**Physiological and Molecular Responses of Feeding Ractopamine to Yearling Heifers Across Days on Feed**

by

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## Abstract

Heifers comprise 30% of the annual beef harvest in the United States. Production pressures over the past several years have lead to a need to increase production efficiency. One mode currently available is the use of the β-agonist ractopamine. Ractopamine is designed to be fed 28 - 42 d prior to harvest and has been shown to increase REA and feed efficiency while decreasing yield grade. Seventy-one crossbred yearling heifers were allotted for days on feed (DOF) including 79 (n = 16), 100 (n = 16), 121 (n = 16), 142 (n = 16), and 163 (n = 7). Half of the heifers in each DOF were assigned to a ractopamine treatment of 300 mg/hd/d for 35 d prior to harvest (treatment phase) and half served as control. Leptin concentrations (LC) were determined for all animals at regular intervals. Real-time PCR was used to determine $\beta_2$-receptor expression for comparison of treatment and control groups. Ractopamine had no significant effects on growth or carcass traits across DOF. During the treatment phase, animals fed ractopamine were found to have increased ADG ($P < 0.05$) (1.24 kg/d) compared to control animals (1.10 kg/d); ($P < 0.02$). Dry matter intake during the treatment phase across control and treatment groups were equal (5.53 kg/d). However, ractopamine significantly improved dry matter feed efficiency ($P < 0.05$) during the treatment phase when compared to control (7.57, 9.86 kg feed/kg gain, respectively). Ractopamine had no effect on leptin concentrations ($P > 0.6$). Mean LC were determined to be 24.9 ± 3.13 ng/mL 24 hrs prior to harvest. Correlations were found between leptin concentration and

HCW (r = 0.27, $P < 0.05$), yield grade (r = 0.20 $P < 0.05$, and ADG (r = 0.28, $P < 0.05$). No differences were seen in expression of the $\beta_2$-receptor or plasma IGF across treatments ($P > 0.4$).

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# Introduction

Heifers comprise 30% of the annual United States beef harvest (USDA, 2009). In the present day beef cattle industry, overall efficiency has become a key to being successful. In general, heifers have lower feed efficiencies, lower HCW, and produce beef that is less tender than steers of the same age (Choat et al., 2006; Tatum, 2007). Therefore, in most cases heifers are not as desirable as steer counter parts in a feedlot. In 2003 the β-adrenergic agonist ractopamine hydrochloride (**RAC**) was approved for use in beef cattle for the final 28 - 42 d prior to harvest. In heifers, RAC has been shown to improve ADG while not affecting DMI (Gruber et al., 2007; Walker et al., 2006). Feed intake has been shown to be correlated with the protein hormone leptin (Pelleymounter et al., 1995) discovered by Zhang et al. (1994). Additional metabolic factors such as insulin, glucose, NEFA, and IGF-I play important roles in overall carcass composition. Reports of gene expression of $\beta_2$-adrenergic receptors in response to RAC are mixed. Winterholler (2007) determined RAC has a tendency to increase gene expression whereas Walker (2007) reported down regulation of the β-adrenergic receptor.

Given recent market conditions with corn and soybeans prices recovering from all time highs in May and June of 2008, days on feed (**DOF**), has become an important consideration in beef production. There were three objectives for this study (1) to determine if a relationship between leptin and RAC exists; (2) to determine if a relationship exists between leptin, growth, and carcass characteristics across DOF and

treatments; and (3) to determine the effect ractopamine has on gene expression of $\beta_2$-receptor, IGF-1, and myostatin.

The overarching hypothesis for this project was as DOF increases, animals will reach a higher degree of adiposity causing an increase in leptin in those animals not receiving RAC. In animals receiving RAC, leptin levels should decrease as fat is mobilized via lipolysis and energy is converted toward protein accretion.

Literature Review

**Heifers and days on feed:**

Over the past 30 years heifers have comprised 28 - 33% of the annual United States beef harvest (USDA, 2009). In general, heifers have lower feed efficiency, lower HCW, and produce beef that is less tender than steers of the same age (Choat et al., 2006; Tatum, 2007). Tenderness of beef ranks among the most important factors to consumers (Platter et al., 2003; Tatum, 2007) . Reviewing studies conducted between 1985 and 2006 (Tatum, 2007), steaks from heifers have a higher mean WBSF value (0.25 kg higher) when compared to WBSF values in steaks from steer counterparts. This difference in WBSF values is across multiple aging periods, from 6 to 18 d. The decrease in heifer performance provides a basis to value feeder heifers lower than contemporary feeder steers.

Management problems can also affect meat quality of heifers. Generally, heifers are more excitable than steers as Lanier et al. reported (2000). Lanier and coworkers (2000) found heifers to have increased sensitivity to environmental stimuli. Motion, sound, and tactical stimulation were measured. Data showed 84.21% of heifers responded to a combination of motion and sound stimuli, compared with 68.89%, 50.00%, and 67.57% of cows, steers, and bulls respectively. This contradicts the findings of Tulloh (1961) who demonstrated no significant behavior differences between sexes. However, it was determined lighter cattle were significantly more excitable than heavier cattle. This

excitable temperament can lead to negative effects on carcass characteristics such as darker color, higher calpastatin activity, and higher final muscle pH (Wulf et al., 1996).

Heifers also exhibit estrus. Heifers expressing estrus are considerably more active than heifers fed melengestrol acetate (**MGA**). Melengestrol acetate suppresses estrus, thereby limiting physical activity, which in turn can improve performance (Busby et al., 2001). As a consequence 63.2% of female cattle on feed were administered MGA (USDA, 2000).

Anabolic steroids have been used in beef production for many years. Denussion and others (1950) first published the effects of diethylstilbesterol (**DES**) on growth rate of heifers (Montgomery et al., 2001). Typical of anabolic steroids, DES increased rate of gain (12 - 17%) and increased feed conversion efficiency (4 - 11%). These effects come from increased protein accretion (Montgomery et al., 2001). Diethylstilbesterol was linked to cancer in 1971, and shortly afterwards the FDA banned the use of DES in cattle administered both orally and as implants (Herbst, 1999). Since the ban of DES, many other anabolic steroids have been approved by the FDA for use in beef cattle. Currently there are 16 compounds under 39 name brands on the market up from 26 name brands in 2001(Duckett and Andrae, 2001; FDA, 2009). Overall, use of anabolic implants is high in finishing of beef cattle. In 1994 and 1999, implant use at feedyards in cattle weighing more than 318 kg was reported at 98.9% and 97.2% respectively (USDA, 2000).

The advantages of anabolic steroid use have been well described in heifers. Improved ADG, HCW and feed:gain ratios have been seen in heifers (Boles et al., 2009; Duckett and Andrae, 2001; Popp, 1997). Anabolic steroids have been approved for all phases of cattle production. Duckett and Andrae (2001) reported a possible benefit of $93

per animal if implants were used across all phases of production. However, there are negative impacts to anabolic implants.

Anabolic implants have been shown to negatively affect tenderness in heifers (Schneider et al., 2007). Schneider and others (2007) showed a lower percentage of heifers grading prime or choice when receiving two implants over the feeding period, as compared to those receiving one or no implant. USDA (2000) reported 30.4% of cattle were implanted twice in the feedyard. Regardless of some negative data, anabolic steroids continue to be a viable option in beef cattle production, given current production pressures.

Current market conditions, have forced producers to increase the efficiency of operation. The number of days on feed (**DOF**) has become an important factor in efficiency and cost of production in the finishing phase of beef cattle. Winterholler et al. (2007) reported ADG, G:F, and DMI decreased and HCW, USDA Quality Grade, and Yield Grade increased as DOF increased from 150 to 192 d.

Anderson and Gleghorn (2007) conducted a serial slaughter project using heifers. Assigned DOF were 110, 124, 137, and 152. Increased DOF resulted in decreased ADG, increased HCW, increased occurrence of premium quality grades but not in choice, and increased the occurrence of USDA Yield Grade (**YG**) YG 3's and 4's. It was also determined profitability increased as DOF increased, however higher feed prices would negate the advantage (Anderson and Gleghorn, 2007). Over the past three years feed prices have increased substantially. Corn and soybean prices reached all time highs in May – June 2008 (USDA, 2008).

With increased economic pressures, producers continually look for ways to improve production efficiency. One method to improve efficiency is through the use of β-adrenergic agonist (**BAA**) and anabolic implants. Beta-agonists and anabolic implants are sometimes confused. While both are growth promoting agents used in the beef industry, time and administration are different, along with the mode of action.

**Ractopamine**

*Beta Adrenergic Agonists Structure and Effects*

Approved for use in beef cattle by the FDA in 2003, ractopamine hydrochloride (**RAC**), under the trade name Optaflexx®, is a well described BAA. Developed and patented in 1986 by Eli Lilly; subsidiary Elanco Animal Health® (Greenfield, IL), currently administers the rights to the patent. Ractopamine is known as a repartitioning agent, shifting metabolism away from fat deposition to protein accretion as an animal reaches the apex of its growth curve.

Mills and Mersmann (1995) list criteria for controlling exogenous agents. Specifically for RAC, these controlling factors are oral absorption, degradation in the liver, excretion, distribution to the tissues, concentration at receptor, specificity to receptor, capable of biological effects, and counters other systems (Mills and Mersmann, 1995).

Mills (2002) provides the best characterization of RAC and BAA in farm animals to date. Ractopamine and BAA are classified as phenethanolamines, which can be compared to endogenous catecholamines, epinephrine and norepinephrine (Beermann, 2002; Hancock et al., 2006). Phenethanolamines are classified by a substituted aromatic ring with an ethanolamine side chain with various substitutions (Figure 1); (Mills, 2002).

Ractopamine along with other BAA derive their biological activity from this unique structure. Substitutions on the base structure can occur in four places. Most substitutions occur at the *meta*-(2) and *para*- positions on the aromatic ring in relation to the β-carbon and to the R group adjacent to the aliphatic nitrogen (Smith, 1998). There are rarely substitutions at the *ortho* position. These substitutions give phenethanolamines their biological characteristics and the variability of plasma half-life and bioavailability (Smith, 1998).

Easson and Stedman (1933) proposed β-agonists bind β-receptors (**BR**) at three points: the β-hydroxyl group, the aliphatic nitrogen, and the aromatic ring. Easson and Stedman (1933) reported differing "enantiomorphs" bind to the receptor differently, and only one binds completely. Evidence of this was demonstrated by Hieble (1993) by using site directed mutagenesis of the BR (Smith, 1998). Natural catecholamines were discovered in the early 1900's paving the way for the classification of α and β receptors (Mills and Mersmann, 1995). By 1967, BR1 and BR2 subtypes had been identified. It was not until 1984 BR3 were classified in brown adipose tissue (Mills and Mersmann, 1995).

Beta-receptors are seven transmembrane cell surface receptors whose action is mediated through a G-stimulatory protein, activating adenylate cyclase, production of cAMP, followed by protein kinase activation, and the subsequent phosphorylation of specific protein molecules (Figure 2); (Hancock et al., 2006; Mills and Mersmann, 1995; Mills, 2002). Amino acid homology between subtypes in a species ranges from 80 - 90%, molecular weights of 42 - 51 kDa, with highly conserved transmembrane sequences (Mills and Mersmann, 1995). Beta one receptor and BR2 subtypes are found in most

major tissues of the body, and BR3 receptors are found primarily in adipose tissue (Mersmann, 1998).

As with most BAA, RAC has a higher affinity to a particular receptor sub-type. In the case of RAC the BR1 receptor is bound with a greater affinity than BR2 (Hancock et al., 2006). Alpha-receptors can also bind some β-agonists, such as natural catecholamines (Mills, 2002). This is not the case for RAC, which binds almost exclusively to BR (Mills, 2002).

Chirality of BAA determines biological activity. In the case of RAC, the β-hydroxyl group provides one chiral carbon and a second chiral carbon as seen in the R group. The chirality produces four different stereoisomers RR, RS, SR, and SS (Ricke et al., 1999). The RR and RS stereoisomers are levorotatory while SR and SS stereoisomers are dextrorotatory (Ruffolo, 1991). Ricke and others (1999) determined the RR stereoisomer was responsible for effects seen when RAC is administered. This is in line with the study by Ruffalo (1991) suggesting levorotatory isomers are responsible for biological effects.

Ractopamine was first approved for use in swine by the FDA in 1999, followed by approval for use in beef cattle in 2003. In swine, the BR1 subtype is the primary subtype comprising 80% of BR in adipose, 72% in heart, 65% in lung, 60% in skeletal muscle and 50% in liver (Mills, 2002). Comparably in cattle, BR2 comprises a majority of BR with 75% of BR in skeletal and adipose tissue being BR2 subtype (Vanliefde et al., 1994).

The effects of RAC are well documented. However, a debate existed for a short time over whether the mode of action was direct or indirect. Indirect actions would

include changes in concentration in circulating hormones and/or change in sensitivity to these hormones. Direct actions include responses mediated by the receptor (Beermann, 2002). Most literature to date indicates a direct receptor-mediated response (Beermann, 2002; Walker et al., 2007; Winterholler et al., 2007).

Physiological responses have been reported for some BA, namely reduced insulin and increased glucose concentrations (Beermann, 1987; Beermann et al., 1987). Cimaterol was shown to reduce insulin concentration by 41% two weeks after administration Beermann (1987); (see Table 1 for a partial list of β-agonists and original manufacture). In contrast, to the effects reported by Beermann (1987), Eisemann and Bristol (1998) reported no differences in insulin or glucose concentrations in cattle treated with RAC after 15 d as compared to the control. A lesser effect was reported by Walker et al. (2006). In Holstein steers fed RAC at a rate of 200 mg/hd/d, plasma glucose concentrations tended ($P = 0.08$) to decrease and plasma insulin levels tended to increase ($P = 0.13$); (Walker et al., 2007).

