# INSULIN SENSITIVITY IN TROPICALLY ADAPTED CATTLE WITH DIVERGENT RESIDUAL FEED INTAKE

A Thesis

by

GENTRIE LYNN SHAFER

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

August 2011

Major Subject: Physiology of Reproduction

Insulin Sensitivity in Tropically Adapted Cattle with Divergent Residual Feed Intake

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# ABSTRACT

Insulin Sensitivity in Tropically Adapted Cattle with Divergent Residual Feed Intake.

(August 2011)

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Residual feed intake (RFI) is one method to identify feed efficient animals; however, this method is costly and time consuming therefore, identifying an indirect measure of RFI is important. Evaluating the glucoregulatory mechanisms in cattle selected for divergent RFI may provide insight into metabolic processes involved in feed efficiency.

This study evaluated the effect of a glucose (GLUC) challenge on efficient (LRFI) and inefficient (HRFI) tropically adapted bulls and heifers. Insulin (INS) secretion was determined by radioimmunoassay (RIA) and GLUC was determined by colorimetry. Insulinogenic index (IIND) was calculated as the ratio of INS to GLUC (I/G).

Bonsmara heifers were evaluated in two experiments. Similar results were observed in both experiments. RFI affected ($P < 0.05$) INS response; with LRFI heifers having a greater INS response than HRFI heifers. Similarly, RFI affected ($P < 0.05$) IIND; with LRFI heifers having a greater IIND than HRFI heifers.

In Santa Gertrudis bulls, RFI did not affect ($P > 0.05$) GLUC conc. or Ins. response; however, numerically HRFI bulls had a greater INS response than LRFI bulls. RFI affected ($P < 0.05$) IIND; with LRFI bulls having a lower IIND than HRFI bulls.

In Brahman heifers (Exp 1), RFI did not affect ($P > 0.05$) GLUC concentration or INS. response; however, numerically HRFI heifers had a greater INS response than LRFI heifers. RFI affected ($P < 0.05$) IIND; with LRFI heifers having a lower IIND than HRFI heifers. In Brahman bulls (Exp 2), RFI affected ($P > 0.05$) INS response; with HRFI bulls having a greater INS response than LRFI bulls. RFI affected ($P < 0.05$) IIND; with LRFI bulls having a lower IIND than HRFI bulls.

Bonsmara cattle evaluated for RFI had a response to an influx of exongenous glucose that was opposite to that observed in the Brahman and Santa Gertrudis cattle evaluated for RFI. Insulinogenic index was significantly different between RFI groups in each experiment. The lower amount of INS required for clearance of the GLUC from the circulation of the Brahman and Santa Gertrudis cattle fits with our hypothesis that more efficient cattle would require less INS than the less efficient cattle. Further research and studies need to establish glucoregulatory differences between breeds and sexes of cattle evaluated for RFI.

# DEDICATION

To the loving and cherished memory of my hero, my Dad, Roger Shafer (1957-2009), the greatest man I will ever come to know and who kindled my passion for agriculture, red tractors, and Elvis. Looking up to him, I learned the value of hard work, having compassion for others and keeping faith in God. I will forever cherish the time spent being gate girl and checking cows with him; learning how to care for animals and the land. I will continue to trail in his big footsteps, while also blazing a path of my own. As promised, I will never stop being your little Nern-head…no matter my age; and I know that I am never alone in this journey of life.

"I thank my God every time I remember you." - Philippians 1:3

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# CHAPTER I

# INTRODUCTION

In a given production system, a greater profit margin can be achieved by minimizing inputs and maximizing outputs. A greater profit margin equates to an improvement in profitability. Production of beef cattle is no exception. Traditionally the selection of cattle for a breeding herd has been based on maximizing output traits such as reproductive performance, growth rates, and carcass characteristics (Archer et al., 1998). The beef industry has many genetic indicators that focus on these production (output) traits, but recently due to the increasing prices of feed, fuel, and fertilizer utilized for cattle production , more attention has been granted to production costs (inputs) as a major factor of profitability (Crews et al., 2005). Feed is the most expensive and widely utilized input associated with producing beef cattle. USDA (2011) reported in 2009 and 2010 feed costs comprised 71 and 68%, respectively, of the annual operating costs of maintaining a bred beef cow in the United States. Profitability of beef cattle operations is predominately reliant on the producer's ability to reduce feed expenses.

Identifying animals that are more efficient in feed conversion, thus requiring less feed to achieve the same level of performance as their cohorts, may be one method to help reduce feed expenses and improve profitability. Simply put, feed efficiency can be described as the amount of feed consumed by an animal per amount of weight gained. However, accurately measuring feed efficiency has proven to be a much greater

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This thesis follows the style of Journal of Animal Science.

challenge. While many methods attempting to measure feed efficiency have been developed, the industry has historically utilized feed to gain ratio (F:G) as the most common method (Nkrumah et al., 2004). This method was favored in the industry because of the simplicity and low costs associated with the equation. However, this method tends to lead to the selection of cattle that are larger at maturity and therefore have greater maintenance requirements; due to increasing prices of grain and fuel larger framed cattle at maturity are undesirable in today's economic climate. As an alternative method to calculating feed efficiency, residual feed intake (RFI) was introduced by Koch et al., (1963). Although this method has proved to identify more appropriately sized feed efficient beef cattle, it is still costly and time consuming. For these reasons, selection of efficient animals using RFI has not been widely adopted by commercial beef cattle producers.

There is great interest within the animal production community to find an indirect measure of RFI. One component of the equation used to calculate RFI is metabolic body weight (BW); consequently, a variation in RFI among a cohort of animals signifies differences in how those animals utilize energy for maintenance and growth (Kennedy et al., 1993). Evaluating the glucoregulatory mechanisms in efficient animals compared to inefficient animals may give us insight into metabolic differences between the two groups. If differences in glucoregulatory mechanisms can be linked to the different RFI groups, then a more affordable method of determining feed efficiency may be established. In turn, this would lead to a positive impact on beef cattle profitability. The response of insulin to an influx of glucose was evaluated in an effort to investigate the glucoregulatory

mechanisms and insulin sensitivity of tropically adapted cattle evaluated for RFI. Differences in insulin sensitivity between efficient and inefficient animals would suggest a glucose tolerance test may be utilized to accurately identify efficient animals in a herd.

# CHAPTER II

# LITERATURE REVIEW

Traditionally, maximizing output traits such as reproductive performance, growth rates, and carcass characteristics have been the basis for selecting cattle (Archer et al., 1998). The beef industry has many genetic indicators that focus on these production (output) traits; such as carcass traits, weaning or yearling weights, and scrotal circumference. However, more attention is being given to production costs (inputs) as a major influence on profitability due to increasing prices of feed, fuel, and fertilizer utilized for cattle production (Crews et al., 2005). Feed is the most expensive and unavoidable input associated with producing beef cattle, estimated to account for 68-71% of total costs of production (USDA, 2011). Because of the progressing upward trend in price of feedstuffs, it is important for producers to identify more efficient cattle. In addition to reducing Dry Matter Intake (DMI), efficient animals also have been shown to have reduced manure production and reduced emission of methane; therefore, reducing the environmental footprint print of beef cattle production (Nkrumah et al., 2006; Hegarty et al., 2007). Feed to gain ratio (F:G) was traditionally the method of choice to determine efficiency, but this measure tends to lead to the selection of larger framed cattle that are less desirable in today's beef industry. Currently, residual feed intake (RFI) is deemed to be a more accurate method, but it is costly and labor intensive. Evaluating glucoregulatory mechanisms may offer a less expensive, more convenient indirect measure of RFI, allowing producers to identify more efficient animals within a cohort.

**Tropical Adaptation**

Breed diversity in the beef industry allows producers to raise beef cattle in various regions of not only the United States, but also the world. Various breeds naturally adapt to and flourish in certain environments. Areas that are unsuitable for crop production, due to climate or soil composition, are great opportunities for beef cattle producers to maximize on forage availability. Producers must be selective when choosing the breed of cattle to use for production in such areas because cattle that are acclimated to temperate climates experience a loss in production when moved to a tropical or subtropical climate (Bonsma, 1949). Temperate acclimated cattle experience this loss in production performance when transferred to a tropical environment due to the animal's inability to efficiently transfer or exchange heat. Finch (1986) reveals that the tropically adapted *Bos indicus* breeds are more efficient at transferring or exchanging heat with the environment in comparison to temperate *Bos taurus* breeds. *Bos indicus* breeds are equipped with mechanisms that allow them to transfer heat such as slicker hair coat, sweat glands and rate of respiration. These mechanisms are vastly different in tropically adapted breeds of cattle in comparison to temperate breeds of cattle (Cartwright, 1955; Shrode et al., 1960; Yeates et al., 1975; Finch, 1986). As a result, tropically adapted breeds are able to withstand the hot and humid conditions of tropical and subtropical regions and are usually the breeds of choice in such climates due to their ability to perform well under these environmental conditions.

Tropically adapted breeds have a flat coat of hair with shallow follicles, allowing them to maintain optimal body temperature by exchanging heat with the environment and

providing resistance to environmental heat-flow. The smooth coat of hair protects from environmental heat gain by also acting as a reflector of radiation (Finch, 1986). This is not the case for temperate breeds as their hair coat is thick and deep, working to deflect heat loss by serving as a barrier to outward movement of heat. Finch (1986) reports that the thick hair coats of temperate breeds act as a limiting factor to evaporative cooling which is essential under hot, humid conditions. The adaptive thermoregulation ability of *Bos indicus* cattle also involves mechanisms of the tissue directly beneath the hair coat. There is a difference in the shape of the sweat glands of tropically adapted cattle in comparison to temperate cattle (Yeates et al., 1975). *Bos indicus* cattle have a baggy-0-shaped gland which promotes a higher level of activity in comparison to traditional coiled glands found in *Bos taurus* breeds. Increased activity of sweat glands results in a greater amount of sweat being produced, thus promoting evaporative cooling. The third mechanism for adaptation is rate of respiration (Shrode et al., 1960) in tropical breeds. It has been shown that heat tolerant breeds have a lower rate of respiration which aids in thermoregulation (Shrode et al., 1960; Carvalho et al., 1995). *Bos indicus* cattle are imperative to the beef industry in the southern United States and countries with tropical or subtropical climates, therefore it is economically important to investigate feed conversion mechanisms in these animals in order to capitalize on gains in productivity.

**Feed:Gain Ratio**

The initial and most common method used for identifying feed efficient animals was introduced in 1945 by Samuel Brody as the feed:gain ratio (F:G). Brody (1945)

defines this ratio as the amount of feed required to produce one unit of weight gain in an animal. An animal with low F:G requires less feed to achieve a unit of gain than an animal with a high F:G. The simplicity and low overhead costs associated with this equation make it appealing to the beef industry and, consequently, it is used extensively as a selection tool to improve feed utilization. However, after further scrutiny of this method, it was found that using F:G as a measure of feed efficiency comes with negative consequences to the beef herd. Feed:gain ratio has been negatively correlated with composition of gain, growth rate and body size in young, growing cattle (Mrode et al., 1990; Koots et al., 1994; Herd and Bishop, 2000; and Arthur et al., 2001b) The implication of these correlations is that selection for feed efficiency using F:G produces similar results to selecting for increased growth rate (Mrode et al., 1990), which is associated with an increased mature size (Herd and Bishop, 2000). Cattle that are larger at maturity have greater nutrient demands and energy maintenance requirements (Barlow, 1984), thus contradicting the initial goal of reducing feed costs by making it more expensive to maintain those cattle at maturity.

