THE INFLUENCE OF BREED AND TEMPERAMENT ON CIRCULATING CONCENTRATIONS OF INSULIN-LIKE GROWTH FACTOR-I (IGF-I) AND ITS RELATIONSHIP TO FEED EFFICIENCY IN BEEF CATTLE

A Thesis

by

LISA CAROL CALDWELL

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2009

Major Subject: Physiology of Reproduction

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Approved by:

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ABSTRACT

The Influence of Breed and Temperament on Circulating Concentrations of Insulin-like Growth Factor-I (IGF-I) and Its Relationship to Feed Efficiency in Beef Cattle. (May 2009)

Lisa Carol Caldwell, B.S., Texas A&M University

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Insulin-like growth factor–I (IGF-I) is a growth hormone that acts as a key modulator of the growth axis. Serum and plasma concentrations of IGF-I have been linked to economically important traits in beef cattle.

In order to determine whether concentrations of IGF-I differed among breeds of beef cattle, plasma samples from purebred and crossbred animals were analyzed. Two calf crops were derived from three-breed diallel matings using temperate and tropically-adapted breeds of cattle. The breeds consisted of temperate *Bos taurus* (A; Angus), tropical *Bos indicus* (B; Brahman), and tropical *Bos taurus* (R; Romosinuano). Plasma samples were obtained from 10 heifers and 10 steers of each breed-type at weaning, and two dates post-weaning. Concentrations of IGF-I were determined by radioimmunoassay (RIA). Breed differences were observed ($P < 0.001$). Relative to the temperate *Bos taurus* breed, IGF-I was greater in tropically-adapted breed-types.

In an effort to select for the improvement of economically important traits, experiments were performed to explore the possible use of concentration of IGF-I and

temperament assessment as tools for selection. Using a Calan gate system, 3 Brahman heifer crops were fed during70-day trials. Performance and feed intake data were collected to determine feed efficiency. Temperament, determined by exit velocity and pen score, was evaluated at weaning. Serum samples were taken at weaning and days 0 and 70 of each trial. Concentrations of IGF-I and cortisol were determined by RIA. Correlations including IGF-I were weak ($P > 0.05$). Temperament had no significant effect on RFI but may affect ADG.

In an attempt to examine the relationship between IGF-I and RFI, body weight and feed intake data were collected during individual finishing phase feeding trials, on steers at El Reno, OK. The breeds consisted of temperate *Bos taurus* (A; Angus), tropical *Bos indicus* (B; Brahman), and tropical *Bos taurus* (R; Romosinuano). Plasma samples were obtained from 10 steers of each breed-type at weaning and days 0 and 60 of each finishing phase. Concentrations of IGF-I were determined by RIA. Correlations between IGF-I and RFI were weak ($P > 0.05$). Breed and year significantly influenced RFI ($P < 0.01$).

DEDICATION

To the loving memory of my second cousin, Mrs. Sarabeth Caldwell Waller (1934 –2007), for whom you may all thank for encouraging and boosting my keen sense of humor and wit. She gave me an appreciation for the past and a drive for the future. You truly were an angel flying close to the ground.

To the loving memory of my father-in-law and one of the most intelligent men I have ever met, Mr. Jameson "Jim" Mapel (1946 –2008). He was my mentor for practical joking, my fishing buddy and my friend. He could always say so much with so few words. I'll keep the yellow bellies cold until we meet again.

ACKNOWLEDGEMENTS

I would like to thank my committee co-chairs, Dr. Randel and Dr. Riggs, and my committee members, Dr. Welsh and Dr. Chase, for their guidance and support throughout the course of this research. Thanks to all of our outside collaborators for having such great senses of humor and for making my time in the graduate program fun and exciting. Thanks to Dr. Forbes for his assistance in matters of feed efficiency. Thanks to Dr. Forrest for the opportunity to teach ANSC 433. Extra thanks go to Dr. Welsh for accepting his nickname with honor and for always having candy.

Thanks also go to my fellow graduate students and colleagues. In particular, I would like to extend thanks to Mr. Kevin "Booby" Curley and Ms. Andrea "Beeby" Loyd for countless meaningful discussions. Your friendship has meant more to me than words can express.

Finally, thanks to my best friend and fiancé, Mr. Steven Mapel, for his encouragement to pursue this degree, his continued support and his ability to answer every question I ever asked him.

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CHAPTER I

INTRODUCTION

Insulin-like growth factor I (IGF-I), also known as somatomedin-C, is a polypeptide growth hormone structurally related to insulin. As a growth promoting hormone, IGF-I is responsible for regulating a variety of cellular processes and acts as a signaling molecule between cells. The tissues targeted by IGF-I range from bone, muscle and adipose to several organ systems. IGF-I functions as a key modulator in the growth axis of several species by stimulating glucose uptake and protein synthesis. This stimulation is made possible by the transport of IGF-I to target tissues and the binding of IGF-I to the transmembrane insulin-like growth factor-I receptor (IGFR) with subsequent activation of phosphorylation cascades.

The concentration of circulating IGF-I is a quantitative and heritable (h = ~0.4) trait (Herd et al., 1995) that has been used successfully as a selection tool for improvement of economically important traits in both pigs (Bunter et al., 2002) and sheep (Blair et al., 2002). Serum and plasma concentrations of IGF-I have been linked to many economically important traits in beef cattle as well.

This thesis follows the style of Journal of Animal Science.

Johnston et al. (2001) found favorable correlations between the circulating concentration of IGF-I and traits such as live and carcass weight, carcass fatness and the feed efficiency measurements of average daily gain (ADG) and residual feed intake (RFI). This favorable link between IGF-I and feed efficiency has led to publications that involve the use of IGF-I concentration as an indirect predictor of RFI (Wood et al., 2004). With the rising costs of both feed stuffs and feed intake measurements, an inexpensive predictor of feed utilization and efficiency would be of great importance. Determination of concentrations of IGF-I is relatively inexpensive, can be measured earlier in life and can be utilized on much larger numbers of animals (Moore et al., 2003), thus justifying the need for experiments to determine the efficacy of this prediction or selection method.

Research has shown that circulating concentrations of IGF-I differ between breeds of beef cattle. Simpson et al. (1997) notes that concentrations of IGF-I differ between Brahman and Angus cows, with the Brahman breed exhibiting higher concentrations of the growth hormone. Our experiments will examine the differences in circulating concentrations of IGF-I between different breed-types and sexes of beef cattle, while also exploring its use as a predictor of feed efficiency. Temperament or reaction to a stressor has also been implicated in altering the concentrations of IGF-I. Studies in mice have proven that circulating concentrations of IGF-I decrease as a result of repeated stress (Laugero and Moberg, 2000). Among Brahman females, individual animal temperament and concentration of cortisol will be compared to circulating

concentrations of IGF-I, feed efficiency measurements and ultrasound traits in order to assess any effects that temperament may have upon these economically important traits.

CHAPTER II

LITERATURE REVIEW

IGF-I Structure

When first discovered by Salmon and Daughaday (1957), the peptide was termed sulphation factor for its role in the incorporation of sulfate into chondroitin sulfate of cartilage. Then, in 1972, this factor, along with two other peptides thought to be members of the same family, were renamed somatomedins, as a joining of two terms characteristic to their function. The first being "somato," for its association with somatotrophin or growth hormone (GH), and the second being "medin," for its intermediary role in the actions of GH (Daughaday, 1972). In 1978, the protein and its homolog IGF-II were purified and sequenced (Rinderkneckt and Humble, 1978). Further purification and structural determination by Baxter (1986) led to the conclusion that the three somatomedin family members were indeed the same protein and the peptide was termed insulin-like growth factor I for its insulin-like function in glucose uptake.

The *IGF-I* gene spans more than 45 kilobases of genomic DNA and consists of 6 exons, 5 introns and two promoters (Rotwein, 1991). *IGF-I* encodes a 70-amino acid, single-chain, low molecular weight polypeptide that is responsible for promoting cellular mitosis and differentiation in a variety of systems and acts as a key modulator of the growth axis. Bioassays have established that all mammalian and some non-mammalian

serum contains IGF-I (Van den Brande et al., 1974). The amino acid sequence of IGF-I has been determined in more than 50 different species and all show a substantial degree of conservation (D'Ercole et al., 1984). Cattle, pig and human amino acid sequences of IGF-I are identical (Bishop et al., 1989, Francis et al., 1989).

While the majority of circulating IGF-I originates in the liver, other organs such as the lung, kidney, heart, stomach and gonads, as well as muscle and bone, can also produce significant quantities of this growth promoting hormone (Daftary and Gore, 2005). The synthesis of IGF-I at these multiple locations suggests that along with its endocrine role it may also function in an autocrine or paracrine fashion (D'Ercole et al., 1984).

The regulation of the life processes controlled by IGF-I are achieved through the inner workings of the growth hormone releasing hormone (GHRH)-growth hormone(GH)-insulin-like growth factor -I axis (Figure 1). This axis is commonly referred to as the GH/IGF-I, growth or somatotrophic axis. Within the axis, GH, released in a pulsatile manner, is the primary stimulator of IGF-I production (D'Ercole et al., 1984). Newbold et al. (1997) was able to demonstrate that treatment of dairy cattle with exogenous bovine somatotrophin (bST) resulted in an increase in plasma concentrations of IGF-I. He also found that nutrient density had a significant effect on circulating concentrations of IGF-I by increasing the quantity of hepatic binding sites for bST or GH. Both studies proved the ability of GH to stimulate the production of IGF-I. The production of GH within the anterior pituitary is regulated by GHRH release from the hypothalamus. Release of GHRH is able to stimulate the synthesis of GH by

increasing both the amount and rate of GH gene transcription (Barinaga et al., 1985). Also shown in Figure 1 is IGF-I's negative feedback on GH release. Upon stimulation and secretion of IGF-I, somatostatin (SRIF) is activated within the hypothalamus which in turn inhibits the release of GH. Somatostatin inhibits the response to GHRH within the anterior pituitary. IGF-I has also been shown to inhibit the release of GH at both the hypothalamus and anterior pituitary (Gluckman and Breier, 1986).

The IGF-I binding proteins (IGFBPs) coordinate and regulate the activities of IGF-I. The binding proteins prolong the half-life of IGFs by protecting them from degradation. In addition, they act as storage reservoirs for controlled delivery of the peptide to target cells and facilitate transport to target tissues (Giudice, 1992). Six different IGFBPs have been identified that regulate the bioavailability of IGF-I. Both IGFBP-1 and IGFBP-3 are known to be the most active in adult life, while the others aid in fetal development and early growth. Of the six binding proteins, IGFBP-3 is the most prominent. Insulin-like binding protein-3 has the highest binding affinity, and is responsible for the formation of the circulating complex that allows for the presence of IGF-I in serum (Hadley, 2000). This 150-kD ternary complex circulates nearly 85% of the IGF-I found in serum. It also serves as the reservoir that allows the freeing of IGF-1 by binding protein proteolysis. The complex contains IGF-I itself, IGFBP3, and an acid-labile subunit (ALS). This glycoprotein subunit is manufactured in the liver by the stimulation of GH. It is responsible for the stability and the extension of half-life for the complex (Baxter, 1990). The dependence upon GH for the formation of the complex was discovered in hypophysectomized rats. Zapf et al., (1989) found that the ternary

complex will not form in the absence of GH. Upon infusion of GH, the complex was formed.

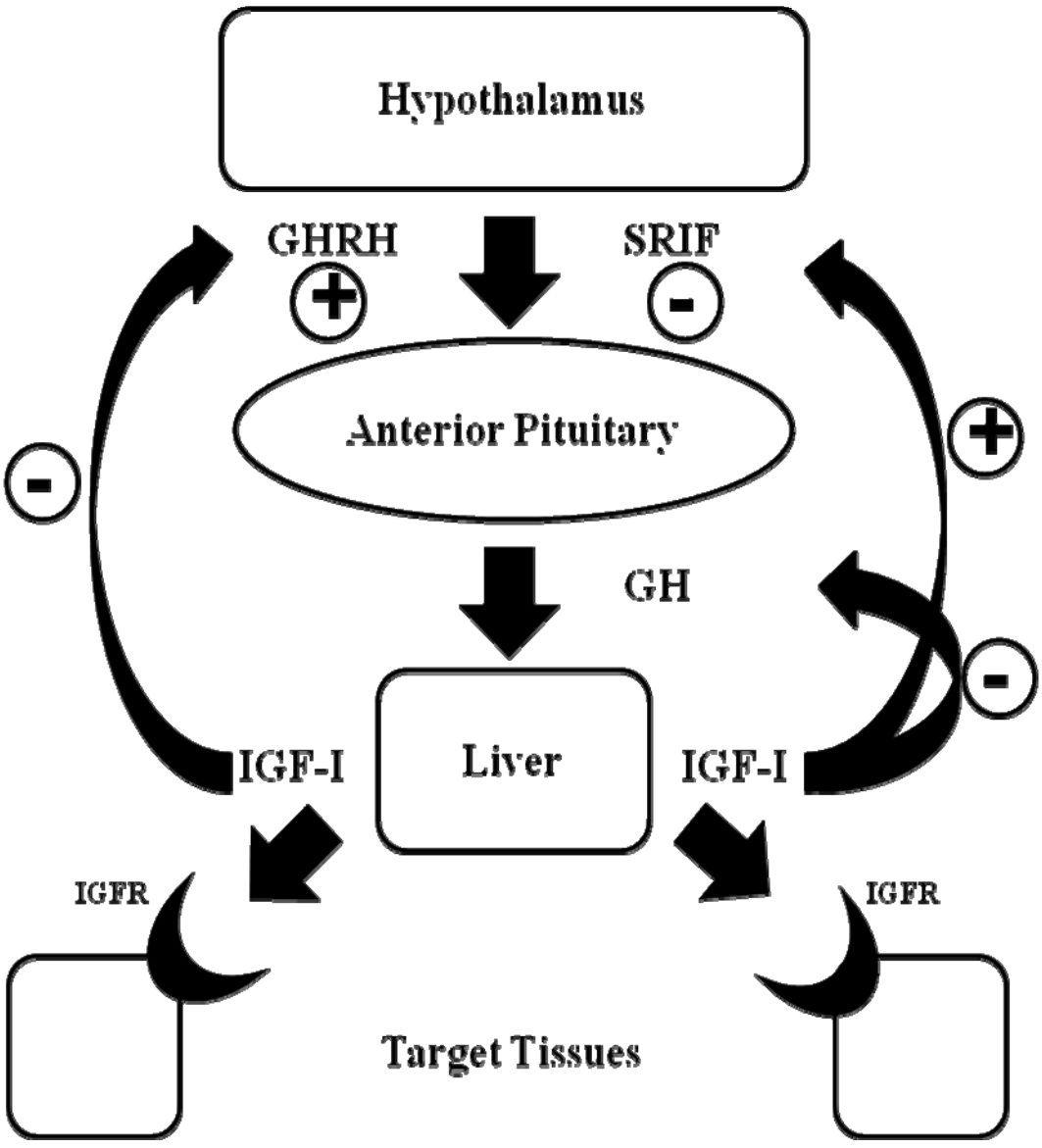


Figure 1. Schematic diagram of the growth axis.

Once produced through GH stimulation and transported by binding proteins to target tissues, the primary mechanism behind the initiation of the signaling pathway is the interaction of IGF-I with its specific cell surface receptor tyrosine kinase (RTK). The IGF-receptor is a tetramer glycoprotein comprised of two α-β heterodimers linked together by interchain disulfide bonds. The α-chains reside outside the cell and make up most of the binding surface while the β-chains are intracellular and contain the transmembrane segment along with the tyrosine kinase domain. In response to ligand binding to the α-subunit, the heterodimers join and the β-subunit RTK is activated (Li and Hristavo, 2006). Upon RTK activation and phosphorylation of insulin receptor substrate-I (IRS-I), adapter proteins bind to and convert the GTPase, Ras, from an inactive state to an active state by the exchange of GDP for GTP. This exchange leads to a conformational change in Ras that activates the map kinase (MAPK) cascade and allows for phosphorylation of transcriptional activators within the nucleus (Watson et al., 2008).

IGF-I Function

The actions of IGF-I upon transcriptional activation can vary depending upon the target tissue. Most importantly, IGF-I is involved in somatic growth acting as the mediator of GH dependent building of body mass. This effect was first demonstrated by Van Buul-Offers and Van den Brande (1982) in mice. Snell dwarf mice were administered IGF-I over a 4-week period during which increases in both body weight and length were observed. This study is of importance when combined with previous

results from Dev and Lasley (1969) who determined that concentrations of GH did not differ between dwarf Hereford cattle and normal Hereford cattle. The similar concentrations of GH between the distinctly different types of cattle suggest that GH alone does not promote growth.