*Ractopamine Effects*

In heifers, RAC has improved ADG while having either no affect on DMI (Gruber et al., 2007; Walker et al., 2006) or decreasing DMI (Avendano-Reyes et al., 2006). Improvement in ADG without an increase in DMI improves overall efficiency in production. This improvement in efficiency makes RAC a viable growth promotant in the finishing of feedlot heifers.

Schroder and others (2004) determined steer ADG was improved ($P < 0.001$) by an average of 25.7% when compared to steers receiving no Optaflexx®. In the same study a 20.4% improvement in ADG was observed in heifers ($P < 0.003$). In both cases

no difference in feed intake was found. These effects were seen across three treatment levels of Optaflexx (100, 200, and 300 mg/hd/d) across two treatment durations (28 and 42d). Schroder and others (2003) reported ADG in heifers as 1.24, 1.34. 1.46, and 1.49 kg/d across treatment levels of 0, 100, 200, and 300 mg/hd/d, respectively. Hot carcass weights were found to be significantly higher in heifers fed 200 and 300 mg/hd/d Optaflexx. On a pen basis, feed efficiency was significantly improved ($P < 0.03$) compared to control, with heifers receiving 300 mg/hd/d exhibiting a 20.5% increase.

Quinn and coworkers (2008) examined the effects of RAC in feedlot heifers for treatment length and dosage. Ractopamine treatment length is currently approved for 28 to 42 d prior to harvest at a rate of 70 to 430 mg/hd/d. Quinn and others (2008) used four different treatment groups to elucidate possible strategies of RAC administration; ( (A) RAC fed at 200 mg/hd/d for 28 d; (B) RAC fed at 300 mg/hd/d for 28 d; (C) RAC fed at 200 mg/hd/d for 42 d; and (D) Step-up RAC fed at 100 mg/hd/d for 0-14 d, 200 mg/hd/d for 15-28 d, 300 mg/hd/d for 29 - 42d). Dry matter intake was the only significant difference observed among groups, with group B and D having decreased DMI compared to A and C (Quinn et al., 2008).

Winterholler et al. (2007) observed similar effects from the administration of RAC. Ractopamine fed at a rate of 200 mg/hd/d for 28 d increased HCW by 8 kg and ADG by 4.6% without negatively impacting marbling score (Winterholler et al., 2007). In addition to reporting growth and carcass characteristics, mRNA expression of the three BR subtypes were reported.

Another area of concern with β-agonists is the down regulation of β-receptors (Sillence, 2004). Beta-receptor down regulation has been shown in chronic dosing of

BAA (Hausdorff et al., 1990; Walker et al., 2007). Winterholler and others (2007) reported no difference in BR1 and BR3 mRNA when compared to control animals. However, a tendency of RAC to increase BR2 mRNA was reported by Winterholler and others (2007). This is in contrast to Walker et al. (2007) who reported a decrease in BR1 and BR2 mRNA when steers were fed RAC compared to controls. Additionally, Sissom et al. (2007) reported the effect of RAC on BR across implant strategy. Similar to Winterholler et al. (2007), BR2 has a tendency to increase with the administration of RAC.

This same decrease in BR2 has been observed when RAC was fed to finishing pigs (Gunawan et al., 2007). A 4 week serial harvest was utilized to determine the change in BR2 mRNA over the course of the trial. There was a significant decrease in BR2 expression by week 2 of the trial (Gunawan et al., 2007). It is currently unknown if these same effects would be seen in cattle during the RAC administration period.

Other BAA, such as clenbuterol have been shown to have adverse side effects in research trials such as increased heart rate and decreased feed intake (Brockway et al., 1987). Sumano and coworkers (2002) documented problems associated with administering clenbuterol to food animals. Clenbuterol is considered a bronchodilator, as are most BAA, however clenbuterol has a higher affinity for β-receptors when compared to other BAA (Sumano et al., 2002). The cardiostimulative activity of clenbuterol is 2,000 times higher than zilpaterol, another FDA approved BAA (Sumano et al., 2002). This high affinity for β-receptors and high activity has led to clenbuterol being banned in most countries, with the exception of therapeutic use in Europe (Kuiper et al., 1998). Clenbuterol has also been shown to remain in tissues for an extended period of time

(Kuiper et al., 1998; Sauer et al., 1995). The high retention rate of clenbuterol has been shown to cause hand numbness, nervousness, headaches, and muscle tremors in humans after the consumption of tainted meat (Mitchell and Dunnavan, 1998).

Strydom and coworkers (2009) compared the effects of three different BAA, clenbuterol, RAC, and zilpaterol. All three BAA were fed for 30 d followed by a 2 d withdrawal period for all treatments along with a control group. Zilpaterol was fed at a rate of 75 mg/hd/d, which is within FDA guidelines for dosage. Ractopamine was fed over the FDA approved guidelines, at a rate in excess of 430 mg/hd/d. While RAC was fed over the FDA approved guidelines, Strydom and others (2009) performed the research in South Africa prior to the approval of zilpaterol in the United States. Clenbuterol, not approved by the FDA, was fed at a rate of 24 mg/hd/d, a rate typical of previous clenbuterol studies. Strydom and others (2009) found the only treatment differing ($P < 0.05$) in live weight from the control group were the animals fed RAC. While no visual signs of distress were observed, it was determined animals fed clenbuterol had reduced feed intake (70% compared to other groups) and poor growth for seven d following start of administration (Strydom et al., 2009). These findings were similar to those of Ricks et al. (1984) and Brockway et al. (1987) who reported increased heart rates in animals fed clenbuterol.

Several other non-endogenous β-agonists exist and have been studied for their inclusion in the beef industry. Between 1984 and 1994 most major animal health companies had a form of β-agonist (Sillence, 2004); (Table 1). Currently of all the β-agonists developed in the 1980's and 1990's, RAC and zilpaterol are the only FDA approved BAA for use in beef cattle.

Zilpaterol, in comparison to RAC, is a β-2 agonist (Hancock et al., 2006). Just as with RAC, zilpaterol functions as a repartitioning agent. Approved in 2006 by the FDA, there is not the extent of published data on zilpaterol compared to RAC. This lack of data presents problems when comparing RAC and zilpaterol.

In contrast to RAC, zilpaterol has a three day withdrawal time. It is during this withdrawal the anabolic effects seen during use are quickly mitigated (Sillence, 2004). Robles-Estrada and coworkers (2009) reported the withdrawal effects of zilpaterol. Animals were fed zilpaterol for 30 d and then withdrawn 3, 6, or 12 d prior to harvest. Robles-Estrada and others (2009) determined a prolonged withdrawal period decreases the positive effects seen from zilpaterol of increased HCW and dressing percentage. A linear relationship ($P = 0.11$) to withdrawal period was reported for carcass dressing percentage, lean yield, and carcass adjusted ADG (Robles-Estrada et al., 2009). Hilton et al. (2009) found zilpaterol increased yield in some primal cuts, decreased trimmable fat, and decreased tenderness based on results with a consumer panel.

There are some concerns over the excitability of animals receiving RAC. It was reported by Marchant-Forde et al. (2003) that pigs receiving RAC were more difficult to handle and had increased heart rates. This has not been seen in cattle, possibly due to swine having a higher percentage of BR1 than BR2. Baszcazak and coworkers (2006) reported behavioral effects of RAC administered at 200 mg/hd/d in steers. Entry scoring, entry speed, chute scoring, and exit scoring were recorded. Entry speed of animals administered RAC was significantly higher than control animals. Entry scoring, chute scoring, and exit scoring were not different (Baszczak et al., 2006).

## Leptin

The discovery of *ob/ob* mice, which are genetically obese and sterile, in the 1950's led to multiple hypotheses surrounding the role of adipose tissue depots and the regulation of food intake. A key mediator of these roles is the protein hormone leptin. Leptin was discovered in 1994 by Jeffery Friedman and his lab group at Rockefeller University (Zhang et al., 1994). Leptin has been the catalyst for a great deal of research. On the human front, leptin research deals primarily with adipose tissue and effects attributed to leptin. Two leptin attributes, appetite and weight gain have led to the understanding of leptin's possible benefits to beef production (Lusk, 2007).

### *History of Leptin and Food Intake Research:*

In the early 1950's, animal caretakers in Jackson Laboratories discovered *ob/ob* mice. Researchers recognized a difference between normal and obese mice beginning at four to six weeks of age. At three months of age, normal mice weighed half the amount of obese mice. The maximum weight of obese mice during the study was four times heavier than normal mice. Fat comprised 50% of body composition of the obese mice at their heaviest weight (Ingalls et al., 1950). It was also determined *ob/ob* mice were sterile, leading to the hypothesis of leptin controlling reproduction along with food intake.

Kennedy (1953) provided insight to the history of leptin research. While the discovery of leptin was 45 years in the future, multiple hypotheses existed on the role fat played in food intake and reproduction. Two main hypotheses existed for the control of food intake via the hypothalamus. Brobeck (1948) suggested the hypothalamus was controlled by sensitivity to heat released during the metabolism of food. Mayer, Vitale,

and Bates (1951) and Bruce and Kennedy (1951) followed, suggesting chemo-sensitivity to circulating metabolites could be responsible for regulating energy intake. The theory of Bruce and Kennedy (1951) is closest to the present day knowledge of the sensitivity of the hypothalamus to circulating leptin concentrations.

Kennedy (1953) suggested these two hypotheses could be used together. Both temperature and chemo-sensitivity could play a role in energy intake. Wade (2004) suggested pressure receptors on feet may play a role in body weight. Two examples given by Wade (2004) were long distance runners and whales and seals. Long distance runners are normally thin and have the least amount of body fat, while whales and seals have no feet and have high proportions of body fat.

The *ob* gene product was first described in detail in 1994 as being a part of a signaling pathway acting to regulate fat deposition (Zhang et al., 1994). The following year three papers (Halaas et al., 1995; Pelleymounter et al., 1995; Rentsch et al., 1995) were published showing the *ob* protein, now known as leptin, when administered to mice eliminated obesity of *ob/ob* mice (Houseknecht et al., 1998).

*Leptin Description and Function:*

Upon the identification of leptin in 1994 (Zhang et al., 1994), there was a need to fully characterize and understand the functions of this molecule. The name leptin is derived from the Greek word *leptos* meaning thin. Using positional cloning, leptin was determined to be 167 amino acids long, with a molecular weight of 16 kDa, synthesized primarily in adipose tissue (Friedman and Halaas, 1998). A 19 kDa variant has been reported in the stomachs of rats. The form and function of this variant remain unknown (Halaas et al., 1995).

The *ob* gene has been shown to be highly conserved across species, with an 83% homology between mice and humans. The *ob* gene is 15,000 bp long located on chromosome four in cattle and mice and on chromosome seven in humans. Leptin shares structural similarities with many of the interleukins and growth hormone, being a helical cytokine (Zieba et al., 2005).

The *ob/ob* genotype is incapable of producing active leptin. The mutant *ob* gene is formed when a 5 kb transposon is inserted into the first intron of the gene resulting in mRNA not being synthesized. A nonsense mutation has also been described resulting in a truncated protein (Friedman and Halaas, 1998) . In the case of the nonsense mutation, mRNA continues to be produced at high levels. This continuous production led to the exploration of negative feedback on the *ob* gene (Friedman and Halaas, 1998). Without the negative inhibition, adipose cells will continue to produce leptin.

Initial research focused on the ability of leptin to limit energy intake which in turn limited fat accretion (Friedman and Halaas, 1998). The focus of most early leptin research was on the potential of leptin as an agent to treat obesity. Over the course of this work it was determined administering leptin to *ob/ob* mice, which are sterile, returned normal reproductive function (Zieba et al., 2005). This involvement of leptin in reproduction has been the subject of studies in cattle (Garcia et al., 2002; Zieba et al., 2005).

*Leptin Secretion, Regulation, and Targets:*

Leptin is produced by adipose, stomach, skeletal, and mammary tissue, fetal cartilage, the pituitary, and the placenta (Zieba et al., 2005). In cattle Ji et al (1998) determined fat depot location did not play a role in leptin production in cattle (Altmann

and Von Borell, 2007). Once produced, leptin enters circulation in both free and bound forms. Physiological status and species often determine the binding proteins and binding rate. In the human, leptin has a half life of approximately 30 minutes with the kidneys being responsible for 80% of the clearance (Zieba et al., 2005).

Leptin levels in blood plasma are highly correlated with adipose tissue mass (Geary et al., 2003). A greater amount of adipose tissue results in higher concentrations of leptin in circulation. Leptin is considered a long term regulator of adipose tissue, not a short term regulator like glucose, insulin, or body temperature (Friedman and Halaas, 1998). Daniel et al. (2002) reported the effects of feeding and fasting ewes. Both thin and fat ewes were utilized. It was determined leptin concentrations were also highly correlated with ultrasound estimates of fat thickness. No diurnal rhythm was detected, however, leptin was secreted in an episodic pattern. Although these findings were reported in sheep, cattle should follow a similar pattern. These findings in ruminants contrast the findings in non-ruminants by Licinio and others (1998). It was found in humans a circadian rhythm does exist with lowest concentration occurring in early morning and an increase throughout the day with a peak between 24:00 and 2:00 h. (Zieba et al., 2005).

Leptin is transported across the blood brain barrier (**BBB**) by a mechanism yet to be determined. It has been suggested a leptin receptor isoform may be responsible for transport across the BBB (Friedman and Halaas, 1998). In the absence of leptin, neropeptide Y (NPY) stimulates appetite and originates from the hypothalamus. This is in line with the finding of increased NPY RNA in *ob/ob* mice (Friedman and Halaas, 1998; Houseknecht et al., 1998). Thus, leptin inhibits the NPY neuron to reduce feed intake.

To date, six leptin receptor isoforms have been discovered, Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd, Ob-Re, and Ob-Rf (Bjorbaek et al., 1997). Of these six isoforms only Ob-Rb contains the necessary protein motifs to activate the Janus kinase 2 and signal transducers and activators of transcription (JAK-STAT) pathway (Uotani et al., 1999). Mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase (PI-3K) have also been implicated in leptin receptor signaling. One emerging research area of leptin involves leptin resistance in humans. Leptin resistance is associated with hyperleptinemia (Friedman and Halaas, 1998). Over time this can lead to physiological problems. Leptin has also been linked to other physiological processes through receptors (Zieba et al., 2005).

