. (Capper, 2011). Despite the improved efficiency in beef production, there is still a need to become more efficient in higher quality beef carcass production. Smith et al. (2000) reported that common carcass production problems producers face include excessive carcass weights, excessive back fat, low marbling scores, and lack of uniformity within harvest groups. Inadequacies in beef marbling and quality grades were also reported as the number one quality challenge facing the beef industry (Smith et al., 2006). Those inadequacies have a direct negative impact on beef cattle production profitability (DiCostanzo and Dahlen, 2000; Hogan et al., 2009; Cruz et al., 2012). Therefore, the beef industry is investigating methods to improve carcass quality.

Both the beef industry and beef cattle producers could benefit by improved nutritional and management strategies to increase carcass quality at harvest and therefore meet consumer demands (Sharman, 2012). Management strategies that are implemented prior to feedlot entry can influence the quality of the carcass (Anderson and Gleghorn, 2007) as adipogenesis has been shown to be active within skeletal muscle much earlier than can be visually detected (Wegner et al., 1998). The management practice known as early weaning has been shown to improve carcass quality through an increase in marbling scores (Harvey et al., 1975; Scheffler et al., 2014) and a decrease in Warner-Bratzler shear force values (Meyer et al., 2005). Early weaning extends the time producers can

influence marbling through nutritional supplementation, as the effectiveness of increasing the number of intramuscular adipocytes decreases after 250 days of age (Du et al., 2010). Traditionally, supplemental dietary fat was investigated to understand its influence on physiological processes in beef cattle like thermogenesis in newborn calves and female reproduction (Lammoglia et al., 1999 a, b; Hess, 2002). Recently, the effects of fat have been investigated in the feedlot phase, with hopes to increase marbling (Zinn et al., 2000; Gillis et al., 2004). Supplementing fats in the form of calcium salts has been shown to decreases the level of biohydrogenation of fatty acids in the rumen and therefore increase the amount of unmodified fatty acids available for absorption in the small intestines (Zinn et al., 2000; Huang et al., 2009). With the growing need to improve carcass quality in an efficient and consistent way, investigation into the combined efforts of effective nutritional strategies and pre-feedlot based management systems might bring the beef industry closer to its goals.

# CHAPTER II

# LITERATURE REVIEW

## Introduction

Beef quality and carcass price are primarily determined by the amount of marbling (USDA, 1997). One of the major goals of the beef industry is to increase the quality of beef carcasses and thereby the value to help increase profit margins. Intramuscular adipose tissue/marbling is positively associated with meat quality and therefore, an increase in the amount of marbling would increase carcass price. Meat quality is not only measured by the amount of intramuscular adipose tissue but can also be measured by consumer appeal based on palatability (Platter et al., 2005; Garmyn and Miller, 2014). Intramuscular adipose tissue is mainly comprised of adipocytes, which operate primarily to store excess energy in the form of triglycerides (Azain, 2004). Prior to becoming adipocytes, these cells begin their life as mesenchyme stem cells (Scanes, 2003), which have the potential to develop into preadipocytes, as well as myocytes, chondrocytes, and fibroblasts (Hausman et al., 1980; Gesta et al., 2007). The transformation process of preadipocytes into mature adipocytes is known as adipogenesis. Adipogenesis is affected by many positive and negative influential factors but this review will focus on some of the mechanisms that positively impact adipogenesis such as: peroxisome proliferator activated receptors (PPARs), retinoid X receptors (RXRs), and CCAAT/ enhancer binding proteins (C/EBPs). Also, nutrition can influence the development of intramuscular adipose tissue (Pethick et al., 2004). An animal's diet can provide adipocytes and their molecular mechanisms with the necessary components

to initiate, inhibit, and conserve all pathways involved in the transformation of preadipocytes into mature adipose cells. Once these intramuscular adipocytes grow into visible adipose tissue within the meat, it is then known as marbling (Harper et al., 2001). The amount of marbling in a beef carcass is under the influence of many factors including genetic, environmental, and nutritional factors (Harper and Pethick, 2004). Recently, nutritional influencers, such as fat supplementation, have been investigated to understand the effects on marbling in feedlot cattle (Zinn and Plascencia, 2004; Nelson et al., 2008; Araujo et al., 2010). Furthermore, certain management practices, such as creep feeding, early weaning, and different nutritional strategies implemented during the backgrounding, stocker, and finishing stages, have also proven to be influential. Therefore, it is important to understand the factors that influence carcass quality and palatability, and the influence quality has on consumer willingness to purchase and thereby, the economic value. This review will focus on the links between adipose tissue development, its effects on carcass quality and consumer appeal, and also the effects of early-weaning on carcass quality.

## Adipogenesis

Preadipocytes, a type of stromo-vascular cell, and mature adipocytes, also known as fat cells, are the two types of cells that predominantly make up adipose tissue (Cornelius et al., 1994). The mature adipocytes take on a unique rounded shape as they fill with lipids in the form of triglycerides (Cianzio et al., 1985). Adipocytes, in general, can range in diameter from 10 – 250 μm. However, intramuscular adipocytes are smaller cells ranging from 40 – 90 μm in diameter (Cianzio et al., 1985). Adipose tissue is

capable of growth by both an increase in number of cells, hyperplasia, and size of cells, hypertrophy (Hood and Allen, 1973; Cianzio et al., 1985; Robelin, 1986). Intramuscular adipocytes grow in a biphasic pattern, first by a wave of hyperplastic growth, followed by a wave of hypertrophic growth, and this pattern persists throughout growth. (Allen, 1976).

Adipocytes primary function in mature animals is to act as a site for energy utilization and energy storage. Etherton and Walton (1986) showed that 80 - 90 % of the total body energy stores are located in the adipose tissue of animals at market weight. Adipose tissue undergoes lipogenesis, synthesis and storage of triglycerides when energy intake exceeds the metabolic energy needs of the animal. But when the animal experiences a negative energy balance, then lipolysis, the breakdown of triglycerides and release of free fatty acids, occurs (Azain, 2004; Nafikov and Beitz, 2007). In ruminants, lipogenesis de novo substrates are acetate and glucose/lactate (Pethick et al., 2004). Smith and Crouse (1984) showed that glucose/lactate is the main substrate in intramuscular depots and acetate for subcutaneous depots during lipogenesis. Adipocytes also function as a storage site for fat soluble vitamins and play a role in the immune and reproduction systems (Cornelius et al., 1994). Through the secretion of growth hormone, leptin, insulin-like growth factor 1, and other compounds, adipose tissue also acts as an endocrine gland (Doglio et al., 1987; Siiteri, 1987; Klein et al., 1996).

Adipogenesis produces brown adipose tissue (BAT) and white adipose tissue (WAT). Both white and brown adipose tissues are present in fetal tissue (Casteilla et al. 1987, Devasker et al. 2002). Brown adipose tissue contains a large amount of the unique

uncoupling protein 1 (UCP1) and mitochondria (Alexander et al., 1975; Landis et al., 2002; Cannon and Nedergaard, 2004). Although BAT only comprises a small percentage of fetal tissue, it is a major contributor in energy balance of fetuses (Smith and Horwitz, 1969; Cannon and Nedergaard, 2004). Energy is utilized from BAT through non-shivering thermogenesis (Poissonnet et al., 1984; Landis et al., 2002), which is the primary method of heat production to prevent hypothermia by most precocial mammals (Symonds et al., 1995; Klingenspor, 2003). Most visceral fat is composed of mainly BAT before birth, but after birth there is a transition from BAT to WAT due to an increase in the nutritional environment (Gemmell et al., 1972; Gemmell and Alexander, 1978; Clarke et al., 1997). White adipose tissue is involved in energy balance through lipid storage and metabolism, and it is the major site of energy storage in mammals (Gregoire et al., 1998).

Adipocytes are originally derived from either mesenchymal stem cells (MSC) (Kubota et al., 1989) or from a small percentage of satellite cells (Asakura et al., 2001). Very little research has been conducted to further understand this portion of the process of adipogenesis. However, *in vitro* research using the 3T3-L1 and 3T3-F442A preadipocyte cell lines show that these cells must evolve through multiple phases of mitosis and growth arrest before committing to terminal differentiation (Cornelius et al., 1994; MacDougald and Lane, 1995; Hwang et al., 1997; Gregorie et al., 1998). It is the development of established immortalized preadipocyte cell lines that enabled details of adipocyte differentiation to be studied (Smas and Sul, 1995). First, preadipocytes undergo proliferation by mitosis. The cells then reach confluence and are limited by cell-

to-cell contact. Once this contact occurs, the preadipocytes cease to proliferate and consequently undergo growth arrest. Smas and Sul (1995) state that it is the growth arrest rather than the act of confluence that is the necessary action to begin the path to differentiation. After growth arrest, cells will undergo several rounds of mitotic division (mitotic colonal division) under the influence of hormones including growth hormone (GH) and insulin-like growth factor-1 (IGF-1) (Green et al., 1985; MacDougald and Lane, 1995; Smas and Sul, 1995). In the 3T3-F442A cell line, GH has been shown to be necessary for differentiation to mature adipocytes and increase the sensitivity to mitogenic effects of IGF-1 for colonal expansion (Green et al., 1985; Corin et al., 1990; Guller et al., 1991). This response to hormones is a great example of the similarities between mature adipocytes produced *in vitro* and those produced *in vivo* (Rubin et al., 1977). The replication of DNA and the doubling of cells have been attributed to the clonal amplification of committed cells (Pairault and Green, 1979). During mitotic division, the continued replication of DNA is also thought to change the availability of promoter/enhancer regions to transcription factors for the genes involved in the start of differentiation (Cornelius et al., 1994). The preadipocytes terminally differentiate by expressing certain transcription factors that terminate mitosis and initiate the conversion of preadipocytes to mature adipocytes through the transcription of adipogenic genes (Christy et al., 1989; Freytag and Geddes, 1992; Lin and Lane, 1994; Tontonoz et al., 1994a; Bruns et al., 1996). Although preadipocyte cell lines have many of the same characteristics as *in vivo* adipose tissue, these culture cells are aneuploidy and subsequently could express different genes from that of *in vivo* adipocytes and/or

preadipocytes (Smas and Sul, 1995). However, there are studies showing that implantation of the differentiated culture cells into subcutaneous tissue of mice produce adipocytes that are histologically indistinguishable from the animal's WAT (Green and Kehinde, 1979; Vannier et al., 1985).

Over 100 proteins are involved in terminal differentiation of adipocytes (Sidhu, 1979). The two most well-known and influential transcription factors that regulate adipogenesis are peroxisome proliferator activated receptor γ (PPARγ) (Green, 1995; Schoonjans et al., 1996) and CCAAT/enhancer binding proteins α (C/EBPα) (Cao et al., 1991; Yeh et al., 1995). There are also several indirect promoters that influence differentiation such as insulin, cortisol, and FA; they need to be in combination with another promoter to truly influence adipocyte differentiation (Spiegelman and Green, 1980; Hauner et al., 1987; Teboul et al., 1995). Furthermore, PPARγ and C/EBPα play a role in the transcription of many genes and the expression of certain proteins including adipocyte protein 2 (aP2), glucose transporter 4 (GLUT-4), stearoyl-CoA desaturase-1 (SCD1), phosphenolpyruvate carboxykinase (PEPCK), and leptin (Tontonoz et al., 1994a, 1995; Long and Pekala, 1996; Miller and Natambi, 1996; Hollenberg et al., 1997).

Peroxisome proliferator-activated receptors is a family of transcription factors that are involved with gene expression and lipid metabolism (Issemann and Green 1990; Tontonoz et al., 1994, 1995; Bruns et al., 1996; Chawla et al., 1994; Hu et al., 1995). Peroxisome proliferator-activated receptor γ is a critical player in adipocyte differentiation because it can independently induce adipogenesis and it is required for adipogenesis (Tontonoz et al., 1994a; Tontonoz et al., 1994b; Barak et al., 1999; Kubota

et al., 1999; Rosen and Spiegelman, 2001). There are two isoforms of PPARγ, PPARγ1 and PPARγ2 (Tontonoz et al., 1994a; Zhu et al., 1995). Although both PPARγ1 and PPARγ2 isoforms are highly expressed in adipose tissue and promote the transition of preadipocytes to adipocytes, PPARγ2 is more highly expressed in adipocytes and with the addition of 30 N-terminal amino acids, PPARγ2 enhances the ability for PPAR ligands to bind (Tontonoz et al., 1994a; Zhu et al., 1995; Vidal-Puig et al., 1996). Tontonoz (1994b) used retroviral expression of PPARγ2 in addition to PPAR ligands to show that the combination could initiate adipogenic differentiation in fibroblasts. Therefore, the binding of these ligands to PPARγ leads to the downstream effects of gene transcription that initiates adipogenic cell differentiation.

Specifically, differentiation can be activated by PPARγ ligands such as fatty acids (Gottlicher et al., 1992; Banner et al., 1993; Kliewer et al., 1995; Schopfer et al., 2005), but oxidized fatty acids are a more effective ligand (Nagy, 1998). Polyunsaturated fatty acids such as linoleic, linolenic, arachidonic and eicosapentaenoic acid (EPA) are some natural ligands that bind to PPARγ (Kliewer et al., 1997; Xu et al., 1999). Various synthetic ligands also activate PPAR γ, one being thiazolidinediones (TZDs) that belongs to a class of antidiabetic agents that show a high-affinity for PPARγ (Lehmann et al, 1995). In cell culture, the use of TZDs induces the expression of PPARs and enhances adipocyte differentiation (Kletzien et al., 1992; Ibrahimi et al., 1994). Other synthetic ligands come from the J series of prostaglandins that are derived from $PGD_2$ especially, 15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$ (Yu et al., 1995; Forman et al., 1995; Kliwer et al., 1995).