Growth is defined as the normal expansion of size as produced by the accretion of tissues similar in constitution to that of the original. It can be achieved by cell proliferation and by cell enlargement (Gerrard and Grant, 2007). Most often, IGF-I mediates growth through cell proliferation and DNA synthesis. Early reports note the synthesis of DNA and the multiplication of cells in varying tissues during growth. Kato et al. (1981) found that DNA synthesis in cultured bovine chondrocytes could be greatly enhanced by the addition of IGF-I, then termed somatomedin-C, purified from bovine cartilage. This same cell proliferation-stimulating activity has been noted among fibroblasts, lymphocytes, Sertoli cells, fetal brain cells and myoblasts. The mitogenic behavior of IGF-I is made possible by its ability to stimulate incorporation of thymidine into DNA and by promoting the phosphorylation of proteins responsible for cell replication (Baxter, 1986).

Named for its insulin-like properties, IGF-I is active in stimulating glucose metabolism and protein synthesis (Baxter, 1986). It is known that in states of malnutrition or fasting, concentrations of IGF-I significantly decrease. Breier et al. (1986) investigated the relationship between circulating concentrations of GH and IGF-I in steers fed at three different planes of nutrition. The underfed steers experienced great increases in concentration of GH with reduced concentrations of IGF-I. These

conflicting concentrations could be explained by GH insensitivity within the liver and other tissues. The GH increase could also be in part due to the lack of negative feedback on somatostatin within the hypothalamus resulting from the lowered IGF-I. While in a state of nutrient deficiency, animals will experience reduced glucose uptake and protein synthesis and instead rely on the breakdown of fat stores for energy (Bauman and Currie, 1980). An excellent way to observe the actions of IGF-I in glucose metabolism is among underfed animals. Douglas et al. (1991) observed that IGF-I infusions in fasted lambs could reduce concentrations of plasma glucose by increasing the rate of uptake. The same study also demonstrated that the infusions were responsible for conserving protein by promoting protein synthesis. The normal actions of IGF-I were restored by the addition of rIGF-I to the lambs thus proving the importance of this factor in glucose metabolism.

IGF-I is also recognized as a signal that controls reproductive function. Reports indicate that the IGF-I system and its interaction with the reproductive axis may account for increased reproductive efficiency. Cows with better reproductive performance have been found to have higher concentrations of circulating IGF-I (Meikle et al., 2004**)**. IGF-I is believed to work together with follicle stimulating hormone (FSH) to directly regulate ovarian follicular development (Spicer et al., 2002). Webb et al. (2002) detected mRNA encoding IGFR in regions of the bovine corpus luteam (CL) specific to steriodogenic luteal cells. His studies found that IGF-I was capable of stimulating progesterone secretion from the CL as well as activation of both StAR and P450scc enzymes in steroid production. Findings also indicate that IGF-I may be a direct

regulator of gonadotropin-releasing hormone (GnRH) and aid in the onset of puberty (Daftary and Gore, 2005).

Temperament, Stress and the Hypothalamic-Pituitary-Adrenal Axis

Temperament in cattle can be characterized as a fear or avoidance response to interactions with humans (Murphy et al., 1994). This fear response is often termed a stress response and its induction is not limited to human-animal interactions (Burrow, 1997). Minton (1994) notes that management as well as environmental conditions can induce a stress response. The term stressor encompasses such practices as weaning, branding, dehorning and vaccination; transportation and the mixing of unfamiliar animals, and climate extremes (Minton, 1994). Moberg (1999) described this biological response to stressors as a coping mechanism used to return an animal to homeostasis. The level of response and thus the degree of stress incurred is dependent upon the nature or temperament of the animal. Response to stress increases the rate of metabolism which leads to energy consumption through the activation of the hypothalamic-pituitary-adrenal (HPA) axis or stress axis (Figure 2) (Brockman and Laarveld, 1986). The stress response results in production and secretion of glucocorticoids from the adrenal cortex and catecholamines, epinephrine and norepinephrine, from the adrenal medulla. Hydrocortisone, or cortisol, is the primary glucocorticoid steroid involved in the stress response. The pro-opiomelanocortin (POMC) product, adrenocorticotropin hormone (ACTH), is the key modulator of cortisol secretion from the adrenal cortex. Release of ACTH from the anterior pituitary is triggered primarily by corticotrophin-releasing

hormone (CRH) and partly by vasopressin (VP). Both CRH and VP are synthesized by parvicellular neurons of the hypothalamic paraventricular nucleus and are secreted upon activation of the HPA axis in response to a stressor (Carrasco and Van de Kar, 2003). Multiple negative feedback loops regulate the axis. Production of CRH is mediated by its own release along with feedback from the release of ACTH. Cortisol acts upon both the hypothalamus to stop secretion of CRH as well as the anterior pituitary to prevent release of ACTH (Calogero et al., 1988).

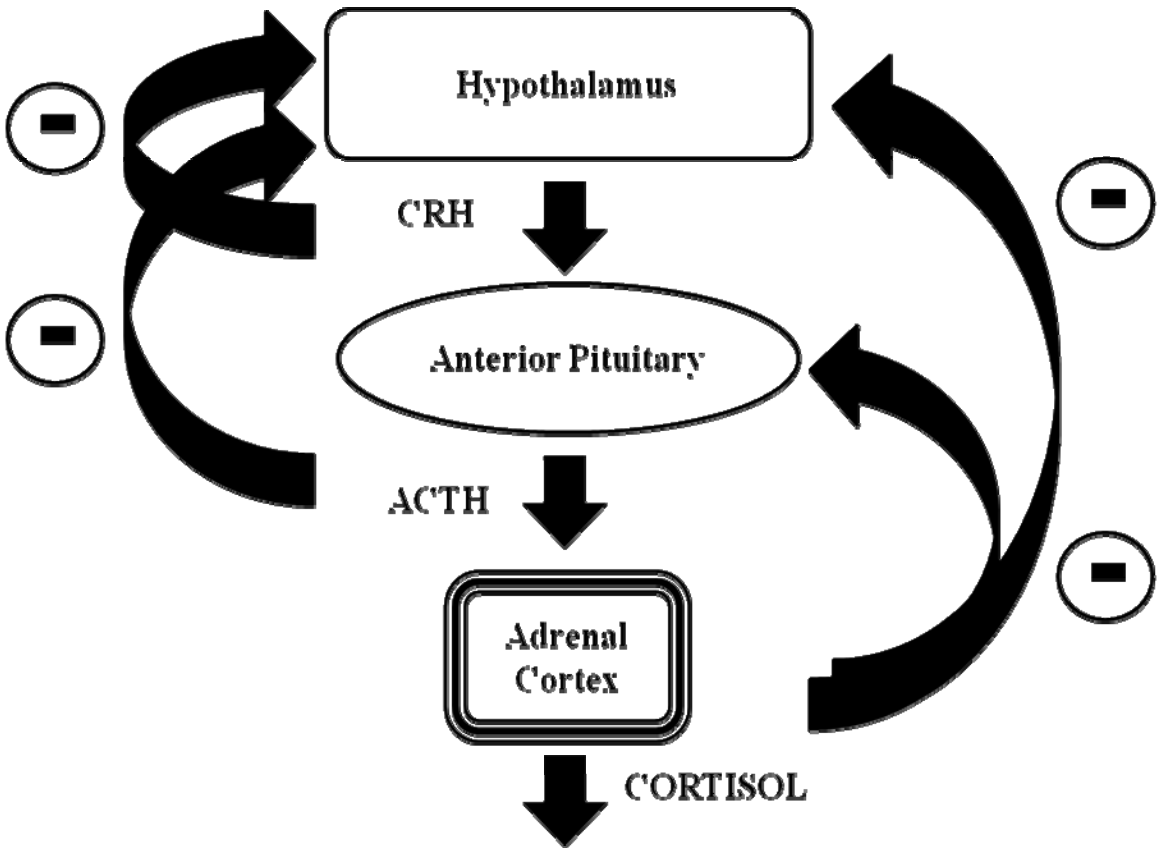


Figure 2. Schematic diagram of the HPA axis.

High concentrations of cortisol have been associated with stressed animals (Minton, 1994) and have a reported link to decreased growth rate (Obst, 1974). Feedlot heifers with access to shade reached their target slaughter weight 20 days sooner than heifers without access to shade and exhibited higher gains (Mitlohner et al., 2001). Cortisol can inhibit bone formation by decreasing the expression of endothelial growth factor in growth plate chondrocytes (Koedam et al., 2002). Knott et al. (2007) found a highly significant correlation between feed efficiency and serum concentrations of cortisol following an ACTH challenge in rams. He found that sheep with high residual feed intake had a greater response to the challenge than did the more efficient animals. Cattle with poor or excitable temperaments have also been reported to experience reduced ADG (Fell et al., 1999; Voisinet et al., 1997a), lighter slaughter and carcass weights (Burrow and Dillon, 1997), lower body condition scores and dressing percentage (Petherick et al., 2002), increased incidence of dark cutters (Voisinet et al., 1997b), as well as a compromised immune system (Fell et al., 1999).

Assessment of Temperament

Early assessment of temperament was done in order to maintain a safe working environment for animal handlers with methods varying between different operations. Once evidence was uncovered that proved poor temperament was detrimental to economically important traits, more reliable and consistent measures were adopted. Measurements such as exit velocity and pen score are used to determine temperament in beef cattle. Both are designed to allow observation of the degree of aggressiveness

exhibited from an animal towards its handlers. The measurement of exit velocity (Burrow et al., 1988; Curley et al., 2006) is used to measure the rate (m/sec) at which an animal exits a squeeze chute and traverses a fixed distance of 1.83 m. Infrared sensors attached to panels along the chute exit path are used to remotely trigger the start and stop of a timing device used to record time, in seconds, between crossing the first and second sensors. This method is objective and is positively correlated with cortisol concentrations (Curley et al., 2006). Cattle that react mildly to handling and exit slower are considered calm and have lower concentrations of cortisol relative to the cattle that exit more rapidly. Specifically, those that react aggressively and exit rapidly are said to have an undesirable temperament and possess increased concentrations of cortisol (Curley, 2004). Curley et al. (2006) reported that although exit velocity did decrease over time, individual animal rank remained constant, thus proving exit velocity as a functional assessment of temperament. Pen scoring (Hammond et al., 1996) is a more subjective measurement and is given based upon the reaction of the animal to the presence of a handler inside its pen or corral. Scores are given on a 1 (calm) to 5 (excitable) scale based upon the degree of aggressiveness exhibited towards a handler located inside a pen with a small group of animals (n=5). For experiments described in this thesis, all pen scoring was performed by the same observer to maintain consistency in temperament assessment. Each animal had its EV and pen score recorded at weaning. These values were then averaged to assign an overall temperament score.

Feed Efficiency

Feed efficiency can be defined as the gain in body weight ensuing from consumption of a known amount of feed (Koch et al., 1963). As the cost of feed plays a major role in the economics of a livestock operation, feed efficient animals have a positive economic impact. Several feed efficiency traits have been used to describe and categorize efficient and inefficient animals. The traits covered by the scope of these experiments include feed conversion ratio (FCR), partial efficiency of growth (PEG) and residual feed intake (RFI). All are calculations based upon dry matter intake (DMI), body weight (BW) and gain (ADG). Dittmar (2007) reviewed each trait as seen in Table 1.

Table 1. Review of feed efficiency traits[a]

Feed Efficiency Trait	Definition	Calculation	Desired Phenotype
Feed conversion ratio (FCR)	Feed intake required to produce one unit of weight gain.	DMI ÷ ADG	Low
Partial efficiency of growth (PEG)	Efficiency of BW gain after maintenance requirements have been accounted for.	ADG ÷ (Actual DMI – DMI required for maintenance) when maintenance is calculated as $0.077 \times BW^{0.75} \div NE_m$ concentration of the diet	High
Residual feed intake (RFI)	Difference in expected DMI for maintenance and growth at a given level of production and actual DMI	DMI – Expected DMI, where expected DMI is obtained by the regression of DMI on mid-test metabolic BW and ADG	Low

[a]Adapted from Arthur et al. (2001b), Hennessy and Arthur (2004), Brown (2005) and Dittmar (2007)

Feed conversion ratio is the most commonly used measure of feed efficiency among beef cattle producers (Nkrumah, 2004). This measure is calculated as the ratio of DMI to weight gain. Cattle exhibiting low FCR are considered efficient. They require less DMI for gain compared to those with high FCR. Although used often, FCR fails to take into account the requirements needed for maintenance. For true analysis of feed efficiency, calculations should include the requirements needed for both maintenance and growth (Arthur et al., 1996).

Partial efficiency of growth was introduced by Kellner (1909). Similar to FCR, PEG is a ratio of intake to gain but takes into account the requirements needed for maintenance. High PEG indicates that an animal had a greater increase in BW per unit of available energy accounting for both maintenance and gain (Hennessy and Arthur, 2004). Archer et al. (1999) states "although PEG does account for maintenance requirements, estimates of the requirements are not reliable due to assumptions that are made when predicting the DMI needed for maintenance between different animals."

Residual feed intake was developed in 1963 (Koch et al., 1963) and is defined as the actual feed intake minus the expected feed intake of the animal. Arthur et al. (1996) describes RFI as a measure of net feed conversion efficiency. The amount of expected feed intake is calculated based on the energy requirements needed for maintenance of body weight while also allowing for weight gain (Koch et al., 1963). Predicted DMI is determined by linear regression. Regressions of weekly BW are used to determine ADG and mid-test metabolic BW ($MBW^{0.75}$). Predicted DMI is determined by regressing actual DMI on ADG and $MBW^{0.75}$. RFI is then determined as the residual between

actual DMI and predicted DMI (Arthur et al., 1996). Low or negative RFI is desirable as it indicates an animal's ability to efficiently gain on less DMI intake than predicted (Archer et al., 1999). Since the measure is performed on an individual basis, feeding trials that accommodate one animal per feed bunk are necessary. The duration of the feeding trials is also important. In a set of tests, Archer et al. (1997) attempted to assess the optimal trial duration for RFI and the two components of the measure, ADG and DMI, in *Bos taurus* cattle. The results demonstrated that a minimum of 70 days was required in order to minimize variability in ADG. Results also indicated that following d 70 there was minimal decrease in the variation of RFI. Koch et al. (1963) found RFI to be moderately heritable ($h = 0.4$). Findings from Herd et al. (2003) support the idea that performance can be improved through genetic selection for RFI. Wood et al. (2004) also concluded that testing and selecting for RFI increased profits.

Recent reports have presented data supporting a link between IGF-I and RFI (Wood et al., 2004; Moore et al., 2005). Data collected by Johnston et al. (2002) suggest that circulating concentrations of IGF-I may be associated with the prediction of RFI in beef cattle. Tests revealed that IGF-I was genetically positively associated with RFI among *Bos taurus* cattle. This correlation suggests that selection for reduced IGF-I will result in lower RFI. However, when tested among Brangus females, Lancaster et al. (2007) failed to find a correlation between concentration of IGF-I and RFI.

Tropical Adaptation

Tropically adapted breeds of beef cattle are able to withstand the hot and humid conditions common to the Gulf Coast regions of North America and the tropical regions of South America. This adaptation refers mainly to an animal's ability to cope with the heat stress associated with the harsh climate. While tropically adapted breeds perform well under these conditions, more temperate breeds experience a loss in production (Bonsma, 1949). The cause behind this loss in production involves the animal's ability to efficiently transfer or exchange heat. Finch (1986) reports that the tropically adapted *Bos indicus* breeds can more efficiently transfer heat compared to temperate *Bos taurus* breeds. The mechanisms behind this transfer of heat include hair coat, sweat glands and rate of respiration. All of which differ between the tropically adapted and temperate breeds of beef cattle (Finch, 1986; Yeates et al., 1975; Shrode et al., 1960).

An animal's hair coat must be resistant to environmental heat-flow in order to maintain optimal body temperature. The thick and deep hair coats associated with temperate breeds act as a barrier to outward movement of heat while the flat coat with shallow follicles of the adapted breeds allows for heat exchange. The smooth coats among the adapted breeds also serve as reflectors of radiation to protect from environmental heat gain (Finch, 1986). Finch also reports that evaporative cooling under humid conditions is limited by the thick hair coat in temperate breeds. The tissues directly beneath the hair coat also aid in thermoregulation. Tissue resistance among the temperate breeds is greater than that of the adapted breeds thus making the rate of metabolic heat exchange lower (Finch, 1986). Yeates et al. (1975) reports that the

difference in the shape of sweat glands between the adapted versus temperate cattle plays a role in heat exchange as well. The baggy shaped glands of *Bos indicus* breeds have greater activity than the coiled glands of the *Bos taurus* breeds. The higher amount of activity of *Bos indicus* sweat glands leads to more sweating and therefore more evaporative cooling. The rate of respiration between the breeds also differs (Shrode et al., 1960). Studies show that the lower respiration rates seen in heat-tolerant breeds aid in thermoregulation (Shrode et al., 1960; Carvalho et al., 1995).