*Leptin in Animal Science*

Leptin may play multiple roles in animal science: reproduction, predicting carcass composition, and controlling feed intake. Leptin concentrations have been linked to the onset of puberty in heifers (Garcia et al., 2002) along with being used to predict carcass composition (Geary et al., 2003). A third and novel approach to the investigation of leptin is the use of leptin to increase feed intake (Sillence, 2004).

Sillence (2004) suggested possible immunoeutralisation of leptin could be used when circumstances exist to warrant artificially increased feed intake. One example of this circumstance would be a decreased growth due to poor quality diet (Sillence, 2004). While this approach is novel, Sillence (2004) mentions the trend in animal production is for animals to become more efficient, not necessarily to eat more.

In most mammals, gonadotropin releasing hormone (GnRH) mediates gonadotropin secretion (Zieba et al., 2005). By administering leptin to sterile ob/ob mice,

normal reproductive function returned. Leptin has also been linked to the onset of puberty in mice and cattle (Ahima et al., 1997; Chehab et al., 1997; Garcia et al., 2002).

Garcia et al. (2002) observed leptin concentrations in spring-born heifers. Leptin concentration was measured 16 weeks prior to pubertal ovulation. Simple linear regressions were performed on BW and leptin concentrations to puberty. Body weight (r = 0.99) and leptin (r = 0.73) were correlated to the onset of puberty. There was no evidence of leptin binding proteins as seen in other species (Garcia et al., 2002).

Garcia and others (2002) also recorded leptin concentrations in mature cows. Over four seasons, leptin concentration was reported to be highest (~8 - 9 ng/mL) in the summer and lowest in the winter (~4 - 5 ng/mL) (Garcia et al., 2002). This effect may not be based entirely on temperature but also on other mechanisms, such as BW which may mediate leptin concentrations.

Another area of research involving leptin is the prediction of carcass composition. Geary and others (2003) examined if serum leptin concentrations could be used to determine carcass composition in feedlot cattle. Two different groups of cattle were utilized, 88 ( ½ Red Angus, ¼ Charolais, and ¼ Tarentaise) (**CGC**) steers and 91 steers and heifers from a Lean Beef (**LB**) project. The author's hypothesized growth occurs through adipose tissue once cattle reach mature size. If this is the case leptin concentration would increase at maturity. Through multivariate analysis Geary and others (2003) determined leptin concentrations predicted overall carcass fatness ($P < 0.01$). For individual carcass traits, leptin was reported separately for CGC and LB. Leptin was positively correlated with quality grade (CGC r = 0.36, $P < 0.01$; LB r = 0.49, $P < 0.01$), KPH (CGC r = 0.42, $P < 0.01$; LB r = 0.54, $P < 0.01$), $12^{th}$ rib fat depth (CGC r = 0.34, *P*

< 0.01; LB r = 0.46, $P < 0.01$), and marbling score (CGC r = 0.35, $P < 0.01$; LB r = 0.50, $P < 0.01$). The differences between the two groups can most likely be associated with management practices (Geary et al., 2003). Fat depths over the 12$^{th}$ rib were reported as 0.76 cm in the CGC steers and 0.94 cm in the LB steers and heifers. The CGC steers were castrated at one y of age. Generally bulls have lower leptin concentrations that steers or females. Marino et al. (2009) reported Podolian bulls to have leptin concentrations between 6 and 8.5 ng/mL at approximately 600 d of age.

Brandt et al. (2007) reported leptin concentrations from random cattle selected after exsanguination on four different dates. This method was designed to represent a random sampling of the general population. No bull or bullock carcasses were chosen. Serum leptin concentrations were found to be positively correlated with 12$^{th}$ rib fat depth (r = 0.37, $P < 0.001$), USDA Yield Grade (r = 0.32, $P < 0.001$), marbling score (r = 0.28, $P < 0.001$), and KPH (r = 0.23, $P < 0.001$). These values are similar to those reported by Geary and others (2003); (Table 2). As leptin concentrations increased USDA Quality Grade also increased (Figure 3).

With leptin's effect on appetite and overall adiposity, the question arises on whether leptin could be used in selection and marketing. Lusk (2007) reported on the economic value of selecting and marketing by leptin genotype in cattle. As with most genes of importance SNP's have been identified. These SNP's have been shown to have an effect on performance and leptin concentration (Nkrumah et al., 2005; Schenkel et al., 2005). Lusk (2007) reported on seven different leptin genotypes, Type 1-7 (Table 3). Using production prices from 2004, selecting heifers and steers for the correct feeding program may increase profit by up to $22/hd.

## Materials and Methods

### *Animal Care and Use*

All procedures were approved by the Auburn University Institutional Animal Care and Use Committee (2007-1273), for the use of live animals in experiments.

### *Animal handling and sample collection*

Seventy-one crossbred heifers were group purchased via tele-auction in 2007. Heifers were co-mingled from seven individual Alabama beef producers. Birth date, source, genetic makeup, and vaccination record were known. Sire breeds included Angus, Simmental, Charolais, and Angus-Simmental or Simmental-Angus composites. Dam breeds were comprised of 16 different individual and combinations of Angus, Simmental, Charolais, Gelbvieh, Limousin, Brangus, Santa Gertrudis, and Barzona.

After purchase, animals were backgrounded for a period of 66 days on a summer perennial pasture mix (bermudagrass: *Cynodon dactyloncl* and bahiagrass: *Paspalum notatum Flusga*) and soyhull pellets at a rate of 3.2 kg/hd/d.

After backgrounding, heifers were transported to the Auburn University Beef Cattle Evaluation Center where they remained for the duration of the project. Weights and hip heights were recorded on each heifer upon arrival. Heifers were assigned to 1 of 6 pens based on height and weight to minimize social dominance. Each pen of cattle had inside and outside access with a capacity of 12 cattle per pen. Pens were 6.1 by 9.1 meters inside and 18.3 by 92.7 meters outside. The outside portion of each pen was

divided into three 6.1 meter strips. Heifers were allowed access to a different strip weekly to maintain groundcover (common bermudagrass: *Cynodon dactyloncl*) which served to minimize erosion and promote hoof health. Each pen contained 12 Calan Gates® (American Calan, Northwood, NH) to measure individual feed intake and cattle had continuous access to automatic water toughs.

Treatment groups were allotted for DOF stratifying across weights. Assigned days on feed were 79 (n = 16), 100 (n = 16), 121 (n = 16), 142 (n = 16), and 163 (n = 7). Half of each DOF group were assigned to receive 300mg/hd/d of ractopamine hydrochloride (Optaflexx®; Elanco Animal Health, Greenfield, IN) 35 d prior to harvest. The remaining half of each DOF group served as controls.

A 21 d warm-up period was utilized to train heifers to the Calan Gates®. During this period, heifers were fed a corn-based diet at 2% of their body weight (Table 4) and hay (bermudagrass and bahiagrass mix). Heifers were fed by hand twice daily. After the initial 21 d warm up period, hay access was removed and heifers were hand fed ad libitum amounts of the corn-based diet. MGA was added to the diet at a rate of 0.5 mg/hd/d to suppress estrus. Orts were measured and recorded daily.

Heifer weights and hip heights were measured and recorded weekly. To measure levels of hormones, blood samples were collected every 28 d until 35 d prior to harvest. Blood samples were then taken every 14 days until harvest (Table 5). Plasma samples were taken via jugular venipuncture using 10 mL EDTA lined vacuum tubes. Serum blood samples were taken via jugular venipuncture using non-treated vacuum tubes. Blood samples were stored at 4°C until transport to the laboratory at which time plasma was collected after centrifugation at 2500 x *g* for 15 min. Samples were aliquoted into 1.5

mL microcentrifuge tubes and stored at -20°C until needed. Blood samples for serum were stored at 4°C for 24 hours then centrifuged at 3500 x *g* for 20 minutes. Serum was then aliquoted into 1.5 mL microcentrifuge tubes and stored at -20°C until needed.

Days on feed group 163 was removed from the study due to small samples size. One heifer calved at d 110 of the study and another was untrainable to the Calan Gates®. Two other heifers (control from DOF 79 and 142) were untrainable to the Calan Gates®, and they were also removed from feed analysis.

Ultrasound BF, rump fat, percent intramuscular fat, and longissimus dorsi area were taken three times during the project on all heifers. An initial ultrasound was collected on all heifers on d 15 of the feeding period. A second set of ultrasound data were recorded for heifers at the start of RAC administration. A third and final set of ultrasound data were recorded 24 h prior to harvest. All ultrasound data were collected by the same Ultrasound Guidelines Council certified technician using an Aloka 500 (Aloka America, Wallingford, CT) with a 17 cm transducer using CUP Lab image capture software.

*Carcass data and Hormone Analysis:*

Twenty-four hours prior to harvest, heifers were weighed, measured for hip height, bled for hormone analysis, and feed removed. Heifers were transported to the Lambert-Powell Meats Laboratory. Heifers were harvested under USDA inspection and humane harvesting procedures. Prior to carcasses entering the cooler, HCW was recorded and livers and lungs were checked for abscesses.

Tissue samples were taken from the gastrocnemius muscle, rump adipose, longissimus dorsi, subcutaneous fat, and liver at the earliest possible time post-harvest.

Two samples were taken from each location. Approximately 0.5g of each tissue was placed in 5 mL of TRIzol (Invitrogen, Carlsbad, CA) and stored at -80°C. The remainder of the tissue (approximately 0.75 to 1.25 g) was placed in aluminum foil and flash frozen in liquid nitrogen and stored at -80°C.

Carcass characteristics, which included 12$^{th}$ rib back fat (**BF**), LM area, percentage KPH, and marbling score (**MS**) were recorded 24 hours post mortem. Marbling score was evaluated with a scale of 100 to 999 (100 = practically devoid$^{00}$, 200 = trace$^{00}$, 300 = slight$^{00}$, 400 = small$^{00}$, and 500=modest$^{00}$)

Blood samples collected during the project were analyzed for glucose, insulin, NEFA, IGF-1, and leptin. Serum glucose concentrations were analyzed using a Wako Autokit Glucose (Cat. No. 439-90901, Wako Chemicals USA, Inc. Richmond, VA). Serum NEFA concentrations were analyzed using Wako NEFA-HR(2) assay (Wako Chemicals USA, Inc. Richmond, VA). Plasma insulin concentrations were determined by utilizing a Coat-A-Count Insulin RIA (Siemens, Los Angeles, CA). Serum IGF-I analysis was a collaboration between Dr. Ted Elsasser (Growth Biology Laboratory, USDA, ARS, Beltsville, MD) and the authors, using procedures described by Elsasser et al. (1988). Plasma leptin samples were sent to Dr. Duane Keisler (Department of Animal Science, University of Missouri, Columbia, MO) for analysis using procedures described by Delavaud and others (2000).

*Gene Expression*

Using TRIzol reagent (Invitrogen, Carlsbad, CA) RNA was extracted from the gastrocnemius muscle and rump adipose tissues collected during harvest. Purified RNA was produced using an RNeasy mini kit (Kit 74102); (Qiagen, Vencia, CA). Purified

RNA was eluted into 40 μL of nuclease free water and stored at -80 °C. To determine the best sample storage method, RNA was extracted from samples stored in TRIzol (Invitrogen, Carlsbad, CA) and samples frozen in liquid nitrogen. Integrity of the RNA was determined on an agarose gel stained with ethidium bromide and RNA concentration was determined on a Beckmann Coulter DU-640 Spectrophotometer. Samples stored in liquid nitrogen were found to have the highest concentration of RNA and used for RNA isolation.

A total of 3 μg of RNA was reverse transcribed (iScript cDNA synthesis kit, Bio-Rad, Hercules, CA) for each tissue. Primers for PCR were designed using Vector NTI version 9 (Table 6); (Invitrogen, Carlsbad, CA). Primers were verified by using Reverse Transcription-Polymerase Chain Reaction (**RT-PCR**), cloning the product into pCRII (Invitrogen, Carlsbad, CA) and plasmids were transformed in INVαF competent cells. Plasmid DNA was extracted and quantified using a Wizard Plus SV Minipreps DNA Purification Kit (Promega, Madison, WI). Using agarose gels, transcript size was determined and sequences were verified by sequencing at the Auburn University genomics lab.

To determine optimum annealing temperature and standard curve range of primers, temperature gradients (50 – 65 °C) and standard curve determinations were made using 10-fold dilutions. Real-time quantitative RT- PCR was utilized to determine gene expression of IGF-1, myostatin (**MSTN**), and $\beta_2$-adrenergic receptor in isolated RNA. Housekeeping genes were determined by using the same optimization and sequencing steps as with the genes of interest, in conjunction with the GeNorm Excel spreadsheet to assure no effects from RAC existed (GeNorm Excel Spreadsheet uses

principles described by Vandesompele et al. (2002)). Housekeeping genes were glyceraldehyde phosphate dehydrogenase and β-actin. Table 5 contains the primer sequence and Genebank accession numbers. A Bio-Rad MyIQ thermocycler was used. Reaction volume totaled 30 μL with SYBR Green (Bio-Rad, Hercules, CA) as the flourophore.

*Statistical Analysis*

Performance, carcass, and blood data were analyzed using the Proc Mixed procedure in SAS (SAS Institute Inc, Cary, N.C.). Data were arranged as a 4 x 2 factorial randomized block design with animal as the experimental unit. Fixed effects included DOF and treatment (RAC or Control). Covariates were HCW and adjusted BF measured at the $12^{th}$ rib. Age was not used as a covariate because age was found not to be a significant source of variation in the model. Orthogonal contrasts (linear, quadratic, and cubic) were used to compare DOF means for growth and carcass characteristics. For simple correlation analysis between leptin concentrations, ultrasound data, growth, and carcass traits, the Proc Corr procedure of SAS was used. For gene expression, thermocycler data were analyzed using Bio-Rad gene expression excel spreadsheet. Gene expression data were then analyzed in SAS using the Proc Mixed procedure in SAS using the fixed effects of DOF and treatment (RAC or control).