Prior to ligand binding, these PPAR proteins form heterodimers with retinoid X receptor alpha (RXRα) allowing the PPAR/RXR complex to bind to a DNA sequence (Kliewer et al., 1992, Rodriguez de la Concepcion, 2004). Hausman (2003) stated that PPARγ binds to DNA in the nucleus. The PPAR/RXR heterodimer binds with the peroxisome proliferator response elements located on a target gene sequence (Ijpenberg, 1997; Tontonoz, 1995; and Schoonjans, 1997). Peroxisome proliferator-activated receptor γ begins differentiation by removing transcriptional regulators from DNA coding genes that controls with cell growth and cyclin-dependent kinase inhibitors (Rosen, 2000). Within the PPAR/RXR complex, RXRα is activated by its own ligand as is PPAR (Kliewer et al., 1992) giving RXRα some control over PPARγ (Heyman et al., 1992).

The C/EBP family consists of three isoforms known as, C/EBPα, C/EBPβ, and C/EBPδ, all of which play a vital role in adipogenesis. Two of the three isoforms, C/EBPβ and C/EBPδ, are precursors for the expression of C/EBPα and PPARγ (Cao et al., 1991; Yeh et al., 1995; Farmer, 2006; Rosen and MacDouglad, 2006; Lefterova and Lazar, 2009). Wu et al. (1996) conducted studies on C/EBPβ and C/EBPδ that showed observable adipocyte differentiation when C/EBPβ was overexpressed or was expressed normally in conjunction with the ectopic expression of C/EBPδ in adipocytes. From these results, it is suggested that adipogenesis can be credited to the expression of C/EBPα and PPARγ, following the expression of C/EBPβ and C/EBPδ. However, in the absence of PPARγ ligands, and if C/EBPα and PPARγ are expressed together, they can activate the transformation of preadipocytes into mature adipocytes (Tontonoz, 1994b). These findings support the concept that CEBPα and PPARγ work synergistically and

reciprocally to activate the transcription of one another and the downstream promoters of adipogenesis. A loss-of-function study was conducted by Rosen et al. (2001) using fibroblasts lacking PPARγ to investigate if C/EBPα's effect on adipogenesis is completely dependent on PPARγ. The study concluded that C/EBPα is necessary for adipogenesis, but only through the stimulation and maintenance of PPARγ; proving that PPARγ is the true direct effector of adipocyte differentiation.

**Linoleic Acid**

Omega 6 polyunsaturated fatty acids' (e.g. linoleic (18:2) and arachidonic (20:4) acid) effect on adipogenesis has been described as both pro-adipogenic (Okuno et al., 1997; Matsuo et al., 2002) and anti-adipogenic (Cleary et al., 1999; Massiera et al., 2003; Prentice, 2001). Both effects have been attributed to the level of cyclic adenosine monophosphate (cAMP) (Petersen et al., 2003). Arachidonic acid is responsible for triggering cAMP production through the synthesis and activation of prostacyclin (Gilliard et al., 1989, Negrel et al., 1989; Vassuax et al., 1992). Prostacyclin acts as a ligand to the cell-surface receptor of prostacyclin receptor. Together, they can activate the expression of C/EBP-β and C/EBP-δ (Catalioto et al., 1991; Aubert et al., 1999; Belmonte et al., 2001).

Linoleic acid (18:2) is the precursor for arachidonic acid (20:4; Rett and Whelan, 2011). Dietary sources of fatty acids can act as adipogenic hormones by binding to the PPARs to initiate adipogenesis and thereby, offers a link between excessive fat intake, increase in accretion of fatty acids into adipocytes, and increased fat mass (Alihaud, 1999). When activated C/ EBP-β and C/EBP-δ initiate the increased expression of

PPAR-γ (Cao et al., 1991; Yeh et al., 1995; Belmonte et al., 2001; Farmer, 2006; Rosen and MacDouglad, 2006; Lefterova and Lazar, 2009) which is known as the ultimate promoter of adipogenesis (Tontonoz et al., 1994a,b; Barak et al., 1999; Kubota et al., 1999; Rosen et al., 2001). Therefore, under the right circumstances, an increase in linoleic acid could ultimately increase the expression of PPAR-γ and therefore, adipogenesis.

## Adipose Depots

Beef cattle production profitability due to carcass characteristics is directly affected by adipose tissue and its quantity in different adipose depots (Cruz et al., 2012). For example, the amount of intramuscular fat affects the quality of the meat and therefore, the price of the carcass; while the amount of subcutaneous fat affects the lean meat yield, also known as cutability, of the carcass (Dikeman et al., 1998; Killinger et al., 2004). Bovine adipose depots differ in adipocyte size (Moody and Cassens, 1968) and time of depot development and growth. There are four main adipose depots in ruminants: intermuscular, intramuscular, visceral (including mesenteric, omental, and perirenal), and subcutaneous.

Many factors influence the amount and timing of fat accretion throughout the body but they are still not fully understood. It has been suggested that the partitioning of fat across different depots is effected by breed (Ledger, 1959; Charles and Johnson, 1976; Kempster et al., 1976; Williams, 1978) and that certain breeds have a higher potential for marbling (Pickworth et al., 2011). Differences in adipocyte deposition due to breed in cattle have even been shown to occur *in utero* (Taga et al., 2011). Nutrition can also

influence fat deposition. For example, Morris and Zemel (2005) investigated the effect of energy content of the diet on adiposity and determined it has an influence on the size of adipocytes. They reported that a diet with a greater energy content or more readily available energy can increase the size of adipocytes. Furthermore, Corah and McCully (2006) showed that management practices and nutrition can either work together or impede each other to alter where fat accretion occurs. Age has also been identified as a factor that influences when lipids begin to accumulate within adipocytes (Cianzio et al., 1982). This is important because unlike bone and muscle, the deposition of fat in animals continues to develop with age (Hood and Allen, 1973). Although much research has already been conducted to describe these factors, situations where fat deposition and accretion differ in cattle of the same breed and in similar management conditions (Pickworth et al., 2011) or when fat development differs within depots (Cianzio et al., 1985) highlight the need for further, more in-depth investigation.

The general order of adipose depot development is visceral, intermuscular, subcutaneous, and intramuscular (Vernon, 1981; Pethick et al., 2004). Although visceral depots develop earliest, only 20% of the fat in beef steers is associated with the viscera while the remaining 80 % is associated with the meat product of the carcass (Cianzio et al., 1985). Cianzio et al. (1985) conducted an experiment using steers from 11-19 mo of age investigating the adipocyte depot changes during growth. They classified the adipocyte size from largest to smallest as follows: kidney, pelvic, and heart fat (KPH), mesenteric, subcutaneous, intermuscular, and intramuscular.

Fat that is in close association with the viscera of an animal is known as the visceral fat depot. The perirenal fat of the visceral fat depot along with the fat associated with the heart and pelvic regions, in production, is converted into a carcass characteristic known as KPH (McPhee et al., 2008). The percent KPH of empty carcass weight is a factor that helps determine the carcasses' yield grade and therefore, value. Visceral depots have a much higher triglyceride turnover rate, indicating a higher metabolic rate, compared to subcutaneous (Murphy, 2006). Furthermore, fat cells in visceral depots have a higher leptin expression compared to subcutaneous fat (Chilliard et al., 2001; Ren et al., 2002). Many studies have been and are still being conducted to try to understand the deposition of visceral fat due to its relationship with many diseases in humans (Matsuzawa et al., 1995; Despres, 2007).

Intermuscular fat, also known as seam fat, refers to the fat between muscles. Throughout post-natal growth, the ratio of intermuscular to total fat decreases and thereby, we can conclude that it is an early developing depot (Cianzio et al, 1982). This depot is not directly related to carcass quality, but it would still be ideal for producers to reduce the fat deposition in the intermuscular depot (Hood and Allen, 1973; Du et al., 2011).

The subcutaneous fat depot is located beneath the surface of an animal's skin or hide. Hood and Allen (1973) suggested that subcutaneous adipose tissue is an earlier developing depot and that hyperplasia is nearly complete within that depot by 8 mo of age. However, Robelin (1981) concluded that during the growth period (15 - 65 % of mature weight) subcutaneous tissue experienced much more hyperplasia than other

adipose tissues. He further noted that subcutaneous fat is known to be a late developing tissue. For larger-framed steers, subcutaneous fat accumulated at a faster rate than total fat (Cianzio et al, 1982).

The visible flecks of white fat that are located within the muscle of an animal are known as marbling and make up the intramuscular fat depot (Harper et al., 2001). It is traditionally believed that intramuscular fat is a late developing depot due to hyperplasia still being active as late as 14 mo of age (Hood and Allen, 1973). However, Wegner et al. (1998) reported that adipogenesis is active within muscle as early as 6 months of age, much earlier than is evidenced by marbling. Therefore, intramuscular depots can instead be considered a late maturing depot. Oddy et al. (2000) reported that the intramuscular fat percentage at the end of the finishing period was related ($r = 0.364$) to the intramuscular fat percentage at the beginning of the feedlot period. Therefore, adipocytes that are present upon entering the feedlot continue to grow by fat accretion (Hood and Allen, 1973) and by the addition of new adipocytes (Leibel et al., 1989). The intramuscular depot increases at a similar rate to total fat in beef steers (Cianzio et al, 1982). Also, the largest adipocytes within the intramuscular depot are within the largest flecks of intramuscular tissue in a particular muscle (Moody and Cassens, 1968). This is another depot that is translated into a carcass characteristic. Intramuscular fat is converted into a percent of fat within the muscle to help predict the quality grade of meat product (Jost et al., 1983; McPhee et al., 2008).

According to Cianzio et al. (1985), the majority of fat produced in an animal is found in the carcass. In the current beef industry, there is a desire to increase the amount

of intramuscular fat while decreasing the amount of subcutaneous, intermuscular, and visceral fat. This would be ideal since the subcutaneous, intermuscular, and viscera depots contribute to excess fat trim and economic loss instead of a positive effect on meat quality (Hood and Allen, 1973). Through continued investigation and a greater understanding of the processes of the development of these fat depots, scientists and producers might better be able to effectively increase the quality of beef while decreasing excess fat.

## Marbling

Marbling, the visible white flecks of adipose tissue, is located within the muscle fibers of skeletal muscle (Harper et al., 2001). Marbling is often referred to as intramuscular adipose tissue. Adipocytes are not the sole component of intramuscular adipose tissue, which also includes connective tissue and a blood supply (Harper and Pethick, 2004). The intramuscular adipose tissue is visible to the human eye when there is a cluster of 10-15 cells (Cianzio et al., 1985; May et al., 1994; Lee et al., 2000). It is the white flecks of fat that USDA graders visually assess to assign the carcass a USDA Quality Grade (Greiner, 2002). The amount of marbling, through the quality grade, has an influence on the value of the carcass as well (USDA, 1997). However, there is a base value (price) for beef that involves carcass weight, quality grade, and yield grade and then accounts for any premiums or discounts (i.e., USDA Premium grade, USDA Standard grade, dark cutter, ect.). The base price usually falls on carcasses weighing between 249.5 and 408.2 pounds, grading USDA Choice and having a USDA Yield Grade of 3 (DiCostanzo and Dahlen, 2000; Hogan et al., 2009). Like many other beef

industries, in the United States an increase in marbling leads to an increase in the carcasses quality (Japan Meat Grading System Association, 1988; USDA, 1997; Canadian Beef Grading Agency, 2006).

Furthermore, marbling influences the carcass quality through means other than the USDA grade. The amount of intramuscular fat influences the tenderness, juiciness, and flavor of meat, also known as palatability (Jost et al., 1983). Tenderness is generally assigned by consumers as the greatest contributor to overall palatability (Smith, 1972; Savell et al., 1987; Miller et al., 1995, 2001). Tenderness is influenced by multiple traits including the actomyosin effects- the contractile state of actomyosin and/or integrity of the Z line, the amount of connective tissue within the meat, and the amount of intramuscular fat that replaces protein in the meat along with the intramuscular distribution throughout the meat (Smith et al., 1973). Wood (1990) attributed tenderness to the location of the adipose tissue between muscle fiber bundles; it aids in breaking up the muscles structure allowing for easier breakdown in the consumers mouth. The tenderness of meat is often measured by the Warner-Bratzler shear force (WBSF) value and a negative correlation has been shown between the WBSF value and the age or growth of cattle (French et al., 2001; Purchas et al., 2002). Juiciness, however, is a product of initial fluid release and retained juiciness that occurs due to fat's influence on salivation (Weir 1960; Bratzler, 1971). Pearson (1966) reported that the initial fluid release is affected by the method of cooking and the level of doneness, but the retained juiciness is due to the intramuscular fat content. The variations in juiciness can be due to a particular meat's adipose content, moisture content, and water-holding capacity (Berry,

1972). The water-holding capacity of meat can be attributed to moisture being stored within muscle by being trapped by lipids within the adipose tissue and subsequently lubricate the muscle fibers during heating (Smith and Carpenter, 1974; Wood et al., 2004). When tenderness is deemed acceptable, flavor becomes the next important factor to consumers (Goodson et al., 2002; Killinger et al., 2004; Behrends et al., 2005a, 2005b). The combination of taste and odor make up the flavor in beef (Legako et al., 2015). The fat within meat can influence flavor through the effects of fatty acids on oxidation and the storage of odorous compounds (Hornstein, 1971). Many of the influential compounds include: hydrocarbons, aldehydes, ketones, alcohols, furans, thriphenes, pyrrols, pyridines, pyrazines, oxazols, thiazols, sulfurous compounds, and others released during heating (Hornstein, 1971; Ho et al., 1994; MacLeod, 1994). The influence of fatty acid content on meat flavor differs between ruminants and non-ruminants due to differences in digestion of fatty acids (Calkins and Hodges, 2007).