Heterosis

Heterosis, or hybrid vigor, is defined as the increased strength of the F_1 cross over the mean of the parents or the better parent. An F_1, or first filial generation is the first generation offspring of two parents (Hayes et al. 1955). Heterosis is considered to be the combined effects of gene dominance and epistasis, or interactions between genes (Springer and Stupar, 2007). Although the mechanism of heterosis has yet to be completely determined, two century old theories give explanations of how the vigor among hybrids is achieved. The two models used to explain heterosis are dominance (Jones, 1917) and overdominance (Shull, 1908). The dominance model suggests that each of the parental lines contain slightly harmful alleles that reduce their strength (Springer and Stupar, 2007). By suppression of undesirable recessive alleles from one parent by the dominant allele of the other parent, the virtues of each parent are combined resulting in genetically superior heterozygous offspring. The overdominance model suggests that the heterozygous combination of alleles at any given locus is superior to

either of the homozygous combinations for the same locus (Springer and Stupar, 2007). The allelic interactions that occur in the hybrid allow the heterozygote to outperform the homozygote. Although the ideas behind each model differ slightly, both are non-additive in effect. Non-additive effects, or expression patterns, occur whenever the F_1 hybrid expression level differs from the mean parental value (Springer and Stupar, 2007).

CHAPTER III

THE INFLUENCE OF TROPICAL ADAPTATION ON PLASMA INSULIN-LIKE GROWTH FACTOR-I IN PUREBRED AND CROSSBRED BEEF CATTLE

Introduction

Due to an abundance of available forage, a large majority of the US cattle population is located in the hot and humid regions near the Gulf of Mexico (Chase, 2002). While many temperate breeds struggle, tropically adapted breeds of cattle thrive in these areas (Turner, 1980). Tropically adapted *Bos indicus* Brahman cattle have been reported to have higher concentrations of GH and IGF-I compared to the temperate *Bos taurus* Angus despite their lower ADG and growth rates (Simpson et al., 1997; Turner, 1980). Large amounts of heterosis are seen among Brahman crossbreds although some growth and performance issues still remain a problem. In order to retain tropical adaptation while eliminating poor performance qualities, alternative breeds are being utilized in crossbreeding programs. One such breed is the tropically adapted *Bos taurus* Romosinuano (Riley et al., 2007). In an effort to determine the effect of tropical influence on the growth parameter IGF-I, three-breed diallel matings were conducted using temperate *Bos taurus* (A; Angus), tropical *Bos indicus* (B; Brahman), and tropical *Bos taurus* (R; Romosinuano) cattle. Purebred and crossbred steers and heifers were evaluated in calf crops from the years 2003 and 2004.

Materials and Methods

Breeding Stock

All management and procedures involving cattle were performed at the USDA-ARS Subtropical Agricultural Research Station (STARS) in Brooksville, Florida as described by Riley et al., (2007). Romosinuano cattle were imported as embryos from the Centro Agronomico Tropical de Investigacion y Ensenanza (CATIE) in Turrialba, Costa Rica, and from purebred breeders in Venezuela during the 1990s. The STARS Brahman herd was begun in the late 1940s. Some herd bulls were raised in the herd, but most were purchased or borrowed from various herds. Approximately once every decade, females were purchased from various herds. Several prominent bloodlines are present in the herd. The Angus herd was started in the early 1950s from other herds within Florida. Some herd bulls were raised in the herd, but most were produced from purchased semen. A total of 42 bulls sired the calves used in this project. Romosinuano bulls (n = 16) were a result of Venezuelan embryo transfer. Brahman bulls (n = 12) were obtained from outside herds, primarily from Florida breeders. Of the Angus bulls (n = 14), 6 were born and raised in the STARS herd with the rest being obtained from Florida or Georgia breeders.

Breeding Design

Purebred Romosinuano, Brahman, and Angus cows were randomly assigned to each of the 3 management units comprising STARS and then separated into breeding herds of 25 to 30 cows (6 herds at each location, each herd consisting of cows of all 3

breeds). Purebred bulls of each breed were randomly assigned to the single-sire breeding herds. Each year, the 90-d breeding season began on or near March 20. Each breeding herd was exposed to a different breed of bull every year. The mating design produced 9 breed groups of calves (R = Romosinuano, B = Brahman, and A = Angus). Purebreds (AA, BB, RR) and crossbreds (AB, BA, AR, RA, BR and RB) from the 2003 and 2004 calf crops were evaluated.

Post-weaning Management and Sample Collection

Calves were born from late December to early April in each year. All calves were weighed and tagged within 24 hr after birth. Male calves were also castrated at this time. Each year, weaning occurred on unique dates during 3 consecutive weeks in September with each week being 1 of the 3 STARS management units. Calf age at weaning ranged from 154 to 274 d with an average of 229 d. Blood samples were taken from each animal at weaning. Approximately 9 mL of whole blood was collected via venipuncture using Sarstedt tubes containing EDTA (Sarstedt AG and Co., Germany). Sample tubes were refrigerated on ice and subsequently centrifuged at 4ºC at 2000 x g for 30 minutes. Plasma was collected and stored at -20º C. Following adaptation from weaning, the calves were separated by sex.

Heifers

All heifers were managed on Bahia grass pastures. Mixed Bahia grass and perennial peanut hay was offered ad libitum. A mixture of soybean hulls and molasses

(2.26kg/head/day) was given as a supplement to the roughage. This feeding regime was continued through the winter and spring until early summer when forage availability was adequate to support the herd. Blood samples were collected at the start of the study and at 28-d intervals thereafter. Approximately 9 mL of whole blood was collected via venipuncture using Sarstedt tubes containing EDTA (Sarstedt AG and Co., Germany). Sample tubes were refrigerated on ice and subsequently centrifuged at 4ºC at 2000 x g for 30 minutes. Plasma was collected and stored at -20º C.

Steers

Upon separation, all steers were shipped to the USDA-ARS, Grazinglands Research Laboratory in El Reno, OK. The steers were then grouped and placed into traps containing native grasses and given ad libitum access to bermudagrass hay. A 20% CP supplement (0.9 kg/head/day) was given in addition to the hay. At 24 hr post-arrival, the supplement was changed to 1.6 kg per head per day. After 5 d, each steer was fed its weekly CP supplement in five meals (Monday-Friday). This feeding regime was continued until the end of the 28-d receiving phase. Steers were then regrouped and allowed to graze for approximately 5 months during the grazing phase. Upon completion of spring grazing, steers were sorted to feedlot pens for the finishing phase (6 head/pen X 14 pens). Utilizing a Calan gate system (American Calan, Northwood, NH), individual performance was measured. Blood samples were collected at the beginning of the finishing phase and at 28-d intervals until slaughter. Approximately 9 mL of whole blood was collected via venipuncture using Sarstedt tubes containing EDTA

(Sarstedt AG and Co., Germany). Sample tubes were refrigerated on ice and subsequently centrifuged at 4ºC at 2000 x g for 30 minutes. Plasma was collected and stored at -20º C.

IGF-I RIA

Plasma samples (n = 90) from 10 heifers and 10 steers of each purebred and crossbred breed-type from each calf crop, at weaning and 2 dates post-weaning, were removed from storage for determination of concentration of IGF-I by radioimmunoassay. The post-weaning dates for the heifers were d 0 and d 84 of the study periods. Day 0 and d 60 of the finishing phase were utilized for the steers. Concentrations of IGF-I were determined by radioimmunoassay (Appendix A) as described by Bilby et al. (1999). The protocol included two modifications. The final primary antibody was diluted 1:120,000 and the goat-anti-rabbit secondary antibody was diluted 1:60. The IGF-I antibody used was anti-hIGF-I (AFP4892898, A.F. Parlow, National Hormone and Peptide Program, Torrance, CA). Unknown concentrations of IGF-I were calculated using Assay Zap software (Biosoft, Cambridge, UK) using counts per minute (cpm) obtained from a Cobra II auto-gamma-counter (Perkin Elmer, Waltham, MA). Interassay and intraassay coefficients of variation for heifer assays were 16.85% and 5.59%, respectively. Interassay and intraassay coefficients of variation for steer assays were 6.52% and 6.09%, respectively.

Statistical Analysis

Repeated measures ANOVA was conducted using the MIXED model procedure of SAS (2002), for analysis of year and breed effects on concentrations of IGF-I. The model included year, breed and year X breed interaction. The MIXED model procedure of SAS(2002) was also used to compare the effect of sex class on weaning concentrations of IGF-I. The Least Squares Means (Appendix B) for effect of breed derived from the GLM procedure as SAS (2002) showed no significant difference between the crossbred animals ($P > 0.05$) also, no significant interaction was observed for the effect of breed by year ($P > 0.05$). Therefore, the crossbred data were grouped by diallel cross and the calf crops were pooled. Purebreds (AA, BB, RR) and crossbreds (ABX, ARX, BRX), both male and female were analyzed.

Results and Discussion

Differences in heifer plasma concentrations of IGF-I at weaning are shown in Figures 3 and 4. Breed was an important source of variation ($P < 0.003$). Purebred temperate AA exhibited the lowest values of IGF-I compared to all other breeds ($P < 0.02$). Crossbred heifers (ARX and ABX) did not differ in concentrations of IGF-I ($P = 0.38$). Tropically adapted BB and RR did not differ ($P = 0.93$) but both were higher than temperate AA ($P < 0.0001$). The crossbred BRX had the highest circulating concentration of IGF-I. Although BRX did not significantly differ from BB ($P = 0.40$) or RR ($P = 0.46$), the tropically adapted crossbred was higher than all three temperate crosses ($P < 0.0006$). These values show that all tropically adapted purebred and

crossbred heifers exhibited higher concentrations of IGF-I at weaning. An effect of sex class on weaning concentrations of IGF-I was also observed ($P < 0.0001$). The steers exhibited higher concentrations of IGF-I (Appendix B) compared to the heifers throughout all breeds ($P = 0.0001$). Mean concentration of IGF-I at weaning was 139 ng/mL among steers compared to the heifer average of 106 ng/mL.

Results from d 0 of the studies (Figures 5 and 6) varied slightly from those at weaning. Breed was an important source of variation ($P < 0.001$). Temperate AA had lower concentrations of IGF-I compared to all breeds ($P < 0.02$) except the *Bos taurus* crossbred ARX ($P = 0.50$). The purebred RR and BB along with crossbred ABX and BRX did not differ but all were higher than both *Bos taurus* AA and ARX ($P < 0.02$).

Mean concentrations of IGF-I among the heifers at d 84 of the post-weaning studies are shown in Figures 7 and 8. Results from this sampling date vary compared to data seen at both weaning and d 0 of the post-weaning studies, although breed was an important source of variation ($P < 0.001$). All purebred breeds have lower concentrations compared to the crossbreds. The temperate AA did not differ from BB ($P > 0.05$) but was lower than all other breeds ($P < 0.03$).

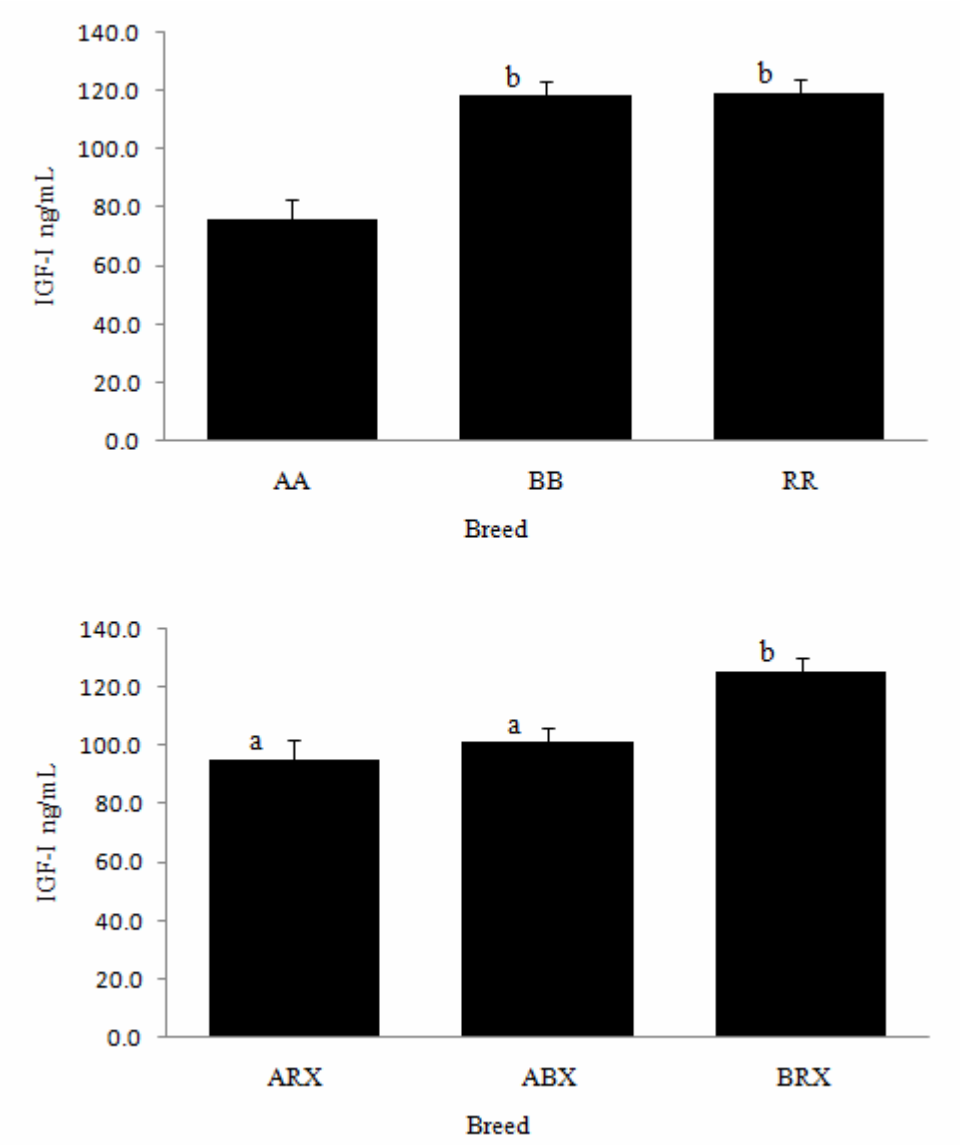


Figure 3. Mean concentrations of IGF-I from purebred and crossbred heifers at weaning. [a, b, c] Means with unlike superscripts differ ($P < 0.0006$).

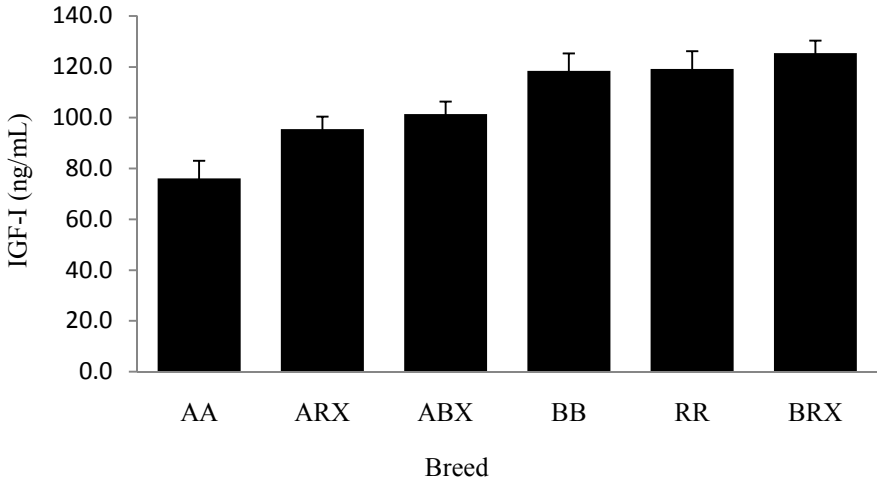


Figure 4. Mean concentrations of IGF-I from heifers of all breed types at weaning.