## Results and Discussion

*Days on feed*

Heifers had an initial age of 398.43 ± 34.0 d and initial mean BW of 434.87 ± 36.0 kg going on test. There were no differences in initial age ($P > 0.3$) or initial weights ($P > 0.1$) across DOF groups (Table 7). Days on feed group 79 had the lightest final BW (532.4 kg). No contrasts were seen in final BW across DOF ($P < 0.15$) (Table 8). Days on feed groups 121 and 142 were reported to be similar to each other but have significantly heavier BW compared to DOF group 79. Final BW for DOF group 100 was not different from DOF groups 79, 121, or 142. Age at harvest increased linearly as DOF increased, as would be expected, with only DOF groups 100 and 121 having similar ages at harvest (linear; $P < 0.001$). Overall ADG was greatest in DOF group 100, with DOF groups 79 and 121 having similar overall ADG ($P > 0.05$); (1.31, 1.22, 1.24 kg/d respectively) when compared to DOF group 100. DOF group 142 had the lowest overall ADG (1.09 kg/d), which was statistically lower when compared to DOF group 100 ($P < 0.05$) and not different from DOF groups 79 and 121 ($P > 0.05$). A linear relationship was found between ADG and DOF, as DOF increased ADG decreased (linear, $P = 0.001$). No relationships were found for dry matter feed efficiency (**DMFE**); ($P > 0.2$) and no differences were observed across DOF groups for DMFE ($P > 0.1$).

A cubic relationship existed for HCW and DOF ($P < 0.001$). DOF group 79 had a significantly lower HCW (321 kg) than DOF groups 100, 121, and 142 (342, 365, and 360 kg respectively); ($P < 0.05$); (Table 7). This is in contrast to reports by May (1992)

who determined HCW increased linearly as DOF increased ($P < 0.01$). Steers used by May et al. (1992) were approximately 480 d old starting the feeding period, approximately 80 d older than the heifers in the current project. Six steers were slaughtered on d 0 of the feeding period with a mean HCW of 345.9 kg. From d 0 to d 196 steers were slaughtered at 28 d intervals with HCW of 413, 431, 481, 506, 544, 563, and 622 kg across 28, 56, 84, 112, 140, 168, and 196 DOF, respectively.

A quadratic relationship was found for marbling score across DOF ($P < 0.03$), similar to the report by May et al. (1992). Days on feed group 79 had the highest marbling score which was significantly higher than DOF group 121 and similar to DOF groups 100 and 142. It should be noted as DOF increased, less variation was seen in marbling scores. May et al. (1992) reported no significant increases in marbling score past 84 DOF. Brethour (2000) reported extending days on feed may be counterproductive from a quality grade stand point. Our data demonstrates, while marbling score may not increase as DOF increase, yield grade increased as DOF increased (Figure 4).

USDA yield grade was significantly higher for DOF groups 121 and 142 than DOF groups 79 and 100 ($P < 0.05$); (Table 7). Yield grade was found to be highly correlated with $12^{th}$ rib back fat ($r = 0.91$, $P < 0.001$) which is similar to values reported by May (1992) ($r = 0.94$, $P < 0.05$). This correlation was also demonstrated by Boleman et al. (1998) and Garcia et al. (2008) in the 1995 and 2005 National Beef Quality Audit. Back fat has been shown to increase exponentially during the feeding of a high concentrate ration (Brethour, 2000). Exponential increases in back fat coupled with the slow rate of intramuscular fat deposition, also shows increased DOF may not provide a benefit to the production system, from the producer to the consumer.

Similar trends were seen in 12th rib back fat across DOF. Back fat measured at the 12th rib is accounted for in the USDA Yield Grade equation. DOF groups 79 and 100 had significantly lower 12th rib back fat thickness than DOF groups 121 and 142 ($P < 0.05$). When considering the USDA yield grade equation after accounting for back fat and HCW, REA and percent KPH fat are the remaining two variables. It was determined DOF group 142 had a significantly larger REA when compared to DOF groups 79 and 100 ($P < 0.05$). Days on feed group 121 did not differ significantly from DOF groups 79, 100, or 142. Percent KPH was found to be significantly higher in DOF groups 100, 121, and 142 when compared to DOF group 79 ($P < 0.05$).

When considering DOF, our data indicates feeding yearling heifers more than 100 d may not be beneficial. Data shows yearling heifers fed over 100 d have increased USDA yield grades, lower ADG, and increased back fat. Similar to reports in steers by May et al. (1992) who found USDA yield grades and back fat to significantly increase past 112 DOF.

*Ractopamine*

Ractopamine dose and duration of administration in beef cattle varies in the literature. In the current project RAC was administered at 300 mg/hd/d for 35 d preceding harvest. Other studies fed RAC at 396 mg/hd/d for 30 d with a 2 d withdrawal (Strydom et al., 2009), 200 mg/hd/d for 28 d (Gruber et al., 2007; Sissom et al., 2007; Walker et al., 2006; Winterholler et al., 2007), and in a step-up dosage (100 mg/hd/d for 14 d, 200 mg/hd/d for 14 d, and 300 mg/hd/d for the final 14 d); (Quinn et al., 2008). It is important to note, most published reports involve animals administered steroidal implants at some point during the feeding period. Sissom et al. (2007) reported possible interactions

between steroidal implants and RAC. In the current study no implants were used, in attempt to isolate the response to RAC.

The data analysis indicates RAC had no significant effect on any growth or carcass traits measured over the course of the full feeding duration (*P*-values > 0.16); (Table 7). The lack of response to RAC could be due to age, weight, and fat cover of heifers at the start of the feeding period. Average ultrasound fat cover of the heifers at d 15 of the feeding period was 101.7 mm. Based on previous reports, gender may play a role in the lack of effects seen in heifers in the present study. Schroeder et al. (2004) reported the effects of RAC in heifers were less than those seen in steers. At a rate of 300 mg/hd/d, RAC was found to increase ADG 25.7% in steers and 20.4% in heifers. This trend was also seen at RAC concentrations of 100 and 200 mg/hd/d, with steers improving ADG by 17.1 and 19.6% respectively and heifers improving ADG 8.0 and 17.5% respectively. In line with Quinn et al. (2008), this data seems to indicate differences in the response to RAC may be related to sex. In this project, control (n = 32) and treatment (n = 32) heifers were not different in respect to initial weight and age. Final live weights and HCW were not different across control and treatment groups.

Both control and treatment group heifers in the current study exhibited higher initial BW, adjusted back fat thickness, and lower ADG compared with heifers in other studies (Quinn et al., 2008; Schroeder et al., 2004; Sissom et al., 2007). Similar to the heifers in this project, Sissom et al. (2007) found only feed efficiency to be improved during the treatment phase and not for the duration of feeding period. No significant improvement in feed efficiency was observed over the duration of the feeding period (*P* > 0.05). However, these results are different than those reported by Schroder et al. (2004).

Ractopamine fed at a rate of 200 mg/hd/d in heifers was found to improve feed efficiency, ADG, and increase HCW, while not affecting marbling score or feed intake (Schroeder et al., 2004).

Significant differences in growth traits were observed between the control and treatment heifers during the 35 d prior to slaughter (treatment phase); (Table 9). In heifers fed RAC during the treatment phase, ADG and overall treatment gain increased without affecting DMI, leading to an increase in DMFE ($P < 0.02$). These results are in contrast to the increase in DMI of 3.5% found by Winterholler et al. (2007) in steers during a 28 d RAC feeding period.

One premise of this study was to determine effects of RAC on yearling heifers across DOF. As expected with an increase in DOF, ADG decreased and HCW, BF, and YG increased. These effects were seen regardless of RAC administration ($P > 0.2$), similar to results previously reported by Sissom et al. (2007) in heifers. Sissiom et al. (2007) reported RAC administered at 200 mg/hd/d for the final 28 days to heifers fed 129, 150, and 170 days had a tendency (P = 0.1) to increase HCW while no significant effects were seen in BF or YG. Results in the current study and Sissom et al. (2007) are contrary to results seen by Winterholler et al. (2007) who reported RAC effects were observed without respect to DOF in steers ($P < 0.05$). Compared to the current study, Winterholler et al. (2007) used three DOF groups at 140, 171, and 192 d with an initial BW of 314 kg with final BW of 575, 586, and 608 kg, respectively. While final BW were similar to those in the current project, these data indicate a conclusion similar to Sissom et al. (2007) who reported gender differences may exist due to endogenous hormones.

In the present study, RAC effects may have been mitigated by gender, age, weight, and fat thickness. Both gender and age directly affect fat thickness when feeding a concentrated diet and heifers in the present study were at an advanced state on their growth curve and compositional endpoint. Additional research may be in order to compare interrelations of gender, age, weight, and beginning fat thickness. Results from the present work indicate RAC effects on growth, efficiency, and composition in backgrounded yearling heifers may not be as beneficial as seen in other studies.

*Leptin and blood analysis*

Plasma leptin concentrations have been considered as a possible predictor of carcass composition in beef cattle (Geary et al., 2003; Marino et al., 2009) and overall body fatness (Chilliard et al., 2005). Average leptin concentrations of the heifers 24 hrs prior to harvest (24.73 ± 3.16 ng/mL) across treatment groups were within the ranges reported by Geary et al. (2003) for steers and heifers (18.71 to 27.03 ng/mL) and above the concentrations reported by Marino et al. (2009) in bulls (6.42 ng/mL). Plasma leptin concentrations were not significantly affected by ractopamine administration ($P = 0.62$) (Table 9). The simple means of leptin concentrations 24 hr prior to harvest in control and treatment heifers were found to be 24.9 ± 3.13 ng/mL and 24.5 ± 3.22 ng/mL respectively.

Across DOF groups, heifers in the142 DOF group was found to have a significantly lower leptin concentration (22.4 ng/mL) than the heifers in the 79, 100, and 121 DOF groups (25.4, 26.3, 24.7 ng/mL respectively); (Table 9). Body fat has been shown to have a positive effect on the circulating leptin concentrations (Delavaud et al., 2000). This was not the case in the current study. Heifers in DOF group 142 had

significantly higher BF and KPH and lower leptin concentrations when compared to DOF groups 79 and 100 ($P < 0.05$). Significantly lower leptin concentrations in DOF group 142 were also observed at 21 and 7 d prior to harvest. Chilliard (2005) and Altmann and Von Borell (2007) give several factors other than adiposity influencing leptin synthesis, including gender, physical activity, age, breed and feed intake.

Differences in feed intake were hypothesized to be a possible explanation for the differences in leptin concentration across DOF observed in the current study. Delavaud (2000) reported fatness and feeding level accounted for 52 - 64% of the variation of leptin concentration. The DMI for the 35 d preceding harvest in heifers fed 142 d was 292.1 kg. Dry matter intake 35 d prior to harvest for heifers fed 142 d was significantly lower ($P < 0.05$) than heifers fed 79, 100, and 121 d (363.8, 331.6, 316.2 kg respectively). Overall, DMI was not correlated with leptin concentration ($r = 0.13$, $P > 0.2$). However, during the 35 d preceding harvest DMI was found to have positive correlation ($r = 0.38$ $P < 0.05$) with leptin concentration. This positive correlation is contrary to the general idea leptin is responsible for depressing feed intake. Data indicate leptin concentrations in fat cattle maybe independent of feed intake and adiposity. A possible explanation is the development of leptin resistance. Leptin resistance has been demonstrated in both mice and human models (Galic et al., 2010). With the degree of adiposity seen in the heifers this appears to be a likely explanation.

Correlation coefficients between growth traits and leptin 24 hrs prior to harvest are reported in Table 10. Correlations between leptin concentration and overall ADG were positive ($r = 0.28$ $P < 0.05$) yet lower than the values observed by Marino et al. (2009) ($r = 0.86$ $P < 0.001$). Similar correlations were found between leptin and ADG

during the 35 d prior to harvest (r = 0.27, $P < 0.05$). Collectively, these results indicate leptin may have a positive role in the rate of ADG at least 35 d prior to harvest

Carcass traits were correlated with leptin concentration measured in plasma 24 hrs prior to harvest (Table 11). The correlation between HCW and leptin concentration (r = 0.27 $P < 0.05$) was similar to the correlation reported by Brandt et al. (2007); (r = 0.12, $P < 0.01$) and Geary et al. (2003) (r = 0.27, $P < 0.1$). There was no correlation seen between leptin concentration and marbling score (r = 0.03, P > 0.2). The latter is in contrast to previous studies that indicated a positive correlation existed between leptin concentration and marbling score (Brandt et al., 2007; Geary et al., 2003). This difference could be the lack of variation of marbling score found among heifers within the current project and a small sample size (n = 64) compared to Brandt et al. (2007) (n = 1,682). Consistent with previous reports (Brandt et al., 2007; Geary et al., 2003), a positive correlation between back fat and leptin concentration was found in the present study (r = 0.22, $P < 0.1$). In an effort to further understand how leptin might be used to predict carcass composition, leptin correlation coefficients with carcass traits were reported for 35, 21, 7, and 1 d before harvest (Table 12). Starting at 35 d prior to harvest, leptin concentrations are positively correlated with USDA yield grade, adjusted back fat at the $12^{th}$ rib, and HCW ( r = 0.34, $P < 0.05$, r = 0.37, $P < 0.05$, and r = 0.37, $P < 0.05$). These collective observations confirm the suggestion that plasma leptin has a relationship with carcass adiposity in beef cattle.