**Residual Feed Intake**

As an alternative method to calculate feed efficiency, residual feed intake (RFI) was introduced by (Koch et al., 1963). Residual feed intake is the difference in individual animal feed intake either above or below the predicted amount based upon both metabolic weight and growth rate of the animal (Archer et al., 1999). More simply stated, RFI can be thought of as the difference between an animal's actual feed intake and its expected

feed intake (Arthur et al., 1996). A negative RFI value represents an animal that consumes less feed than expected, equating to a more efficient animal; while a positive RFI value represents an animal that consumes more feed than expected, equating to an inefficient animal. Feed efficiency is not a trait that can be directly measured; therefore, the equation for establishing feed efficiency must be comprised of multiple components. Koch et al. (1963) suggested that feed efficiency should be a function of feed intake, body weight gain, and average weight throughout the course of a feeding trial. Differing from F:G, RFI is phenotypically independent of the animal's body weight (BW) and growth rate (Kennedy et al., 1993; Herd and Bishop, 2000; Arthur and Herd, 2005) suggesting selection for RFI will not result in larger cattle at maturity. Residual feed intake uses linear regression to estimate feed intake from BW and average daily gain (ADG) (Koch et al., 1963). The predicted daily feed intake value is acquired by regressing daily dry matter intake (DMI) on ADG and mid-test-metabolic body weight (MBW; [body weight at test midpoint]$^{0.75}$) or mid-test BW. Residual feed intake is then calculated as the difference between the actual and expected feed intake for each animal (Arthur et al., 1996). Calculating RFI in this manner provides a measure of feed efficiency that is independent of its component traits, BW and ADG and should not result in an increase in mature size or feed requirements (Arthur et al., 2001a; Arthur et al., 2004; Nkrumah et al., 2004; Kelly et al., 2010). Cattle with more negative RFI values consume less feed than expected, being more efficient, and are more desirable than cattle with more positive RFI values. Bingham et al. 2009 reported that high RFI Brangus heifers consumed an average of 1.92 kg/d (22.5%) more feed than low RFI heifers when animals were separated based

on a ± 1 SD from the mean. Additionally, Lancaster et al. (2005) reported that low RFI calves consumed 15% less feed than high-RFI calves when calves were separated based on ± 0.5 SD from the mean.

In order to make great strides in the feed efficiency of an entire cow herd, it is imperative that the selection tool used to identify efficient animals is a heritable trait so that continuous positive selection can be applied over generations. Previous studies have reported heritability estimates for RFI to range from 0.16 (Herd and Bishop, 2000) to 0.47 (Lancaster et al., 2009). Because most studies have reported heritability estimates to fall within this range, RFI is said to be moderately heritable (Arthur et al, 2001a; Robinson and Oddy, 2004; Schenkel et al., 2004). Consequently, selection of a cow herd for RFI should reduce feed costs and allow for production of more efficient progeny without compromising mature size of the cow herd.

## Evaluating Cattle for RFI

### *Sex, Age, and Breedtype*

To date, there is not a standard model established for calculating RFI within a single livestock industry; therefore, it is essential to compare animals within a standard level of nutrition when determining which model best suits a group of cohorts to be tested (Knott et al., 2008). Herd et al., (2004) suggested that feed efficiency is relative to the type and amount of feed consumed, the sex and breed of that animal, and environmental conditions in which that animal is managed. Each of these components must be taken into consideration when designing a protocol to determine feed efficiency of a cohort utilizing

RFI. Residual feed intake is calculated as an index, making it important to select an appropriate group of cattle to evaluate. It is well-established that cattle of different sexes perform unequally (Brinks et al., 1961; Bogart et al., 1963; Wilson et al., 1969). Due to these established differences, the industry typically manages cattle by sex class or makes adjustments to performance records. These differences must be taken into consideration by grouping cattle of the same sex together as a cohort.

The RFI index has the advantage of attempting to account for variation in maintenance requirements among cattle of different ages and breedtypes by including metabolic BW in the model (Arthur et al., 2001b). This would indicate that cattle differing in breed composition or age could be compared for RFI in the same cohort. However, further scrutiny and research have since shown that this theory may not be accurate. Schenkel et al. (2004) and Riley et al. (2007) have reported breedtype differences in RFI and shown that comparisons across breedtypes are not valid. Limited studies have addressed age as a factor. Crews et al. (2003) reported a moderate correlation ($r = 0.55$) for RFI of calves evaluated in the same cohort at 2 different ages. A similar correlation ($r = 0.59$) was reported by Johnston (2007) between post-weaning RFI and feedlot RFI of calves evaluated in the same cohort. Bradbury et al., (2011) examined the relationship between postweaning RFI and mature RFI in *Bos inidicus* females. Sire, cow age, heifer pen, lactation status, and pen were all included as random effects in this study. Results from this study indicate that heifer RFI may not be an accurate predictor of RFI as a mature cow. This leads to a more scientific indication that feed efficiency and RFI results may be dependent on stage of maturity as well as breed of the animal (Arthur

and Herd, 2005). To most accurately determine RFI, evaluation should be conducted on cattle of similar breedtypes and age (Herd and Aurthur, 2009).

*Feed*

Variation in type and amount of feed provided to animals during the RFI evaluation period may alter or affect the results of an RFI evaluation. There are contrasting results supporting both aspects of this statement. Fan et al. (1995) evaluated Angus and Hereford bulls for RFI using 2 different diets, and found significant differences in RFI values calculated. Those fed a high concentrate diet had more positive RFI than those bulls fed a high roughage diet (0.36 ± 0.12 vs. -1.67 ± 0.12 kg/d intake, respectively). However, Goonewardene et al. (2004) reported contrasting results when evaluating crossbred steers for RFI. Residual feed intake became more positive as the proportion of roughage increased; whereas RFI became more negative as the proportion of grain was increased. Type of diet provided may affect performance of animals being evaluated.

Amount of feed provided to the animal during period of evaluation may also impact RFI evaluation. When animals are offered feed *ad libitum* during the RFI evaluation period appetite should be considered as a variable; alternatively, that variable is removed when animals are offered a limit-fed ration. *Ad libitum* access to the ration more appropriately reflects a feedlot setting and is the more common ration allocation when evaluating cattle for RFI. However, providing feed *ad libitum* is not always the best option when evaluating growing heifers, later to be incorporated into the breeding herd, as

the increased weight gain during this time period may hinder reproductive performance as maturity is achieved. The initial idea and basis behind RFI determinations leads to the thought that RFI calculations determined in a feedlot setting would also be applicable to the breeding herd (Arthur et al., 2001a; Arthur and Herd, 2005). Herd et al., (1998) evaluated Angus heifers for post-weaning RFI, providing *ad libitum* access to a high concentrate ration. The most efficient and least efficient heifers from that cohort were later re-evaluated for RFI as 3-yr-old cows on pasture; utilizing intra-ruminal alkane capsules and fecal samples to estimate DMI. Results from this study showed no differences in pasture DMI between the two groups. It should be noted that cows were in their third month of lactation at the time of DMI determination, and both groups had similar subcutaneous fat stores and reared calves of similar weight. It can be concluded that the amount of diet or type of diet could influence the outcome of an RFI evaluation test.

*Estimating Feed Intake*

Correctly estimating expected feed intake becomes an essential role in determining feed efficiency and evaluating cattle for RFI. Two methods have been proposed. Koch et al., (1963) proposed using linear regression of actual feed intake on growth rate and mid-test BW to determine the expected feed intake of each animal. Later, a modification of this model was proposed where metabolic BW would be used instead of actual BW (Arthur et al., 1996; Knott et al., 2008). More recent studies have adopted this modification. By utilizing this modification RFI can account for the reported wide

variation in maintenance requirements observed between animals at similar production levels (Montano-Bermudez et al., 1990).

Fan et al. (1995) suggested a model to determine expected feed intake for RFI evaluation that used an equation rather than actual data. The equation considered BW and growth rate using NRC estimates to establish the net energy required for maintenance and growth. From there, nutrient content of the feed is considered when calculating expected feed intake. Accurately estimating feed intake becomes problematic for studies that have utilized this method. Knott et al., (2008), performed a study using this method to calculate expected feed intake and the model over estimated feed consumption in 6-mo-old sheep while it under-estimated intake in 13-mo-old sheep. Additionally, when utilizing this system to estimate feed intake, correlations between BW and RFI and growth rate have been observed. Similarly, Fan et al. (1995) reported correlations between RFI and average daily gain (ADG) of -0.50 in Hereford bulls and -0.58 in Angus bulls. In addition, correlations between RFI and yearling weight of the Hereford (r= -0.44) and Angus (r = -0.53) bulls were also observed (Fan et al., 1995). In conclusion, estimating feed intake for RFI calculation using the linear regression model seems to produce more accurate and appropriate results.

*Test Duration*

Altering the test duration of the feeding trial is a factor that could affect the results of an RFI evaluation. Feeding trials are expensive and labor intensive, thus the duration of feeding period used to evaluate RFI in cattle has been the focus of many subsequent

studies. Due to the large expense associated with feeding trials, a shorter testing period is economically beneficial to producers and researchers alike. Initially, 168 d for the feeding trial was suggested as the required duration necessary to get accurate results (Koch et al., 1963). Eventually this feeding period was reduced to 140 d and then 112 d; neither reduction had a negative effect on accuracy (McPeake et al., 1986). To establish a more precise time frame Archer et al. (1997) conducted an extensive study to accurately assess the ideal number of days required for the RFI feeding period using Angus, Hereford, and Shorthorn heifers and bulls. By progressively increasing number of days in each feeding trial from 7 to 119 d, it was determined that little decrease in the variation of RFI is observed after d 70. Therefore, it was proposed that reducing the feeding period to 70 d provided an adequate level of accuracy in measuring feed efficiency and RFI in British breeds of cattle.

This was a novel breakthrough that reduced the necessary feeding period by over half of the initially proposed required days, but unfortunately this trial only took British breeds into consideration. Robinson (1997) verified that there were distinct differences in feeding behaviors between *Bos taurus* and *Bos indicus* cattle when managed in the same feedlot environment. That led Archer and Bergh (2000) to conduct a similar study to investigate appropriate RFI feeding trial duration for cattle of diverse biological breedtypes. Using Angus, Hereford, Simmental, Afrikaner, and Bonsmara young bulls, they concluded that a 70 d feeding trial was sufficient for these breeds as well. The findings of these studies suggest that the feeding period can be reduced to as little as 70 d without affecting accuracy in determining RFI.

## Sources of Variation in RFI

### *Composition of Gain*

Differences in body composition and RFI have been reported in young, growing cattle. Richardson et al. (2001) reported a correlation (r = 0.43) between whole-body chemical composition and genetic variation in RFI; additionally, increased fatness was associated with steer progeny born to high RFI parents. Furthermore, differences in fat composition have been reported as well. There is a trend for low RFI steers to have decreased amounts of intramuscular fat (P= 0.06) and dissectible carcass fat (P = 0.08) in comparison to medium and high RFI steers (Basarab et al., 2003). While these data suggest that body composition has an effect on energetic efficiency but, differences in RFI and body composition have been estimated to only account for 5 (Richardson and Herd, 2004) to 9 (Lancaster et al., 2009) percent of total variation in RFI.