Studies have identified the relationship between marbling and palatability as low-to-moderate (r = 0.24 - 0.84) (Briskey and Bray, 1964; Jeremiah et al., 1970; Smith et al., 1987, Emerson et al., 2013). Smith et al. (1984), however, reported that the correlation (r = 0.24 – 0.34) was strong in specific muscles such as the *Longissimus dorsi* (LD) from young (A-maturity) carcasses. Marbling scores, which are determined from the 12$^{th}$ rib LD muscle area, are used to assign beef carcass quality grades. The USDA has eight Quality Grades for beef including the following: USDA Prime, Choice, Select, Standard, Commercial, Utility, Cutter, and Canner. In addition to the marbling within the area of the 12$^{th}$ rib LD, the age of the carcass is determined. The physiological maturity is

assessed by the color of lean in the LD and the amount of ossification in the vertebral column of each carcass (USDA, 1997).

Consumer satisfaction is a major contributor in the demand for beef (Moeller and Courington, 1998). Gaining more insight into consumer willingness to purchase beef of different quality grades would help the industry understand the level of economic incentive available for improving the quality and palatability of beef products (Platter et al., 2005). According to The Beef Customer Satisfaction Survey, although many factors affect the overall like ratings of cuts including top round, top sirloin, and top loin steaks, they found that flavor and tenderness contribute equally high influence on the overall like ratings (Neely et al., 1998, 1999; Lorenzen et al., 1999; Savell et al., 1999). In addition, recent studies have shown consumers favor the taste of meat with a higher marbling score, according to the current grading system (Platter et al., 2003; Killinger et al., 2004; Emerson et al., 2013), and that consumers take quality grades into account when selecting steaks for purchase (Mennecke et al., 2007). Platter et al. (2005) conducted a consumer's willingness-to-pay study where they investigated the influence of palatability traits (in the form of marbling scores and WBSF) on a consumer's willingness to pay. They utilized an experimental method known as auction bidding that is believed to produce a more reliable estimate of consumers' willingness to pay compared to the more traditional hypothetical willingness-to-pay survey method (Davis and Holt, 1993; Kagel and Roth, 1995). Platter et al. (2005) concluded that consumers were more willing to bid on steaks with a high marbling score or a low WBSF value. They also observed that as WBSF values increased it was more likely that consumers would not bid on that steak. The

authors stated that consumers were more likely to refuse to bid on steaks in the Low Choice and Select grades than Premium Choice or Premium grades.

It is evident that marbling plays a major role in the quality and value of meat. The primary determinant for carcass price, for the USDA beef grading system, is the abundance of marbling (USDA, 1997). Marbling is primarily composed of intramuscular fat and is valued at the 12$^{th}$ rib LD muscle. The amount of marbling influences the tenderness, juiciness, and flavor of the beef product. Those three meat characteristics influence the consumers' level of acceptance of the product. Altogether, marbling is a visual measure of meat quality, an influencer of meat palatability, and a determinate of consumers' willingness to pay.

## Early Weaning

Beef calves are traditionally weaned between 180-250 days of age in the United States (Williams et al., 1975; Neville et al., 1977; Richardson et al., 1978; Pickworth et al., 2012). When calves are weaned prior to 180 days, this practice is known as early weaning (Bellows et al., 1974; Harvey et al., 1975). Weaning calves early can be implemented to aid producers in situations where cows lack optimal body condition at the time of calving (Lusby et al., 1981) or when forages are insufficient in quality and/or quantity (Peterson et al., 1987). Under those circumstances, early weaning is a tool that can be used to reduce the nutrient requirements of the lactating female and will allow her to more easily gain weight and body condition (Story et al., 2000). Also, as a dam's milk production begins to decline around two to three months of lactation, the calf is not receiving a sufficient amount of nutrients from the dam anymore and the dam is

expending a lot of energy to continue supplying that milk (Robinson et al., 1978; Scheffler et al., 2014). Therefore, weaning calves early can benefit the calf by offering a more nutrient dense diet and the dam by decreasing her energy output and transitioning her energy utilization to improving her own weight and body condition. As for feed availability, early weaning can greatly reduce the amount consumed by both calf and cow while also improving the calf's feed efficiency (Peterson et al., 1987).

Whatever the motivation is for using an early weaning system, this production method potentially poses both positive and negative effects for a producer. Shortening the lactation period can improve the dam's reproductive efficiency by returning her to cyclicity sooner following parturition and increasing her average daily gain (ADG) (Myers et al., 1999). Dams also benefit from early weaning through an increase in conception rate, reduced postpartum period, reduced feed intake, and expansion of cull cow market (Bellows et al., 1974; McKee et al., 1977; Lusby et al., 1981). Additionally, a first-calf heifer can increase her body condition score (BCS), improve pregnancy rate, and decrease her calving interval (Arthington and Kalmbacher, 2003). Ciminski et al. (2002) reported that for every 2 weeks a calf suckles on a spring-calving cow, that cow loses one tenth of a BCS. Therefore, weaning at 150 days rather than 210 days can save a cow almost half a BCS. However, this effect on BCS could be due to region and production schedule as this study was performed on the sandhills range where the forages available are very different. It has also been stated that even if a cow has a low BCS at calving, if her calf is weaned before or early in the breeding season, the cow's

reproductive performance can still be improved (Laster et al., 1973; Lusby et al., 1981; Barnes et al., 1996).

Early weaning also offers many benefits to calf production such as an increase in ADG and overall body weight (BW), heavier calves at time of normal weaning, decrease in grazing pressures, improved feedlot performance, and decreased WBSF value prior to aging (Arthington et al., 2005, Meyer et al., 2005; Scheffler et al., 2014). Furthermore, there are many economic benefits through improved reproductive performance of dams, expansion of calf marketing options, production of younger calves at slaughter, improved carcass quality, higher marbling scores, larger longissimus muscle area (LMA), and a greater feed efficiency (Lusby et al., 1981; Whittier, 1995; Fluharty et al., 2000; Meyer et al., 2005; Scheffler et al., 2014).

From a nutritional standpoint, early weaning can benefit the calf by enhancing the effects of nutritional treatments. For instance, Harper and Pethick (2004) explained that, because younger animals are more likely to have a greater number of multipotent stem cells and preadipocytes, it would be most effective to apply a nutritional treatment early on in the animal's life. One study that involved supplying high starch vs. low starch diets to early-weaned steers concluded that the diets effects were greatest during the early growing phases (Grauganard et al., 2010). Vernon (1981) stated that intramuscular fat develops late in life; however, Wegner et al. (1998) reported that the early stages of adipocyte development within the muscle occurs much earlier than can be detected by macroscopic expression of the traits. Therefore, depending on what the producer is hoping to accomplish, it is important to keep in mind that the age of the animal has a

great impact on adipocyte development and that developmental programs involving growth and age can influence significant changes in the distribution of fat and overall fat accumulation (Vernon, 1981; Kirkland et al., 2002).

Methods of weaning calves will vary from producer to producer but there are three weaning systems that are the most generally used weaning styles. Rasby (2007) stated that the most common method was to move calves away from any sort of contact with the dam. Another practice is to move the dams away from the calves for an initial 3-5 days. After that time period the calves' and the dams' locations are reversed. This method is said to reduce stress of the calves by maintaining a familiar environment during the first part of weaning. Price et al. (2003) described a method known as fence-line weaning. This method involves placing calves and dams in adjacent pastures that allows them nose-to-nose contact through the fence-line. This method is thought to minimize stress through maintained contact but still prevents suckling.

There have been numerous studies conducted to investigate early weaning methods and the effects of early weaning on both calf and dam. However, most early weaning studies are done in conjunction with a nutrition study, making it impossible to determine the effects of early weaning without the nutritional influences. Therefore, we will look into a few typical feeding practices of early weaned calves and their positive and negative effects.

Scheffler et al. (2014) conducted a study using 24 Angus-sired steers; half were early weaned (EW) at 105 ± 6 days of age and the other half were weaned normally (NW) at 253 ± 6 days of age. The early-weaned steers were fed a high-forage diet for

148 days while the normal-weaned steers remained on pasture with their dams. After the 148 days of treatment, both groups were put on pasture for 154 ± 6 days. Finally, both groups were moved to a feedlot and transitioned to a high-concentrate diet. This study showed that the EW steers were heavier than the NW steers at normal weaning age, the EW steers had a greater ADG from 105-253 days and were heavier entering the feedlot, and the EW steers finished with heavier carcasses, and higher marbling scores (12 scoring Prime). However, not all benefits were in favor of early weaning. The NW steers actually gained more during the pasture grazing phase. No difference was shown between NW and EW steers in feedlot performance, yield grade, 12$^{th}$ rib fat thickness, or carcass adjusted ribeye area.

Another early weaning study performed by Fluharty et al. (2000) used 78 Angus crossed cow-calf pairs. Thirty-nine of the calves, from the cow-calf pairs, were weaned early at the age of 103 ± 3 days, while the other 39 calves remained on pasture with their dams. The early-weaned calves were placed in a single pen and fed a high-concentrate diet for 100 ± 3 days. At 203 ± 3 days, the other 39 calves were weaned from their dams and were shipped to a feedlot along with the EW calves. The calves were given 29 days to adjust and for quarantine. After 29 days, the calves were assigned to one of four treatment groups. The four treatment groups were 1) *ad libitum,* 100 % crude protein (CP); 2) *ad libitum,* 120 % CP; 3) step-up program, 100 % CP; and 4) step-up program, 120 % CP. The step-up program worked such that the calves allotted were fed 85% the amount of feed that the *ad libitum* group received for 55 d. From day 56-112 the calves were fed 92.5 % of *ad libitum* and from 113 d until slaughter, they were fed *ad libitum.*

All calves were harvested at 545 ± 10 kg BW. In this experiment, EW steers had a 56 % higher ADG prior to normal weaning, but NW calves had a higher ADG for the first 55 days of finishing and there was no statistical difference for the remainder of the experiment. Early-weaned calves showed a higher BCS prior to normal weaning, heavier weights at normal weaning and upon entering the feedlot, and greater gain from early weaning to normal weaning. Additionally, the EW calves reached market weights 33 days earlier, on average, than NW calves.

In addition, Myers et al. (1999) conducted a two year study to evaluate differences between EW and NW steers while applying a creep-feeding phase to half of the NW steers. The second year also compared different breeds of cattle. The same three treatments were used in both years of the experiment. The treatments were as follows: 1) early weaned (EW) and put on a finishing diet; 2) supplemented for 55 d while nursing on dam in pasture and then weaned at a normal age and put on a finishing diet (NWC); and 3) unsupplemented but remained on pasture to nurse on dam until normal weaning and then placed on a finishing diet (NW). The first year study started with 84 Angus × Hereford steers and allocated 28 steers per treatment. The EW steers were weaned at 177 ± 9 d of age, while the NWC and NW steers were weaned at 231 ± 9 d of age. The second year study had 167 steers (83 = Angus × Hereford, 40 = Angus × Simmental, 44 = Angus × Wagyu) and allocated 56 steers were allocated per treatment. The EW steers were weaned 158 ± 21 d of age, while the NWC and NW steers were weaned at 213 ± 21 d of age. All steers were fed until they reached the desired BCS end point. Results from this study revealed that the EW steers were 19 kg heavier at harvest than the average

harvest weight of NWC steers with creep feed and NW steers without creep feed for both year one and two. Early-weaned steers also had 11 kg heavier carcasses than the average of NWC and NW for year one and 9 kg heavier carcasses in year two. The EW steers were more efficient for both years of the study compared to the average of NWC and NW. During the first year's study, EW had a greater height change than the average of NWC and NW. Also, EW steers exhibited a slightly higher yield grade and an increased marbling score for both years compared to the average of NWC and NW, although there was no difference in the percentage of assigned yield grades between treatments. The marbling scores showed improved percentage of steers grading USDA average Choice or higher in both years but in the second year the EW steers also had a greater percentage that graded USDA Prime. Despite these benefits, this study showed the EW steers had to be fed for a longer amount of time compared to the average length of NWC and NW for both years, although age at slaughter was similar. Early weaned steers had an increase in percentage of kidney, pelvic, and heart fat compared to NWC and NW for both years. In addition to changes in calf performance and carcass traits, this experiment showed improved performance of cows that had calves that were weaned early. This improved performance was shown through a heavier body weight at the time of normal weaning, increased ADG, higher BCS, and improved pregnancy rates. However, it is important to note the wide range of weaning ages in this experiment which could lead to skewed and inaccurate results.

In conclusion, early weaning can be implemented for many reasons and can be tailored to a producers' situation. Early weaning offers many benefits including;

increased calf feed efficiency, BW, ADG, and marbling score, along with increased dam conception rate and ADG and decreased energy output and postpartum period. However, there are some drawbacks to early weaning such as an increase in cost of production and management time and effort. Results between studies differ by both benefits and drawbacks. Therefore, it is ultimately up to the producer to assess their particular situation and determine if early weaning is right for their herd.