Marino et al. (2009) suggested leptin concentration could be useful in predicting intramuscular fat one month prior to harvest. Results also indicate leptin could be a useful tool in predicting overall adiposity which generally has a positive correlation with

intramuscular fat. Geary et al. (2003) reported 12$^{th}$ rib fat depth had a positive correlation with marbling score (r = 0.41. $P < 0.01$). However, in the present study, no correlations existed for leptin concentrations 24 hrs prior to harvest and marbling score in carcasses at 35, 21, or 7 d prior to harvest (r = 0.03, $P > 0.2$, r = -0.04, $P > 0.2$, and r = 0.05, $P > 0.2$, respectively). An explanation for the lack of correlations between leptin concentrations and marbling is likely due to lack of variation in marbling scores with a majority of the heifers grading low or average choice (simple mean of 615 ± 104 USDA marbling score); ( 300= slight$^{00}$, 400= small$^{00}$, 500= modest$^{00}$ 600= moderate$^{00}$).

USDA yield grade was positively correlated with leptin concentration 35, 21, 7 and 1 d prior to harvest (r = 0.34, $P < 0.05$, r = 0.36, $P < 0.05$, r = 0.34, $P < 0.05$ and r = 0.20, $P < 0.05$ respectively). These observations are similar to correlations reported by Brandt et al. (2007) (r = 0.32, $P < 0.01$) for leptin concentrations and yield grade. Slightly lower correlations were reported by McFadin et al. (2003) and Geary et al. (2003); (r = 0.19, $P < 0.1$ for both). The ability to use leptin concentration in predictive equations for USDA yield grade could have a beneficial effect on marketing.

Comparing the current study with the three previous studies demonstrating positive correlations between leptin concentration and yield grade, a wide range of yield grades and leptin concentrations have been reported. McFadin et al. (2003) reported the mean yield grade of steers (n = 84) to be 2.29 ± 0.79, with a mean leptin concentration of 8.82 ng/mL. Geary et al. (2003) reported the yield grade on two groups of cattle to be 2.12 ± 0.47 in steers (n = 88) and 2.31 ± 0.76 in steers and heifers (n = 91) with leptin concentrations of 18.71 and 27.03 ng/mL, respectivly. Brandt et al. (2007) reported on four groups of cattle with yield grades ranging from 2.43 to 3.22 and leptin

concentrations ranging from 8.23 to 14.03 ng/mL (n = 995 steers, n = 757 heifers). Heifers in the present study were found to have a mean USDA yield grade was 3.55 ± 1.07 and a mean leptin concentration of 24.73 ± 3.16 ng/mL. Given the range of yield grades and leptin concentrations seen in studies reporting positive correlations between leptin concentration and yield grade, it is possible leptin concentration might be used as a predictor of yield grade through the USDA yield grade range.

The use of ultrasound to determine carcass characteristics (% intramuscular fat, longissimus muscle area, and back fat) has been in use for over two decades. Recently, Griener and others (2003) reported the high correlation between ultrasound measured back fat and actual carcass back fat (r = 0.89, $P < 0.001$). A positive correlation existed between 12$^{th}$ rib back fat measured postmortem to ultrasound back fat measured 35 and 1 d prior to harvest (r = 0.82, $P < 0.001$ for both). Correlations between ultrasound data and leptin concentrations for 35 and 1 d prior to harvest are summarized in Tables 13 and 14 respectively. Thirty five days prior to harvest plasma leptin concentrations were found to be positively correlated with ultrasound back fat and longissimus dorsi muscle area (r = 0.33, $P < 0.05$ and r = 0.31, $P < 0.05$ respectively). The correlation of leptin concentration to percentage subcutaneous fat has been previously reported in mutton. Similarly, Altmann et al. (2005) found percentage subcutaneous fat to be correlated with ultrasound fat thickness and leptin (r = 0.53, $P < 0.01$ and r = 0.59, $P < 0.01$ respectively) in mutton. A practical application of these relationships could be in the use of ultrasound measurements and leptin concentration one month prior to harvest to benefit marketing decisions of cattle through a grid pricing system based on composition and expected yield. For example, Lusk (2007) reported selecting cattle through leptin

genotype for the correct feeding system may lead to an increase of $22 in profit per animal.

Other blood analysis data included IGF-1, insulin, NEFA, and glucose shown in Table 15. Non-esterified fatty acids were not different ($P > 0.7$) at the onset of RAC of administration. During RAC treatment NEFA concentrations were significantly lower at 21 and 7 d prior to harvest but not different 24 hr prior to harvest. No significant differences were seen in serum IGF-1 concentrations across the treatment period. Walker et al. (2007) reported a tendency ($P = 0.06$) for RAC to lower IGF-1 concentrations in steers. Insulin concentrations were not different ($P > 0.1$) at the start of RAC treatment. At 21 d prior to harvest, insulin concentrations tended ($P = 0.08$) to be lower in heifers receiving RAC. At 7 and 1 d prior to harvest, heifers receiving RAC were found to have significantly lower insulin concentrations ($P \leq 0.01$). A previous report by Walker et al. (2007) found only a tendency for RAC to reduce insulin. Glucose concentrations were lower ($P < 0.01$) at the start of RAC administration. No difference in glucose concentrations were observed at 21, 7 and 1 d prior to harvest. These results are similar to those reported by Walker et al. (2007).

*Gene Expression*

Gastrocnemius muscle and rump adipose tissue were used to determine effects of RAC on $\beta_2$-receptor and IGF-1 mRNA expression in both adipose and muscle tissue. The gastrocnemius muscle was chosen for ease of sampling access and also used to determine the expression of myostatin mRNA. Myostatin mRNA was not observed in high enough concentrations to warrant further exploration in the adipose tissue samples.

Cattle possess primarily $\beta_2$-receptors. In comparison swine have a higher percentage of $\beta_1$-receptors. Ractopamine effects are mediated through β-receptors (Figure 2); (Hancock et al., 2006). There were no differences observed in mRNA expression of $\beta_2$-receptor in the gastrocnemius muscle between control and RAC treatments ($P > 0.05$) (Figure 5). To date, results are mixed in studies examining $\beta_2$-receptor mRNA in response to RAC. Sissom (2007) and Winterholler (2007) both showed RAC to have a tendency ($P < 0.1$) to increase mRNA expression of the $\beta_2$-receptor in the semimembranosus. Also in contrast to the present study, Walker (2007) determined RAC decreased $\beta_2$-receptor mRNA expression in the longissimus dorsi in Holstein steers. Across DOF, group 142 had greater $\beta_2$-receptor mRNA expression in muscle compared to DOF groups 79, 100, and 121 ($P < 0.05$).

The role of IGF-1 and the IGF-1 axis in muscle growth is well understood. IGF-1 response to anabolic steroids has been well described. Serum data from the current study suggest IGF-1 gene expression may not change in response to the administration of RAC (Table 9). Grant (1993) reported RAC had no effect on IGF-1 mRNA in the liver and longissimus dorsi in pigs. Similar results were seen in the heifers, IGF-1 was not significantly different between control and treatment groups or across DOF (Figure 6). These results are different than data reported by Sissom (2007). They reported there was an implant by RAC interaction in heifers receiving anabolic implants ($P = 0.03$). It was determined IGF-1 mRNA decreased ($P < 0.05$) when RAC was administered in conjunction with 200 mg of trenbolone acetate. In contrast, there was only a numerical increase in IGF-1 mRNA in heifers receiving RAC and 20 mg of estradiol-17β ($P = 0.15$) in the Sissom study.

Myostatin is known as a potent negative regulator of muscle mass discovered by McPherron (1997). Given the propensity of myostatin to negatively affect muscle growth, it could be hypothesized the administration of RAC would down regulate myostatin gene expression. Data showed no clear evidence of down regulation of myostatin gene expression (Figure 7) between control and treatment heifers ($P > 0.05$). DOF groups 79 and 100 were not different when compared to DOF groups 121 and 142. However, DOF group 142 had a significantly higher myostatin mRNA expression when compared to DOF group 121 ($P < 0.05$). While the role of myostatin in muscle regulation and animal breeding is well understood, literature does not exist to fully understand the involvement of myostatin in finishing cattle. Future research could yield possible biological mechanisms to increase production efficiency.

Ractopamine was not found to affect mRNA expression in adipose tissue between treatment and controls or across DOF in regards to IGF-1 or $\beta_2$-receptor (Figures 8 and 9, respectively). Few data have been published regarding the effect of RAC administration on gene expression in adipose tissue of cattle. Spurlock (1994) showed a reduction in $\beta_2$-receptor density in subcutaneous adipose of pigs fed RAC while no change was observed in the density of $\beta_2$-receptors in muscle.

Conflicting reports and lack of data in the literature about the effect of RAC on $\beta_2$-receptor mRNA expression in both muscle and adipose tissue leave future work to be done. It has been hypothesized β-agonists may be muscle specific (Strydom et al., 2009) and tissue specific. Current data supports this hypothesis by showing when beef animals are fed RAC, $\beta_2$-receptor mRNA increases in the semimebranosus (Sissom et al., 2007;

Winterholler et al., 2007), decreases in the longissimus (Walker et al., 2007), and had no effect on the gastrocnemius.

## Conclusions

The number of days an animal spends on feed has become extremely important. In the current project, feeding yearling heifers beyond 100 d may not be beneficial, with yield grades increasing significantly past 100 days on feed. The price of any animal sold on the grid regardless of quality grade will be docked above yield grade 3. With the positive relationships found between leptin and measured of fatness, such as back fat, KPH, and YG, leptin may provide a better understanding and improving the efficiency of feeding cattle. While the effects of ractopamine were not seen to the extent normally observed, due to age and fat cover, it remains a viable growth promotants in the beef industry.

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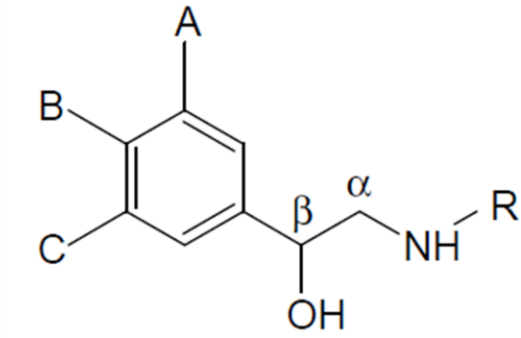
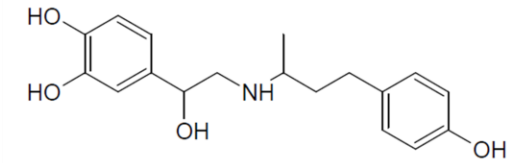
Tables and Figures

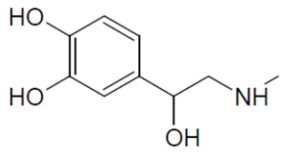
**Figure 1.** Overall structure of a beta-adrenergic agonist including structures of ractopamine, epinephrine, zilpaterol, and clenbuterol *. Substitutions normally occur at the *para* (B), *meta* (A and C), and R positions. In the case of ractopamine substitions occur at the B, C, and R positions. Also, steroisomers are formed base at the α and β carbon. In the case of ractopamine RR, RS, SR, and SS stereoisomers can be formed.



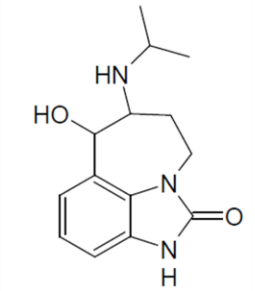

Phenethanolamine Backbone.



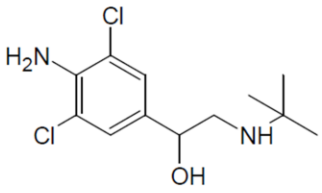

Ractopamine




Epinephrine




Zilpaterol




Clenbuterol

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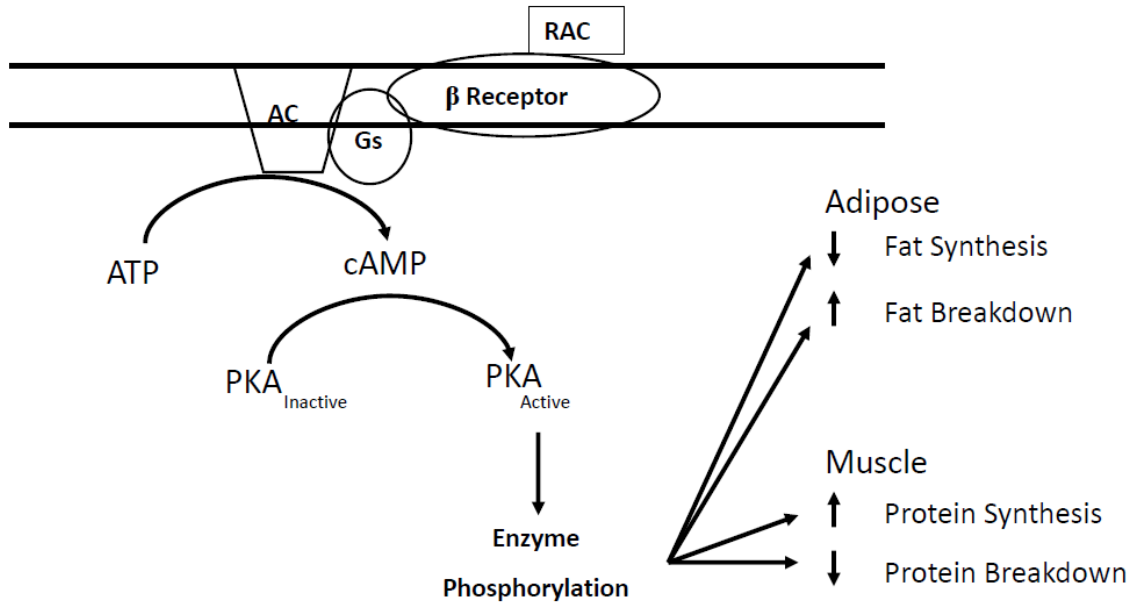
** Drawn in ChemSketch, Version 12.01, ACDlabs.com*
Adapted from (Hancock et al., 2006; Smith, 1998)

**Figure 2.** Beta-receptor pathway and general sequence of events for ractopamine effects. Ractopamine binds to the β-receptor (seven transmembrane cell surface receptor), response is mediated through a G-stimulatory protein, followed by the cascade of activation of adenylate cyclase, production of cAMP, followed by protein kinase activation, and the subsequent phosphorylation of specific protein molecules.