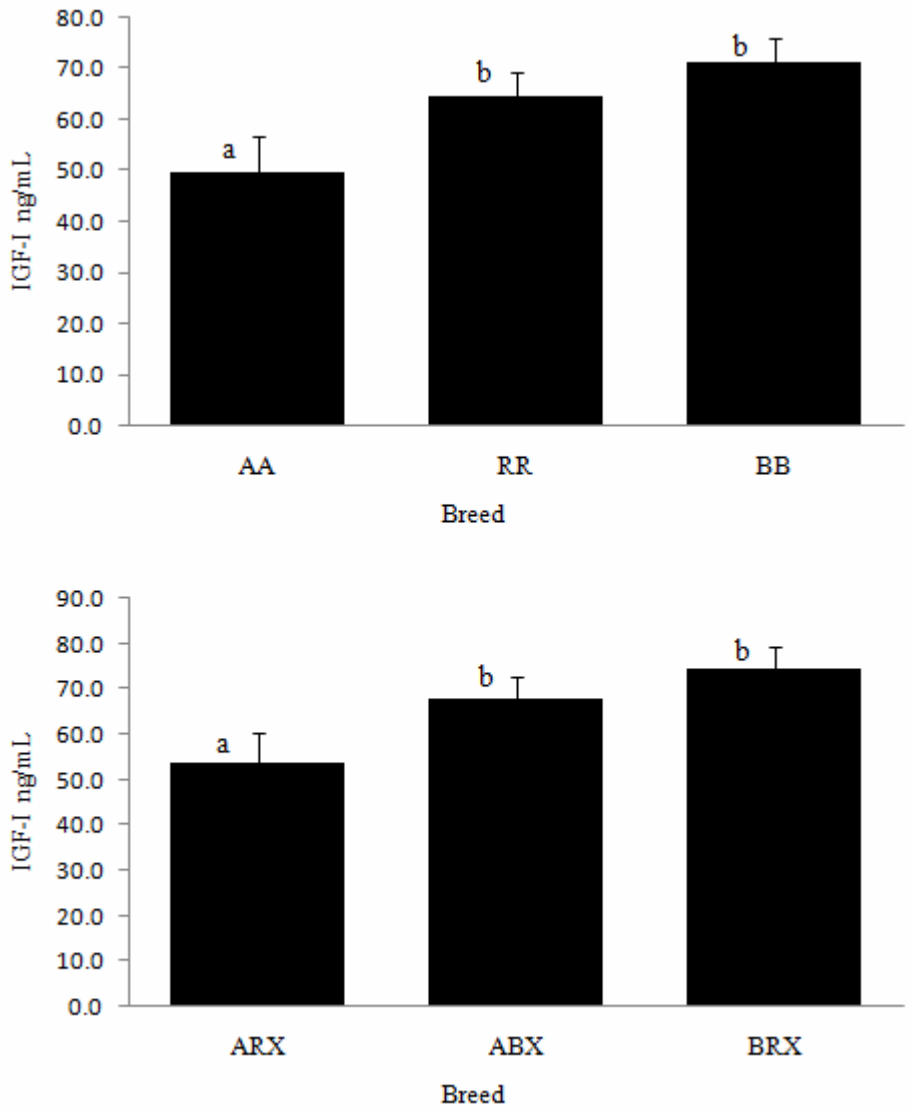


Figure 5. Mean concentrations of IGF-I from purebred and crossbred heifers at d 0 of post-weaning studies. [a, b, c] Means with unlike superscripts differ ($P < 0.003$).

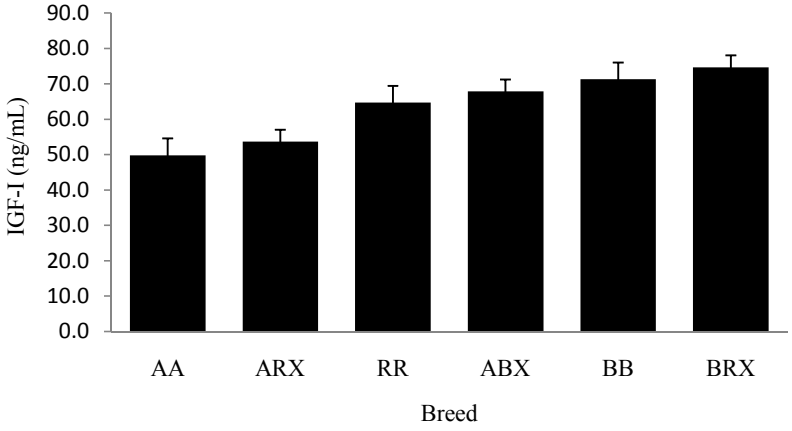


Figure 6. Mean concentrations of IGF-I from heifers of all breed tpes at d 0 of post-weaning studies.

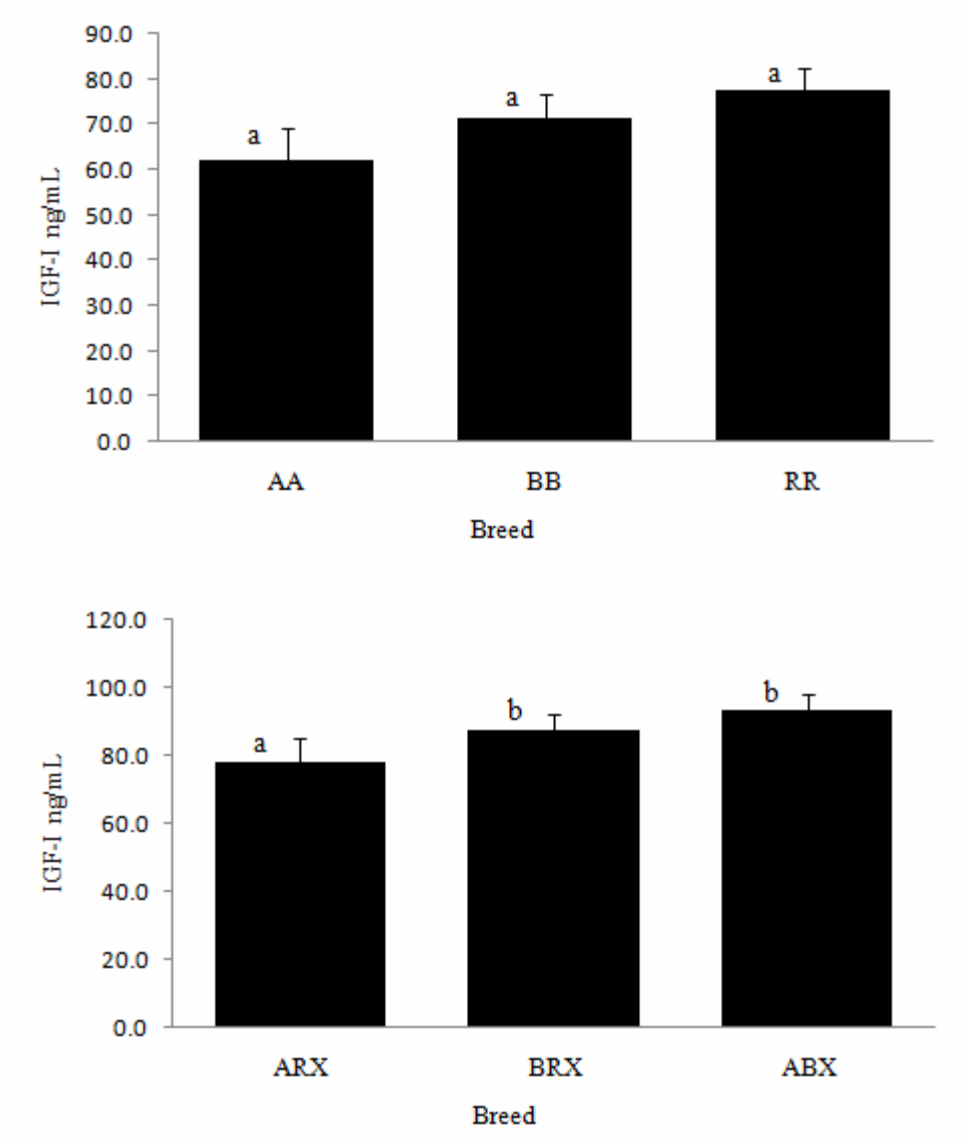


Figure 7. Mean concentrations of IGF-I from purebred and crossbred heifers at d 84 of post-weaning studies. [a, b, c] Means with unlike superscripts differ ($P < 0.01$).

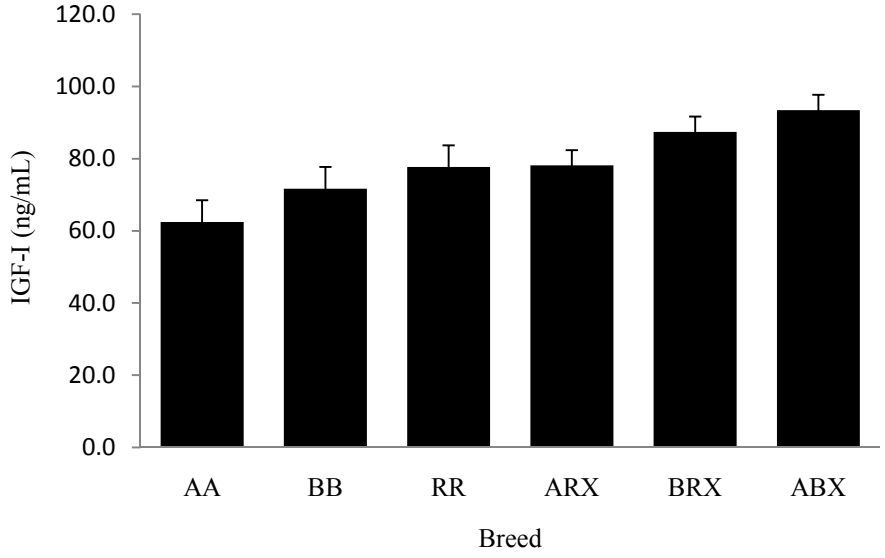


Figure 8. Mean concentrations of IGF-I from heifers of all breed types at d 84 of post-weaning studies.

Although the order of breeds does change over time, a trend is easily observed that shows the temperate, *Bos taurus* AA having the lowest concentraions of IGF-I throughout all of the sampling dates. Similar results were observed among the steers. Differences in steer plasma concentrations of IGF-I at weaning are shown in Figures 9 and 10. Breed accounted for an important source of variation ($P < 0.007$). Again, temperate AA had a lower concentration of IGF-I compared to all other breeds ($P < 0.003$). The crossbred ABX and ARX did not differ ($P = 0.14$) although both were lower than the tropically adapted crosses ($P < 0.003$). There was no difference observed between the tropically adapted purebred; BB, RR and crossbred BRX ($P > 0.05$).

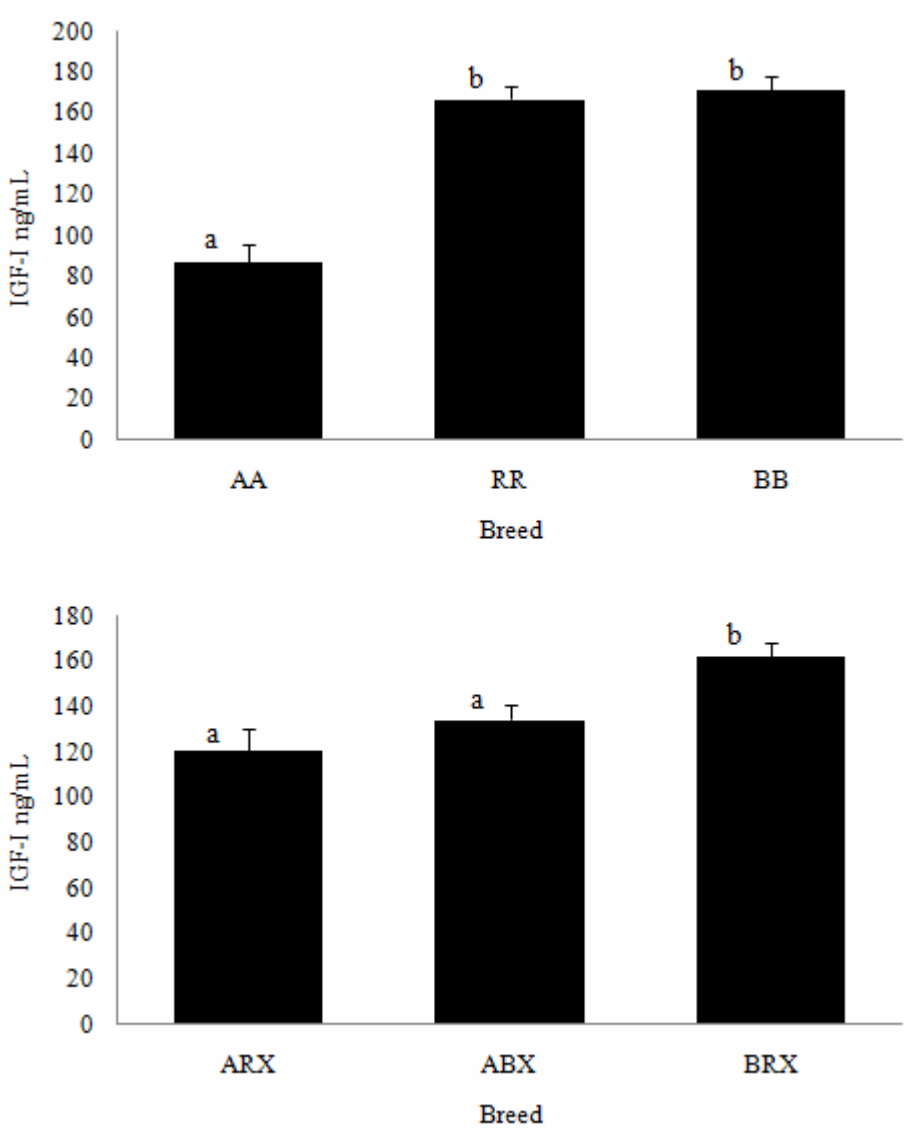


Figure 9. Mean concentrations of IGF-I from purebred and crossbred steers at weaning. [a, b, c] Means with unlike superscripts differ ($P < 0.003$).

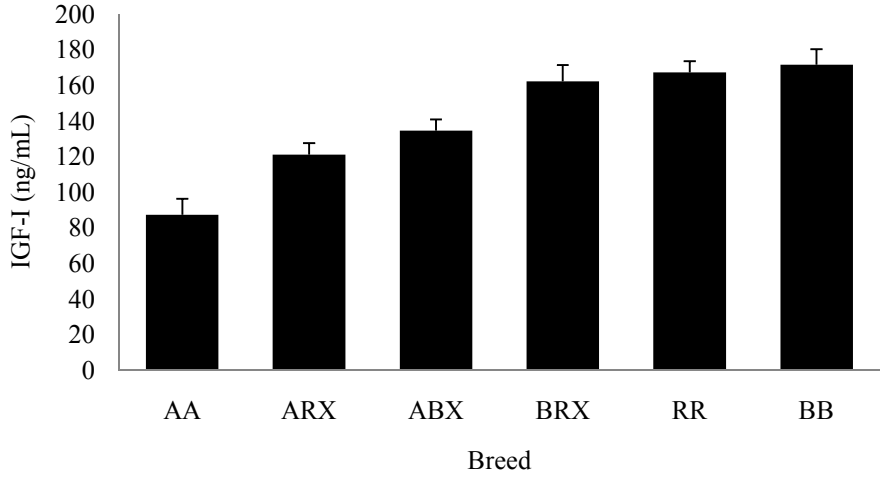


Figure 10. Mean concentrations of IGF-I from steers of all breed types at weaning.

No differences were observed in concentrations of IGF-I at d 0 of the individual finishing phase feeding trials (Figures 11 and 12). Mean IGF-I in ng/mL ranged from 176.23 to 200.09. Standard errors within each breed were high. The lack of difference in concentration of IGF-I is perhaps due to individual variabilty at the time of sampling. Following the trend, temperate AA again exhibits lower concentrations of IGF-I compared to the tropically adapted breeds ($P > 0.05$).