### *Feeding Behavior*

Physical activity results in expending energy and production of heat. Cohorts should be exposed to the same basic feeding conditions to reduce variance caused by energy requirements for locomotion or competing for bunk space. Golden et al., (2008) reported that lower RFI animals expressed a lower duration of daily feeding activities compared to high RFI animals within a cohort. Additionally, feeding time per day, number of eating sessions per day, and eating rate has been positively correlated with RFI (Robinson and Herd, 2004). Cattle fed an *ad libitum* diet are allowed to express

differences in appetite and feeding behaviors; this activity could potentially cause loss in energy and influence digestibility in comparison to animals consuming a restricted diet.

*Feed Digestibility*

Some studies provide evidence that more efficient cattle may possess a greater ability to digest consumed DM. Grovum and Hecker (1973) reported that as feed intake increases, ruminal passage rate accelerates thus; decreasing the time period feed remains in the rumen for digestion. Therefore, the increased daily feed consumption and passage rate of high RFI cattle may result in a reduction in digestibility. Nkrumah et al. (2006) reported a negative correlation (r = -0.44; $P < 0.05$) between RFI and metabolizable energy in a study where high RFI steers recovered 10% less metabolizable energy than low RFI steers. Low RFI heifers have been reported to have a higher ($P < 0.05$) dry matter (731 vs. 705 ± 12 g/kg dry matter) and crude protein (691 vs. 657 ± 13 g/kg dry matter) digestibility than high RFI heifers (Krueger et al., 2008). Richardson et al. (1996) reported a trend ($P < 0.10$) for low RFI cattle to have increased nutrient digestibility of 1% difference compared to high RFI cattle; however, the authors speculated this 1% difference may account for as much as 14% of the observed difference in feed efficiency.

**Indirect Measures of RFI**

Regardless of the proposed reduction of RFI trials to as little as 70 d, feeding trials still come with an expensive overhead and large economic burden. Basarab et al. (2002) estimated that testing a single animal for RFI could cost as much as $188. In addition to

costs associated with the intense feeding period, proper equipment, facilities, and labor are required to evaluate cattle for feed efficiency using RFI. While evaluating sires for RFI is becoming more popular within the industry, these limitations provide an economical challenge that prevents wide scale use of this selection tool. Finding an indirect measure of RFI that is less laborious and relatively inexpensive for producers to incorporate into their production system could speed the rate of genetic change in feed efficiency (Davis and Simmen, 1997).

### *Insulin-like Growth Factor-I*

Insulin-like growth factor-I (IGF-I), a hormone related to growth and development, received some attention by researchers who hypothesized a possible correlation between IGF-I and feed efficiency. Circulating IGF-I primarily originates in the liver; however, the lungs, kidneys, heart, stomach and gonads, as well as muscle and bone, also produce a substantial quantity of this hormone (Daftary and Gore, 2005). The growth hormone releasing hormone (GHRH)-growth hormone (GH)-IGF-I axis regulates many life processes. The feedback mechanisms involved with this axis (commonly referred to as the GH/IGF-I, growth or somatotrophic axis) are outlined in Figure 2.1 (Caldwell, 2009). Johnston (2001) reported IGF-I to be correlated with RFI in *Bos taurus* cattle. From this, IGF-I was suggested as a selection tool for cattle to be evaluated for RFI; however, Johnston (2001) insisted that more extensive research needed to be conducted to accurately determine the correlation between IGF-I and RFI. In growing bulls and heifers, plasma IGF-I concentrations have been reported to be genetically

correlated (0.56) (Moore et al., 2005) and phenotypically correlated in a positive manner with RFI; conversely, Kelly (2010) showed no correlation between overall plasma IGF-I and RFI in yearling beef heifers. Kelly (2010) hypothesized that the explanation for this inconsistency may be the result of differences in age and diets between studies; suggesting that younger animals on trial consuming a roughage based diet may be expected to have a greater rate of lean tissue gain and reduced carcass fatness thus altering the IGF-I concentrations found. In efforts to more clearly establish the relationship between RFI and IGF-I, Lancaster (2007) evaluated a group of Brangus cattle and found no correlation between IGF-I and RFI. However, this discrepancy could be due to differences in breed types evaluated. Caldwell (2009), also reported no relationship between circulating concentrations of IGF-I and RFI among varying purebred and crossbred breeds of beef cattle. This study evaluated Romosinuano, Brahman, and Angus purebreds and various crosses between these breeds of cattle. Obviously, a clear correlation between IGF-I and RFI has not been established within the beef industry to this date.

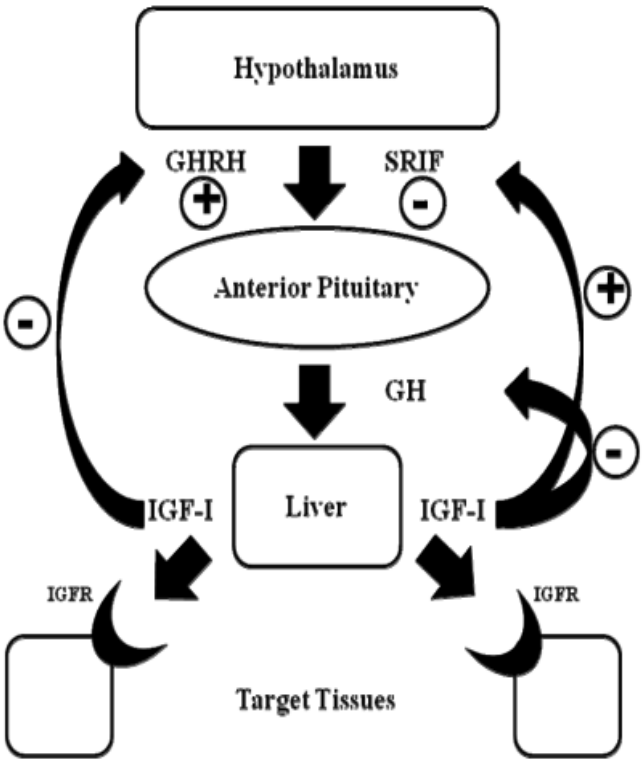


Figure 2.1 Schematic diagram of the growth axis.

*Genetic Markers*

Genetic correlations exist between RFI and growth, intake, and energy partitioning traits which are controlled by a variety of genes (Sherman et al., 2008). Single nucleotide polymorphisms (SNP) have been evaluated to identify genes that are responsible for RFI variations. A whole-genome study of feedlot cattle of diverse breedtypes and varying RFI values, revealed 161 SNP that were significantly associated with RFI (Barandse et al., 2007). The 20 most significant SNPs accounted for 76% of the genetic variation in RFI, suggesting that genetic markers may be more accurate biomarkers than circulating analytes (Moore et al., 2009). Genetic tests, such as IGENITY® (Merial Limited, Duluth,

GA) and GeneSTAR® (Pfizer Animal Health, New York, NY), are currently available to identify feed efficiency differences in individual animals. Both products report genetic correlations between markers and RFI; however, these tests only account for less than 15% of the variation in feed intake. As the beef industry is constantly striving to produce genetically superior animals, modifications of genes are constantly occurring. This would suggest that variances in genes over time may lead to a changing genetic profile, making it difficult to concentrate on a specific gene for selection. When utilizing these tests to quantify relative feed efficiencies in beef cattle, caution should be taken.

*Estimated Breeding Values*

Estimated breeding values (EBV) are indexes given to quantify the genetic worth of an animal for a given trait and are utilized by producers when selecting animals for their breeding herds. Estimated breeding values exist for RFI; designed with the capability to compare animals within and across herds individually evaluated for RFI (Sherman et al., 2008). Positive correlations ($r = 0.35$; $P < 0.05$) have been reported in Angus steers to their respective sire's RFI EBV by Richardson et al. (2004), suggesting that EBV may be a fairly reliable method to predict RFI. However, selecting prospective cattle for a breeding herd should never been done by using single trait selection; this holds true when utilizing RFI EBV's as well. Instead, Crews et al. (2005) urges producers to utilize RFI EBV's merely as a component of a multiple trait selection.

## Glucoregulatory Mechanisms

### *Relationship Between Glucose and Insulin*

Glucose is a carbohydrate that is used as a short-term energy source by an organism. It functions as a metabolite that is utilized for energy in every cell of the body. Maintenance of plasma glucose concentrations is critical to survival as glucose is the most utilized metabolic fuel by the central nervous system. Ruminants differ in glucose production in comparison to monogastrics. The rumen of ruminants contains microorganisms that are capable of digesting fibrous material; the microorganisms enable ruminants to eat and partly digest plant cellulose and hemicelluloses (Hocquette and Abe, 2000). The major products of the fermentation of dietary carbohydrates are volatile fatty acids, primarily acetate, propionate and butyrate. The low dietary absorption of glucose in the ruminant leads to lower blood glucose concentrations and adaptation of glucose metabolism regulation (Brockman, 1993).

Hormones that control glucose concentrations include insulin, glucagon, epinephrine, cortisol and growth hormone. Insulin serves as the primary glucose lowering hormone. Insulin is a metabolic hormone, synthesized and secreted from beta (β) cells in the pancreatic islets. It is then released into the hepatic portal circulation and serves to affect the liver and peripheral tissues. Insulin functions as a key component in intermediary metabolism and is primarily released in response to elevated blood concentrations of glucose; from this response insulin inhibits glycogenolysis and gluconeogenesis and promotes glucose uptake by the liver as well as other tissues such as muscle and fat (Hocquette and Abe, 2000).

The glucose transporter Glut 2, facilitates diffusion of glucose into the beta cells when there are elevated concentrations of glucose in the extracellular fluid (ECF). When high concentrations of glucose enter the beta cell, subsequent depolarization of the membrane occurs stimulating an influx of extracellular calcium (Hocquette and Abe, 2000). The increased intracellular calcium is thought to be one of the primary triggers for exocytosis of insulin, enabling the insulin to act on target cells. The effects of insulin on glucose metabolism vary depending on the target tissue; however, the overall goal of insulin is to clear glucose from the blood circulation through one of two methods; 1) by facilitating the entry of glucose into muscle, adipose, and several other tissues or 2) by stimulating the liver to store glucose in the form of glycogen (Schenk et al., 2008). In general terms, ruminant tissues are considered less sensitive to insulin than non-ruminant tissues. Brockman (1983) reported it took 50-60 uIU/mL to cause a 50% reduction in glucose production; in contrast in humans this value is 30 uIU/mL (Rizza et al., 1981). Because of the robust relationship between glucose and insulin and their role in energy metabolism, this biological mechanism may contribute to RFI and feed efficiency.