## Fat Supplementation

Fat is primarily used as an energy source in ruminant diets because it contains 2.25 times the energy value compared with carbohydrate and protein sources (NRC, 2007). Dietary fat can also be used to decrease the dustiness of feed and improve palatability. However, Hess et al. (2007b) stated that there is an increase in the interest in fat supplementation due to its influence on physiological processes and/or alteration of fatty acid composition of ruminant food products. Diets that contain > 5 % of tallow or animal and vegetable fat blends have shown to decrease DM intake and fiber digestibility (Byers and Schelling, 1993; Coppock and Wilks, 1991); however, the 2001 Dairy NRC stated that fat could be included in the diet up to 6 - 7 %.

Common fat sources utilized in ruminant diets include: canola, cottonseed, safflower seed, soybeans, and sunflower seed (Staples et al., 1998; Williams and Stanko, 1999). Beef cattle supplemental fat also includes calcium soaps of fat, FFA, fishmeal, flaked fat, hydrogenated fat, medium-chain, prilled fat, tallow, triglycerides, and yellow grease (Staples et al., 1998; Williams and Sanko, 1999). Fats from oilseeds are mostly comprised of polyunsaturated fatty acids (PUFAs) while rendered animal fats (tallow and

yellow grease) and refined fats (prilled fats and calcium soaps) are mostly comprised of monounsaturated (MUFAs) and saturated fats, respectively (Coppock and Wilks, 1991)

Upon entry into the rumen, dietary fat undergoes hydrolysis, the process of separating fatty acids from glycerols found in triglycerides, phospholipids, and galactolipids (Funston, 2004). The free fatty acids then undergo biohydrogenation. During biohydrogenation, the microorganisms within the rumen remove the double bonds of unsaturated fatty acids and sometimes shift their orientation (Mattos et al., 2000) as unsaturated fatty acids are toxic to the microorganisms (Maia et al. 2010). Intestinal digestibility of supplemental fat is low, due to 65 % of dietary fatty acids undergoing complete ruminal biohydrogenation (Plascencia et al., 1999). Though few unprotected fatty acids escape the rumen without undergoing complete biohydorgenation, the small intestines are the major site of fatty acid absorption. High concentrate diets that result in a decreased ruminal pH (Van Nevel and Demeyer, 1995; Van Nevel and Demeyer, 1996), diets high in fat that negatively impact the microorganisms (Jenkins, 1993), and supplemental fat in the form of calcium salts can all decrease the rate of biohydorgenation within the rumen (Jenkins and Palmquist, 1984 and Wu et. al., 1991).

Despite the high percentage of biohydrogenation of fatty acids in the rumen, many studies have shown that fat supplementation can have a positive influence on carcass composition and performance (Zinn, R. A., 1989; Zinn, R. A., 1992; Zinn, R. A., and A. Plascencia, 1996; Zinn et al., 1998; Andrae et al., 2001; Zinn, R. A., and A. Plascencia, 2004; Pavan et al., 2007; Nelson et al., 2008). These studies reported that supplemental fat at certain levels ($\leq$ 6 %) can increase feed efficiency, dietary $NE_m$ and

dressing percentage of cattle (Zinn, 1989, Zinn and Plascencia, 1996; Zinn et al., 2000; Zinn and Plascencia, 2004; Pavan et al., 2007; Nelson et al., 2008). In diets with an energy density up to 1.90 Mcal/kg $NE_m$, the addition of fat to the diet can increase energy intake and subsequently increase ADG and feed efficiency (Plegge et al., 1984). Increasing the supplemental fat level has been reported to decrease ADG, DMI, feed efficiency, and dietary NE (Zinn and Plascencia, 2004). However, the method of supplementation does not affect the dietary NE (Zinn and Plascencia, 2004). Studies reported an increase in marbling score due to fat supplementation (Zinn, 1989; Zinn and Plascencia, 1996; Andrae et al., 2001). However, other adipose depots (KPH and subcutaneous) increased with fat supplementation as well (Zinn, 1989; Pavan et al., 2007; Nelson et al., 2008). Fat supplementation has been reported to alter the fatty acid content of the meat (Andrae et al., 2001; Nelson et al., 2008).

Although the majority of dietary fat undergoes major alterations within the rumen through hydrolysis and biohydrogentaiton; it still has the potential to positively influence carcass composition and performance. The effects of fat supplementation may differ due to fat source, level of fat provided, or main diet composition. The full effect of fat supplementation on beef carcasses is not fully understood and therefore, requires additional research.

## Summary

The beef industry's search for improved quality has led to many methods of improving carcass quality. However, a greater understanding of the development of the adipocytes within the muscles of the carcass is necessary. First, a better understanding of

the pathways of MSC and their development into adipocytes is needed (Kubota et al., 1989). Continued use of established immortalized preadipocyte cell lines, as well as conducting more *in vivo* studies will further our understanding of the process of adipogenesis. The two most influential transcription factors involved with adipogenesis, PPARγ and C/EBPα, are also the most well-known (Cao et al., 1991; Yeh et al., 1995; Green, 1995; Schoonjans et al., 1996) and can continue to further our understanding. Linoleic acid's influence on adipogenesis, as PPAR ligand and precursor to downstream adipogenic reactions also plays a major role (Gaillard et al., 1989; Negrel et al., 1989; Vassaux et al., 1992; Kliewer et al., 1994). Additional work needs to be done with PPARγ ligands, as PPARγ is the ultimate promoter of adipogenesis (Tontonoz et al., 1994a,b; Barak et al., 1999; Kubota et al., 1999; Rosen et al., 2001) and is dependent on its ligands to initiate it. If we can understand the substances that influence the ultimate promoter of adipogenesis, then we can potentially control the occurrence and rate of adipocyte development and growth.

In addition to searching for a better understanding of adipogenesis, there is a desire to develop ways to influence adipogenesis within specific depots. For example, the intramuscular fat depot has a positive influence on carcass quality (Jost et al., 1983; McPhee et al., 2008). It would be advantageous to increase the amount of marbling and subsequently, the consumers' willingness to pay (Platter et al., 2003; Killinger et al., 2004; Emerson et al., 2013). In contrast, subcutaneous and visceral fat depots have a negative effect on yield grade, and therefore carcass price (USDA, 1997; Pickworth, 2009). An increase in subcutaneous and visceral fat leads to a decrease in cutability

(USDA, 1997; Dikeman et al., 1998; Killinger et al., 2004); therefore, it would be advantageous to decrease the amount of adiposity in these depots.

Although marbling has traditionally been thought of as a late-developing adipose depot (Hood and Allen, 1973; Cianzio et al., 1985; May et al., 1994; Schoonmaker et al., 2004a), the investigation into the early-weaning production practice has shown an improvement in carcass quality through increased marbling in calves that are weaned early and fed a high-concentrate diet (Loy et al., 1999, Schoonmaker et al., 2003; Schoonmaker et al., 2004ab). Improved marbling, as well as feedlot performance, has also been reported from the supplementation of fat (Zinn, 1989, Zinn and Plascencia, 1996; Zinn et al., 2000; Andrae et al., 2001; Zinn and Plascencia, 2004; Pavan et al., 2007; Nelson et al., 2008). The findings from early-weaning studies combined fat supplementations and the understanding that final intramuscular fat percentage is related to the intramuscular fat percentage at the beginning of the feedlot phase (Oddy et al., 2000) leads to the conclusion that the early stage of growth is a critical period for the deposition of intramuscular adipose tissue despite it being a late maturing depot (Pethick et al., 2004; Wegner et al., 1998).

# CHAPTER III

## THE EFFECT OF SUPPLEMENTING RUMEN UNDEGRADBALE UNSATURATED FATTY ACIDS ON MARBLING IN EARLY-WEANED STEERS


### Abstract

The objective of this study was to determine if supplementation with Megalac-R, rumen undegradable unsaturated fatty acids (FA), improved marbling in early-weaned steers. All steers (Angus, n = 23 and Angus × Hereford, n = 24) were weaned at 150 ± 5 d of age. Steers were blocked by BW and breed then randomly assigned to either control (CON; 1.5 kg of corn gluten feed (CGF), n = 23) or isocaloric supplementation of a rumen undegradable fat source (RUF; 200 g of Megalac-R in 1.06 kg of CGF, n = 24) for 110 d (fed 5 d/wk). All steers had *ad libitum* access to pastures throughout treatment. Steer BW and blood samples were collected at 0, 55, and 110 d of supplementation, and real-time ultrasound measurements were collected at d 110. Following treatment, steers were transported to Oklahoma State University for finishing and subsequent harvesting at a commercial plant. All data were analyzed using PROC MIXED procedure of SAS either as a repeated measures or ANOVA depending on parameters. There were no significant changes in BW from beginning of treatment to harvest due to treatment. Ultrasound data showed that RUF steers tended ($P = 0.08$) to have more intramuscular fat than CON at d 110. Serum concentrations of FA showed a treatment × day interaction ($P < 0.02$) for 16:0, 18:0, 18:1*c*-9, 18:2, 20:4 and total FA. These specific FA concentrations slightly increased in CON steers, but there was a more pronounced increase in the concentration of these FA across the supplementation period in EFA steers. Serum triglyceride and cholesterol concentrations were increased ($P < 0.01$) on d

55 and 110 in RUF steers compared with CON steers. Serum leptin concentration in RUF steers was greater ($P < 0.01$) than CON steers at d 110. After slaughter, yield grades of the RUF carcasses were greater ($P = 0.04$) than CON carcasses. Marbling scores of the RUF carcasses tended ($P = 0.09$) to be higher than CON carcasses. There was a tendency ($P = 0.09$) for the percentage of total lipids to increase for RUF steaks compared with CON. There was also a tendency ($P = 0.06$) for RUF to have a greater percentage of 20 - 30 μm adipocytes in their intramuscular depot than CON. The results of this study may indicate that supplementation of unsaturated FA can positively impact marbling deposition in early-weaned steers.

## Introduction

Currently, one of the interests of the beef industry is finding methods to improve the quality of carcasses. One of the most common ways to increase carcass quality is by increasing the amount of intramuscular (IM) fat, also known as marbling. By increasing marbling, one increases the palatability traits including tenderness, juiciness, and flavor (Jost et al., 1983) while also increasing the carcass price (USDA, 1997). A production practice known as early weaning has been shown to improve carcass quality by increasing marbling scores (Harvey et al., 1975; Scheffler et al., 2014) and decreasing the Warner-Bratzler shear force values (Meyer et al., 2005). This may be due to adipogenesis being active within the muscle much earlier than is evident by marbling (Wegner et al., 1998). The utilization of supplemental fats in cattle diets has also been investigated for many years (Ashes et al., 1992; Moallem et al., 1997; Staples et al., 1998; Duckett et al., 2002). Producers have begun to expand their investigation of fat

supplementation from reproduction to the influence on fatty acid (FA) content within the meat (Hess et al., 2007a). Furthermore, calcium salts have been shown to decrease biohydrogenation of FA within the rumen which will increase the amount of unsaturated fats available for postruminal absorption (Zinn et al. 2000; Huang et al., 2009). For this study, we combined nutritional and environmental influences with an objective to investigate the effects of supplemental rumen undegradable unsaturated fatty acids and early weaning on marbling in beef steers.

## Materials and Methods

All procedures were approved by Clemson University's Institutional Animal Care and Use Committee (AUP# 2013-013).

### Animals

Forty-seven steer calves with an average weaning weight of 210.5 kg were utilized for this experiment. All steers were castrated within 24 h of birth. All steers were Angus sired out of an Angus (AN) or an Angus × Hereford (AN/HP) dam between 2 to 17 yr of age. Steers were weaned at 150 ± 5 d and were stratified into 6 blocks according to time of weaning. Following weaning, the steers underwent a 14 d adaptation period in a pen separated from their dams. Seven d post weaning, the steers were introduced to individual feeding using a 20:80 ration of corn gluten feed (CGF) and oats. Immediately after the adaptation period, the steers began treatment. Steers were assigned to one of two treatment groups for 110 d based on BW and breed. The treatments were either the control group (CON; n = 23) fed 1 kg of the 20:80 ration or the

rumen undegradable fat group (RUF; n = 24) fed 0.56 kg of the 20:80 ration plus 0.2 kg of Megalac-R (Church & Dwight Co., Inc., Princeton, NJ). These treatments were formulated to be isocaloric and were fed to the steers individually 5 d/wk. The fatty acid profile and composition of Megalac-R is shown in Table 1. During the fourth wk, the steers were gradually advanced from a 20:80 ration to an 80:20 ration, by increasing the ratio each day of 5 d of supplementation in the following sequence: 20:80, 30:70, 50:50, 70:30, and 80:20. After 30 d on treatment, CON steers were fed 1 kg of CGF and RUF steers were fed 0.56 kg of CGF plus 0.2 kg Megalac-R. At d 55 of treatment, CON steers received 1.5 kg CGF and RUF steers received 1.06 kg CGF plus the 0.2 kg of Megalac-R. With 18 d left on treatment, all supplement availability was increased; CON steers received 2.0 kg of CGF while RUF steers received 1.56 kg CGF plus 0.2 kg Megalac-R. Throughout the experiment, the steers had *ad libitum* access to bermudagrass, novel endophyte tall fescue, and wild type endophyte infected tall fescue pasture (average of 8000 kg/ha, 10 % CP, 55.7 % TDN, 1.3 Mcal/kg of $NE_m$, ADF of 37.9 % and NDF of 71.6 %), shown in Table 2. When forage availability was less than 5500 kg/ha, steers were offered *ad libitum* supplemental bahia/bermudagrass hay (average of 9.4% CP, 54.4 TDN, 1.3 Mcal/kg of $NE_m$, ADF of 39.3 % and NDF of 74.6 %). All steers remained at Edisto Research and Education Center (Blackville, SC) through the duration of treatment. Blood samples and BW were collected and recorded at 0, 55, and 110 d of treatment. Blood was collected via jugular venipuncture using Serum Z/9 mL Luer Monovette collection tubes (Sarstedt Inc., Newton, NC) and 18 G × 1½ in. Precision Glide needles (Becton, Dickinson and Company, Franklin Lakes, NJ). The blood was refrigerated at

4ºC for 24 h and then centrifuged at 1200 × g for 30 min. Serum was collected and stored at -20ºC. Real-time ultrasound carcass measurements were collected on 110 d of treatment to assess intramuscular fat, muscle depth, and backfat thickness. Longitudinal scans for intramuscular fat content, muscle depth, and backfat thickness were obtained between the 11th and 13th rib using an Aloka 500 V machine with a 17 cm, 3.5 MHz linear probe (Corometrics Medical Systems, Wallingford, CT). Images were interpreted using BioSoft Toolbox (Biotronics, Ames, IA) by a trained individual. Upon completion of treatment, all steers were transported by a commercial hauler 1700 km to Oklahoma State University's Willard Sparks Beef Research Center to begin the finishing phase. Steers were blocked into 4 pens per treatment based on BW. The total feed consumed per pen and refusal was measured daily. Subsequently, pen DM intake and individual ADG were calculated.