RAC= Ractopamine Hydrochloride, Gs= G-Stimulatory Protein, AC= Adenylate Cyclase, ATP, cAMP=Cyclic Adenosine Monophosphate, and PKA=Phosphokinase A. Adapted from (Hancock et al., 2006).

**Table 1.** A partial list of β-Agonists with original manufacturer and status of FDA approval

| B-agonist | Original Manufacturer | FDA Approved |
|---|---|---|
| BRL-47672 | SmithKline Beecham | NO |
| Cimaterol | American Cyanamid | NO |
| Clenbuterol | Boehringer Ingelheim | NO |
| L-644,969 | Merck Sharp and Dohme | NO |
| Ractopamine | Eli Lilly | YES |
| Zilpaterol | Intervet | YES |
| Ro 16-8714 | Roche Pharmacuticals | NO |
| Salbutamol | Glaxo | NO |

Adapted from Sillence M. N. 2004

**Table 2.** Pearson correlations of leptin with 12th rib back fat, kidney pelvic and heart fat, USDA quality grade, and marbling score across two studies*

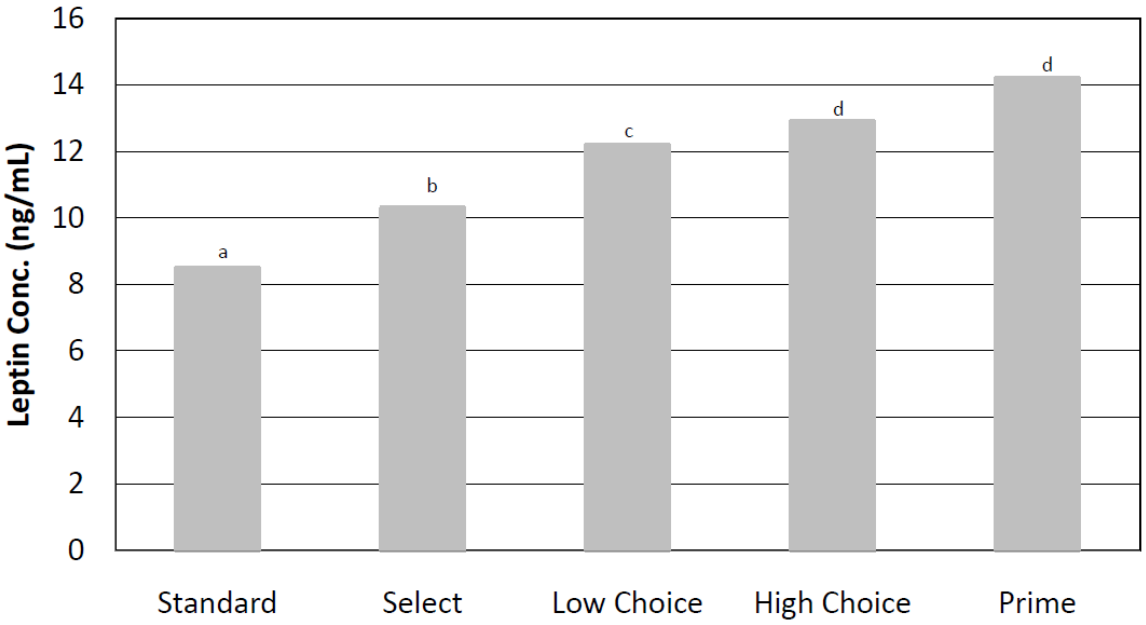
| Study† | Brandt et al., 2007 | | Geary et al., 2003 LB | | Geary et al., CGC | |
|---|---|---|---|---|---|---|
| | ng/mL | SD | ng/mL | SD | ng/mL | SD |
| Leptin Conc. (ng/mL) | 10.80 | 5.7 | 27.03 | 8.24 | 18.71 | 7.4 |
| | | | | | | |
| Pearson Correlation of leptin with: | | | | | | |
| 12th rib back fat | 0.37 | | 0.46 | | 0.34 | |
| KPH | 0.23 | | 0.54 | | 0.42 | |
| USDA QG | N/A | | 0.49 | | 0.36 | |
| USDA MS | 0.28 | | 0.35 | | 0.50 | |

† Brandt et al. 2007 involved four groups of commercially slaughtered cattle (n = 995 steers and n = 757 heifers). The four groups were pooled to arrive at a representative sample of commercial cattle. Geary et al. 2003 used two different groups of cattle. LB (Lean Beef project cattle) (n = 91). CGC ( ½ Red Angus, ¼ Tarentaise, and ¼ Charolais) (n = 88).

KPH = Kidney, Pelvic, and Heart Fat, USDA QG = USDA Quality Grade, USDA MS = USDA Marbling Score .

* All P-values < 0.01.

**Figure 3.** Serum leptin concentration at harvest compared to USDA Quality Grade.




[a-d] Bars lacking similar subscripts differ ($P < 0.05$).
Adapted from Brandt (2007).

**Table 3.** Leptin genotype distribution among 1,668 head of cattle

| | Type 1 | Type 2 | Type 3 | Type 4 | Type 5 | Type 6 | Type 7 |
|---|---|---|---|---|---|---|---|
| Head | 134 | 269 | 128 | 392 | 408 | 308 | 29 |
| Percent | 8.03% | 16.13% | 7.67% | 23.5% | 24.46% | 18.47% | 1.74% |

(Lusk, 2007)

**Table 4.** Diet Composition†

| Ingredient | Percentage |
|---|---|
| Corn | 38.5 |
| Corn Gluten Pellets | 17.5 |
| Cottonseed hull pellets | 10 |
| Dried Distillers Grain | 9.5 |
| Wheat Midds | 6.5 |
| Soyhulls | 6.5 |
| Cottonseed hulls | 5 |
| Molasses | 2.5 |
| Limestone | 1.25 |
| BICARB | 1 |
| Fat | 1 |
| Salt | 0.5 |
| Vitamins A,D,E | 0.1 |
| Trace Minerals | 0.1 |
| Rumensin 80 | 0.019 |

†As fed; Dry Matter = 90%, CP = 13.7%, $NE_{gain}$ = 0.78 Mcal/Kg, NDF = 32.2%, ADF = 15.7%.

**Table 5.** Blood sampling and ultrasound measurement days during each feeding period

| DOF | Day of Blood Draw | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 79 | 1 | 27 | 44** | 58* | 72* | 78** | | | |
| 100 | 1 | 27 | 57 | 65** | 79* | 93* | 99** | | |
| 121 | 1 | 27 | 57 | 86** | 100* | 114* | 120** | | |
| 142 | 1 | 27 | 57 | 85 | 107** | 121* | 135* | 141** | |
| 163 | 1 | 27 | 57 | 85 | 114 | 128** | 142* | 156* | 162** |

* = Blood drawn during RAC administration.
** = Blood drawn and ultrasound measurements.

**Table 6)** Real-time Polymerase Chain Reaction primers used for gene expression of β-actin, Glyceraldehyde phosphate dehydrogenase (GAPDH), $\beta_2$ adrenergic receptor, and myostain

| Primer | Sequence |
|---|---|
| β-actin (accession no. BC142413) | |
| Forward | TGGACTTCGAGCAGGAGATG |
| Reverse | CGTTGCCGATGGTGATGAC |
| GAPDH* (accession no. U85042) | |
| Forward | TGACCCCTTCATTGACCTTCA |
| Reverse | GATGGTGATGGCCTTTCC |
| $\beta_2$ adrenergic receptor (accession no. BC_114132) | |
| Forward | CAGCTCCAGAAGATCGACAAATC |
| Reverse | CTGCTCCACTTGACTGACGTTT |
| IGF-1 (accession no. X15726) | |
| Forward | TTGGTGGATGCTCTCCAGTTC |
| Reverse | GCACTCATCCACGATTCCTGT |
| Myostatin (accession no. NW_001494561) | |
| Forward | GGCCATGATCTTGCTGTAACCT |
| Reverse | GCATCGAGATTCTGTGGAGTG |

**Table 7.** Least squares means for growth and carcass characteristics for days on feed and treatment[1] with covariates of HCW and BF

| | Days on Feed (n=64) | | | | | Treatment (n=64) | | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | 79 (n=16) | 100 (n=16) | 121 (n=16) | 142 (n=16) | SEM | Control (n=32) | Ractopamine (n=32) | SEM | P-Value[5] |
| Age on Test (d) | 391 | 407 | 389 | 407 | 8.36 | 404 | 393 | 5.91 | 0.189 |
| Initial weight (kg) | 436.3 | 435.6 | 434.3 | 433.4 | 9.3 | 432.8 | 436.9 | 6.6 | 0.364 |
| Final weight (kg) | 532.4$^{a}$ | 566.1$^{a,b}$ | 584.1$^{b}$ | 588.7$^{b}$ | 12.3 | 565.7 | 570.2 | 8.7 | 0.166 |
| Age at harvest (d) | 470$^{a}$ | 508$^{b}$ | 509$^{b,c}$ | 549$^{d}$ | 8.36 | 514 | 503 | 5.91 | 0.189 |
| ADG (kg/d) | 1.22$^{a,b}$ | 1.31$^{a}$ | 1.24$^{a,b}$ | 1.09$^{b}$ | 0.06 | 1.21 | 1.22 | 0.04 | 0.778 |
| DMFE (f:g) | 7.88 | 7.59 | 7.90 | 8.60 | 0.40 | 8.08 | 7.91 | 0.28 | 0.654 |
| HCW[2] (kg) | 321$^{a}$ | 345$^{b}$ | 365$^{b}$ | 360$^{b}$ | 7.7 | 349 | 347 | 5.7 | 0.934 |
| MS[3] | 649$^{a}$ | 610$^{a,b}$ | 558$^{b}$ | 640$^{a,b}$ | 27.6 | 618 | 612 | 18.1 | 0.789 |
| YG[4] | 2.91$^{a}$ | 3.17$^{a}$ | 4.1$^{b}$ | 4.02$^{b}$ | 0.24 | 3.53 | 3.57 | .17 | 0.555 |
| BF[2] (mm) | 18.5$^{a}$ | 16.4$^{a}$ | 23.3$^{b}$ | 24.7$^{b}$ | 1.7 | 20.8 | 20.7 | 1.2 | 0.978 |
| REA ($cm^2$) | 84.0$^{a}$ | 83.1$^{a}$ | 86.1$^{a,b}$ | 90.2$^{b}$ | 2.3 | 86.8 | 84.8 | 1.6 | 0.288 |
| KPH (%) | 2.25$^{a}$ | 3.6$^{b}$ | 4.0$^{b}$ | 3.7$^{b}$ | .17 | 3.41 | 3.32 | .11 | 0.628 |

DMFE = Dry matter feed efficiency, BF = back fat measured at the 12$^{th}$ rib, MS = USDA marbling score, YG = USDA yield grade.
$^{a-d}$ Least squares means within a row lacking common superscript differ ($P < 0.05$).
[1] Ractopamine had no significant effects on growth or carcass characteristics.
[2] Covariates were not used for HCW and back fat analyses.
[3] USDA marbling score 300= slight$^{00}$, 400= small$^{00}$, 500= modest$^{00}$ 600= moderate$^{00}$
[4] USDA yield grade 1-5.
[5] *P*-Value for Control vs. Ractopamine.

**Table 8.** Orthogonal contrast for days on feed[1] with covariates of HCW and BF

| Trait | Linear | Quadratic | Cubic |
|---|---|---|---|
| ADG[3] | 0.001 | NS | NS |
| DMFE | NS | NS | NS |
| HCW[4] | NS | NS | < 0.001 |
| MS | NS | 0.026 | NS |
| YG | NS | 0.041 | NS |
| BF[4] | 0.002 | NS | 0.045 |
| REA | 0.038 | NS | NS |
| KPH | 0.001 | < 0.0001 | NS |

ADG = Average Daily Gain, DMFE = Dry matter feed efficiency, MS = USDA Marbling score, YG = USDA Yield Grade, BF = adjusted 12$^{th}$ rib back fat, KPH = Percent kidney, pelvic, and heart fat.
NS = Contrast were not considered significant is P > 0.05.
NR = Not reported in May et al. 1992.
Linear = $P < 0.01$
Quadratic = $P < 0.05$
[1] Days on feed were 79, 100, 121, and 142 d.
[2] DOF were 0, 28, 56, 84, 112, 140, 168, 196 d.
[3] ADG in days on feed groups were not different ($P > 0.05$) except d 28.
[4] Covariates were not used for HCW and back fat analyses.

**Figure 4.** Simple means comparison of USDA marbling score and USDA yield grade across 79, 100, 121, and 142 days on feed.