*RFI and Glucoregulatory Mechanisms*

In testing this hypothesis many studies have measured circulating insulin and glucose concentrations in the blood at various stages of growth and evaluated their relationship with feed efficiency traits and RFI. Greater insulin concentrations were reported in inefficient (high RFI) Angus influenced steers at the end of a feedlot trial (Brown, 2005; Richardson et al., 2004). The higher insulin concentrations found in high

RFI steers could be associated with a greater carcass fat composition because of the role insulin plays in stimulating lipogenesis in adipose tissue (Hocquette et al., 1998). In contrast Kelly (2010) found no relationship between plasma insulin concentrations and RFI in developing heifers throughout the feeding period used to evaluate animals for feed efficiency. Glucose concentrations at the time of weaning showed no correlation to RFI in growing heifers or steers (Richardson et al., 2004; Kelly, 2010, respectively). Kolath et al. (2006) observed a higher plasma glucose concentration in high RFI steers compared to low RFI steers. However, this difference was accredited to greater feed intake seen in high RFI steers and was not deemed significant as no differences were observed in the ratios of glucose to insulin between the two groups. The ratio between glucose and insulin at a specific time (glucose: insulin) has been used in the scientific community as an indicator of glucose metabolism. The ratio glucose to insulin has not been found to be correlated with RFI (Kelly, 2010; Kolath et al., 2006).

Despite the fact that previous research efforts have not yet established a secure relationship between RFI and glucose concentrations or insulin concentrations or even glucose:insulin at any given time, previous literature demonstrates that a relationship may still exist. Insulin sensitivity can be an indicator of metabolic efficiency in many species. For example, low RFI animals tend to be leaner; an association has been shown between leanness in farm animals and insulin sensitivity in muscle tissue and increased glycolytic muscle energy metabolism (Hocquette et al., 1998; Oddy et al., 1995). This may suggest that high RFI steers may have developed decreased insulin sensitivity in muscle tissue; therefore, lessening the response of insulin to glucose. Previous studies have evaluated

the relationship between glucose, insulin, and RFI at a set time such as post-weaning or at the end of a feeding trial but failed to look at the response of insulin to glucose; therefore, failing to explain the effect of insulin sensitivity.

*Glucose Tolerance Test*

A glucose tolerance test (GTT) involves the administration of glucose to increase blood glucose concentrations, then monitoring the response of insulin to the sudden influx of glucose. The hyperglycemic effect of the infused glucose should cause an adaptive increase in insulin secretions (Abdelmannan et al., 2010). After the infusion of glucose, blood samples are collected in increments over an established period of time to evaluate the concentrations of glucose and insulin that are produced and the rate at which insulin clears glucose from the circulation. From the analysis of these samples at various time points, it is possible to calculate peak insulin concentration, time insulin achieved peak concentration, as well as concentration of glucose half life and time glucose half life was achieved, giving insight into the metabolic differences that may exist in regards to insulin sensitivity. This test also provides an enhanced method for evaluating the relationship between glucose and insulin as opposed to the ratio of glucose:insulin. Insulinogenic index (IIND) is calculated by dividing the change in insulin from baseline insulin concentration by the change in glucose from baseline glucose concentration (I/G) for each collection time during the GTT (Guerrero-Romero et al., 2001; Abdelmannan et al., 2010). Baseline insulin and glucose concentrations are determined by collecting blood samples prior to glucose infusion after a period of fasting for twelve hours. This equation

provides us with an index that evaluates the change in glucose and insulin concentrations that occurs during glucoregulation.

The most common use of a GTT today is on humans as a "stress test" in efforts to identify those individuals which have an increased risk of developing Type II diabetes. However, glucose tolerance tests have been administered to dairy cattle for many decades in an effort to evaluate the glucoregulatory mechanisms in ruminants. Glucose Tolerance Test results have allowed dairy scientists to gain a better understanding of the effects that nutrition, exercise, medication, and disease have on glucose concentrations. For example, GTT has given insight into effects of early lactation (Herbein et al., 1985), nutritional status (Knut, 1978), and diabetes in cows (Kuneko and Rhodes, 1964). Glucose tolerance tests have also been utilized in buffalo, as a treatment option to support energy balance in sick buffalo (Liu et al., 2004). Currently, there are no previous studies in the literature reporting administering a GTT to beef cattle to determine a relationship between glucose metabolism and feed efficiency. More efficient animals that have increased insulin sensitivity should clear the influx of infused glucose at a faster rate than less efficient animals that have developed decreased insulin sensitivity; however, the exact mechanism has yet to be elucidated.

*Insulinogenic Index*

Abdelmannan et al., (2010) utilized an insulinogenic index (IIND) to try to gain a better understanding and elicit a specific response from a single dose of dexamethasone on healthy adults. This study calculated IIND by dividing the change in insulin from

baseline by the change in glucose from baseline ($\Delta I/\Delta G$) for each collection time during the GTT (Abdelmannan et al., 2010). Baseline insulin and glucose concentrations are determined by collecting blood samples prior to GTT after a period of fasting. This equation evaluates the change in glucose and insulin concentrations during metabolic activity and presents an index for comparison. Guerrero-Romero et al. (2001) utilized a fasting insulin-to-glucose ratio (FIG) to determine glucose intolerence in humans. In this model, baseline is not adjusted for and it is simply the ratio of insulin to glucose after fasting. A glucose tolerence test is administered after a period of fasting to establish insulin sensitivty to an influx of glucose. From this test, we can determine insulin sensitivy and create an index for comparison by creating at the insulinogenic index.

## Effect of Cortisol on Glucoregulatory Mechanisms

### *Cortisol*

Common to all endocrine systems, there are many factors and pathways utilized to maintain homeostasis. Stress is one disruptor of glucoregulatory homeostasis, and disruption of glucose balance initiates a plethora of reactions in an effort to re-establish homeostasis. Stress can be due to a number of factors including nutrition, the environment, and fear associated with human or predator interactions. In response to a stressor, activation of the hypothalamic-pituitary-adrenal (HPA) axis facilitates a cascade of endocrine responses that allows for coping with the stressor (McDowell, 1983). Corticotrophin-releasing hormone (CRH) and vasopressin (VP) are released from the hypothalamus to stimulate the corticotroph cells of the anterior pituitary (Minton, 1994).

The activation of the anterior pituitary gland stimulates the subsequent release of adrenocorticotropic hormone (ACTH) into the circulation, where it then acts on the adrenal cortex. This stimulation then induces the adrenal cortical tissue to release glucocorticoids (GC) (Burdick, 2007). Although many GCs have been recognized, cortisol is the primary GC associated with the stress response in domestic livestock species. Therefore, measuring concentrations of cortisol can be a physiologic indicator of the stress level of a particular animal.

Glucocorticoids (cortisol) and glucose have an intricate relationship. Cortisol can disrupt glucose homeostasis as additional amounts of energy may be needed to cope with a stress response. Cortisol is one of the hormones associated with elevating the concentrations of glucose. Decreased concentrations of circulating glucose results in an influx of cortisol in an effort to increase energy in the body. Since glucose and insulin are so intimately related, a shift in glucose concentrations during a stress response may elicit an exaggerated insulin reaction, (Munck et al., 1984). Insulin mediated glucose uptake in certain tissue types is inhibited by catecholamines and glucagon which function as the first responders of the stress response. Under normal conditions insulin facilitates cellular uptake of glucose through receptor mediated actions; however, during the stress response insulin receptors are down regulated resulting in GCs counteracting the actions of insulin (McDowell, 1983). Turnbow et al. (1994) observed this as dexamethasone decreases insulin receptor substrate-1 in adipose tissue. Long term stress and cortisol production can cause a continuous increase in insulin production and alter glucose uptake.

Breedtype and gender have been found to have an effect on basal cortisol concentrations and secretions in beef cattle. Welsh et al. (2009) evaluated Angus (*Bos taurus)* and Brahman (*Bos indicus)* bulls, steers and heifers for basal cortisol secretion; results from this study support the concept that adrenocortical function is influenced by breedtype and that sexual dimorphism in basal and induced cortisol secretion can occur across genetic strains or breeds. Breedtype differed ($P < 0.01$) between bulls, steers and heifers in serum cortisol. Additionally, Brahman bulls differed ($P < 0.01$) from Brahman steers and heifers and Angus differed ($P < 0.01$) between all three categories (bulls, steers and heifers) in serum cortisol with concentrations (ng/mL) of 21.8, 37.1, and 40.6, respectively. Similar results were found during a study that evaluated the response of cortisol to an ACTH challenge as a useful predictor of RFI in Brahman cattle. While this was not found to be a useful predictor of RFI in Brahman cattle, a sexual dimorphism in the cortisol response to an ACTH challenge was observed (Welsh et al., 2009).

Temperament in cattle can be defined as the response of an animal to being handled by a human (Fordyce et al, 1982). There are multiple ways to access temperament of cattle including evaluating the animal's cortisol concentration or utilizing objective and subjective measures to obtain a temperament score. Exit velocity (EV) is an objective measure that can be obtained on each individual animal. This measurement can then be used in an equation, along with pen score (a subjective measure) to classify an animal as calm, intermediate, or temperamental compared to a set group of cohorts. Animals of different temperaments have diverse functional characteristics of their HPA axis and therefore react to stress differently (Curley et al., 2008). Cattle that are more

excitable (termed temperamental) have higher concentrations of stress hormones such as cortisol and epinephrine than calm cattle, which are correlated to temperament (Schuehle et al., 2005; King et al., 2006; Curley et al., 2006, 2008). High concentrations of cortisol are linked to stressed (temperamental) animals; whereas cortisol concentrations rise as an animal experiences higher amounts of stress (Minton, 1994). Thus it would be expected that more excitable cattle experience a higher peak in insulin concentrations as cortisol rises.

*Exit Velocity*

Curley et al. (2006) found that exit velocity can be an effective tool to predict temperament and was found to be positively correlated with temperament. Exit Velocity is the rate (m/s) at which the animal travels 1.83 meters after being released from a working chute (Curley et al. 2006). It is best to collect exit velocity data at an early time point of a calf's life. Typically, cattle producers move their cattle through chutes for vaccination and sorting at time of weaning. This provides a convenient time point to collect exit velocity data on the animal. Exit velocity is then used with pen score to access the animal's temperament score. Increased exit velocity has been correlated with higher concentrations of serum cortisol in cattle (Fell et. al. 1999, Curley et. al, 2006); which could disrupt the glucoregulatory mechanisms. This suggests that EV should be a variable to consider when evaluating animals for glucoregulatory mechanisms.

As cortisol is a glucocorticoid, it blocks the transport of glucose into tissues such as adipose and muscle. If this is true, then cortisol concentrations and stress may disrupt

the results of a glucose tolerance test. Bradbury et al. (2011) conducted a study to evaluate glucose utilization in temperamental cattle. Cattle were evaluated for temperament and then a glucose tolerance test was performed on calm and temperamental cattle; glucose, insulin, and cortisol were analyzed. It was observed that higher concentrations of cortisol decrease glucose utilization and increase the blood glucose concentration. Cattle that were more temperamental had a numerically higher insulin/glucose ratio; therefore, more temperamental animals exhibit greater insulin sensitivity to an influx of glucose (Bradbury et al., 2011).