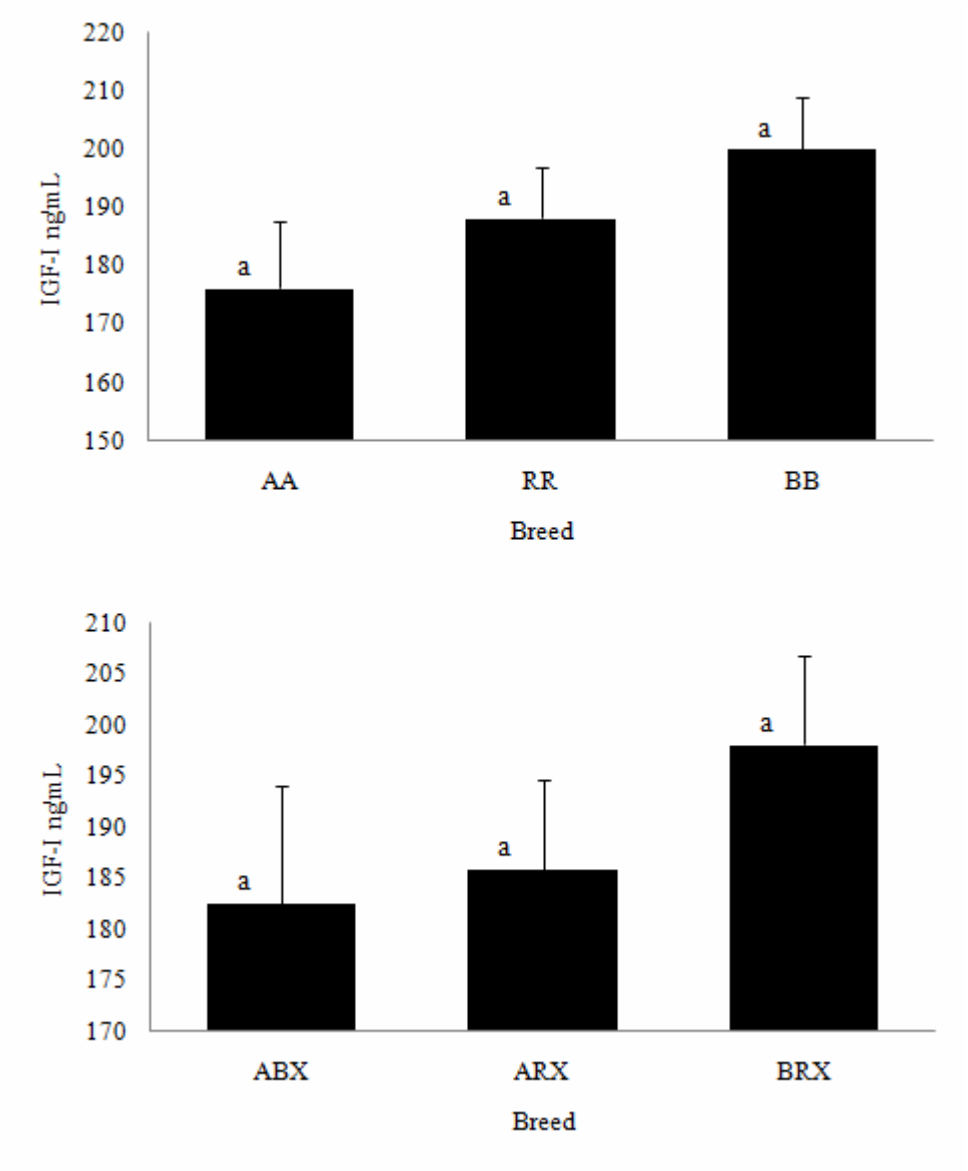


Figure 11. Mean concentrations of IGF-I from purebred and crossbred steers at d 0 of individual finishing phase feeding trials. [a, b, c] Means with unlike superscripts differ ($P < 0.05$).

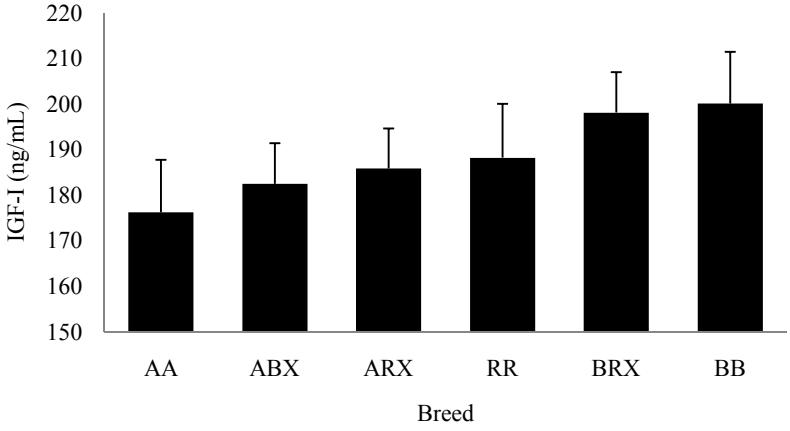


Figure 12. Mean concentrations of IGF-I from steers of all breed types at d 0 of individual finishing phase feeding trials.

Mean concentrations of IGF-I among the steers at d 60 of the individual finishng phase trials are shown in Figures 13 and 14. Temperate AA and the temperate-tropically adapted crosses, ABX and ARX, did not differ ($P > 0.05$). All three had numerically lower average values compared to purebred BB but only tended to differ ($P = 0.06$). The tropically adapted BRX and RR did not differ from BB but were both significantly higher than the temperate AA ($P < 0.01$).

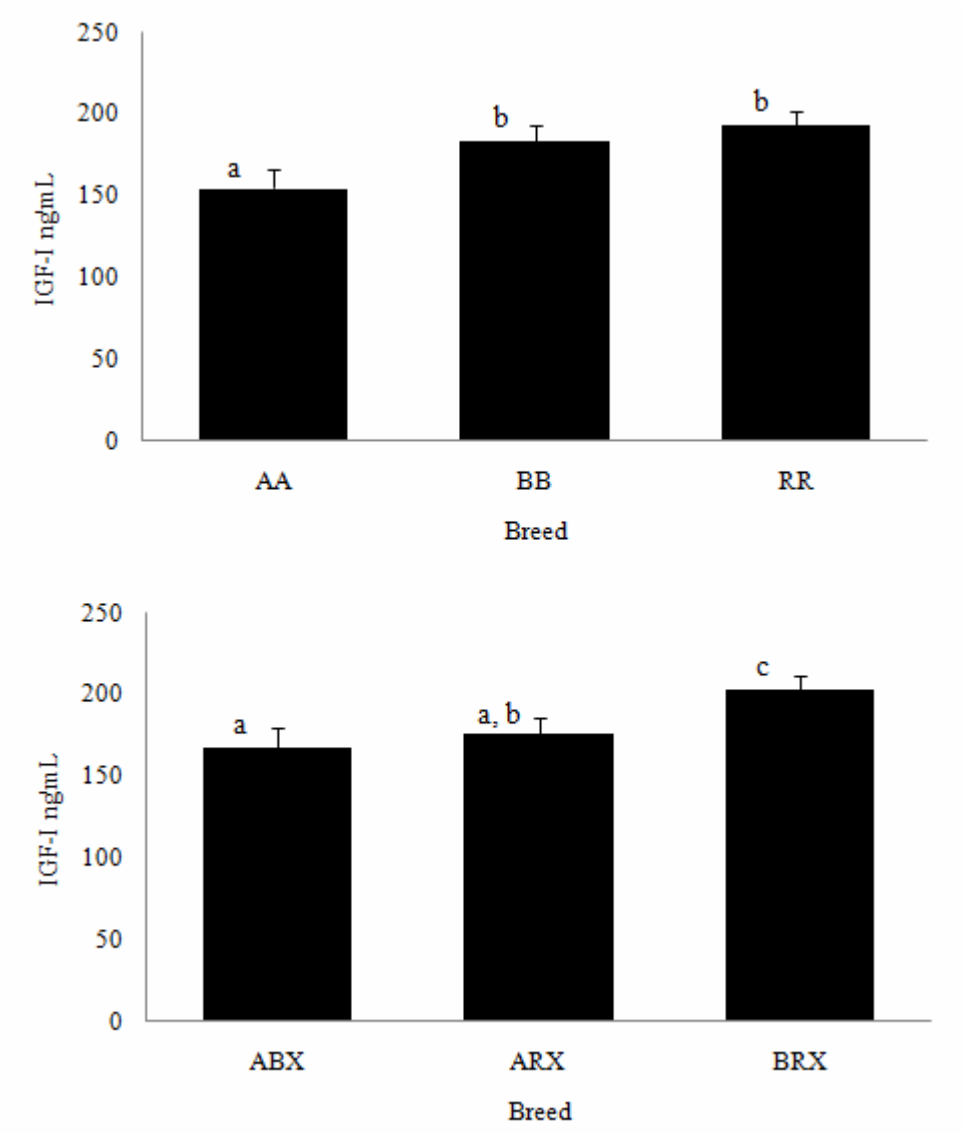


Figure 13. Mean concentrations of IGF-I from purebred and crossbred steers at d 60 of individual finishing phase feeding trials. [a, b, c] Means with unlike superscripts differ
($P < 0.02$).

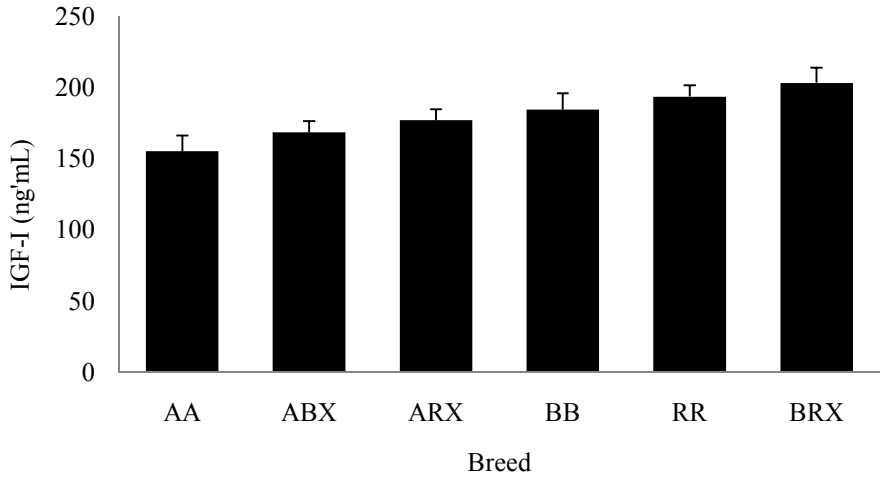


Figure 14. Mean concentrations of IGF-I from steers of all breed types at d 60 of individual finishing phase feeding trials.

It is apparent from the trends observed within the data that, relative to the temperate *Bos taurus* breed, plasma concentrations of IGF-I were greater in male and female tropically-adapted breed-types. Simpson et al. (1997) found similar results that revealed that Brahman have greater concentrations of IGF-I compared to Angus cows. This difference in concentration of IGF-I could possibly, in part, be due to a greater quantity of IGFBP3. Simpson et al. (1997) also demonstrated that Brahman cows had greater IGFBP3 binding activity compared to Angus. Since IGF-I has not been demonstrated to be stored in tissue, the pool being circulated by IGFBPs is the only form of storage for the growth promoting peptide. The IGFBP3 has a very high binding affinity to IGF-I, higher even than that of the type I IGFR (Baxter, 1986). This high binding affinity has been known to act in an inhibitory manner on the actions of IGF-I

(Baxter, 1988). Presumably, the high concentrations of IGF-I seen among the tropically adapted breeds are resultant from greater concentrations of IGFBPs.

Greater concentrations of GH among the tropically adapted breeds could also explain the increase in circulating IGF-I. This theory is less likely due to research that has demonstrated that concentrations of GH do not differ between cattle of varying frame size (Verde and Trenkle, 1986). In the same study, cattle of varying frame size did have differing concentrations of IGF-I. Perhaps the differences observed in concentrations of IGF-I are due to unstable numbers of hepatic GH receptors or inconsistent transcription post binding; thus explaining why cattle with similar concentrations of GH exhibit inconsistent growth patterns. It seems the mechanisms controlling concentrations of circulating IGI-I are unclear.

CHAPTER IV

EVALUATION OF INSULIN-LIKE GROWTH FACTOR-I AND TEMPERAMENT AS SELECTION TOOLS IN BRAHMAN HEIFERS

Introduction

Throughout the beef industry, one of the best ways for a producer to optimize profit is by minimizing production costs. The largest input for all operations is typically the expense of feed. Feed costs account for 60-65% of total production costs (Sainz and Paulino, 2004). As the cost of feed plays a major role in the success of an operation, feed efficient animals have a positive economic impact. In contrast, negative economic impacts are associated with temperamental animals. Early detection of efficient animals would benefit producers. Measurement of feed efficiency tends to be expensive and time consuming (Archer et al., 1997) while determination of IGF-I concentration is less costly than individual feeding test, can be measured earlier in life and on larger numbers of animals (Moore et al., 2003). Recent reports have presented data supporting a link between IGF-I and RFI (Wood et al., 2004; Moore et al., 2005). Data collected by Johnston et al. (2002) suggest that circulating concentrations of IGF-I may be associated with the prediction of RFI in beef cattle. However, when tested among Brangus females, Lancaster et al. (2007) failed to find a correlation between concentration of IGF-I and RFI. In an attempt to utilize IGF-I in the search for efficient cattle, breeds outside the current realm of study should be tested for a possible relationship between concentration

of IGF-I and RFI. Therefore, the objective of this experiment was to evaluate the relationship of concentration of IGF-I and temperament to economically important traits among Brahman females.

Materials and Methods

Animals and Experimental Design

Three separate 70-d individual feeding trials were conducted on three separate groups of spring-born Brahman heifers at the Texas A&M AgriLife Research Center in Overton, TX as described by Dittmar (2007). Utilizing a Calan gate system (American Calan, Northwood, NH), individual performance was measured in the fall of each year. Heifers from the 2005 (n = 50; 10.5 to 13.5 mo) and 2006 (n = 56; 5 to 8 mo) calf crops were limit-fed a 12% crude protein (CP) ration (Table 2) at 2.2% of their gross BW for 7 d prior to entering the Calan gate system. Heifers from the 2007 (n = 50; 5 to 8 mo) calf crop were limit-fed a 12% CP ration (Table 2) at 2.5% of their gross BW. Upon entry, heifers were assigned to a pen based on BW and fitted with an electronic key, worn around their neck, which allowed access to their individual feed bunk within the system. Before the start of each trial, the heifers were given a 4-d training period to learn to eat from the Calan gates. Heifers that were unable to learn to eat from the Calan gate bunks were removed from the trial. On d 0, BW was determined and feed amounts for each heifer were calculated (2005-2006: 2.2% BW; 2007: 2.5% BW) for that week. Heifers were fed their ration once daily in the morning (0800 h). Weekly BW was determined on the first day of each new week, prior to feeding, and daily feed allocation was adjusted

for the following week. Orts (if any) were collected and weighed at the end of each week, following BW determination, to adjust actual feed intakes for the previous week.

Table 2. Ingredients and nutrient content

Diet	Year 2005	2006	2007
Ingredients (As-fed-basis):	% of Diet	% of Diet	% of Diet
Cotton seed hulls	25.00	37.50	25.00
Cotton seed hulls, pelleted	-	-	30.00
Soybean hulls	20.00	-	7.45
Corn, ground	10.00	6.37	-
Corn, crimped	-	-	2.00
Alfalfa, dehy 20%	8.73	12.50	12.50
Wheat midds	7.35	5.53	-
Rice bran	6.25	8.50	10.00
Cottonseed meal, 41%	6.01	4.33	-
Corn gluten feed	5.00	5.00	-
Corn, cracked	5.00	5.00	-
Binder molasses	2.00	2.00	-
Calcium	1.25	1.27	0.70
Whole cotton seed	0.93	-	-
Salt	0.62	0.67	0.85
BIOFOS 21P 18CA	0.56	0.51	-
Soybean meal, 48%	0.50	4.75	10.47
Dynamate	0.26	0.25	-
BGY 28	0.25	0.25	-
Xtra-bond	0.15	0.15	-
T/M for dairy	0.05	0.05	0.02
Dairy (ADE)	0.04	0.04	0.04
Vitamin A-30	0.04	0.04	0.04
Zinpro 100	0.03	0.03	0.01
Equine T/M	-	0.01	-
Rice hulls	-	5.25	-
Nutrients (Dry-matter-basis):			
DM, %	90.33	90.24	
CP, %	13.40	13.41	12.00
NE_m, Mcal/kg	1.59	1.41	
NE_g, Mcal/kg	0.90	0.68	
ADF, %	34.36	38.17	
NDF, %	48.34	48.34	
Calcium, %	1.00	1.00	0.70
Phosphorus, %	0.55	0.55	0.30

Determination of Feed Efficiency

Individual performance and feed intake data were collected for each animal in order to determine variation in feed efficiency. Following each 70-d feed efficiency evaluation, heifers were ranked based on RFI by determining the residual between the actual and expected dry matter intake (DMI) for each heifer. Expected DMI was determined by linear regression of actual DMI, ADG, and mid-test $BW^{0.75}$. A 3% shrink was applied to all weights in order to achieve a shrunk body weight (SBW). Weekly SBW was regressed against day for each animal. The slope of the regression line was used to determine individual ADG. The intercept of the regression line was used as the initial SBW. The slope of the line and the intercept were used to calculate the final SBW. Mid-test weight was determined by taking the average of the initial and final SBW. Mid-test metabolic BW ($BW^{0.75}$) was calculated as the mid-test SBW to the power of 0.75. Each heifer was ranked as low, medium or high RFI based upon ½ standard deviation above or below mean RFI of each heifer crop. Feed conversion ratio was determined by taking the ratio of DMI to ADG (FCR = DMI kg / ADG kg). Partial efficiency of growth was determined by the ratio of ADG to DMI available for growth. The DMI available for growth is the residual, after the expected DMI for maintenance had been accounted for in the actual DMI. Expected DMI for maintenance was determined by $0.077 \times BW^{0.75}$ / NEm concentration of the diet (NRC, 2000).

Ultrasound Body Composition Traits

Real-time ultrasound measurements for the determination of intra-muscular fat percentage (IMF %) were taken at weaning, d 56 post weaning and yearling dates for each calf crop. Measurements were performed by the same Ultrasound Guidelines Council certified technician using an Aloka 500V real-time ultrasound machine (Corometrics Medical Systems, Wallingford, CT) equipped with a 17.2 cm, 3.5 MHz linear transducer. The transducer was fitted with a Superflab (Designer Genes Technologies, Inc., Harrison, Arkansas) guide for image capture. Animals were curried prior to imaging and 100% vegetable oil was used to obtain proper acoustic contact. Images were stored on a personal computer and interpreted using Beef Image Analysis Pro Plus Software 2.0.3 (Designer Genes Technologies, Inc., Harrison, Arkansas).