After 176 d of finishing, the steers were hauled 118 km to a commercial packing plant for harvest. Carcass measurements including hot carcass weight and ribeye area, back fat thickness, and KPH were collected by trained personnel at harvest or 48 h postmortem, respectively. Additionally, the right rib section of each carcass (112A Rib, Ribeye Roll, Lip-On; NAMP, 1988) was collected, vacuum-packed, put on ice and transported to Clemson University Meat Laboratory. Once at the meat laboratory, the rib sections were stored at 3.5ºC. From each rib section, two steaks approximately 2.54 cm thick were obtained, one for proximate analysis and one for cell size and distribution analysis. Steaks for proximate analysis had all external fat and connective tissue

removed. The steaks were then chopped into cubes and were frozen at -20ºC until analysis. Steaks for cell size and distribution were maintained at 4ºC until analysis.

**Forage and Supplement Analysis**

Samples of available forages and subsamples of CGF and hay were collected for analysis. The fatty acid profile and composition of Megalac-R is shown in Table 1. Forage samples were collected monthly or as the steers were moved to different pastures. Using a 0.09 square meter frame and cutting 2.54 cm from the soil, random pasture samples were collected in triplicate. Hay subsamples were collected prior to being fed *ad libitum* and were then composited. Each pasture sample was weighed after collection and reweighed after drying at 60ºC for 48 h to determine DM. The three forage samples from each cutting, the hay subsamples, and CGF subsamples were pooled and ground using a Wiley Mill (Model 4, Thomas Scientific, Swedesboro, NJ) to pass a 1-mm screen. According to the procedures of Goering and Van Soest (1970), Neutral Detergent Fiber and Acid Detergent Fiber were determined using an Ankom Fiber Analyzer #F200 (Ankom Technologies, Fairport, NY). The concentration of crude protein was determined by the combustion method using a Leco FP-528 Nitrogen Combustion Analyzer (Leco Corp., St. Joseph, MI).

**Proximate Analysis of Steaks**

Steaks were allowed to thaw partially, and were then pulverized in a Blixer 3 Series D (Robot Coupe USA. Inc., Ridgeland, MS). The samples were then stored in ziplock baggies and maintained at -20ºC. Percent moisture and percent total lipid of each

sample was determined in triplicate and in duplicate, respectively, according to AOAC (method 950.46; method 960.39; 1990). Total lipid extraction was determined by washing samples with petroleum ether (EMD Millipore Corporation, Billerica, MA) in a Soxhlet extract apparatus for approximately 24 h. The intra-assay CV for percent moisture and total lipids was 0.9 % and 1.9 %, respectively.

**Cell Size and Distribution**

One steak per animal was used to determine adipocyte cell size of both the subcutaneous and intramuscular adipose depots according to Etherton et al. (1977). All cells were filtered through 20 μm filter mesh to separate out adipocytes and then through a 240 μm filter mesh to remove large debris and the remaining cells were used for analysis. Subcutaneous and intramuscular adipocytes were counted and sized using a particle sizing and counting analyzer (Multisizer 4 Coulter Counter, Beckman Coulter Inc., Brea, CA).

**Serum Fatty Acid Analysis**

Duplicate 1 ml samples of serum from 10 steers per treatment group for all three time points were lyophilized (LabConco, Kansas City, MO) and transmethylated according to Park and Goins (1994). Each sample of fatty acid methyl esters (FAME) was analyzed using an Agilent 6850 gas chromatograph (GC) equipped with an Agilent 7673A automatic sampler. Separations were completed using a 30-m Famewax capillary column (12497, Restek, Bellefonte, PA) according to Duckett et al. (2009). Samples were run at a split ratio of 5:1. Identification of FA was achieved by comparing retention

times of known standards. An internal standard, methyl tricosanoic (C23:0) acid, was incorporated into every sample during methylation in order to quantify the sample as a percentage of weight of total FA. The CV based on total FAs was 12.2 %.

**Biochemical Assays**

Triglyceride and cholesterol concentrations were determined using a colorimetric assay (Pointe Scientific, Inc., Canton, MI). All samples were run in triplicate. The intra-assay and inter-assay CV for cholesterol were 3.8 % and 2.9 %, respectively. The intra-assay and inter-assay CV for triglycerides were 3.6 % and 4.3 %, respectively. This procedure has been previously validated in our laboratory (Tuersunjiang et al., 2013; Long et al., 2014). Leptin concentration was determined using a previously validated RIA (Long and Schafer, 2013; Linco Research, St. Charles, MO). All samples were run in duplicate and in a single assay. The intra-assay CV for leptin was 2.8 %.

**Statistical Analysis**

Calf birth weight, weaning weight, BW at the beginning, middle, and end of treatment and also BW change during treatment were analyzed using the MIXED model of SAS (SAS Institute Inc., Cary, NC) with treatment, breed, and their interaction in the model and weaning block as a random variable. Ultrasound measures, carcass measures, proximate analysis of steaks, adipocyte diameters and size distributions were analyzed using the MIXED model of SAS with treatment, breed, and their interaction in the model statement. Carcass measures were analyzed again using the MIXED model of SAS, but feedlot pen was used as the experimental unit instead of animal; treatment was in the

model statement. Serum FA composition, both concentration and percentage, along with serum concentrations of cholesterol, triglycerides and leptin were analyzed as repeated measures using the MIXED model of SAS with treatment, day of treatment, and their interactions in the model statement. Breed was initially included in serum metabolites and hormone analysis but was found to be non-significant and therefore removed from the model. Feedlot DM intake/steer, calculated from pen DM intake and corrected for number of animals/pen, along with ADG, G:F, and overall gain per steer was analyzed by ANOVA using the GLM procedure of SAS with treatment in the model. Data are presented as least squares means ± SEM and considered significantly different when $P \leq 0.05$ and a tendency was indicated when $P \leq 0.10$.

## Results

Steer BW are provided in Table 3. Steers within both treatments had similar BW at birth and weaning at 150 d of age ($P \geq 0.489$). However, at weaning there was a breed effect, with AN steers being heavier ($P = 0.002$) than AN/HP steers. Steer BW at the start of treatment were similar between CON and RUF steers ($P = 0.716$), but again AN steers weighed more ($P = 0.021$) than AN/HP steers. The difference in steer BW due to breed persisted until the middle of treatment, but there was no difference ($P = 0.229$) by the end of treatment. Steer BW remained similar between treatments throughout the rest of the experiment ($P \geq 0.489$). Overall BW change was similar between treatments ($P = 0.857$), but AN/HP steers had a greater BW change ($P = 0.017$) compared to AN steers.

The ultrasound data from steers at the end of supplementation indicated that RUF steers tended to have a greater ($P = 0.076$) amount of intramuscular fat compared to

CON steers (4.01 ± 0.25 v. 3.36 ± 0.25, respectively). However, there was no significant difference ($P \geq 0.829$) in muscle depth (1.47 ± 0.04 v. 1.46 ± 0.04) or backfat thickness ($P \geq 0.669$; 0.054 ± 0.010 v. 0.048 ± 0.009) between CON and RUF steers, respectively.

Serum FA concentrations in both the CON and RUF steers are given in Table 4 and the serum FA percentages of both treatment groups are given in Table 5. During treatment, there was a treatment × day interaction ($P < 0.02$) for the concentration of palmitic (16:0), stearic (18:0), oleic (18:1 *cis*-9), linoleic (18:2), arachidonic (20:4), acids and total FA. These specific FA concentrations slightly increased in CON steers, whereas there was a more prominent increase in RUF steers across the supplementation period. There was a tendency for the RUF steers to have a greater concentration ($P < 0.1$) of myristic (14:0), pentadecyclic (15:0), and linolenic (18:3) acids, and a greater ($P = 0.043$) concentration of docosapentaenoic acid (DPA) compared to CON. There was a treatment × day interaction ($P \leq 0.02$) on the FA percentage for myristic (14:0), palmitoleic (16:1), elaidic (18:1 *trans*-9), linoleic (18:2) acids, and DPA; with a general trend, excluding linoleic acid (18:2), of the serum FA percentages to decrease throughout the treatment period with the RUF steers experiencing a greater decrease in the FA percentages compared to CON steers. The percentage of 18:2, however, increased $P = 0.001$ during treatment for both CON and RUF with a much greater increase in the percentage for RUF steers. This large increase in the RUF percentage of linoleic acid could account for the greater decrease in the percentage of the other FA of the RUF steers. Also, there was a treatment effect ($P < 0.004$) on 15:0, 18:1 *c*-9, 18:3, and EPA where RUF steers had a smaller percentage of these FAs compared to CON. Similarly,

18:0 showed a tendency ($P = 0.08$) for RUF steers to have a smaller percentage compared to CON.

Serum triglyceride concentrations at d 0 were similar ($P = 0.166$) between treatments, shown in Figure 1. The triglyceride concentration of the CON steers remained similar ($P = 0.261$) from d 0 to d 110 of treatment. For RUF steers, there was an increase ($P < 0.05$) in triglyceride concentration from d 0 to d 55 and this concentration persisted until the end of treatment. Serum cholesterol concentrations were also similar ($P = 0.962$) between treatments on d 0, shown in Figure 2. The cholesterol concentration of the CON steers increased from d 0 to d 55 ($P = 0.0137$) and from d 55 to d 110 ($P < 0.001$). The RUF steers had increased ($P < 0.001$) cholesterol concentrations from d 0 to d 55 and from d 55 to d 110 of treatment ($P = 0.0108$). The concentration of serum leptin (Figure 3) was similar ($P \geq 0.261$) for both CON and RUF steers at d 0 and d 55. However, on d 110, there was an increase ($P < 0.05$) in the leptin concentration for RUF steers compared to CON steers.

The effects of treatment on feedlot performance are shown in Table 6. During the finishing phase, CON steers consumed 10.7 ± 0.2 kg of DM per head per day and the RUF steers consumed 10.6 ± 0.2 kg of DM per head per day ($P = 0.430$). The ADG was similar between treatments ($P = 0.899$). The feed efficiency, G:F, and overall gain were also similar between treatments ($P = 0.447$ and $P = 0.899$, respectively).

Carcass composition is shown in Table 7. There was no difference in HCW between CON and RUF steers ($P = 0.783$). However, carcasses of AN steers had a heavier ($P = 0.05$) HCW compared to AN/HP steers. There was no difference between

treatments in yield grade (YG), longissimus muscle area (LMA), backfat, or KPH. However, LMA was greater in the AN carcasses compared to AN/HP carcasses ($P$ = 0.035). There was a tendency of a treatment × breed interaction ($P$ = 0.094) for backfat thickness. The AN steer in the CON treatment group had the greatest amount of backfat thickness. This could be indicative of a depot specific effect of the rumen undegradable FA supplement and/or breed effect. The marbling score of RUF steaks tended to be greater ($P$ = 0.093) than CON. There was also a tendency for the marbling score to be greater ($P$ = 0.059) for AN than AN/HP steers. There was a tendency for the RUF steers to have a greater ($P$ = 0.085) percentage of total lipids in their steaks compared to CON steers. There was no difference ($P$ = 0.321) in percent moisture of steaks between treatments, however, there was a tendency ($P$ = 0.063) for the steaks of the AN steers to have a lower percent moisture than that of the AN/HP steers.

The carcass composition as determined by feedlot pens in shown in Table 8. There was no difference in HCW between CON and RUF carcasses ($P$ = 0.902). There was no difference in YG, LMA, backfat, or KPH ($P$ = 0.414, $P$ = 0.409, $P$ = 0.900, and $P$ = 0.670, respectively). There was a difference ($P$ = 0.010) in marbling score, where the RUF carcasses have a greater marbling score compared to the CON carcasses.

The average adipocyte diameter of intramuscular adipose tissue was 56.2 ± 0.7 μm for CON steers and 51.9 ± 0.8 μm for RUF steers ($P$ = 0.0003). The size distribution of adipocytes in intramuscular and subcutaneous adipose depots is given in Figure 4. There was a tendency for the intramuscular depot to have a greater percentage of adipocytes in the 20 - 30 μm diameter range for the RUF steer compared to the CON

steers ($P = 0.056$). This is indicative of an increase in hyperplasia of intramuscular adipocytes in RUF steers. The increase in average adipocyte diameter for CON is accounted for by the increase ($P = 0.009$) in the percentage of adipocytes in the 150- 180 μm range and a tendency ($P = 0.073$) for an increased percentage of adipocytes in the 120- 150 μm range. There were no differences between the two treatments for subcutaneous adipocyte diameter size or distribution (Figure 4B).