# CHAPTER III

# INSULIN SENSITIVITY IN BONSMARA HEIFERS WITH DIVERGENT RESIDUAL FEED INTAKE

## Introduction

The Bonsmara breed, comprised of 5/8 Afrikaner, 3/16 Hereford, and 3/16 Shorthorn was developed after reviewing data from extensive climate studies performed on various breeds of beef cattle in order to create a functional breed to maximize growth in tropical climates (Bonsma, 1949). Scientist Jan Bonsma developed this breed while employed at the University of South Africa in an effort to produce high quality beef in tropical regions. Bonsmara cattle have became an important part of beef cattle production in South Africa and other regions with tropical climates due to their ability to dissipate heat while retaining a reasonable rate of growth. South Africa terrain and climate is similar to that found in the Southern United States. In 1997, Texas entrepreneur, G. R. Chapman imported recipient cows carrying Bonsmara embryos into North America. These embryos developed into the first herd of Bonsmara cattle in North America. Matching breed type to climate is an essential part of maximizing profitability in the beef industry. However, once that is achieved it is important to consider other areas of production where producers can minimize production costs. Due to the rising cost of feedstuffs, it is important to evaluate and identify feed efficient animals within a contemporary group; simply put, identification of animals which gain more weight on a lower amount of feed consumed. The Bonsmara's unique ability to produce high quality

beef and impressive weight gain in tropical regions and high forage diets makes this breed a candidate for studying feed efficiency in the Southern United States. As shown in Chapter II, insulin sensitivity can be an indicator of metabolic efficiency in many species. If this is true, low RFI (more efficient) animals would be expected to have reduced insulin sensitivity in response to an increase in glucose concentration, thus being more efficient in the uptake of glucose in comparison to less efficient animals. The objective of this study was to investigate the glucoregulatory mechanisms of Bonsmara cattle previously selected for divergent RFI to investigate a potential indirect method of determining feed efficiency, thus optimizing profitability for producers located in tropical regions. Two cohorts of Bonsmara heifers were evaluated to determine the glucoregulatory mechanisms. The two cohorts were treated as two separate experiments; however in both experiments animals were first evaluated for RFI and then a glucose tolerance test was performed on the most efficient and most inefficient animals of each cohort. All animal procedures were approved by the Texas A&M University System Institutional Animal Care and Use Committee (IACUC).

## Materials and Methods

### *Animals and Experimental Design: Experiment 1*

Bonsmara heifers (n=53) were transported from the Texas AgriLife Research Center in Uvalde to the ASTREC facility at Texas A&M in College Station, TX. Upon arrival, individual BW were collected and animals were assigned to pens according to BW (n=5 head per pen) equipped with Calan Gate feeders. Heifers were fed a high roughage

diet (2.07 Mcal/kg DE, 13.1 g CP/kg DM; Table 3.1). The animals were adapted to the experimental diet and the test facilities during an initial 28-d period. Thereafter, heifers were individually fed ad libitum for 70d. Individual BW was collected weekly and orts, if any, were collected and weighed weekly.

Following the feeding trial, RFI was calculated and a glucose tolerance test was conducted on the most efficient (n=12) and least efficient (n=12) heifers in the cohort. A glucose tolerance test was performed over two days (February 27-28$^{th}$, 2010) with 6 high and 6 low RFI-classified animals being tested each day. Negative RFI and positive RFI heifers had average weights of 380 kg and 378 kg, respectively and were between 12-15 mo of age. Twelve heifers were fitted with a jugular cannula and placed in stanchions in the Ruminant Nutrition and Metabolism facility the evening prior to the glucose tolerance test. Water, but not feed, was available overnight to ensure a fasting period of at least 10 h prior to the challenge. The procedure for fitting the animal with a jugular cannula was as follows. An area over the jugular vein was clipped and scrubbed. After donning sterile gloves, a 14-gauge needle was inserted into the jugular vein. Approximately 15-20 cm of a 1.0 m length of tygon tubing (0.10 cm i.d., 0.18 cm o.d.) was threaded through the needle and into the jugular vein. The remaining tubing was then secured to the heifer's neck using adhesive tape. The end of the tubing was plugged using an 18-gauge needle and a 10 mL syringe. All cannula materials were sterilized by gas sterilization. The tubing was flushed with heparin solution prior to capping.

The morning of the glucose tolerance test, cannula lines were checked for patency and any problems were repaired. After all lines were confirmed to be functional, animals

were allowed a rest period of 2 hrs. Following the rest period, a 50% dextrose solution was infused at 0.5 mL/kg BW via the indwelling jugular catheter. A blood sample (10 mL) was collected at each of the following time points relative to dextrose infusion: -5, 0, +5, 10, 15, 20, 40, 60, 80, 100, 120, 140, 160 and 180 min. A total of 140 mL blood was drawn from each animal. Following collection of blood at each sampling time, 10 mL of sterile saline followed with 4 mL of heparinized saline was delivered via the catheter. After collection of the last blood sample, the catheters were removed and the animals were returned to their original pens. The remaining 12 heifers were catheterized in the same manner the next morning and blood samples were collected after a 2 h resting period beginning after the final heifer was catheterized. Serum was collected in 10 mL Vacutainer® tubes (366430, BD Biosciences; Franklin Lakes, NJ) at each sampling time. Sample tubes were refrigerated on ice and subsequently centrifuged at 4ºC at 2000 x g for 30 min, and then stored at -20ºC until assays could be performed.

Table 3.1 Experiment 1 Diet

| Dietary Composition, (as fed) | % |
|---|---|
| Chopped Alfalfa | 35.00 |
| Cottonseed Hulls | 21.50 |
| Dry Rolled Corn | 20.95 |
| Pelleted Alfalfa | 15.00 |
| Molasses | 7.00 |
| Salt | 0.40 |
| Vitamin E | 0.14 |
| COOP Beef TM | 0.02 |

*Animals and Experimental Design: Experiment 2*

Bonsmara heifers (n=65) were transported from the Texas AgriLife Research Center in Uvalde to the ASTREC facility at Texas A&M in College Station, TX. Upon arrival, individual BW were collected and heifers were assigned to pens (n=5 head per pen) equipped with Calan Gate feeders. Heifers were fed a high roughage diet (2.07 Mcal/kg DE, 13.1 g CP/kg DM; Table 3.1). The animals were adapted to the experimental diet and the test facilities during an initial 28-d period. Thereafter, heifers were individually fed ad libitum for 70 d. Individual BW was collected weekly and orts, if any, were collected and weighed weekly as well.

Following the feeding trial, heifers were evaluated for RFI. Following determination of feed efficiency, a glucose tolerance test was performed on the most efficient (n=6) and least efficient (n=6) heifers in the cohort. A glucose tolerance test was performed over a period of three days (March 2-4$^{th}$, 2011) with 2 high and 2 low RFI-classified animals being tested each day. Negative RFI and positive RFI heifers had average weights of 356 kg and 346 kg, respectively and were between 12-14 mo of age. Four heifers were placed in chutes in a covered area outside of the ASTREC facility after being allowed access to water but not feed for a period of 10 h prior to the challenge. Animals were equipped with a jugular cannula the morning of the glucose challenge using the same method described in Experiment 1 of this chapter. Two blood samples (10 mL each) were collected at -5, 0, 10, 15, 20, 30, 40, 60, 80, 100, 120, 140, 160 and 180 min relative to dextrose (50%) infusion. Blood for plasma was collected into 10 mL EDTA-coated Vacutainer® tubes (366643, BD Biosciences; Franklin Lakes, NJ) and blood for

serum was collected into 10 mL Vacutainer® tubes (366430, BD Biosciences; Franklin Lakes, NJ). A total of 280 mL blood was collected per animal. Following collection of blood at each sampling time, 10 mL of sterile saline followed with 4 mL of heparinized saline was delivered via the catheter. After collection of the last blood sample, the catheters were removed and the animals were returned to their original pens. The remaining two groups (comprised of 2 high and 2 low RFI animals each) were catheterized in the same manner over the next two days and blood samples were collected after a 2 h resting period beginning after the final heifer was catheterized. Plasma and serum samples were refrigerated on ice and subsequently centrifuged at 4ºC at 2000 x g for 30 min then stored at -20ºC until assays could be performed.

*RFI Determination*

The two groups of Bonsmara heifers were fed during two separate feeding trials and were treated as two separate cohorts as they were from different calf crops; accordingly their data were analyzed separately and the two groups were considered as separate cohorts for RFI calculation. Initial BW and ADG were computed from linear regression of BW on day of test using the PROC REG function of SAS (2002). Mid-test BW was estimated using initial BW and ADG and adjusting for a 3% shrink. Considering all females in each cohort, RFI was calculated as the residual from the linear regression of average daily dry matter intake (DMI) on mid-test $BW^{0.75}$ and ADG using the GLM procedure of SAS (2002).

*Glucose Colorimetric Assay*

Concentrations of plasma glucose were determined using the manual protocol of the commercially available enzymatic Autokit Glucose (#439-90901; Wako Chemical USA, Inc., Richmond, VA). Resulting inter-assay and intra-assay coefficients of variation for Experiment 1 were 0.49 and 2.45%, respectively. Experiment 2 inter-assay and intra-assay coefficients of variation were 2.69 and 1.58%, respectively.

*Insulin RIA*

Serum insulin concentrations were determined in a single assay using a commercially available radioimmunoassay Coat-A-Count kit (Siemens Healthcare Diagnostic, Los Angeles, California). The concentration of insulin was 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). The intra-assay coefficient of variation was 6.85%.

*Cortisol RIA*

Serum cortisol concentrations were determined using a commercially available radioimmunoassay Coat-A-Count kit (Siemens Healthcare Diagnostic, Los Angeles, California). The concentration of cortisol was calculated using Assay Zap software (Biosoft, Cambridge, UK) using CPM obtained from a Cobra II auto-gamma-counter (Perkin Elmer, Waltham, MA). The inter-assay and intra-assay coefficients of variation were 13.44% and 9.38%, respectively.

*Exit Velocity*

Exit velocity (EV) was calculated on individual animals. Exit velocity is a rate reported as meters per second (m/s) and is calculated from the time it takes an animal to travel 1.83 m. after being released from a working chute. In Experiment 1, animals were evaluated for initial exit velocity (IEV) after the 28d adaptation period prior to start of the RFI feeding period. Animals in Experiment 2 were evaluated for arrival exit velocity (AEV) the day the animals arrived at the ASTREC facility at Texas A&M University in College Station, TX prior to being sorted and penned for the RFI feeding period.

*Statistical Analysis*

A repeated measure ANOVA was conducted using the MIXED model procedure of SAS (2002) for analysis of RFI group, time, and the RFI group x time interactions on insulin concentration and glucose concentration for each experiment individually. Insulinogenic index was calculated by dividing the concentration of insulin by the concentration of glucose (I/G) for each collection time for each experiment individually. A repeated measures ANOVA was conducted using the MIXED model procedures of SAS (2002) for analysis of insulinogenic index for each experiment individually. Time to peak concentration of insulin and half-life concentration of glucose were determined using the GLM procedure of SAS (2002) for each experiment. Area under the curve (AUC) was calculated using the trapezoidal rule for glucose, insulin, and cortisol concentrations and IIND. Exit velocity of each RFI group was determined using the GLM procedure of SAS (2002) for each experiment.

## Results

*Experiment 1*

Residual feed intake groups did not differ ($P > 0.05$) in EV. Negative RFI and positive RFI heifers had mean EV (m/s) of 1.88 ± 0.15 and 2.10 ± 0.15, respectively (Table 3.2). Residual feed intake groups did not differ ($P > 0.05$) in cortisol concentrations (Figure 3.1) There was no RFI group x time interaction affecting ($P > 0.05$) cortisol concentrations. Time relative to glucose infusion, had a significant affect ($P < 0.0001$) on cortisol concentrations; this would be expected as cortisol is known to decline during a period of restraint as the animal becomes more adjusted to the surroundings and is assumed to be experiencing less stress throughout the 3 h period. Any extraneous outside variables during the GTT were similar and experienced by both RFI groups. Data reported from cortisol concentrations, coupled with EV data suggest temperament did not likely play a substantial role in glucoregulatory mechanisms between low and high RFI groups for either experiment; consequently, EV was not included as a covariate for any model for statistical analysis.