IGF-I RIA

Approximately 10 mL of whole blood was collected via tail venipuncture using additive free vacutainer tubes (BD, Franklin Lakes, NJ) at weaning and d 0 and 70 of each trial. Sample tubes were refrigerated on ice and subsequently centrifuged at 4ºC at 2000 x g for 30 minutes. Serum was collected and stored at -20º C. Concentrations of IGF-I were determined by radioimmunoassay (Appendix A) as described by Bilby et al (1999). The protocol included 2 modifications. The final primary antibody was diluted 1:120,000 and the goat-anti-rabbit secondary antibody was diluted 1:60. The IGF-I antibody used was anti-hIGF-I (AFP4892898, A.F. Parlow, National Hormone and Peptide Program, Torrance, CA). Unknown concentrations of IGF-I were calculated

using Assay Zap software (Biosoft, Cambridge, UK) using counts per minute (cpm) obtained from a Cobra II auto-gamma-counter (Perkin Elmer, Waltham, MA). Interassay and intraassay coefficients of variation were 10.88% and 7.53%, respectively.

Cortisol RIA

Approximately 10 mL of whole blood was collected via tail venipuncture using additive free vacutainer tubes (BD, Franklin Lakes, NJ) at weaning and d 0 and 70 of each trial. Sample tubes were refrigerated on ice and subsequently centrifuged at 4ºC at 2000 x g for 30 minutes. Serum was collected and stored at -20º C. Concentrations of cortisol were determined by radioimmunoassay (Coat-A-Count, Siemens Medical Solutions Diagnostics, Malvern, PA) (Appendix C). Unknown cortisol concentrations were calculated using Assay Zap software (Biosoft, Cambridge, UK) using counts per minute (cpm) obtained from a Cobra II auto-gamma-counter (Perkin Elmer, Waltham, MA). Interassay and intraassay coefficients of variation were 7.9% and 6.6%, respectively.

Assessment of Temperament

The objective measurement of exit velocity (Burrow et al., 1988; Curley et al., 2006) was used to measure in m/sec the rate at which each heifer exited a squeeze chute and traversed a fixed distance of 1.83m. Infrared sensors were used to remotely trigger the start and stop of a timing device (Farm Tek Inc., North Wylie, TX) used to determine m/sec. Pen scores (Hammond et al., 1996) were also assigned to each heifer. Scores

were given on a 1 (calm) to 5 (excitable) scale based upon the degree of aggressiveness exhibited towards a handler located inside a pen with a small group of heifers (n=5). Both measurements were assessed at weaning. An overall temperament score for each heifer was determined by averaging exit velocity and pen score. Each heifer was then ranked as calm, moderate or excitable based upon ½ standard deviation above or below the mean temperament score of each heifer crop.

Statistical Analysis

Pearson's correlation coefficients were used to determine the strength of relationship between traits. All coefficients were derived using SPSS (2005). Coefficients with a probability value of < 0.05 were considered significant. Analysis of variance using the GLM procedure as SAS (2002) was used to examine the effect of RFI rank on performance and feed efficiency traits. The model included RFI rank as the class variable with the separate traits as the dependent variables.

Results and Discussion

The concentrations of IGF-I for each calf crop grouped by RFI rank (low, medium or high) showed no trend at weaning (Figure 15), d0 (Figure 16) or d70 (Figure 17) of the feeding trials. No significant correlations were observed between concentration of IGF-I and RFI at any sampling date.

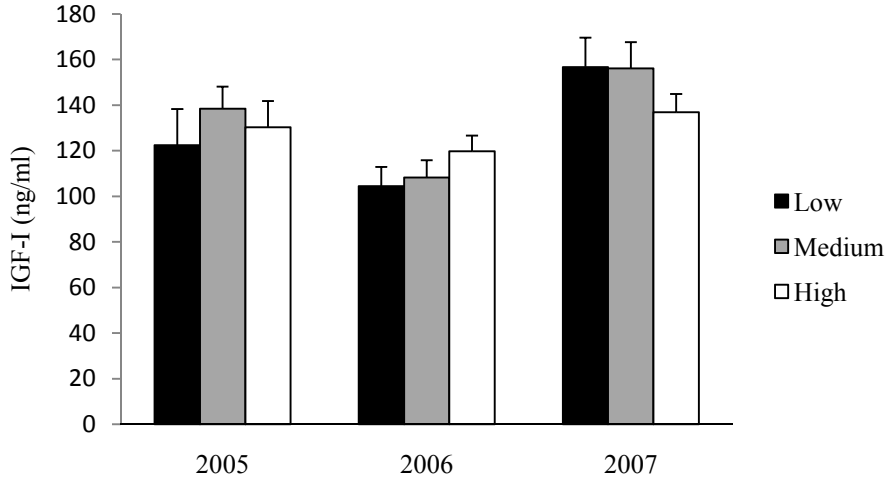


Figure 15. Concentrations of IGF-I at weaning grouped by RFI rank.

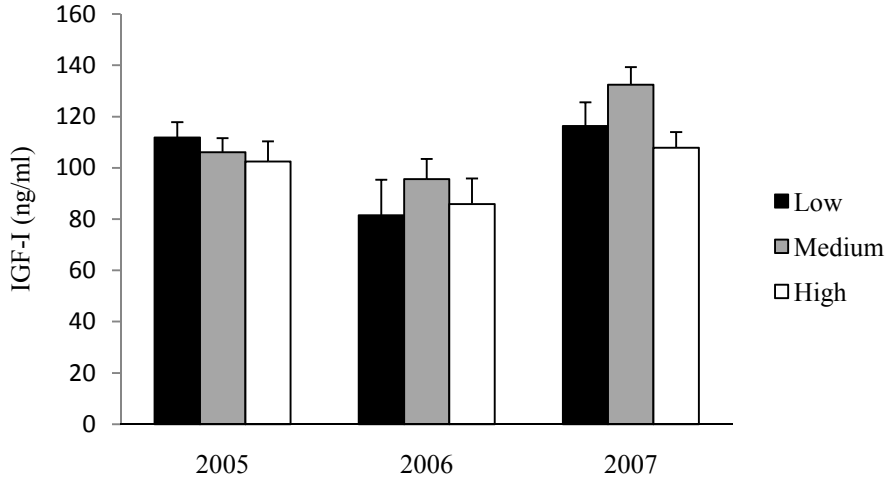


Figure 16. Concentrations of IGF-I at d 0 grouped by RFI rank.

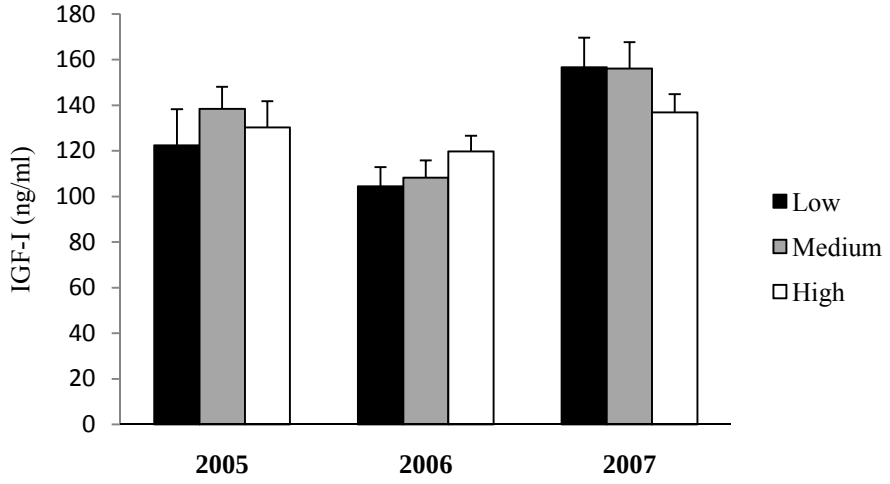


Figure 17. Concentrations of IGF-I at d 70 grouped by RFI rank.

No relationship was observed between concentration of IGF-I and ADG ($P > 0.05$). Correlations were not significant. Results suggest that concentrations of IGF-I at weaning and at both the onset and conclusion of the feeding trials were unrelated to RFI and ADG in Brahman heifers.

Temperament had no significant effect on RFI. Heifers from the 2005 study categorized by temperament as calm, moderate or excitable had mean RFI of -0.001 ±0.01, -0.004 ±0.01 and -0.007 ±0.01 ($P > 0.10$), respectively. The 2006 heifer RFI values averaged -0.002 ±0.02 (calm), -0.01 ±0.02 (moderate) and 0.02 ±0.02 (excitable) ($P > 0.10$). Heifers from 2007 averaged -0.19 ±0.12, (calm) 0.13 ±0.11 (moderate) and 0.07 ±0.11 (excitable) ($P > 0.10$). Although temperament and RFI are unrelated, a relationship was observed between temperament and ADG. Calmer heifers exhibited higher ADG when compared to the more excitable heifers in the 2005 ($P = 0.07$) and

2006 (P = 0.03) calf crops (Figure 18). These results correspond with a study done by Fell et al. (1999) among *Bos taurus* steers where the calmer animals preformed better under feedlot conditions compared to the more nervous or excitable animals. The lowered weight gain observed among the temperamental animals also agrees with previous studies (Obst, 1974).

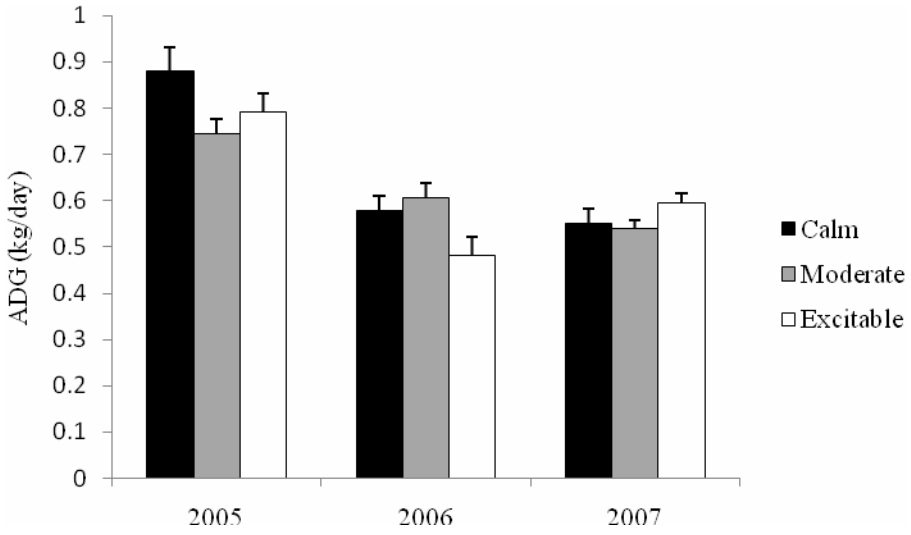


Figure 18. ADG group by temperament.

Once heifers were grouped by low, medium and high RFI, the least squares means for all calculated traits were assembled for overall analysis (Appendix D). Lancaster (2008) found significantly strong correlations among performance and feed efficiency traits in Brangus heifers yet no significant relationships were observed between any of the traits among our tests in Brahman heifers. Phenotypic correlations

that are worth noting in one breed do not necessarily cross over into other breeds. Variance in feed efficiency among cattle has been noted but the causative factors are still under investigation (Richardson and Herd, 2004).

CHAPTER V

EVALUATION OF INSULIN-LIKE GROWTH FACTOR-I AS A SELECTION TOOL AMONG PUREBRED AND CROSSBRED STEERS

Introduction

As mentioned in Chapter III, with in the beef industry, one of the best ways for a producer to optimize profit is by minimizing production costs. As the cost of feed plays a major role in the success of an operation, feed efficient animals have a positive economic impact. Early detection of efficient animals would benefit producers. Reports have presented data supporting a link between circulating IGF-I and RFI (Wood et al., 2004; Moore et al., 2005). Data collected by Johnston et al. (2002) suggest that circulating concentrations of IGF-I may be associated with the prediction of RFI in beef cattle. However, when tested among Brangus females, Lancaster et al. (2007) failed to find a correlation between the traits. In an attempt to utilize IGF-I in the search for efficient cattle, breeds outside the current realm of study should be tested for a possible relationship between concentration of IGF-I and RFI. Therefore, the objective of this experiment was to evaluate the relationship between circulating concentrations of IGF-I and RFI among purebred and crossbred steers of 9 different breed types.

Materials and Methods

Breeding Stock

All breeding management and pre-weaning procedures involving calves were performed at the USDA-ARS Subtropical Agricultural Research Station (STARS) in Brooksville, Florida as described by (Riley et al., 2007). Romosinuano cattle were imported as embryos from the Centro Agronomico Tropical de Investigacion y Ensenanza (CATIE) in Turrialba, Costa Rica, and from purebred breeders in Venezuela. The STARS Brahman herd was begun in the late 1940s. Some herd bulls were raised in the herd, but most were purchased or borrowed from various herds. Approximately once every decade, females were purchased from various herds. Several prominent bloodlines are present in the herd. The Angus herd was started in the early 1950s from other herds within Florida. Some herd bulls were raised in the herd, but most were produced from purchased semen. A total of 42 bulls sired the calves used in this project. Romosinuano bulls (n = 16) were a result of Venezuelan embryo transfer. Brahman bulls (n = 12) were obtained from outside herds, primarily from Florida breeders. Of the Angus bulls (n = 14), 6 were born and raised in the STARS herd with the rest being obtained from Florida or Georgia breeders.

Breeding Design

Purebred Romosinuano, Brahman, and Angus cows were randomly assigned to each of the 3 management units comprising STARS and then separated into breeding herds of 25 to 30 cows (6 herds at each location, each herd consisting of cows of all 3

breeds). Purebred bulls of each breed were randomly assigned to the single-sire breeding herds. Each year, the 90-d breeding season began on or near March 20. Each breeding herd was exposed to a different breed of bull every year. The mating design produced 9 breed groups of calves (R = Romosinuano, B = Brahman, and A = Angus). Purebreds (AA, BB, RR) and crossbreds (AB, BA, AR, RA, BR and RB) from the 2003 and 2004 calf crops were evaluated.

Post-weaning Management and Sample Collection

Calves were born from late December to early April in each year. All calves were weighed and tagged within 24 hr after birth. Male calves were also castrated at this time. Each year, weaning occurred on unique dates during 3 consecutive weeks in September with each week being 1 of the 3 STARS management units. Calf age at weaning ranged from 154 to 274 d with an average of 229 d. Blood samples were taken from each animal at weaning. Approximately 9 mL of whole blood was collected via venipuncture using Sarstedt tubes containing EDTA (Sarstedt AG and Co., Germany). Sample tubes were refrigerated on ice and subsequently centrifuged at 4ºC at 2000 x g for 30 minutes. Plasma was collected and stored at -20º C. Following adaptation from weaning, the calves were separated by sex. Upon separation, all steers were shipped to the USDA-ARS, Grazinglands Research Laboratory in El Reno, OK. The steers were then grouped and placed into traps containing native grasses and given ad libitum access to bermudagrass hay. A 20% CP supplement (0.9 kg/head/day) was given in addition to the roughage. At 24 hr post-arrival, the ration per head was changed to 1.6 kg per day.

After 5 d, each steer was fed its weekly CP supplement in five meals (Monday-Friday). This feeding regime was continued until the end of the 28-d receiving phase. Steers were then regrouped and allowed to graze for approximately 5 months during the grazing phase. Upon completion of spring grazing, steers were sorted to feedlot pens for the finishing phase (6 head/pen X 14 pens). Utilizing a Calan gate system (American Calan, Northwood, NH), individual performance was measured during the finishing phase for approximately 65 days. Expected DMI was determined by linear regression of actual DMI, ADG, and mid-test $BW^{0.75}$. A 3% shrink was applied to all weights in order to achieve a shrunk body weight (SBW). Weekly SBW was regressed against day for each animal. The slope of the regression line was used to determine individual ADG. The intercept of the regression line was used as the initial SBW. The slope of the line and the intercept were used to calculate the final SBW. Mid-test weight was determined by taking the average of the initial and final SBW. Mid-test metabolic BW ($BW^{0.75}$) was calculated as the mid-test SBW to the power of 0.75. Each steer was ranked as low, medium or high RFI based upon ½ standard deviation above or below mean RFI of each steer crop. Blood samples were collected at the beginning of the finishing phase and at 28-d intervals until slaughter. Approximately 9 mL of whole blood was collected via venipuncture using Sarstedt tubes containing EDTA (Sarstedt AG and Co., Germany). Sample tubes were refrigerated on ice and subsequently centrifuged at 4ºC at 2000 x g for 30 minutes. Plasma was collected and stored at -20º C.