## Discussion

To our knowledge, this is the first report investigating the effects of supplemental rumen bypass fat on the carcass quality of early-weaned steers. The data from this experiment shows that the supplemental treatments were in fact isocaloric, evident by no difference in BW between the two treatment groups. The isocaloric supplement containing rumen undegradable essential, unsaturated FA increased the intramuscular fat content of RUF steers and subsequently the marbling scores while the back fat thickness remained similar between treatments. Furthermore, the intramuscular adipocyte diameters and size distribution were altered by supplementation while the subcutaneous adipocyte diameters and size distribution remained unaltered. The FA supplementation also influenced specific blood hormone concentrations similar to the results of the unsaturated FA supplementation from Long et al. (2014). Serum FA concentrations and total lipid content were also affected by supplementation.

Supplementation of the rumen undegradable unsaturated fatty acid source that contained a high percentage of linoleic acid (18:2; 26.8 % of DM) subsequently increased the serum concentration of linoleic acid in RUF steers. Hawkins et al. (1995) and Ryan

et al. (1995) showed that supplementing cattle with dietary lipid increased concentrations of serum lipids. Additionally, the supplemental fatty acids were associated with calcium salts, which are known to decrease the rate of biohydorgenation within the rumen and thereby provide the animal with a greater amount of fatty acids for postruminal absorption (Jenkins and Palmquist, 1984 and Wu et. al., 1991).

Linoleic acid is a precursor for a downstream activation of cell-surface receptor/ligand systems that initiate the expression of C/EBP-β and C/EBP-δ (Gaillard et al., 1989; Negrel et al., 1989; Vassaux et al., 1992). An increase in the expression of C/EBP-β and C/EBP-δ causes an increase in PPARγ (Wu et al., 1995) both directly and through the activation of C/EBP-α (Cao et al., 1991; Yeh et al., 1995; Farmer, 2006; Rosen and MacDouglad, 2006; Lefterova and Lazar, 2009). Peroxisome proliferator-activated receptor-γ and C/EBP-α work synergistically and reciprocally to activate adipogenesis (Mandrup and Lane, 1997).

Adipogensis is the differentiation of preadipocytes into mature adipocytes. Adipocytes can grow by both hyperplasia, increase in cell number, and hypertrophy, increase in cell volume (Hood and Allen, 1973; Cianzio et al., 1985; Robelin, 1986). Intramuscular adipocytes of the RUF steers tended to have an increase in the percent of adipocytes in the smaller diameter range (20 – 30 μm). The RUF steers also tended to have a greater % lipid in the striploin steaks and marbling score. This could indicate that the linoleic acid provided by the rumen undegradable fatty acid supplement could have influenced increased hyperplastic growth of intramuscular adipocytes of the RUF steers. This could also be interpreted as a depot specific effect, as the intramuscular depot

experienced difference in mean diameter and size distribution and the subcutaneous depot did not.

Steer BW remained similar between treatments from birth throughout the supplementation period. This lack of differences in BW could be attributed to the isocaloric supplementation and all steers grazing on the exact same pastures throughout treatment, similar to many other studies that supplemented an isocaloric fat (Lammoglia et al., 1999b; Whitney et al., 2000; Garcia et al., 2003) however, the diets those studies were also isonitrogenous. Conversely, there were some notable differences in BW between breeds. From weaning to the middle of supplementation, AN steers were heavier than the AN/HP steers, though BW between breeds were similar by the end of treatment. Thereby, it was the AN/HP steers that showed a greater change in BW throughout supplementation. The difference in BW at weaning could be attributed to maternal effects. Regardless, AN and AN/HP steers had similar gains until harvested. Similar gains, while in feedlot, indicate that the steers within different pens received the same diets *ad libitum* and consumption per pen was similar.

Although, there were no differences in BW at 110 d due to treatment, RUF steers exhibited increased intramuscular fat content at the end of treatment which was determined by ultrasonography. Harper and Pethick (2004) stated that it is wise to apply nutritional treatment to young animals due to the high possibility that there are a greater number of multipotent stem cells and preadipocytes at a young age. Also, a study using rats showed that with an increase in age there was a decrease in expression in a major regulator of adipogenesis, CCAAT/ enhancer binding protein- α (C/EBP-α)

(Karagiannides et al., 2001). If cattle also experience that decrease in the expression of C/EBP-α, it could serve as another contributing factor in trying to influence adipogenesis earlier in life. By offering the fatty acid supplementation early in the steers' lives, a greater number of undifferentiated stem cells could potentially be driven toward adipogenic confirmation. Also, Wegner et al. (1998) challenged the traditional idea that intramuscular fat is a late developing fat depot (Cianzio et al., 1985) by suggesting that the adiposity of muscle develops much earlier in the animals life than is evidenced by the marbling.

The RUF steers also had greater serum concentration of total FA on d 110 that is supported by the findings of Long et al. (2014) that showed the same results in heifers that were supplemented rumen bypass unsaturated fatty acids. This increase in total FA is indicative of the greater amount of FA available in their diet and the greater amount of unsaturated FA that reached the small intestine due to decreased biohydrogenation (Zinn et al., 2000). The serum concentration of 18:2 increased in the CON steers over the supplementation period but there was an increase in concentration of a much greater magnitude for the RUF steers. Also, 18:3 had a tendency to increase in RUF steers. Our findings are supported by previous studies that reported PUFA supplementation increased plasma concentration of linoleic (18:2) and linolenic (18:3) acids in cattle (Lessard et al., 2003, 2004; Farran et al., 2008). Arachidonic acid (20:4) also had a treatment × day effect similar to linoleic and DPA (22:5) had a treatment effect similar to linolenic acid. This could be attributed to linoleic acid being a precursor for arachidonic acid and linolenic acid being a precursor for DPA. Therefore, the increase in linoleic and linolenic

acid serum concentrations could have led to the conversion into other Omega-6 and Omega-3 fatty acids, respectively. Also, C15:0, C16:0, C18:0, and C18:1 *c*-9 serum FA concentrations had a treatment × day effect where the RUF steers had a greater concentration; further supporting that the RUF diet offered a greater amount of FA.

Serum triglyceride concentrations in the RUF steers were much higher than those of the CON, possibly due to a greater amount of FA being offered in the RUF steer's diet. Therefore, there was an increase in the availability of free fatty acids to be stored as triglycerides within adipocytes. This increase in triglycerides, due to supplementation of rumen bypass unsaturated fatty acids, is supported by Long et al. (2014). The increase in the amount of fatty acids provided in the diet could have also led to RUF steers having a greater increase in cholesterol concentrations. A similar study, that also supplemented rumen bypass fat to beef heifers, suggested that the diet is the most likely explanation for the increase in cholesterol, either directly due to the diet or through an increase in the substrate, acetyl CoA, (Long et al., 2007) since all 27 carbon atoms of cholesterol are derived from acetyl CoA (Berg et al., 2002).

Leptin, a protein hormone produced in adipocytes that travels via the blood, is involved with food intake regulation and energy homeostasis. Therefore, the increase in leptin concentration could be attributed to the increase in rumen undegradable fat intake. A greater concentration of fatty acids bypassed the rumen to the small intestines and thereby a greater amount of energy reached the small intestines. Chilliard et al. (1998) stated that several studies that used multispecies RIA assays have indicated that blood leptin is regulated by the level of energy intake and body condition. Then Chilliard et al.

(2005) reported that the BCS, or adiposity, is the key component in leptin regulation, both tissue and blood, while having a powerful interaction with other factors. However, Ciccioli et al. (2003) showed that blood leptin concentration was greatly affected by a nutritional treatment during a period where BCS remained similar. Additional factors that are believed to influence leptin levels include meal time (Ingvartsen et al., 2001; Delavaud et al., 2002), nutrients (Blache et al., 2000; Delavaud et al., 2000), other hormones (Leury et al., 2003; Block et al., 2003), and environment (Garcia et al., 2002; Kokkonen et al., 2002; Reist et al., 2003). Therefore, since the diet was the only altered effect in our steers, the increase in leptin levels at d 110 could be attributed to a nutrient effect of the rumen undegradable unsaturated fatty acids. Long et al. (2007) had similar findings and also showed that the increase in leptin was correlated with supplementation of FA, although they used saturated FA. Long et al. (2014) showed that even the composition of the supplemental fatty acids could be a contributing factor to increased leptin levels. Furthermore, it has been shown in rodents that FA, specifically linoleic acid, could also influence leptin levels (Takahashi et al., 1999; Rodriguez et al., 2002).

Carcass measurements were not affected by treatment with the exception of the marbling score, which tended to be greater in RUF steers. This tendency to increase marbling took steers from a USDA low Choice grade to an average Choice, thereby, increasing both the quality and value of the carcasses (USDA, 1997). Intramuscular adipocyte distribution was also influenced by the unsaturated FA supplementation. Although adipocytes within the intramuscular depot grow biphasically, first by hyperplasia followed by hypertrophy and so on (Allen, 1976), the increase in the

percentage of intramuscular adipocytes with the diameter of 20 – 30 µm could be indicative of lasting effects on factors effecting hyperplastic growth of intramuscular fat cells. Harper and Pethick (2004) stated that cattle have a certain number of fat cells upon entering the feedlot and that the high energy diets provided during the finishing phase simply fill those adipocytes with lipids. Therefore, the RUF steers could have entered the feedlot phase with a greater number of intramuscular adipocytes, due to increased hyperplastic growth earlier in life, than the CON steers. Then, while in the feedlot, those adipocytes accumulated lipids in the form of triglycerides. This is supported by the fact that RUF steers had a greater percentage of total lipids in the LM. Conversely, there was no difference in the subcutaneous adipose tissue thickness, cell size or distribution between treatments. The lack of difference in subcutaneous adipocytes could be attributed to subcutaneous fat depots early development and subsequent completion of hyperplastic growth by 8 mo. of age (Hood and Allen, 1973); therefore, the hyperplastic growth of the subcutaneous adipose depots could potentially have stopped prior to the end of treatment and only conducted hypertrophic growth while in the feedlot where all steers received the same diets. Furthermore, KPH was similar between treatments, the only other fat depot examined, which agrees with the visceral fat depots developing even earlier than subcutaneous (Vernon, 1981). Therefore, it may be possible that the supplementation of unsaturated FA had a depot specific effect on the intramuscular depot.

The results from this study may indicate that supplementation of ruminal unsaturated bypass FA can positively influence intramuscular adipose tissue deposition in

early-weaned steers. By weaning steers early, we had the opportunity to provide the steers with the unsaturated FA source at a point where there were potentially a greater amount of undifferentiated multipotent stem cell and/or preadipocytes. Therefore, the FA that bypass the rumen, and thereby biohydrogenation, and are then available in the small intestines can be utilized more efficiently and influence undifferentiated stem cells and preadipocytes to commit to becoming adipocytes. Additionally, the supplementation of the rumen undegradable unsaturated FA also appeared to have a depot specific effect on the intramuscular adipose depot. Through early weaning and FA supplementation one could potentially influence the amount of adipose tissue with in the intramuscular depot and therefore the quality and value of the carcass. However, more research is required to determine the full extent of the effects of supplementing FA on meat quality in early-weaned beef steers.

Table 1. Nutrient profile and fatty acid composition of Megalac-R, rumen undegradable unsaturated fatty acid source for early-weaned steers.

| | |
|---|---|
| DM, % | 97.0 |
| Calcium, % DM | 9.0 |
| Ether Extract, % DM | 84.5 |
| C12:0, % DM | 0.0 |
| C14:0, % DM | 0.0 |
| C16:0, % DM | 21.9 |
| C16:1, % DM | 0.0 |
| C18:0, % DM | 3.0 |
| C18:1 *t*, % DM | 0.0 |
| C18:1 *c*, % DM | 27.8 |
| C18:2, % DM | 26.8 |
| C18:3, % DM | 4.0 |
| Other LCFA, % DM | 1.0 |

Data provided by Church & Dwight Co., Inc., Princeton, NJ

Table 2. Chemical analysis of pastures, hay, and corn gluten feed (CGF) available to all steers throughout treatment.

| | | | kg/kg | | %DM | | | | | | Mcal/kg |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | Date | Grazing Days | Allowance | DM | CP | ADF | NDF | Crude Fiber | TDN[1] | Fat | $NE_m$[2] |
| 1 | 7/11/2013 | 754 | 2.4 | 42.9 | 11.3 | 36.4 | 72.9 | 30.9 | 57.3 | | 1.3684 |
| 2 | 7/25/2013 | 452 | 2.1 | 49.6 | 8.5 | 35.6 | 70.9 | 30.3 | 56.8 | | 1.3552 |
| 3 | 8/7/2013 | 1051 | 4.6 | 37.1 | 12.1 | 37.9 | 75.8 | 32.0 | 56.3 | | 1.3420 |
| 4 | 8/30/2013 | 564 | 4.0 | 37.4 | 7.9 | 42.1 | 76.6 | 35.1 | 51.6 | | 1.2056 |
| 5 | 9/11/2013 | 611 | 6.1 | 38.0 | 7.1 | 41.1 | 77.0 | 34.4 | 52.1 | | 1.2188 |
| 6 | 9/24/2013 | 611 | 3.9 | 37.2 | 7.4 | 40.6 | 76.3 | 34.0 | 52.6 | | 1.2342 |
| 7 | 10/7/2013 | 1128 | 3.2 | 35.3 | 15.6 | 36.0 | 63.7 | 30.6 | 59.1 | | 1.4212 |
| 8 | 11/15/2013 | 799 | 3.3 | 48.8 | 12.9 | 33.6 | 59.5 | 28.7 | 60.0 | | 1.4476 |
| Hay | 10/31-11/6 | | | 94.9 | 9.4 | 39.3 | 74.6 | 33.0 | 54.4 | | 1.2837 |
| CGF | | | | 91.7 | 20.1 | 8.0 | 27.8 | | 80.2 | 2.9 | 1.93 |