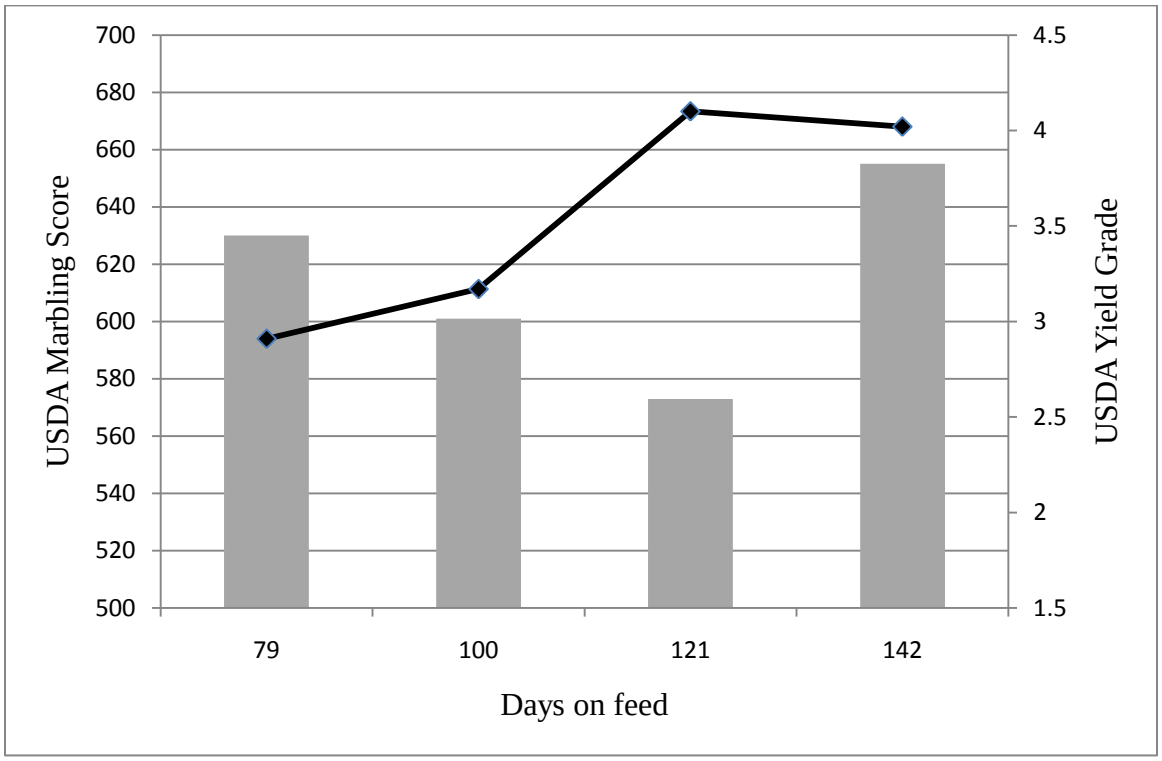


**Table 9.** Least squares means for growth traits during the 35 d ractopamine treatment[1] with covariates of HCW and BF

| Item | Days on Feed (n=64) | | | | | Treatment (n=64) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 79 (n=16) | 100 (n=16) | 121 (n=16) | 142 (n=16) | SEM | Control (n=32) | Ractopamine (n=32) | SEM | *P*- value |
| Trt ADG (kg/d) | 1.22[a] | 1.27[a] | 1.18[a] | 1.01[b] | 0.06 | 1.10 | 1.24 | 0.04 | 0.02 |
| Trt Gain (kg) | 42.8[a] | 44.9[a] | 41.5[a] | 35.5[b] | 2.2 | 38.6 | 43.7 | 1.5 | 0.02 |
| Trt DMI (kg) | 363[a] | 331[b] | 316[b] | 291[c] | 8.42 | 329 | 322 | 5.5 | 0.40 |
| Trt DMFE (f:g) | 9.94 | 7.90 | 7.70 | 9.31 | 1.08 | 9.86 | 7.57 | 0.69 | 0.02 |
| IGF-1 | 223.7 | 261.6 | 239.3 | 257.1 | 14.8 | 253.5 | 237.4 | 13.9 | 0.47 |
| LC (ng/mL) | 25.4[a] | 26.3[a] | 24.7[a] | 22.4[b] | 0.77 | 24.9 | 24.5 | 0.51 | 0.63 |

Trt Gain = Gain 35 d prior to harvest, Trt ADG = average daily gain 35 d prior to harvest, Trt DMFE = dry matter feed efficiency 35 d prior to harvest, Trt DMI = dry matter intake35 d prior to harvest, IGF-1 = IGF-1 concentrations 24 h prior to harvest, LC= leptin concentration 24 h prior to harvest.

[a-e] Least squares means within a row lacking common superscript differ ($P < 0.05$).

[1] Ractopamine administered at 300 mg/hd/d.

**Table 10.** Pearson correlation coefficients for growth traits and final leptin concentrations (n = 64)

| | LC | BW | ADG | DOF | DMFE | DMI | Trt ADG | Trt DMI | Trt DMFE |
|---|---|---|---|---|---|---|---|---|---|
| LC | 1.00 | 0.25** | 0.28** | -0.11 | -0.11 | 0.13 | 0.27** | 0.38** | -0.17 |
| BW | | 1.00 | 0.51† | 0.40† | -0.29** | 0.67† | 0.41† | 0.48† | -0.17 |
| ADG | | | 1.00 | -0.19 | -0.67† | 0.18 | 0.67† | 0.63† | -0.30** |
| DOF | | | | 1.00 | 0.11 | 0.82† | -0.20 | -0.30** | 0.01 |
| DMFE | | | | | 1.00 | 0.10 | -0.60† | -0.15 | 0.71† |
| DMI | | | | | | 1.00 | 0.06 | 0.21* | -0.01 |
| Trt ADG | | | | | | | 1.00 | 0.48† | -0.74† |
| Trt DMI | | | | | | | | 1.00 | -0.02 |
| Trt DMFE | | | | | | | | | 1.00 |

LC= Final leptin concentration, BW=Live Body Weight, ADG= Average Daily Gain, DOF= Days on Feed, DMFE= Dry matter feed efficiency, DMI= Dry Matter Intake, Trt ADG=Average Daily Gain 35d prior to harvest, Trt DMI= Dry Matter Intake 35 d prior to harvest, Trt DMFE= Dry matter feed efficiency 35 d prior to harvest.

†=$P < 0.001$

**=$P < 0.05$

*=$P < 0.10$

**Table 11.** Pearson correlation coefficients for carcass traits and final leptin concentrations (n = 64)

| | LC | KPH | MS | HCW | Adj. BF | REA | YG | DOF |
|---|---|---|---|---|---|---|---|---|
| LC | 1.00 | 0.14 | 0.03 | 0.27** | 0.22* | 0.13 | 0.20 | -0.11 |
| KPH | | 1.00 | -0.25** | 0.44† | 0.36** | 0.11 | 0.48† | 0.55† |
| MS | | | 1.00 | 0.12 | 0.14 | 0.02 | 0.06 | 0.05 |
| HCW | | | | 1.00 | 0.54† | 0.51† | 0.52† | 0.44† |
| Adj. BF | | | | | 1.00 | -0.00 | 0.92† | 0.53† |
| REA | | | | | | 1.00 | -0.27** | 0.29** |
| YG | | | | | | | 1.00 | 0.44† |
| DOF | | | | | | | | 1.00 |

LC= Final Leptin Concentration, KPH=Kidney Pelvic and Heart fat, MS= Marbling score, HCW=Hot Carcass Weight, Adj BF= Adjusted 12$^{th}$ rib back fat, REA=longissimus dorsi area, YG= USDA Yield Grade, DOF= Days on Feed.

†=$P < 0.001$

**=$P < 0.05$

*=$P < 0.10$

**Table 12.** Pearson correlation coefficients for leptin concentrations at 35, 14, 7, and 1 d before harvest

| | LC35 | LC21 | LC7 | LC1 | HCW | BF | REA | MS | YG |
|---|---|---|---|---|---|---|---|---|---|
| LC35 | 1.00 | 0.76† [a] | 0.73† [b] | 0.76† [a] | 0.37** [a] | 0.37** [a] | 0.16[a] | 0.03 [a] | 0.34** [a] |
| LC21 | | 1.00 | 0.81† [a] | 0.80† | 0.36** | 0.37** | 0.13 | -0.04 | 0.36** |
| LC7 | | | 1.00 | 0.80† [a] | 0.45† [a] | 0.39** [a] | 0.27**[a] | 0.05 [a] | 0.34** |
| LC1 | | | | 1.00 | 0.27** | 0.22* | 0.14 | 0.03 | 0.20** |
| HCW | | | | | 1.00 | 0.54† | 0.51† | 0.12 | 0.52† |
| BF | | | | | | 1.00 | 0.00 | 0.14 | 0.92† |
| REA | | | | | | | 1.00 | 0.03 | -.26** |
| MS | | | | | | | | 1.00 | 0.07 |
| YG | | | | | | | | | 1.00 |

LC35= leptin concentration 35 d prior to harvest, LC21= leptin concentration 21 d prior to harvest, LC7= leptin concentration 7 d prior to harvest, LC1= leptin concentration 1 d prior to havest, HCW= hot carcass weight, BF= 12$^{th}$ rib back fat 35 d prior to harvest, REA= longissimus dorsi area, MS= marbling score, YG= yield grade.

[a] = (n=63)

[b]= (n=62)

†=$P < 0.001$

**=$P < 0.05$

*=$P < 0.10$

**Table 13.** Pearson correlations for ultrasound measurements and leptin concentrations taken 35 d prior to harvest (n=64)

| | LC | FUS BF | FUS IMF | FUS REA |
|---|---|---|---|---|
| LC | 1.00000 | 0.33**[a] | 0.11[a] | 0.31**[a] |
| FUS BF | | 1.00 | 0.48† | 0.18 |
| FUS IMF | | | 1.00 | 0.19 |
| FUS REA | | | | 1.00 |

LC= leptin concentration 35 d prior to harvest, FUS BF= Ultrasound 12$^{th}$ rib back fat 35 d prior to harvest, FUS IMF= Ultrasound percent intramuscular fat 35 d prior to harvest, FUS REA= Ultrasound longissimus dorsi area 35 d prior to harvest.
[a] = (n=63)
†=$P < 0.001$
**=$P < 0.05$
*=$P < 0.10$

**Table 14.** Pearson correlation coefficients for final ultrasound and final leptin concentration taken 1 d prior to harvest (n=64)

| | LC | US BF | US IMF | US REA[a] |
|---|---|---|---|---|
| LC | 1.00 | 0.30** | 0.24* | 0.26** |
| US BF | | 1.00 | 0.30** | 0.16 |
| US IMF | | | 1.00 | -0.02 |
| US REA | | | | 1.00 |

LC= Final leptin concentration, US BF= Final Ultrasound 12th rib back fat, US IMF= Final Ultrasound percent intramuscular fat, US REA= Final Ultrasound longissimus dorsi muscle area
[a]N=69 and other n=70
†=$P < 0.001$
**=$P < 0.05$
*=$P < 0.10$

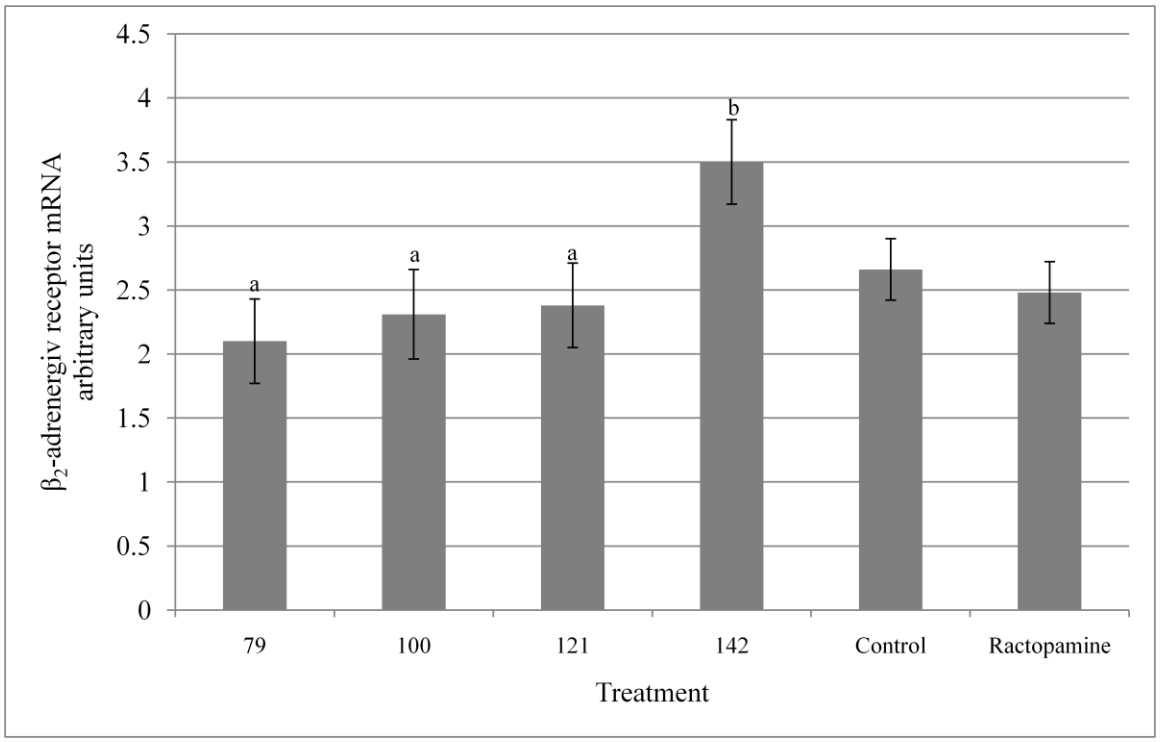
**Table 15.** Least squares means for control and treatment[1] heifer blood metabolite data for NEFA, IGF-1, Insulin, Glucose, and leptin 35, 21, 7 and 1 day before harvest, with covariates of HCW and BF.

| | | Days Prior to Harvest | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 35 d | | 21 d | | 7 d | | 1 d | |
| Blood Metabolite | Treatment | Amount | P-Value | Amount | P-Value | Amount | P-Value | Amount | P-Value |
| NEFA | CON | 0.190 | 0.71 | 0.227 | 0.02 | 0.217 | 0.02 | 0.218 | 0.19 |
| | RAC | 0.186 | | 0.184 | | 0.178 | | 0.189 | |
| IGF-1 (ng/mL) | CON | n/a | n/a | 254 | 0.33 | 244 | 0.74 | 245 | 0.47 |
| | RAC | n/a | | 239 | | 239 | | 232 | |
| Insulin (μIU/mL) | CON | 47.2 | 0.17 | 39.0 | 0.08 | 52.4 | < 0.01 | 61.4 | 0.01 |
| | RAC | 37.6 | | 26.8 | | 26.8 | | 40.4 | |
| Glucose (mg/dL) | CON | 86.2 | < 0.01 | 88.1 | 0.52 | 85.7 | 0.09 | 88.6 | 0.27 |
| | RAC | 76.8 | | 85.1 | | 79.1 | | 84.3 | |
| Leptin (ng/mL) | CON | 23.1 | 0.79 | 23.1 | 0.62 | 24.6 | 0.73 | 24.0 | 0.93 |
| | RAC | 23.4 | | 23.6 | | 24.8 | | 24.7 | |

CON = control heifers (n = 32), RAC = heifers receiving ractopamine at 300 mg/hd/d (n = 32)

[1] Ractopamine administered at 300 mg/hd/d.