IGF-I RIA

Plasma samples (n = 90) from 10 steers of each purebred and crossbred breed-type from each calf crop, at weaning and 2 dates post-weaning, were removed from storage for determination of concentration of IGF-I by radioimmunoassay. Day 0 and d 60 of the finishing phase were utilized. Concentrations of IGF-I were determined by radioimmunoassay (Appendix A) as described by Bilby et al (1999). The protocol included 2 modifications. The final primary antibody was diluted 1:120,000 and the goat-anti-rabbit secondary antibody was diluted 1:60. The IGF-I antibody used was anti-hIGF-I (AFP4892898, A.F. Parlow, National Hormone and Peptide Program, Torrance, CA). Unknown concentrations of IGF-I were calculated using Assay Zap software (Biosoft, Cambridge, UK) using counts per minute (cpm) obtained from a Cobra II auto-gamma-counter (Perkin Elmer, Waltham, MA). Interassay and intraassay coefficients of variation were 6.5% and 6.1%, respectively.

Statistical Analysis

Due to significant differences in RFI between the 6 crossbred breed types, the reciprocal crosses were not combined. Analysis was done on 9 separate breeds. Pearson's correlation coefficients were used to determine the strength of relationship between traits. Due to low sample numbers within each breed, data for all breeds were pooled. All coefficients were derived using SPSS (2005). Coefficients with a probability value of < 0.05 were considered significant. Analysis of variance using the GLM procedure of SAS (2002) was used to assess the effects of breed and year on RFI. The

model included year, breed and year X breed interaction. The same procedure was also used to determine the effect of breed with breed-adjusted NEm as a covariate on RFI among the Brahman, Angus and Brahman X Angus crossbred steers. The model included breed, breed-adjusted NEm and breed X breed-adjusted NEm.

Results and Discussion

As reported in Chapter II, breed did influence concentration of IGF-I ($P < 0.007$). The overall effect of breed on concentration of IGF-I differed for each sample date ($P < 0.007$) except d0 ($P = 0.43$) among the steers. These results prove a significant effect of breed on concentration of IGF-I. No trend was observed when concentrations of IGF-I within the breeds were grouped by RFI rank (Table 3).

Pearson's correlations (Table 4) between concentrations of IGF-I and RFI, pooled over all steer breeds, were weak for all sample dates, with r values ranging from -0.136 to 0.066 ($P > 0.05$). No significant relationship was observed between plasma concentration of IGF-I and RFI. These results suggest that RFI is unrelated to plasma concentration of IGF-I in these breed-types. Results from studies preformed among Brahman females in Chapter III support the lack of relationship between circulating concentrations of IGF-I and RFI.

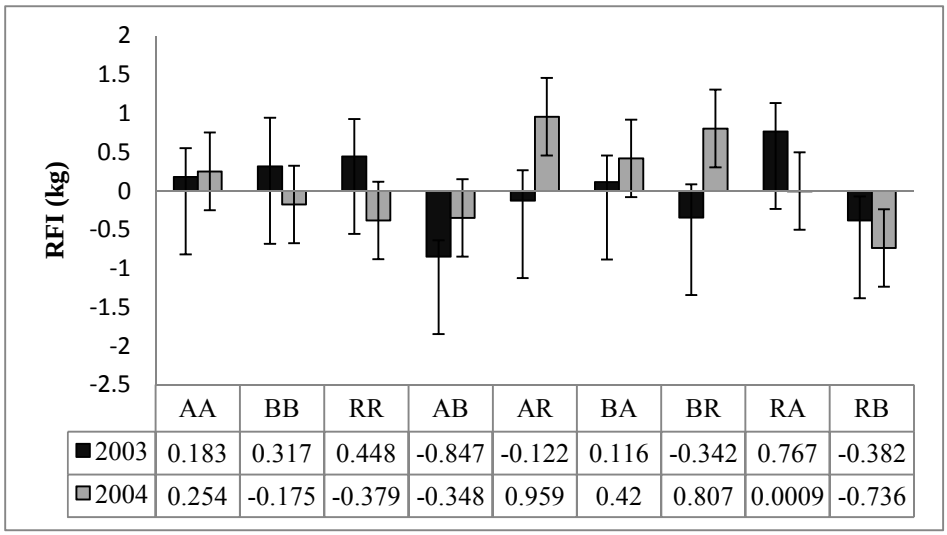
Table 3. Mean concentrations of IGF-I (ng/mL) grouped by RFI rank.

	2003			2004			
Breed	Weaning	d0	d60	Weaning	d0	d60	RFI Rank
	67.49	177.184	100.2395	66.655	171.477	254.92	L
AA	113.6027	152.5083	172.3422	74.7966667	193.4357	141.2092	M
	97.1295	137.7675	129.3005	103.139333	215.8187	168.0117	H
	157.5373	211.201	164.1076	126.69775	184.324	146.839	L
AB	168.831	213.672	166.099	155.7302	188.4992	208.4498	M
	NA	NA	NA	166.754	152.635	204.712	H
	150.9065	167.4585	161.5	NA	NA	NA	L
AR	144.9167	194.384	140.206	102.7615	230.514	172.1328	M
	165.8793	174.585	113.1103	114.846	199.5868	200.4482	H
	154.497	169.536	165.3695	123.107	179.331	147.789	L
BA	104.08	161.68	124.59	104.572333	181.0737	178.4663	M
	115.35	119.65	144.85	113.7066	198.3586	187.019	H
	203.3075	171.782	183.9065	170.23	215.4309	200.2224	L
BB	178.8698	181.706	159.013	216.91	194.332	177.493	M
	112.826	130.406	199.008	154.35	289.953	184.136	H
	139.7484	154.6146	198.7636	NA	NA	NA	L
BR	118.5535	116.488	177.074	147.0635	196.3915	205.5905	M
	139.6935	190.889	204.191	158.716286	225.7317	199.3916	H
	138.661	211.24	321.245	110.57	282.826	178.943	L
RA	111.802	170.9478	190.5473	104.899714	153.6796	170.3053	M
	116.57	192.245	195.9708	112.43	308.929	190.923	H
	146.624	171.3885	181.2205	161.6688	232.6436	258.1046	L
RB	201.714	192.4783	162.0285	153.40475	262.3563	217.3235	M
	237.479	189.154	183.667	230.011	135.549	210.775	H
	176.825	130.71	227.422	139.65	199.1685	187.5743	L
RR	226.713	195.9417	181.1937	128.8515	188.5253	210.3893	M
	182.5056	172.4534	201.9702	158.214	236.9	150.32	H

Table 4. The r values derived from Pearson's correlations between concentrations of IGF-I and RFI.

Sample Date	2003	2004
Weaning	- 0.136	- 0.129
d 0	- 0.064	0.066
d 60	- 0.125	- 0.017

The overall effect of breed on residual feed intake was important (P = 0.01). The interaction of breed and year effects on RFI was also important (P = 0.005). These highly significant effects suggest that RFI values vary between breeds of cattle while also differing between feeding trials (Figure 19). Similar results describing variance among RFI values between diverse breeds of cattle were observed in a study reported by Loyd, et al. (2008). Another source of variation in RFI can be attributed to the differences in requirements of NEm between different breeds of cattle. The maintenance requirement for Brahman cattle is 10% lower than that required for the Angus breed (NRC, 2000). Prior to breed adjustment of NEm, RFI was significantly affected by breed (P = 0.01). Upon the adjustment of NEm among the Brahman, Angus and Brahman X Angus crosses, breed had no significant effect on RFI. This was observed among steers from both the 2003 and the 2004 finishing phase feeding trials. The amount of variation in feed efficiency coupled with the differences in concentrations of IGF-I between breeds may be a reason for the conflicting results observed in studies to determine relationships between IGF-I and RFI.



	AA	BB	RR	AB	AR	BA	BR	RA	RB
2003	0.183	0.317	0.448	-0.847	-0.122	0.116	-0.342	0.767	-0.382
2004	0.254	-0.175	-0.379	-0.348	0.959	0.42	0.807	0.0009	-0.736

Figure 19. Least squares means of RFI separated by breed and year.

CHAPTER VI

CONCLUSIONS

Johnston et al. (2002) states that in order to improve feed efficiency within the beef industry "we need correlated traits that can be easily measured on large numbers of cattle prior to the time when the major selection decisions are made." This reasoning led to studies to determine the correlations between circulating IGF-I and RFI. In two separate studies conducted on large sample populations, moderately strong correlations were observed between the two traits. Although the idea for early selection tools is of importance, results from studies within this thesis refute the findings of Johnston et al. (2002). No relationship was observed between circulating concentrations of IGF-I and RFI among the varying breeds of beef cattle tested within these experiments.

Presumably, this contrast in results may be in part due to the variance seen among circulating concentrations of IGF-I between different breeds of beef cattle. Although the mechanism controlling varying concentrations of circulating IGI-I is unclear, it is apparent from the trends observed within these data that, relative to the temperate *Bos taurus* breed, plasma concentrations of IGF-I were greater in male and female tropically-adapted breed-types. Breed and sex class significantly affected the plasma concentrations of IGF-I at various sampling dates.

Differences in feed efficiency among beef cattle can be attributed to a number of physiological processes. Richardson and Herd et al. (2004) suggested feed intake,

digestion of feed, metabolism, activity of the animal and thermoregulation may all affect efficiency. While these factors are known to contribute to the variation in feed efficiency, nearly 70% of the variation remains unexplained (Richardson and Herd, 2004). The authors speculate that a portion of the unexplained variation involves energy expenditure mechanisms which vary between animals. Energy requirements for maintenance among beef cattle also differ, particularly between breeds. Our results indicate that NEm may account for a larger portion of the variation seen in RFI than previously reported. Prior to breed adjustment of NEm, RFI was significantly affected by breed ($P = 0.01$). Upon the adjustment of NEm among the Brahman, Angus and Brahman X Angus crosses and its covariance with RFI, the breed effect on RFI was no longer significant. Results from Chapter V suggest, due to significant effects of breed on RFI as well as breed specific differences in energy requirements for maintenance, RFI should not be used for comparison across breed-types.

After a review of literature assessing the relationship of IGF-I to feed efficiency, performance and ultrasound composition traits, Lancaster (2008) states that the relationship is complex and can be affected by numerous characteristics unique to each individual animal. Correlations have been observed in the past, however, these data suggest that among the breed-types tested, circulating concentrations of IGF-I cannot be used as a selection tool for feed efficiency. Although results do suggest that animal temperament may have an effect on ADG in Brahman females.

Research has shown that circulating concentrations of IGF-I do indeed influence traits of economic importance to the beef industry. With the relative ease at which the

concentrations can be measured, it might benefit producers if IGF-I assays were developed as a selection tool. It seems that further research is needed to fully understand the variation observed in both concentrations of IGF-I and feed efficiency measurements and the effects that this selection may have on other traits, such as reproductive efficiency. Due to abundant amounts of forage, a large majority of the US cow-calf producers are located in, or near, gulf coastal regions. These producers have a need for selection of feed efficient animals but at the same time cannot afford to negatively affect efficiency of reproduction within their herds. Data from these experiments indicates that tropical adaptation does, in some way, affect circulating concentrations of IGF-I. There is a need for a complete understanding of how breeds differ in the bioavailability of IGF-I, as well as the effect of IGFR activity on the biological mechanisms of this growth factor.

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APPENDIX A

INSULIN-LIKE GROWTH FACTOR-I (IGF-I) RADIOIMMUNOASSAY

PROTOCOL FOR BOVINE SERUM

Reagent Preparation

1. IGF-I Assay Buffer

   0.40 g Protamine (grade II) SO_4 (Sigma S-4380)
   8.28 g Sodium phosphate (monobasic) (Sigma S-0751)
   1.0 ml Tween 20 (Sigma P-1379)
   0.40 g Sodium azide (Sigma S-2002)
   7.44 g EDTA (Sigma E-5134)

   Mix above reagents in double-distilled water (ddH_20). pH solution to 7.5 with NaOH and bring volume to 2.0 liters. Store solution at 4ºC for one month. **(Caution: Sodium azide is highly toxic.)**

2. 1M Glycine

   75.07 g Glycine (Sigma G-8898)

   Mix glycine in approximately 850 ml ddH_20. Using 38% HCl, adjust pH of solution to 3.2 and bring volume to 1.0 liter. Make fresh 1M glycine for each extraction and store at 4ºC.

3. 0.5N NaOH

   Add 50 ml of 2.5N NaOH to 200 ml ddH_20 (1:5 dilution) or dissolve 5.0 g NaOH pellets into 250 ml ddH_20. Store at 4ºC.

4. 12.5% Polyethylene Glycol (PEG)

   250 g Carbowax PEG 8000 (Sigma P-2139)

   Mix PEG in approximately 1800 ml ddH_20, cover and mix at room temperature until solution is clear (~6 hours). Adjust pH of solution to ~8.6 and store at 4ºC overnight. Allow solution to come to room temperature while spinning. Adjust pH of solution to 8.6 and bring volume to 2.0 liters. Store at 4ºC for up to 3 months.

5. Primary Antibody (Anti-Human IGF-I)

(Source: A. F. Parlow, National Hormone and Peptide Program, Harbor-UCLA Medical Center, 1000 West Carson St., Torrance, CA 90509)
Antibody comes lyophilized at a 1:10 dilution in PBS. Use antibody at a final dilution of 1:120,000 in IGF-I Assay Buffer (7.5 µl stock into 90 ml IGF-I Assay Buffer). Prepare fresh daily at least one hour before use and store at 4ºC.

6. Secondary Antibody (GARGG)
(Source: Calbiochem, San Diego, CA; Goat Anti-Rabbit y-Globulin cat#. 539845)Add 667 µl of stock GARGG to 39.33 ml IGF-I Assay Buffer (1:60 dilution). Prepare fresh daily and store at 4ºC.

7. Normal Rabbit Serum (NRS) (IgG Corporation, Cat# IgG-NRS)
Prepare at 1:100 dilution in IGF-I Assay Buffer (500 µl stock into 49.5 ml Buffer).

8. [^{125}I] Tracer
(Source: MP Biomedicals Inc., Cat# 68128)
Calculation of required activity:
1µCi isotope = 22.20 x 10^6 dpm.
2.22 x 10^6 dpm = 1.665 x 10^6 cpm (At 75% counting efficiency estimate [^{125}I]).

(n) RIA tubes x 21,000 cpm x µCi = Required Activity (µCi)
1 tube 1,665,000 cpm
= approx. 15 µCi/1000 RIA tubes (~800 RIA tubes per 10 µCi batch)

Prepare trace at RIA working dilution of 21,000 cpm/100 µl.

Prepare and store in an appropriately labeled HD polypropylene bottle set behind lead-block shielding in 4ºC walk-in. Survey and thaw raw trace shipment under hood. Calculate the final working dilution as above. Make final dilution and store, preferably overnight, before use.

9. IGF-I Standards
Absolute range of IGF-1 standards @1:200 sample dilution is 19.54 through 5000 ng/ml serum. (Dilutions are 1:100 at sera extraction, and 1:2 in RIA; final = 1:200). Expected biological range should be approximately 40 to 250 ng/ml sera, therefore most samples should be represented by the range between the 0.98 and 1.56 ng/ml standards.

hIGF-1, BIO: Lot #01, sample #1168, 134 µg/vial, lyophilized.
Source: A.F. Parlow, National Hormone and Peptide Program,
Harbor-UCLA Medical Center, 1000 West Carson St, Torrance CA

Reconstitute lyophilized standard stock with 1.00 ml ddH_2O (IGF-1 STD Stock I). Note: this resulted in a previous shipment of this specific standard (sample #1168) having a concentration of 134 µg/ml (vial's specific mass listed on same by FJP).
Construct 1 µg/ml IGF-1 STD Stock II. Transfer 74.63 µl (i.e. 10 µg) to a 10 ml volumetric containing approximately 8.0 ml IGF-1 RIA buffer. Bring to volume and allow for equilibration. Aliquot and freeze if not used immediately.

Prepare serial dilutions of IGF-1 standards fresh for each RIA series. Use liquid-to-liquid transfer, and allow for equilibration. The resulting STD A = 25 ng/ml. Continue preparation of serial dilutions by volume. Mix by gentle vortexing then allow to equilibrate for a minute or two before continuing with the next 1:1 by volume dilution (STD B = 12.5 ng/ml). Continue serial dilutions through STD I (0.098 ng/ml).