[1]TDN = 81.38 + (CP x 0.36) - (ADF x 0.77)

[2]$NE_m$ = ((0.029 x TDN) - 0.29)

Table 3. Body weights (kg) from weaning to feedlot placement of steers individually fed isocaloric supplement containing no bypass fat (CON) or fed 200 g of an unsaturated rumen undegradable fat source (RUF) 5 d/wk.

| | Treatment (Trt) | | Breed (Bd) | | P-value | | |
|---|---|---|---|---|---|---|---|
| | CON | RUF | Angus | Angus× Hereford | Trt | Bd | Trt×Bd |
| N | 23 | 24 | 23 | 24 | | | |
| Birth weight | 37.6 ± 1.3 | 36.7 ± 1.2 | 37.8 ± 1.3 | 36.5 ± 1.3 | 0.489 | 0.341 | 0.482 |
| Weaning BW (d 150) | 213.6 ± 5.8 | 219.0 ± 5.7 | 229.5 ± 5.8 | 203.1 ± 5.7 | 0.508 | 0.002 | 0.675 |
| Start weight (d 164) | 217.5 ± 6.1 | 220.5 ± 5.9 | 229 ± 6.0 | 209 ± 6.1 | 0.716 | 0.021 | 0.631 |
| Mid weight (d 241) | 231.8 ± 6.2 | 230.4 ± 5.9 | 239.4 ± 6.1 | 222.7 ± 6.1 | 0.859 | 0.047 | 0.528 |
| End weight (d 318) | 241.5 ± 7.6 | 243.9 ± 7.4 | 248.1 ± 7.7 | 237.3 ± 7.5 | 0.779 | 0.229 | 0.498 |
| BW change | 24.8 ± 3.5 | 24.2 ± 3.4 | 19.9 ± 3.5 | 29.0 ± 3.4 | 0.857 | 0.017 | 0.727 |

Table 4. Serum total and specific fatty acids (mg/ml) of steers individually fed isocaloric supplement containing no bypass fat (CON) or fed 200 g of an unsaturated rumen undegradable fat source (RUF) 5 d/wk.

| | CON | | | RUF | | | SE | P-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d 0 | d 55 | d 110 | d 0 | d 55 | d 110 | | Trt | d | Trt×d |
| n | 10 | 10 | 10 | 10 | 10 | 10 | | | | |
| 14:0 | 5.51 | 6.00 | 7.92 | 7.51 | 10.29 | 12.08 | 1.575 | 0.0785 | 0.0272 | 0.4901 |
| 15:0 | 5.02 | 7.32 | 9.35 | 5.07 | 12.61 | 15.75 | 1.820 | 0.0701 | 0.0001 | 0.0906 |
| 16:0 | 78.14 | 95.42 | 137.03 | 108.9 | 233.82 | 294.81 | 29.856 | 0.0079 | <.0001 | 0.0067 |
| 16:1 | 10.29 | 13.18 | 15.93 | 12.82 | 15.26 | 18.46 | 3.116 | 0.5375 | 0.0621 | 0.9859 |
| 18:0 | 89.67 | 120.39 | 160.34 | 112.82 | 274.51 | 338.24 | 35.346 | 0.0127 | <.0001 | 0.0070 |
| 18:1 *t*-9 | 66.63 | 86.95 | 126.17 | 89.39 | 131.22 | 154.33 | 19.838 | 0.2055 | 0.0007 | 0.5322 |
| 18:1 *c*-9 | 11.59 | 14.61 | 15.24 | 12.70 | 28.32 | 21.39 | 3.009 | 0.0686 | <.0001 | 0.0015 |
| 18:2 | 86.17 | 156.28 | 237.36 | 137.19 | 628.88 | 889.00 | 86.360 | 0.0010 | <.0001 | 0.0004 |
| 18:3 | 35.92 | 44.79 | 52.55 | 43.56 | 69.50 | 84.48 | 10.570 | 0.0977 | 0.0067 | 0.3387 |
| 20:4 | 16.28 | 21.40 | 32.12 | 22.57 | 46.72 | 67.93 | 6.715 | 0.0134 | <.0001 | 0.0140 |
| EPA | 11.87 | 13.94 | 16.68 | 14.39 | 18.59 | 20.39 | 3.178 | 0.3292 | 0.1349 | 0.8723 |
| DPA | 6.12 | 10.02 | 16.25 | 12.72 | 18.72 | 21.74 | 2.839 | 0.0431 | 0.0016 | 0.6652 |
| Total FA | 400.06 | 550.91 | 650.58 | 523.17 | 1255.81 | 1524.36 | 88.30 | <.0001 | <.0001 | 0.0001 |

Table 5. Specific serum fatty acid percentages of steers individually fed isocaloric supplement containing no bypass fat (CON) or fed 200 g of an unsaturated rumen undegradable fat source (RUF) 5 d/wk.

| | CON | | | RUF | | | SE | P-value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | d 0 | d 55 | d 110 | d 0 | d 55 | d 110 | | Trt | d | Trt×d |
| n | 10 | 10 | 10 | 10 | 10 | 10 | | | | |
| 14:0 | 1.16 | 0.90 | 0.80 | 1.23 | 0.62 | 0.57 | 0.063 | 0.0183 | <.0001 | 0.0138 |
| 15:0 | 1.04 | 1.06 | 0.98 | 0.77 | 0.77 | 0.74 | 0.069 | <.0001 | 0.7077 | 0.9538 |
| 16:0 | 16.72 | 14.21 | 14.37 | 17.65 | 14.03 | 13.78 | 0.577 | 0.9146 | <.0001 | 0.4063 |
| 16:1 | 2.14 | 1.88 | 1.65 | 1.96 | 0.92 | 0.85 | 0.133 | 0.0003 | <.0001 | 0.0017 |
| 18:0 | 19.03 | 17.94 | 16.97 | 17.84 | 16.67 | 15.67 | 0.739 | 0.0827 | 0.0238 | 0.9963 |
| 18:1 *t*-9 | 14.21 | 13.05 | 13.50 | 14.96 | 8.05 | 7.32 | 0.729 | <.0001 | <.0001 | <.0001 |
| 18:1 *c*-9 | 2.39 | 2.25 | 1.64 | 2.09 | 1.69 | 1.01 | 0.145 | 0.0003 | <.0001 | 0.5199 |
| 18:2 | 17.39 | 21.27 | 23.67 | 19.52 | 37.36 | 41.21 | 1.493 | <.0001 | <.0001 | <.0001 |
| 18:3 | 7.50 | 6.21 | 5.31 | 6.87 | 4.26 | 4.01 | 0.437 | 0.0039 | <.0001 | 0.2632 |
| 20:4 | 3.38 | 3.13 | 3.61 | 3.54 | 2.76 | 3.23 | 0.166 | 0.1748 | 0.0035 | 0.1906 |
| EPA | 2.57 | 1.92 | 1.89 | 2.01 | 1.12 | 0.95 | 0.185 | <.0001 | <.0001 | 0.5822 |
| DPA | 1.45 | 1.44 | 1.92 | 1.77 | 1.11 | 0.99 | 0.216 | 0.1496 | 0.1974 | 0.0211 |

Table 6. The effects of treatment on live feedlot performance of steers individually fed isocaloric supplement containing no bypass fat (CON) or fed 200 g of an unsaturated rumen undegradable fat source (RUF) 5 d/wk.

| | Treatment (Trt) | | P-value |
|---|---|---|---|
| | CON | RUF | Trt |
| N | 4 | 4 | |
| DMI, kg of DM/hd/d | 10.7 ± 0.2 | 10.6 ± 0.2 | 0.430 |
| ADG, kg | 2.18 ± 0.04 | 2.14 ± 0.04 | 0.899 |
| G:F, kg/kg | 4.94 ± 0.11 | 4.92 ± 0.11 | 0.447 |
| Overall Gain | 380.14 ± 8.45 | 378.54 ± 9.00 | 0.899 |

Table 7. Carcass composition and proximate analysis of steaks from of steers individually fed isocaloric supplement containing no bypass fat (CON) or fed 200 g of an unsaturated rumen undegradable fat source (RUF) 5 d/wk.

| | Treatment (Trt) | | Breed (Bd) | | P-value | | |
|---|---|---|---|---|---|---|---|
| | CON | RUF | Angus | Angus×Herford | Trt | Bd | Trt×Bd |
| n | 23 | 21 | 21 | 23 | | | |
| HCW, kg | 376.9 ± 7.3 | 379.9 ± 7.8 | 389.2 ± 7.8 | 367.6 ± 7.3 | 0.783 | 0.050 | 0.644 |
| Yield Grade | 3.81 ± 0.11 | 3.99 ± 0.12 | 3.93 ± 0.12 | 3.87 ± 0.11 | 0.280 | 0.770 | 0.598 |
| LMA, $cm^2$ | 83.16 ± 1.42 | 81.68 ± 1.55 | 84.71 ± 1.55 | 80.13 ± 1.42 | 0.484 | 0.035 | 0.165 |
| Backfat, cm | 1.62 ± 0.09 | 1.63 ± 0.10 | 1.68 ± 0.10 | 1.57 ± 0.09 | 0.915 | 0.456 | 0.094 |
| KPH, % | 3.35 ± 0.09 | 3.35 ± 0.10 | 3.35 ± 0.10 | 3.35 ± 0.12 | 0.979 | 0.979 | 0.958 |
| Marbling Score[1] | 48.29 ± 1.68 | 52.55 ± 1.81 | 52.82 ± 1.81 | 48.02 ± 1.68 | 0.093 | 0.059 | 0.577 |
| EE,[2] % | 6.29 ± 0.38 | 7.41 ± 0.04 | 7.10 ± 0.43 | 6.59 ± 0.38 | 0.085 | 0.634 | 0.112 |
| Moisture,[2] % | 69.72 ± 0.38 | 69.17 ± 0.40 | 68.92 ± 0.41 | 69.98 ± 0.37 | 0.321 | 0.063 | 0.313 |

[1]Marbling Score: 40 = Small 00; 50 = Modest 00

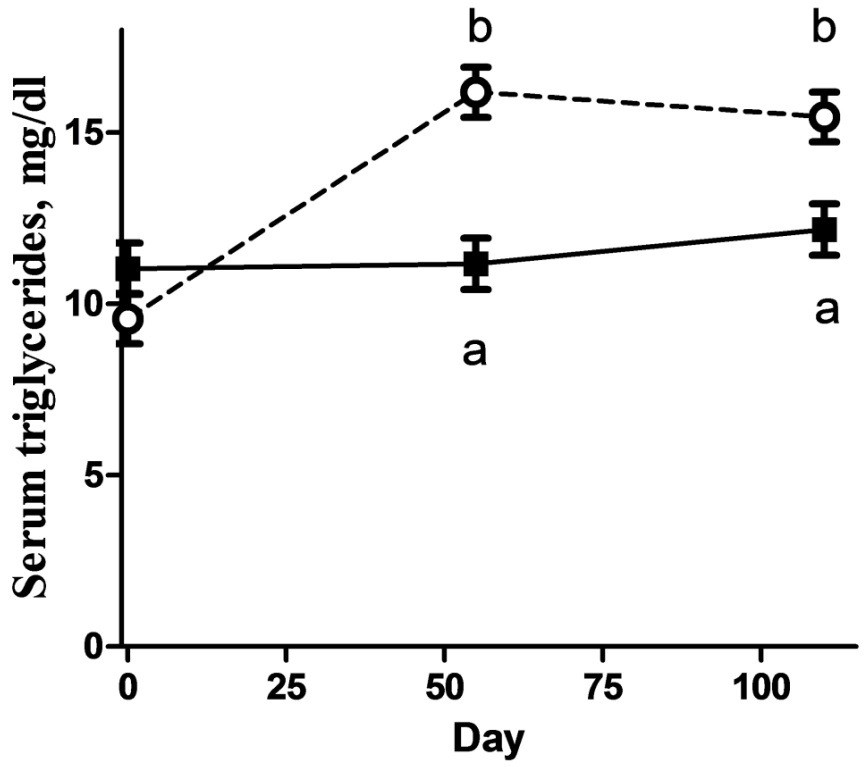
[2]Determined from striploin steak

Table 8. The effects of treatment on carcass composition of feedlot pens of steers individually fed isocaloric supplement containing no bypass fat (CON) or fed 200 g of an unsaturated rumen undegradable fat source (RUF) 5 d/wk.

| | Treatment (Trt) | | P-value |
|---|---|---|---|
| | CON | RUF | Trt |
| N | 4 | 4 | |
| HCW, kg | 375.70 ± 15.30 | 378.48 ± 15.30 | 0.902 |
| Yield Grade | 3.80 ± 0.18 | 4.03 ± 0.18 | 0.414 |
| LMA, $cm^2$ | 83.45 ± 2.01 | 80.93 ± 2.01 | 0.409 |
| Backfat, cm | 1.63 ± 0.14 | 1.65 ± 0.14 | 0.900 |
| KPH, % | 3.35 ± 0.04 | 3.38 ± 0.04 | 0.670 |
| Marbling Score[1] | 48.23 ± 0.82 | 52.48± 0.82 | 0.010 |