Time, relative to glucose infusion, had a significant effect ($P < 0.0001$) on glucose (Figure 3.2) and insulin (Figure 3.3) concentrations. The interaction of RFI group x time affected ($P = 0.01$) insulin concentrations, but there was no RFI group x time interaction affecting ($P > 0.05$) glucose concentrations. Residual feed intake group affected ($P = 0.05$) the insulin response, whereby negative RFI heifers had a greater insulin response than positive RFI heifers. Residual feed intake group did not affect ($P > 0.05$) glucose concentrations. There was a trend ($P < 0.10$) for RFI group to influence peak insulin

concentrations, but RFI group did not affect ($P > 0.05$) time to insulin peak. Negative RFI and positive RFI heifers had insulin peak concentrations (mIU/mL) of 108.1 ± 12.1 and 75.5 ± 12.1, respectively and insulin peak concentration times (minutes) were 16.7 ± 1.8 and 18.6 ± 1.8, respectively. Residual feed intake groups did not differ ($P > 0.05$) in glucose half life concentrations or time that half life was achieved. Negative RFI and positive RFI heifers had glucose half life concentrations (mg/dL) of 80.0 ± 3.1 and 77.0 ± 3.3, respectively and glucose half life times (min) were 33.3 ± 2.5 and 37.0 ± 2.6, respectively. Mean peak insulin concentration and glucose half life concentrations, and their respective times, are presented in Table 3.3. Insulinogenic Index (Figure 3.4) was affected by RFI group ($P < 0.05$), but was not affected ($P > 0.05$) by time or an RFI group x time interaction. Negative RFI and positive RFI heifer IIND (I/G) were 0.44 ± 0.03 and 0.29 ± 0.03, respectively. Insulinogenic index was analyzed from 10 to 60 min for both RFI groups. Area under the curve was calculated using the trapezoidal rule for glucose, insulin, and cortisol concentrations and IIND; no variables were significantly different ($P > 0.05$) in AUC between RFI groups. Table 3.4 outlines the AUC least square means, standard errors and correlated P values for each variable.

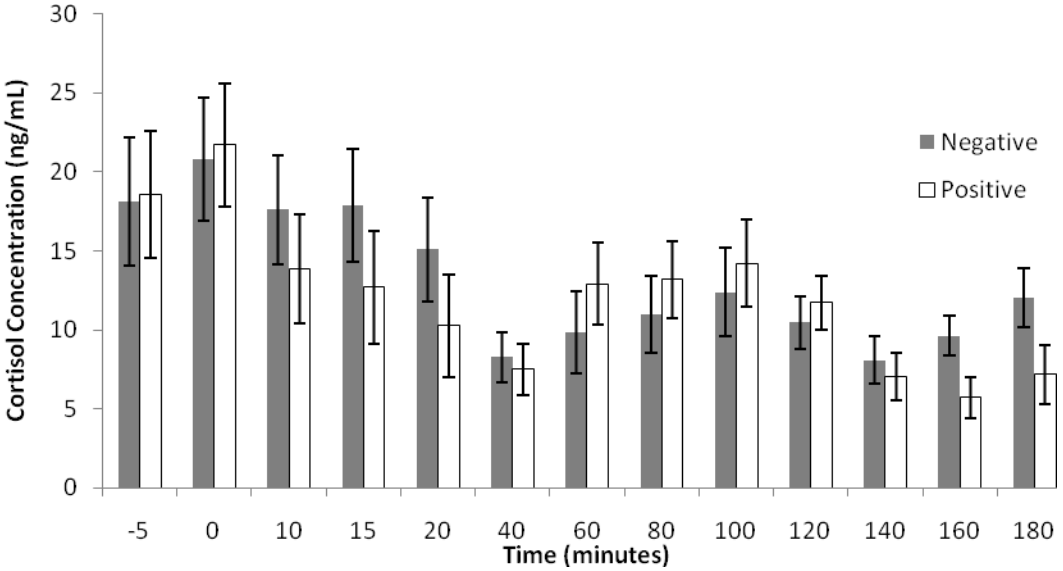


Figure 3.1 Bonsmara Experiment 1. Mean Cortisol Concentration Over Time by RFI Group.

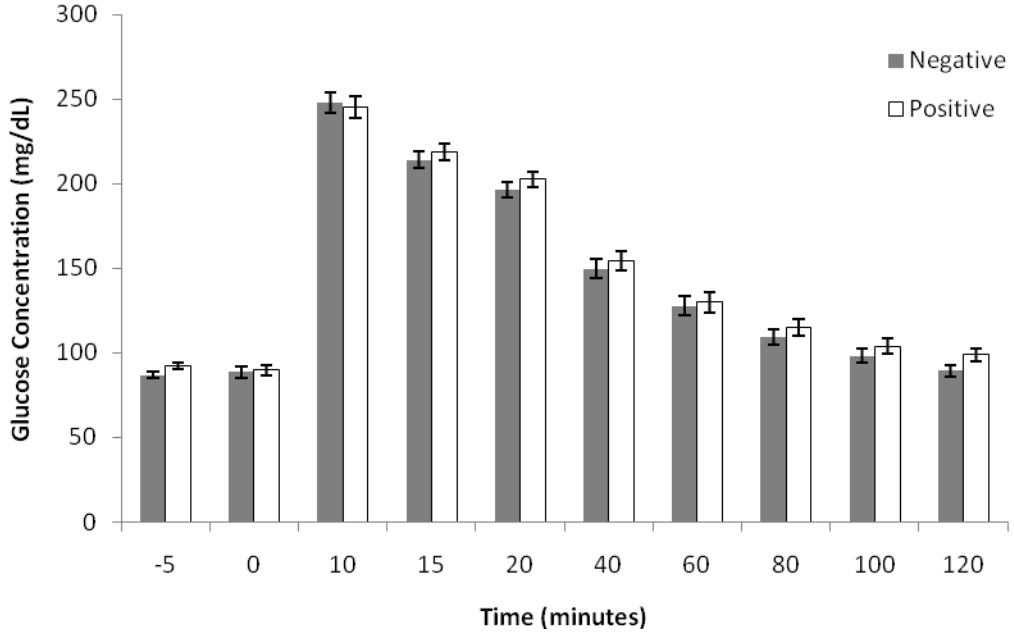


Figure 3.2 Bonsmara Experiment 1. Mean Glucose Concentration Over Time by RFI Group.

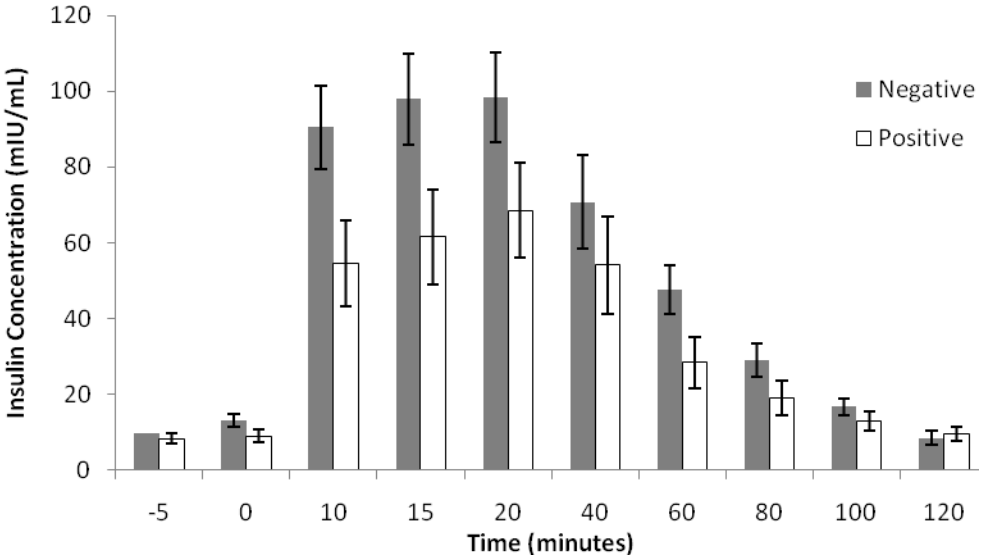


Figure 3.3 Bonsmara Experiment 1. Mean Insulin Concentration Over Time by RFI Group.

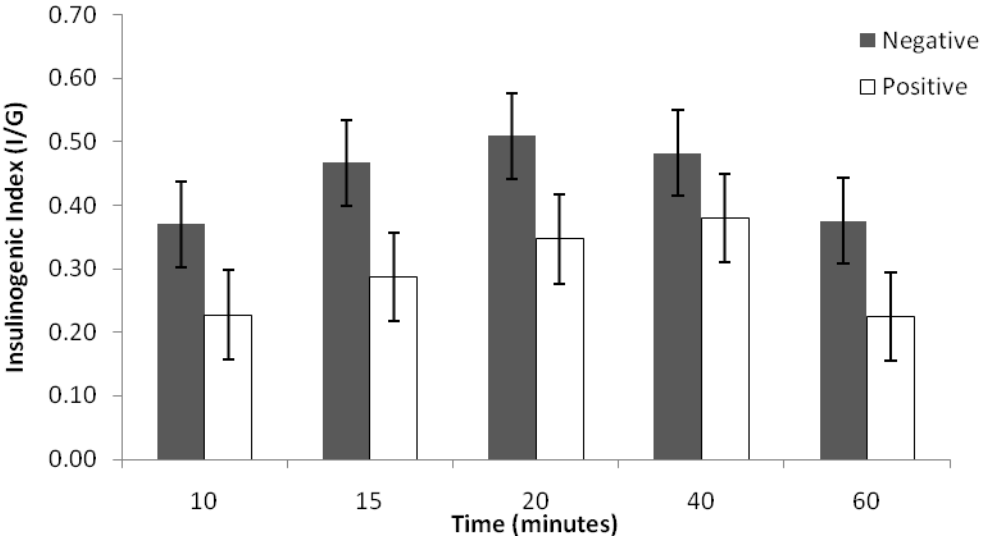


Figure 3.4 Bonsmara Experiment 1. Insulinogenic Index Over Time by RFI Group.