IGF-I Standards chart:

ng/ml	ng/tube		equivalence
IGF-1 STD	at RIA	at RIA	ng/ml sera
STD A	25.000	2.500	5000.00
STD B	12.500	1.250	2500.00
STD C	6.250	0.625	1250.00
STD D	3.125	0.313	625.00
STD E	1.563	0.156	312.50
STD F	0.781	0.078	156.25
STD G	0.391	0.039	78.13
STD H	0.195	0.020	39.06
STD I	0.098	0.010	19.53
STD J	0.000	0.000	0.00

10. IGF-1 Composite Pools for RIA:
For verification of inter-and intra-RIA performance over the expected biological IGF-1 concentration range, construct a "normal" pool and a "high" pool from a composite sub-set of acidified serum samples.
Pool (n = 30) 200 µl aliquots from a random set of acidified serum samples. Pipette 3.00 ml of this to a 13 x 100 mm PP culture tube (to be used for the "high" pool preparation). Aliquot remainder of "normal" pool at 500 µl, freeze and store until use.
For the "high" pool, prepare a 100 ng/ml IGF-1 stock through a 1:10 dilution of 1 µg /ml IGF-1 Standard Stock II. Pipette 20 µl of the 100 ng/ml stock into 2980 µl of the "normal" pool aliquot to produce the "high" pool stock (e.g. back-pipette 20 µl from the 3.0 ml and replace). This results in the addition of 0.667 ng/ml at RIA or, after accounting for the 1:200 final sample dilution following RIA, a

delta at RIA of 133.3 ng/ml. (e.g. "normal" IGF-I concentration plus 133 ng/ml at the serum level). Vortex and aliquot "high" pool at 500 µl, freeze and store until use. Laboratory wide "Welsh" pool should be acid extracted along with unknown samples.

IGF-I Assay Protocol:

A. Acidification of Samples

1. Pipette 10 µl of each bovine serum sample into polypropylene eppendorf tubes. (Polypropylene tubes must be used due to low pH. Number tubes in even numbers so that samples can be assayed in duplicate.)
2. Add 400 µl of 1M glycine to each sample.
3. Add 500 µl of IGF-I Assay Buffer to each sample.
4. Cap tubes and incubate in 37ºC water bath for 48 hours.
5. Add 90 µl of 0.5N NaOH to all samples and vortex to mix. (Continue assay immediately.)
   (Sample dilution is now 1:100)

B. Assay Procedure

1. Each assay should include at least triplicate tubes of total (T), non-specific binding (NSB), zero tubes (B_0), standards, and pools. Single acidified unknown samples should be in duplicate for the RIA.
2. Pipette 400 µl of IGF-I Assay Buffer into NSB tubes.
3. Pipette 300 µl of IGF-I Assay Buffer into B_0 tubes.
4. Pipette 200 µl of IGF-I Assay Buffer into standard tubes.
5. Pipette 200 µl of IGF-I Assay Buffer into all sample tubes and pools.
6. Add 100 µl of each standard to each designated standard tube.
7. Add 100 µl of each acidified serum sample into each designated tube pair.
8. Add 100 µl of acidified pools into control pool tubes.
9. Pipette 100 µl of primary antibody to all tubes except NSB and T.
10. Carefully shake tubes to mix and cover with foil.
11. Incubate for 24 hours at 4ºC.
12. Pipette 100 µl of [^{125}I]-IGF-I Tracer to all tube13. Cover tubes with foil and shake the tubes carefully to mix.
14. Incubate for 16 hours at 4ºC.
15. Pipette 50 µl of NRS to all tubes except totals.
16. Pipette 50 µl of GARGG to all tubes except totals.
17. Pipette 300 µl of PEG to all tubes except totals.
18. Carefully shake tubes to mix and cover with foil.
19. Incubate tubes at room temperature for 30 minutes. (NO LONGER)
20. Centrifuge tubes at 3000 rcf for 25 minutes at 4ºC. (3220 rpm on Sorvall RC3C)
21. Decant tubes (except totals) immediately into radioactive waste container.

22. Allow tubes to remain upside down on absorbent towels for 5 minutes.
23. Remove all visible droplets by tapping tube bottoms.
24. Count tubes on Beckman gamma counter for 1 minute per sample.
25. Use AssayZap to calculate concentrations of unknowns in comparison to a known standard curve. (Final ng/mL concentrations are determined by multiplying mean unknown by 1000)

APPENDIX B

CONCENTRATIONS OF IGF-I SEPARATED BY SEX CLASS

Summary of ng/mL weaning IGF-I Least Squares Means

Breed	ng/mL IGF-I Heifers	ng/mL IGF-I Steers
AA	76.14	83.50
AB	104.85	153.26
AR	100.09	131.11
BA	98.11	115.31
BB	118.41	171.42
BR	111.66	144.75
RA	90.93	111.68
RB	139.19	179.31
RR	119.23	167.00

Summary of ng/mL 1st post-wean IGF-I Least Squares Means

Breed	ng/mL IGF-I Heifers	ng/mL IGF-I Steers
AA	49.97	176.22
AB	63.22	193.23
AR	57.04	186.72
BA	72.45	171.72
BB	71.26	200.09
BR	74.02	184.63
RA	50.24	184.96
RB	75.26	211.57
RR	64.67	188.18

.

Summary of ng/mL 2nd post-wean IGF-I Least Squares Means

Breed	ng/mL IGF-I Heifers	ng/mL IGF-I Steers
AA	62.39	154.96
AB	85.31	178.80
AR	76.09	165.22
BA	101.47	157.38
BB	71.61	184.04
BR	86.13	198.39
RA	80.05	188.09
RB	88.61	207.37
RR	77.62	193.19

APPENDIX C

CORTISOL RADIOIMMUNOASSAY FOR BOVINE SERUM

Intended for use with Coat-A-Count Cortisol Radioimmunoassay (Siemens, TKCO5)

Materials Supplied

1. Cortisol Ab-Coated Tubes
   Protect from moisture by resealing storage bags after use, store at 4ºC.
2. ^{125}I Cortisol
   Stable at 4ºC for 30 days after opening.
3. Cortisol Calibrators (Standards)
   Processed in human serum. Stable for 30 days after opening. Can extend stability by freezing. Aliquot to avoid freeze/thaw.
4. Pooled serum for control sample.

Materials Required But Not Supplied

1. Gamma counter compatible with 12x75mm tubes
2. Vortex
3. 12x75mm assay tubes
4. Micropipettes and compatible disposable tips: p200 and p1000
5. Waterbath that can hold constant 37ºC
6. Foam decanting racks and reservoir and radioactive work space

Sample Collection

1. Collect serum via venipuncture into additive free vacutainer tubes.
2. Separate serum via centrifugation: 4ºC at 2000 x g for 30 minutes.
3. If frozen, thaw at RT and mix by gentle vortex or inversion.

Radioimmunoassay Procedure

1. Allow all components to warm to room temperature.
2. Label four uncoated 12x75 tubes: NSB (nonspecific binding) and T (total counts) in duplicate.
3. Label 12 Ab-coated tubes A-H (2 extra standards) in duplicate for standards.
5. Prepare extra standards:
   0.5ug/dL: Add 50ul of 0ug/dL standard to 50ul of 1ug/dL standard.
   2. 5ug/dL: Add 50ul of 0ug/dL standard to 50ul of 5ug/dL standard.
6. Label pooled control and unknown sample Ab-coated tubes in duplicate.
7. Pipette 25ul of the 0ug/dL standard into the NSB and A tubes. Pipette 25ul of each remaining standard, pooled control or unknown sample into the labeled tubes.

PIPETTE DIRECTLY TO BOTTOM OF TUBE.

8. Add 1mL of ^{125}I Cortisol to every tube and vortex. (Minimum of 10min from start to finish)

9. Cover tubes with foil and incubate for 45min at 37ºC.
10. Decant thoroughly. Remove all visible moisture by patting inverted tubes.
11. Count for 1min on gamma counter.
12. Use Assay Zap (Biosoft, Cambridge, UK) to calculate unknown concentrations against standard curve.

APPENDIX D

LEAST SQUARES MEANS AND STANDARD ERRORS GROUPED BY RFI RANK OF ALL TRAITS COMPARED IN CHAPTER IV

	2005			2006		
RFI Rank	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
n per Rank	14	12	13	11	19	11
Days of Age	361 ± 5.62	363 ± 8.45	372 ± 6.8	213 ± 6.55	212 ± 4.17	210 ± 6.35
IGF-I ng/ml Weaning	122.46 ± 15.77	138.44 ± 9.62	130.36 ± 11.41	104.40 ± 8.48	108.23 ± 7.64	119.76 ± 6.86
IGF-1 ng/mL d0	111.77 ± 5.98	106.14 ± 5.43	102.52 ± 7.82	81.47 ± 13.85	95.62 ± 7.81	85.92 ± 9.85
IGF-1 ng/mLl d70	133.96 ± 10.72	120.22 ± 9.26	120.30 ± 6.77	81.80 ± 8.08	109.37 ± 9.82	98.70 ± 6.28
Cortisol ng/mL Weaning	29.23 ± 5.04	23.87 ± 3.97	28.02 ± 3.34	15.08 ± 1.1	16.23 ± 1.97	15 ± 2.09
Cortisol ng/mL d0	30.7 ± 3.26	26.9 ± 3.81	27.6 ± 3.43	33.13 ± 4.83	25.56 ± 2.59	29.74 ± 3.13
Cortisol ng/mL d70	20.01 ± 4.04	19.74 ± 3.76	20.65 ± 2.57	28.48 ± 3.92	22.46 ± 3.22	22.48 ± 3.2
ADG	0.77 ± 0.02	0.83 ± 0.06	0.79 ± 0.03	0.59 ± 0.03	0.52 ± 0.03	0.57 ± 0.03
Temperament Score	3.43 ± 0.33	2.53 ± 0.24	2.77 ± 0.34	2.38 ± 0.24	2.27 ± 0.19	2.68 ± 0.26
Intake	6 ± 0.11	6.3 ± 0.16	6.2 ± 0.28	5.6 ± 0.13	5.4 ± 0.13	5.6 ± 0.26
FCR	7.92 ± 0.19	8.01 ± 0.59	7.82 ± 0.26	10.1 ± 0.86	11.3 ± 0.94	10.1 ± 0.54
PEG	0.27 ± 0.006	0.27 ± 0.01	0.27 ± 0.01	0.24 ± 0.01	0.22 ± 0.01	0.23 ± 0.01
cm Back Fat_Weaning	0.19 ± 0.009	0.17 ± 0.008	0.22 ± 0.02	0.24 ± 0.03	0.22 ± 0.02	0.25 ± 0.03
cm Back Fat_d56	0.22 ± 0.001	0.19 ± 0.008	0.23 ± 0.02	0.25 ± 0.02	0.27 ± 0.03	0.25 ± 0.02
cm Back Fat_Yearling	0.24 ± 0.05	0.21 ± 0.02	0.26 ± 0.03	0.26 ± 0.04	0.27 ± 0.05	0.27 ± 0.03
cm Rump Fat_Weaning	0.4 ± 0.02	0.38 ± 0.02	0.38 ± 0.04	0.39 ± 0.03	0.37 ± 0.02	0.36 ± 0.05
cm Rump Fat_d 56	0.34 ± 0.03	0.29 ± 0.04	0.38 ± 0.04	0.5 ± 0.03	0.52 ± 0.03	0.52 ± 0.03
cm Rump Fat_Yearling	0.38 ± 0.03	0.36 ± 0.03	0.38 ± 0.05	0.5 ± 0.04	0.45 ± 0.06	0.51 ± 0.04
cm^2 RE Area_Weaning	35.64 ± 1.62	34.59 ± 2.31	38.48 ± 1.05	32.61 ± 1.49	32.48 ± 0.95	35.23 ± 0.77
cm^2 RE Area_d 56	40.45 ± 1.52	38.98 ± 2.28	41.85 ± 1.95	37.47 ± 1.28	36.95 ± 0.89	39.22 ± 1.06
cm^2 RE Area_Yearling	47.17 ± 1.27	46.72 ± 2.08	47.18 ± 1.56	45.8 ± 0.74	44.96 ± 1.26	46.23 ± 2.73
IMF %_Weaning	1.69 ± 0.16	1.55 ± 0.11	1.61 ± 0.14	1.66 ± 0.10	1.63 ± 0.14	1.78 ± 0.17
IMF %_d56	3.09 ± 0.14	2.75 ± 0.13	2.77 ± 0.20	2.98 ± 0.18	2.78 ± 0.19	2.73 ± 0.24
IMF %_Yearling	2.42 ± 0.17	2.32 ± 0.11	2.24 ± 0.19	2.80 ± 0.19	2.29 ± 0.20	2.53 ± 0.28

	2007			ALL		
RFI Rank	**Low**	**Medium**	**High**	**Low**	**Medium**	**High**
n per Rank	14	22	14	39	53	38
Days of Age	174 ± 5.32	188 ± 4.31	197 ± 3.62	252 ± 13.87	236 ± 10.09	261 ± 13.65
IGF-I ng/mLl Weaning	156.75 ± 12.8	156.14 ± 11.45	136.83 ± 8.06	128.96 ± 8.26	134.96 ± 6.49	129.92 ± 5.19
IGF-1 ng/mL d0	116.33 ± 9.24	132.48 ± 6.71	107.84 ± 6.06	104.86 ± 5.91	113.3 ± 4.67	99.67 ± 4.62
IGF-1 ng/mL d70	132.19 ± 6.3	147.3 ± 7.13	127.3 ± 8.5	118.62 ± 6.15	127.57 ± 5.49	116.63 ± 4.61
Cortisol ng/mL Weaning	18.68 ± 2.71	21.04 ± 2.55	22.75 ± 3.06	21.71 ± 2.3	19.7 ± 1.56	22.19 ± 1.88
Cortisol ng/mL d0	31.56 ± 7.19	31.51 ± 3.48	40.98 ± 2.69	31.7 ± 3.06	28.36 ± 1.93	33.13 ± 2
Cortisol ng/mL d70	19.82 ± 2.52	22.71 ± 2.47	30.63 ± 3.76	22.33 ± 2.07	21.98 ± 1.73	25.09 ± 2.02
ADG	0.53 ± 0.02	0.57 ± 0.03	0.55 ± 0.02	0.63 ± 0.02	0.61 ± 0.02	0.63 ± 0.02
Temperament Score	2.05 ± 0.32	2.09 ± 0.17	2.29 ± 0.19	2.65 ± 0.19	2.25 ± 0.11	2.57 ± 0.15
Intake	4.4 ± 0.09	5.04 ± 0.09	5.6 ± 0.11	5.3 ± 0.12	5.44 ± 0.09	5.83 ± 0.13
FCR	8.4 ± 0.32	9.1 ± 0.42	10.4 ± 0.38	8.68 ± 0.31	9.65 ± 0.43	9.43 ± 0.29
PEG	0.25 ± 0.008	0.24 ± 0.01	0.25 ± 0.009	0.25 ± 0.008	0.24 ± 0.01	0.25 ± 0.009
cm Back Fat_Weaning	0.22 ± 0.01	0.22 ± 0.01	0.28 ± 0.02	0.22 ± 0.01	0.21 ± 0.008	0.25 ± 0.01
cm Back Fat_d56	0.24 ± 0.008	0.25 ± 0.01	0.29 ± 0.02	0.23 ± 0.007	0.24 ± 0.01	0.26 ± 0.01
cm Back Fat_Yearling	0.21 ± 0.01	0.21 ± 0.01	0.23 ± 0.02	0.23 ± 0.01	0.23 ± 0.01	0.25 ± 0.01
cm Rump Fat_Weaning	0.56 ± 0.15	0.39 ± 0.02	0.47 ± 0.04	0.45 ± 0.05	0.38 ± 0.01	0.41 ± 0.02
cm Rump Fat_d56	0.43 ± 0.03	0.45 ± 0.03	0.51 ± 0.03	0.41 ± 0.01	0.43 ± 0.02	0.47 ± 0.02
cm Rump Fat_Yearling	0.39 ± 0.03	0.39 ± 0.03	0.47 ± 0.03	0.4 ± 0.01	0.4 ± 0.02	0.44 ± 0.02
cm^2 RE Area_Weaning	31.15 ± 1.22	34.92 ± 0.85	40.81 ± 1.18	33.11 ± 0.87	33.97 ± 0.71	38.44 ± 0.76
cm^2 RE Area_d56	36.09 ± 0.76	41.72 ± 1.25	45.43 ± 1.49	38.04 ± 0.75	39.39 ± 0.83	42.42 ± 0.97
cm^2 RE Area_Yearling	40.98 ± 1.27	44.1 ± 1.21	47.59 ± 1.7	44.48 ± 0.88	45 ± 0.85	47.12 ± 1.06
IMF %_Weaning	2.24 ± 0.18	1.82 ± 0.13	1.70 ± 0.17	1.88 ± 0.10	1.69 ± 0.07	1.69 ± 0.09
IMF %_d56	2.82 ± 0.15	2.40 ± 0.15	2.45 ± 0.14	2.96 ± 0.09	2.61 ± 0.09	2.64 ± 0.11
IMF %_Yearling	2.93 ± 0.16	2.48 ± 0.14	2.74 ± 0.14	2.69 ± 0.10	2.39 ± 0.09	2.50 ± 0.11

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