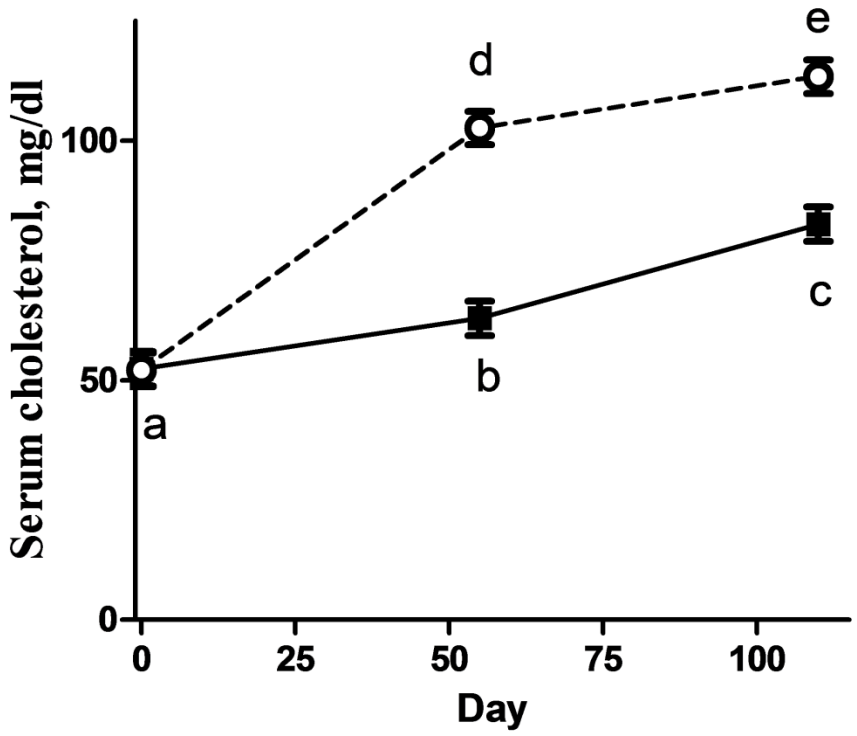
[1]Marbling Score: 40 = Small 00; 50 = Modest 00

Figure 1. Serum triglyceride concentrations for d 0, 55, and 110 of treatment of steers individually fed isocaloric supplement containing no bypass fat (Control, ■, n = 23) or fed 200 g of unsaturated rumen undegradable fat source (RUF, ○, n = 24) 5 d/wk. (Trt, $P = 0.003$; day, $P < 0.0001$; Trt×day, $P < 0.0001$)



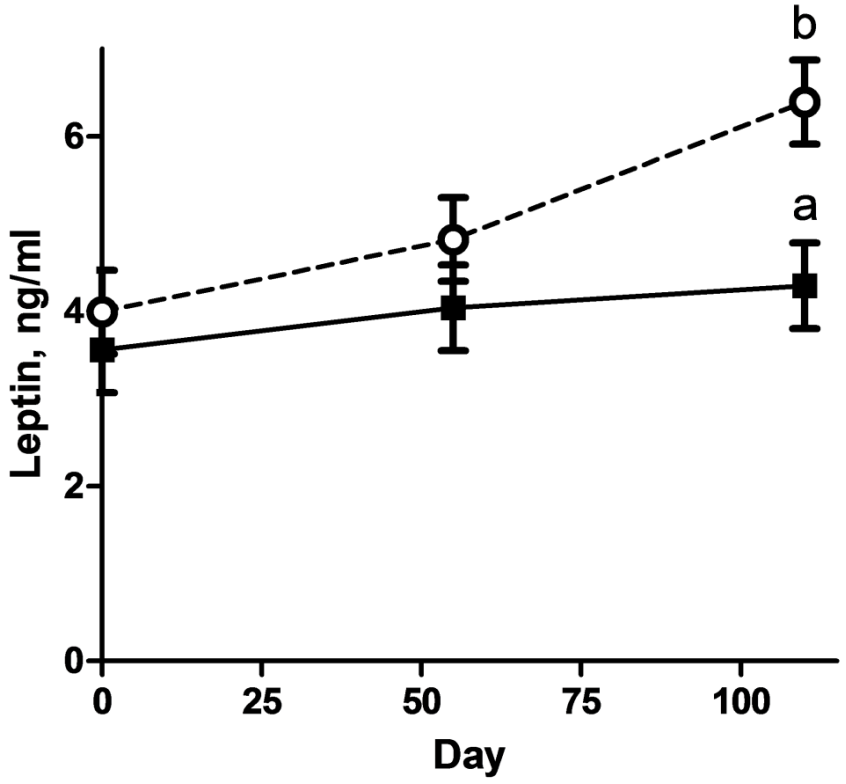

Values are means ± SEM. [a, b] means without a common letter differ ($P < 0.01$).

Figure 2. Serum cholesterol concentrations for d 0, 55, and 110 of treatment of steers individually fed isocaloric supplement containing no bypass fat (Control, ■, n = 23) or fed 200 g of unsaturated rumen undegradable fat source (RUF, ○, n = 24) 5 d/wk. (Trt, $P < 0.0001$; day, $P < 0.0001$; Trt×day, $P < 0.0001$)



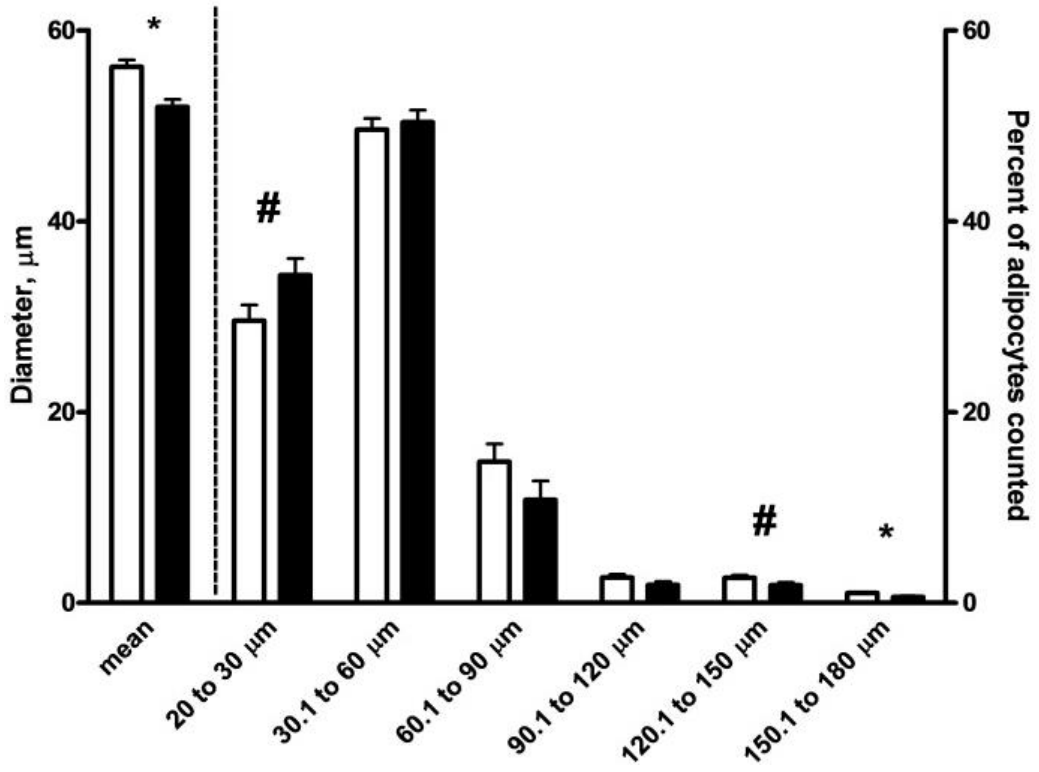

Values are means ± SEM. [a, b, c, d, e] means without a common letter differs ($P < 0.0001$).

Figure 3. Serum leptin concentrations for d 0, 55, and 110 of treatment of steers individually fed isocaloric supplement containing no bypass fat (Control, ■, n = 23) or fed 200 g of unsaturated rumen undegradable fat source (RUF, ○, n = 24) 5 d/wk. (Trt, $P = 0.0238$; day, $P = 0.0048$; Trt×day, $P = 0.1628$)



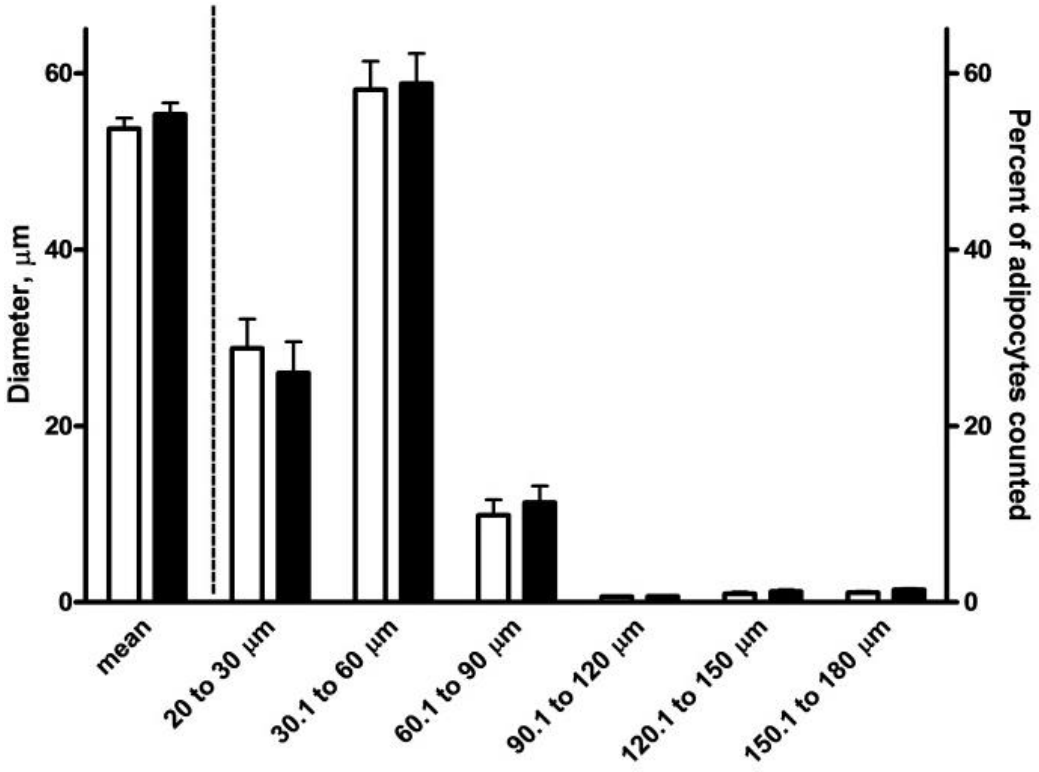

Values are means ± SEM. [a, b] means without a common letter differs ($P < 0.0001$).

Figure 4. Size distribution of intramuscular adipocytes in adipose tissue as a percentage of total adipocytes measured in steers individually fed isocaloric supplement containing no bypass fat (Control, open bar, n = 21) or fed 200 g of unsaturated rumen undegradable fat source (RUF, shaded bar, n = 20) at harvest.




* Means ± SEM differ ($P < 0.05$). # Means ± SEM differ ($P < 1.0$).

Figure 5. Size distribution of subcutaneous adipocytes in adipose tissue as a percentage of total adipocytes measured in steers individually fed isocaloric supplement containing no bypass fat (Control, open bar, n = 21) or fed 200 g of unsaturated rumen undegradable fat source (RUF, shaded bar, n = 20) at harvest.




* Means ± SEM differ ($P < 0.05$). # Means ± SEM differ ($P < 1.0$).

# CHAPTER IV

# CONCLUSION

Treatment of early-weaned steers with supplemental, rumen undegradable unsaturated fatty acids for 110 days tended to increase the marbling score of those steers. In addition to the improved marbling score at harvest, the supplemented steers also showed an increase in intramuscular fat content, via ultrasonography, at the end of the 110 day supplementation period. However, at the end of treatment there was no difference in back fat thickness and at harvest there was only a tendency for the Angus/Control steers to have increased back fat thickness. Furthermore, the treatment of early-weaned steers with supplemental bypass fatty acids decreased the mean diameter of intramuscular adipocytes and increased the percent total lipid within the steaks, indicating an increase in hyperplastic growth and overall adipocyte content in the intramuscular adipose depots of supplemented steers. The subcutaneous adipose depot did not experience an effect on mean diameter or size distribution due to treatment. This could indicate that the supplementation of rumen undegradable unsaturated fatty acids to early-weaned steers has a depot specific effect.

Total fatty acid concentration was increased in supplemented steers. The concentrations of cholesterol, triglycerides, and leptin were all increased in RUF steers by the end of the 110 day supplementation period. There was, however, no difference in BW between treatment groups from the beginning of treatment until harvest. This indicates that the treatments were indeed isocaloric but the supplemental rumen

undegradable unsaturated fatty acids provided the RUF steers with a greater availability of FA, which did not undergo biohydrogenation, for absorption in the small intestines.

The combination of the increased availability of FA for postruminal absorption and the detected increase in intramuscular fat content as early as 274 days of age could indicate positive effects from the bypass fatty acid supplementation on the early stages of adipogenesis. However, the early weaning management practice may not always be ideal in every producer's circumstances. Therefore, depending on the environment and producer's circumstances, supplementation of a rumen undegradable unsaturated fatty acid source to beef steers earlier in life could increase their production of adipose tissue within the intramuscular adipose depots, which may improve the carcasses marbling score and thereby, its consumer appeal and economic value.

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