Table 3.2 Bonsmara Experiment 1 Exit Velocity

| Variable | RFI GROUP | | P Value |
|---|---|---|---|
| | Negative | Positive | |
| Exit Velocity (Initial) | 1.89 ± 0.15 | 2.10 ± 0.15 | 0.3358 |

Table 3.3 Bonsmara Experiment 1 Insulin Peak and Glucose Half Life Data

| Variable | RFI GROUP | | P Value |
|---|---|---|---|
| | Negative | Positive | |
| Insulin Peak Concentration | 108.1 ± 12.1 | 75.5 ± 12.1 | 0.0761 |
| Insulin Peak Time | 16.7 ± 1.8 | 18.6 ± 1.8 | 0.4589 |
| Glucose Half Life Concentration | 80.1 ± 3.1 | 77.0 ± 3.1 | 0.5107 |
| Glucose Half Life Time | 33.3 ± 2.5 | 37.0 ± 2.6 | 0.3191 |

Table 3.4 Bonsmara Experiment 1 Area Under Curve: Glucose, Insulin, Cortisol and Insulinogenic Index

| Variable | RFI GROUP | | P Value |
|---|---|---|---|
| | Negative | Positive | |
| Glucose | 84.5 ± 9.2 | 90.1 ± 9.6 | 0.6818 |
| Insulin | 67.6 ± 8.9 | 45.7 ± 9.4 | 0.1064 |
| Cortisol | 29.4 ± 4.1 | 29.1 ± 4.2 | 0.9612 |
| IIND | 0.38 ± 0.06 | 0.27 ± 0.06 | 0.1900 |

*Experiment 2*

Residual feed intake group did not differ significantly ($P > 0.05$) in EV. Negative RFI and positive RFI heifers had mean EV (m/s) of 2.88 ± 0.26 and 2.77 ± 0.26, respectively (Table 3.5). RFI group did not affect ($P > 0.05$) cortisol concentrations

(Figure 3.5); however, there were significant differences between RFI groups at time -5 and time 0 relative to glucose challenge. This suggests that differences in baseline cortisol concentrations may exist between this cohort's RFI groups. There was no RFI group x time interaction affecting ($P > 0.05$) cortisol concentrations. There was a trend ($P < 0.10$) for time relative to glucose infusion to affect cortisol concentrations; this would be expected as cortisol is known to decline during a period of restraint as the animal becomes more adjusted to the surroundings and is assumed to be experiencing less stress throughout the 3 h period. Any extraneous outside variables during the GTT were similar and experienced by both RFI groups. Data reported from cortisol concentrations, coupled with EV data suggest temperament did not likely play a substantial role in glucoregulatory mechanisms between low and high RFI groups for either experiment; consequently, EV was not included as a covariate for any model for statistical analysis.

Time, relative to glucose infusion, had a significant effect ($P < 0.0001$) on glucose (Figure 3.6) and insulin (Figure 3.7) concentrations. There was no RFI group x time interaction affecting ($P > 0.05$) insulin or glucose concentrations. RFI group affected ($P < 0.01$) insulin response, whereby negative RFI heifers had a higher insulin response than positive RFI heifers. RFI group did not affect ($P > 0.05$) glucose concentrations. RFI group did affect ($P < 0.05$) insulin peak concentration, but not time of insulin peak concentration. Negative RFI and positive RFI heifers had insulin peak concentrations (mIU/mL) of 93. 6 ± 13.4 and 50.25 ± 13.4, respectively and insulin peak concentration times (minutes) were 23.3 ± 3.7 and 15.83 ± 3.7, respectively. RFI group did not affect ($P > 0.05$) glucose half life concentrations or time half life was achieved. Negative RFI

and positive RFI heifers had glucose half life concentrations (mg/dL) of 76.5 ± 2.5 and 77.8 ± 2.5, respectively and glucose half life times (min) were 32.0 ± 2.8 and 26.5 ± 2.8, respectively. Mean peak insulin concentration and glucose half life concentrations, and their respective times, are summarized in Table 3.6. Insulinogenic Index (Figure 3.8) was affected by RFI group ($P < 0.01$), and a trend ($P < 0.07$) for an interaction was found between IIND and time. Negative RFI and positive RFI heifers IIND (I/G) were 0.35 ± 0.02 and 0.17 ± 0.02, respectively. Insulinogenic index was analyzed from 10 to 60 minutes for both RFI groups. Area under the curve was calculated using the trapezoidal rule for glucose, insulin, and cortisol concentrations and IIND; RFI groups differed significantly ($P < 0.05$) in AUC for insulin response and IIND. Table 3.7 outlines the AUC least square means, standard errors and correlated P values for each variable.

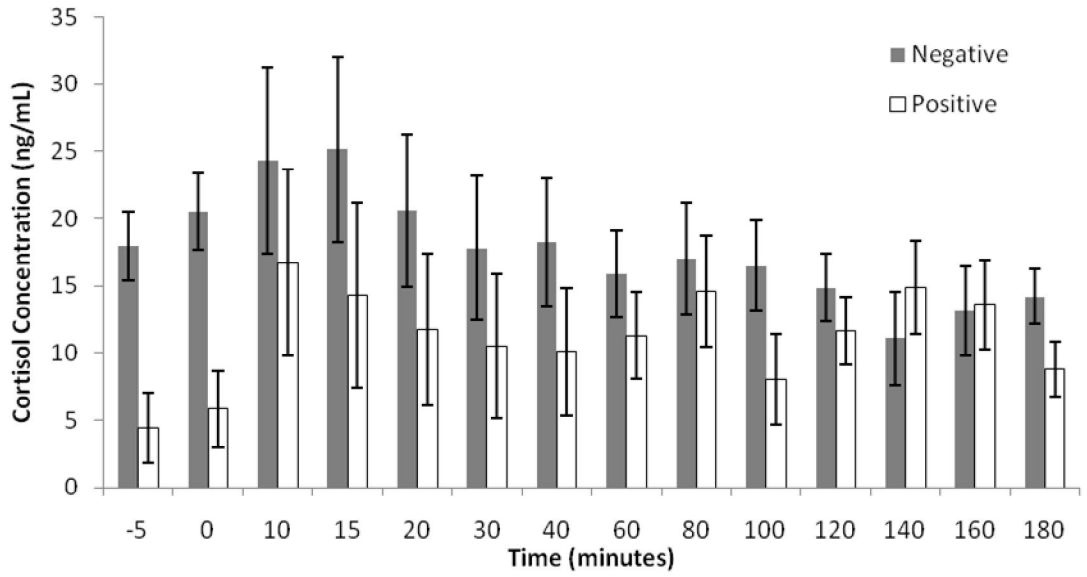


Figure 3.5 Bonsmara Experiment 2. Mean Cortisol Concentration Over Time by RFI Group.

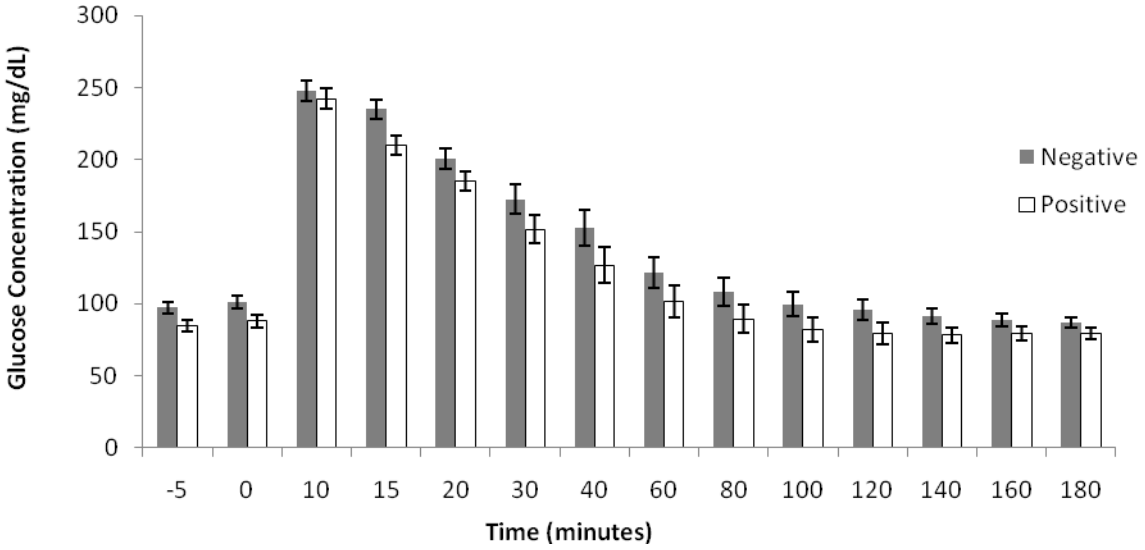


Figure 3.6 Bonsmara Experiment 2. Mean Glucose Concentration Over Time by RFI Group.

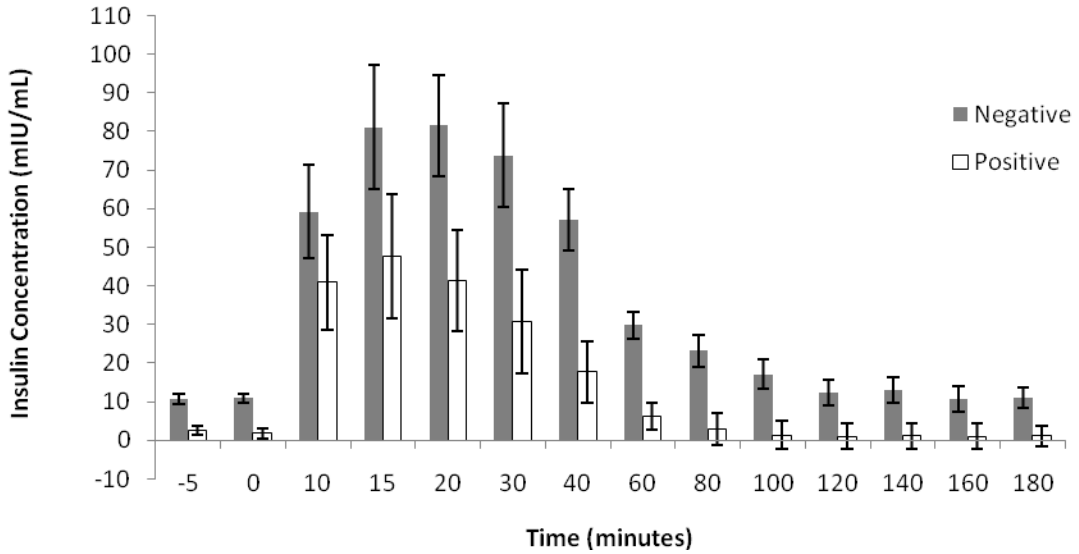


Figure 3.7 Bonsmara Experiment 2. Mean Insulin Concentration Over Time by RFI Group.

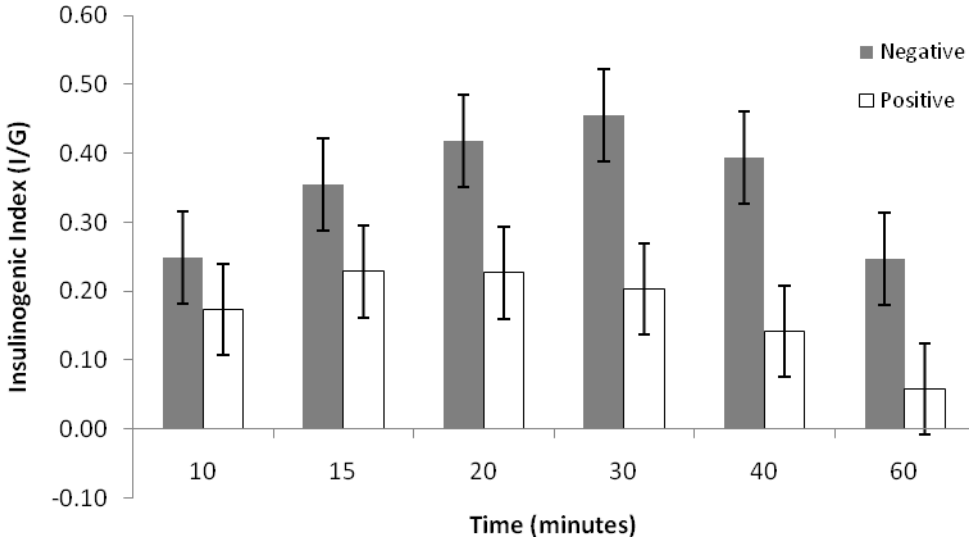


Figure 3.8 Bonsmara Experiment 2. Insulinogenic Index Over Time by RFI Group.