**Figure 5.** $\beta_2$-adrenergic receptor mRNA expression in arbitrary units in the gastrocenimus muscle.

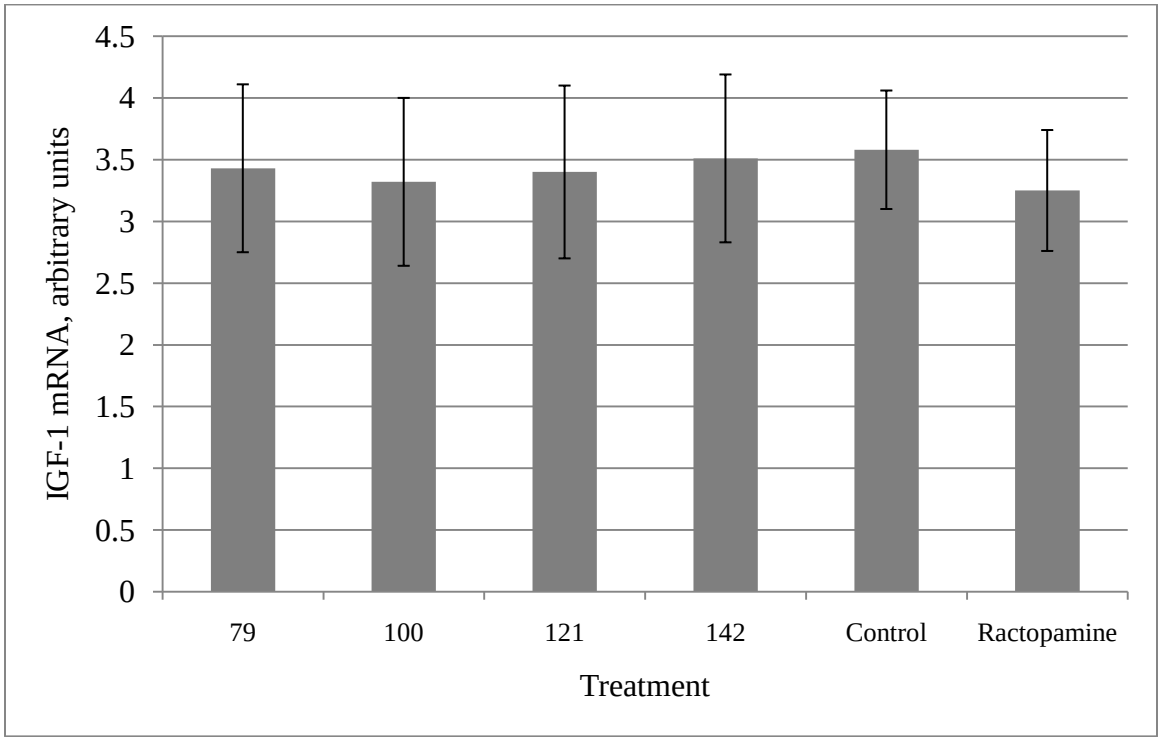



79 (79 d on feed), 100 (100 d on feed), 121 (121 d on feed), 142 (142 d on feed), Control (heifers not fed ractopamine), Ractopamine (ractopamine administered at 300 mg/hd/d 35 d prior to harvest.

[a-b] For days on feed 79, 100, 121 and 142 bars lacking common superscript differ by ($P < 0.05$).

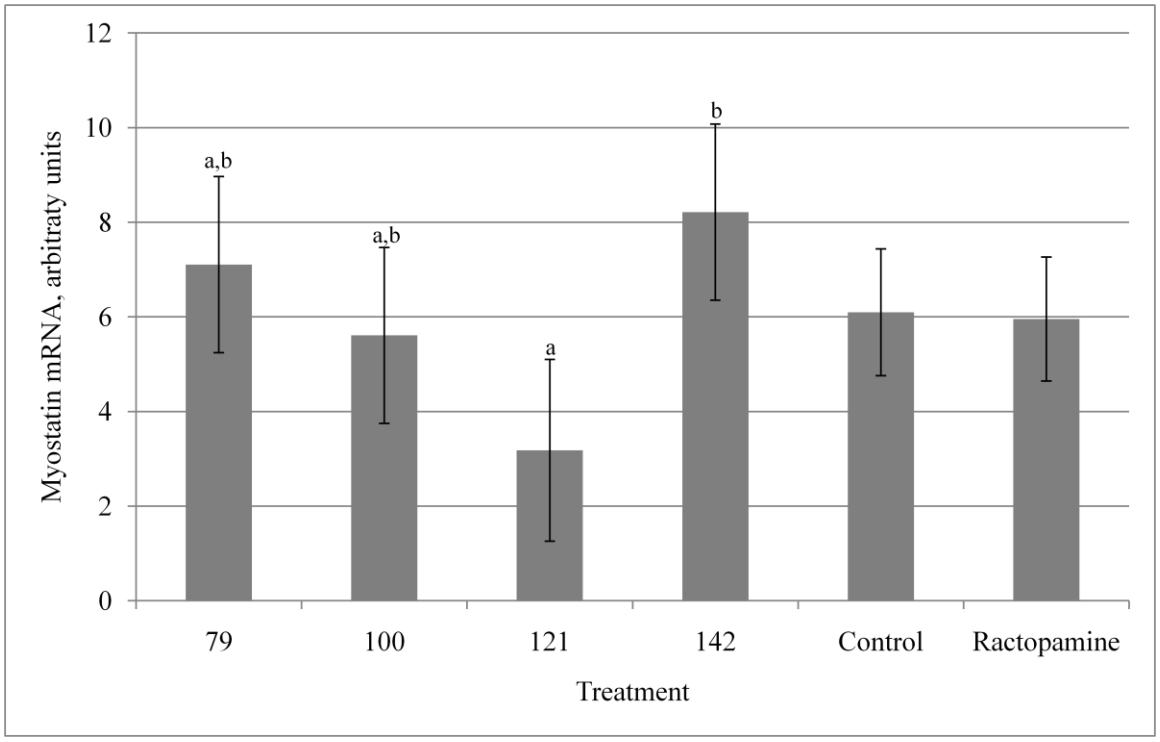
Control and Ractopamine heifers did not differ in $\beta_2$-adrenergic receptor mRNA expression ($P > 0.05$).

**Figure 6.** IGF-1 mRNA expression in arbitrary units in the gastrocenimus muscle.




79 (79 d on feed), 100 (100 d on feed), 121 (121 d on feed), 142 (142 d on feed), Control (heifers not fed ractopamine), Ractopamine (ractopamine administered at 300 mg/hd/d 35 d prior to harvest.
No significant differences across days on feed or between control and ractopamine groups were observed.

**Figure 7.** Myostatin mRNA expression in arbitrary units in the gastrocenimus muscle.

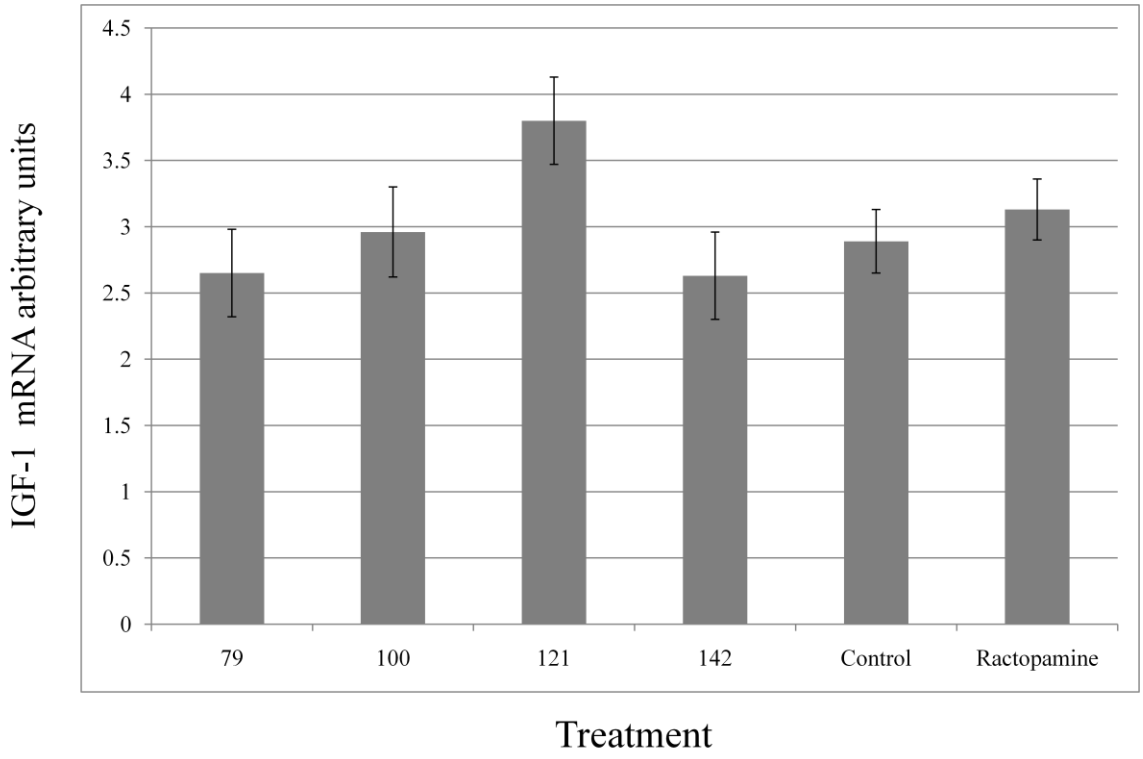



79 (79 d on feed), 100 (100 d on feed), 121 (121 d on feed), 142 (142 d on feed), Control (heifers not fed ractopamine), Ractopamine (ractopamine administered at 300 mg/hd/d 35 d prior to harvest.

[a-b] For days on feed 79, 100, 121 and 142 bars lacking common superscript differ by ($P < 0.05$).

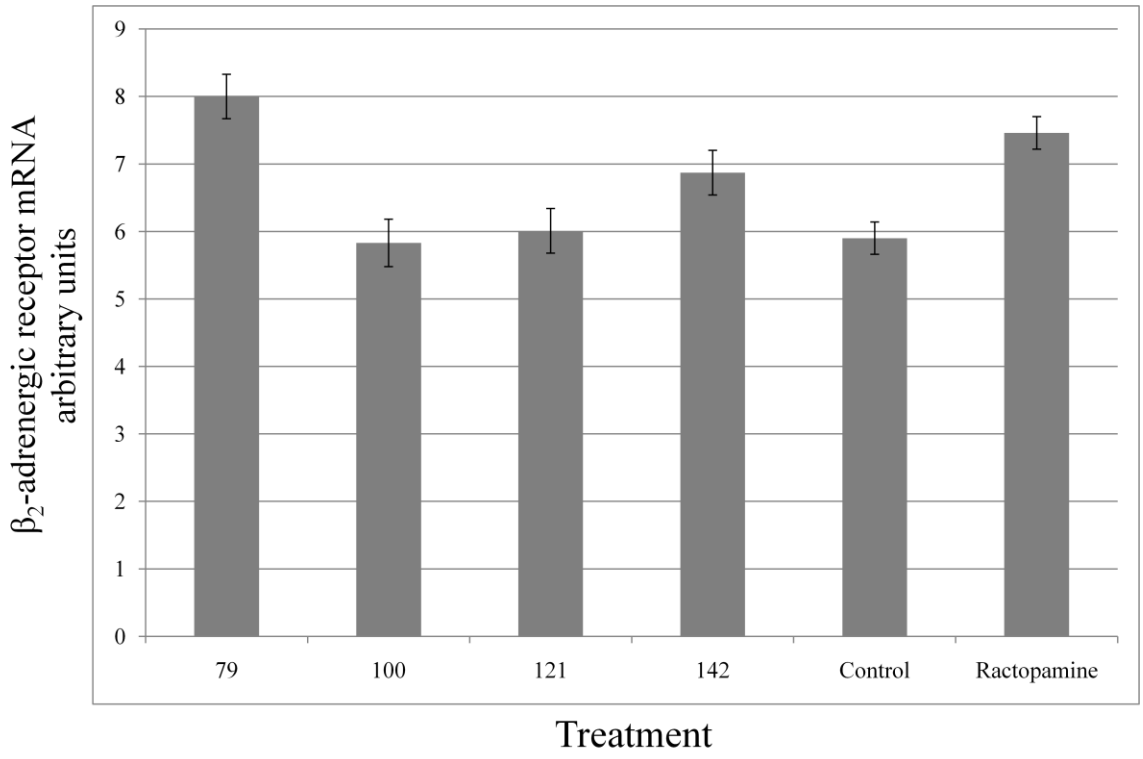
Control and Ractopamine heifers did not differ in myostatin mRNA expression ($P > 0.05$).

**Figure 8.** IGF-1 or mRNA expression in arbitrary units in rump adipose tissue.




79 (79 d on feed), 100 (100 d on feed), 121 (121 d on feed), 142 (142 d on feed), Control (heifers not fed ractopamine), Ractopamine (ractopamine administered at 300 mg/hd/d 35 d prior to harvest.
No significant differences across days on feed or between control and ractopamine groups were observed.

**Figure 9.** $\beta_2$-adrenergic receptor mRNA expression in arbitrary units in rump adipose tissue.




79 (79 d on feed), 100 (100 d on feed), 121 (121 d on feed), 142 (142 d on feed), Control (heifers not fed ractopamine), Ractopamine (ractopamine administered at 300 mg/hd/d 35 d prior to harvest.
No significant differences across days on feed or between control and ractopamine groups were observed.