Table 3.5 Bonsmara Experiment 2 Exit Velocity

| Variable | RFI GROUP | | P Value |
|---|---|---|---|
| | Negative | Positive | |
| Exit Velocity (Initial) | 2.88 ± 0.26 | 2.77 ± 0.26 | . 7750 |

Table 3.6 Bonsmara Experiment 2 Insulin Peak and Glucose Half Life Data

| Variable | RFI GROUP | | P Value |
|---|---|---|---|
| | Negative | Positive | |
| Insulin Peak Concentration | 93.6 ± 13.4 | 50.25 ± 13.4 | 0.0455 |
| Insulin Peak Time | 23.3 ± 3.7 | 15.8 ± 3.7 | 0.1781 |
| Glucose Half Life Concentration | 76.5 ± 2.5 | 77.8 ± 2.5 | 0.7334 |
| Glucose Half Life Time | 32.0 ± 2.8 | 26.5 ± 2.8 | 0.1934 |

Table 3.7 Bonsmara Experiment 2 Area Under Curve: Glucose, Insulin, Cortisol, IIND

| Variable | RFI GROUP | | P Value |
|---|---|---|---|
| | Negative | Positive | |
| Glucose | 56.2 ± 12.7 | 45.9 ± 12.7 | 0.5791 |
| Insulin | 52.5 ± 5.4 | 18.5 ± 5.4 | 0.0012 |
| Cortisol | 13.1 ± 3.3 | 13.6 ± 3.3 | 0.9235 |
| IIND | 0.61 ± 0.05 | 0.18 ± 0.05 | 0.0001 |

**Discussion**

Residual feed intake groups did not differ ($P > .05$) in EV or cortisol concentration throughout the GTT. This suggests temperament did not likely play a substantial role in glucoregulatory mechanisms between low and high RFI groups for either experiment; consequently, EV was not included as a covariate for any model for statistical analysis. Cortisol concentrations were affected ($P > .05$) by time in both experiments; however, this would be expected as the animals are getting acclimated to their surroundings and the situation as the GTT progresses. This response is typical for a period of restraint. Animals representing both RFI groups were subjected to a GTT at the same time; therefore, any extraneous variables during the challenge were similar to both RFI groups.

For Experiment 1, insulin and glucose concentration data were only analyzed through 120 min. After this time point, both circulating concentrations returned to baseline. Baseline was calculated as the mean between time -5 and time 0 relative to glucose infusion. In Experiment 1 and 2, negative RFI and positive RFI heifers had

similar glucose responses over time, demonstrating that the ability to clear glucose from circulation did not differ between RFI groups. However, in Experiment 1 and Experiment 2, negative RFI (more efficient) heifers exhibited a greater insulin response over time than positive RFI (more inefficient) heifers. This implies that more efficient animals produce a higher concentration of insulin in response to an influx in glucose. In Experiment 1, significant differences ($P < 0.05$) were observed between insulin concentrations between RFI groups at 10 and 15 min and tended to differ ($P < 0.10$) at 20 min relative to glucose infusion. This implies that the greatest differences in insulin concentrations occurred within the first 20 min post-glucose infusion. After 20 min relative to glucose infusion significant differences in insulin concentration were not observed between high and low RFI heifers. However, in Experiment 2, significant differences ($P < 0.05$) were observed in insulin concentrations between RFI groups at every time point excluding 10 and 15 minutes relative to glucose infusion. This shows that the greatest effect occurred after the first 20 minutes post glucose infusion. In Experiment 1 there was a significant difference ($P < .05$) in peak insulin concentrations and there was a trend ($P < .10$) for RFI groups to differ in peak insulin concentrations in Experiment 2; although, there were no significant differences in the time it took to achieve half life of glucose in either experiment. In both experiments, RFI group did affect IIND, whereas the negative RFI-classified animals (efficient) had a higher insulinogenic index than positive RFI-classified animals (inefficient). The differences in IIND between RFI groups would be expected as a result of a greater concentration of insulin in response to glucose observed in the negative RFI heifers.

## Conclusion

The two cohorts of Bonsmara heifers had similar insulin responses to the infusion of glucose. In both cohorts, the negative RFI animals had a greater response of insulin to glucose. This suggests that efficient Bonsmara heifers are more sensitive to an influx of glucose and must produce higher concentrations of insulin to clear glucose from the circulation. Diversely, Experiment 1 showed the first 20 min post glucose challenge to produce the greatest affect on insulin concentration, but Experiment 2 suggested the greatest difference occurred after 20 min relative to glucose challenge. The ratio of insulin to glucose may provide more information and be a better indicator of feed efficiency in comparison to actual concentrations of insulin response to a GTT. Due to the differences ($P < 0.05$) observed between RFI groups in IIND, it may be implied that IIND after a glucose tolerance test may be a useful indicator of RFI in Bonsmara heifers. More specifically, these data suggest that the IIND may be a useful predictor 15-30 minutes after a glucose tolerance test and an IIND value of 0.30 (I/G) or greater would equate to a negative RFI (more efficient) Bonsmara heifer.

Heifers in both Experiment 1 and Experiment 2 had insulin responses differing from our hypothesis that more efficient cattle would require less insulin than the less efficient cattle. These data suggest efficient Bonsmara heifers actually have greater insulin sensitivity compared to inefficient Bonsmara heifers. The Bonsmara breed of cattle are designed to put on efficient gains, have substantial resistance to ticks and tick-borne diseases and produce high quality beef in tropical and subtropical regions. These regions provide an environment that is typically associated with loss in productivity for

*Bos taurus* type cattle. The Bonsmaras' unique breed composition and ability to gain efficiently despite harsh climatic conditions (and typically decreased forage availability and quality), may result in differences in energy metabolism in comparison to other *Bos indicus* and *Bos taurus* types of cattle.

# CHAPTER IV

# INSULIN SENSITIVITY IN SANTA GERTRUDIS BULLS WITH DIVERGENT RESIDUAL FEED INTAKE

## Introduction

The Santa Gertrudis breed of cattle was developed by the King Ranch, located near Kingsville, Texas in the southern region of the state. This breed was designed to withstand and adapt to various harsh climates found in southern regions of the United States while exhibiting rapid growth and efficient weight gains. Santa Gertrudis cattle are characterized by their vigor, hardiness, and longevity. The breed is comprised of 3/8 Brahman and 5/8 Shorthorn which gives them the ability to adapt to humid or arid climates while possessing traits for increased production. The unique genetic makeup of the Santa Gertrudis breed provides a thick, loose hide that serves multiple purposes; it protects the cattle from insects, insulates from cold, and provides sweat glands to efficiently dissipate heat. Producers utilize this breed to enhance profitability through either purebred operations or incorporating Santa Gertrudis cattle into crossbreeding systems. Because of Santa Gertrudis' role in beef cattle production located in tropical and subtropical climates, it is economically beneficial to distinguish cattle that utilize feed more efficiently from those who are less efficient. Early detection of efficient animals would benefit producers by minimizing feed costs. As shown in Chapter II, insulin sensitivity can be an indicator of metabolic efficiency in many species. If this is true, low RFI animals would be expected to have reduced insulin sensitivity in response to an

increase in glucose concentration, thus being more efficient in the uptake of glucose in comparison to less efficient animals. The objective of this study was to investigate the glucoregulatory mechanisms of Santa Gertrudis cattle previously selected for divergent RFI to investigate a potential indirect method of determining feed efficiency, thus optimizing profitability for producers located in tropical or subtropical regions. This experiment was conducted to evaluate the glucoregulatory mechanisms of Santa Gertrudis bulls. Animals were first evaluated for RFI. A glucose tolerance test was then performed on the most efficient and most inefficient animals of the cohort. All animal procedures were approved by the Texas A&M University System Institutional Animal Care and Use Committee (IACUC).

## Materials and Methods

### *Animals and Experimental Design*

Santa Gertrudis bull calves (n=50) were transported approximately 300 miles from the King Ranch, Kingsville TX to the Texas A&M University Beef Research Unit in College Station, TX. Upon arrival at the Beef Research Unit, individual BW was collected and animals were fitted with passive radio frequency transponders. Bulls were assigned to pens (n=25 head per pen) based on BW; pens were equipped with GrowSafe bunk units (GrowSafe Systems Ltd., Airdrie Alberta, Canada). Bulls were allowed a minimum of 24 d to adapt to experimental diets. The animals were fed ad libitum twice daily (diet shown in Table 4.1) and individual bunk attendance and feed disappearance was recorded for 77 d using the GrowSafe Data Acquisition software. Procedures for

omitting incorrect daily feed intakes from the GrowSafe feeding system (power outage, equipment malfunction or leak rate) are as reported by Lancaster et al., (2009). Individual BW was collected weekly on bulls.

Following the feeding trial, RFI was calculated and a glucose tolerance test was conducted on the most efficient (n=8) and least efficient (n=8) bulls in the cohort. Two glucose tolerance tests daily were performed over two days (April 13-14$^{th}$, 2010). Bulls were assigned to 4 groups (n=4) to determine day and time of glucose tolerance test so that 2 high and 2 low RFI-classified animals were in each group. Negative RFI and positive RFI bulls had average weights of 383 kg and 373 kg, respectively and were between 14-17 mo of age. Four bulls were placed in chutes in a covered area outside of the ASTREC facility after being allowed access to water but not feed for a period of 10 h prior to the challenge. An area over the jugular vein was clipped and scrubbed. After donning sterile gloves, a 14-gauge needle was inserted in the jugular vein. Approximately 15-20 cm of a 1.0 m length of tygon tubing (0.10 cm i.d., 0.18 cm o.d.) was threaded through the needle and into the jugular vein. The remaining tubing was then secured to the bull's neck using adhesive tape. The end of the tubing was plugged using an 18-gauge needle and a 10 mL syringe. All cannula materials were sterilized by gas sterilization. The tubing was flushed with heparin solution prior to capping. Following the catheterization of the last bull, a rest period of 2 h was allowed and then a 50% dextrose solution was infused at 0.5 mL/kg BW via the indwelling jugular catheter. Two blood samples (10 mL each) were collected at -5, 0, 10, 15, 20, 30, 40, 60, 80, 100, 120, 140, 160 and 180 min relative to dextrose infusion. Blood for plasma was collected into 10

mL EDTA coated Vacutainer® tubes (366643, BD Biosciences; Franklin Lakes, NJ) and blood for serum was collected into 10 mL Vacutainer® tubes (366430, BD Biosciences; Franklin Lakes, NJ). A total of 280 mL blood was drawn per animal. Following collection of blood at each sampling time 10 mL of sterile saline followed with 4 mL of heparinized saline was delivered via the catheter. After collection of the last blood sample, the catheters were removed and the animals returned to their original pens. The next group of 4 (comprised of 2 high and 2 low RFI animals) were catheterized in the same manner that afternoon and blood samples were collected after a 2 h resting period beginning after the final bull was catheterized. The remaining two groups (comprised of 2 high and 2 low RFI animals each) were handled in exactly the same method as bulls tested the previous day and blood samples were collected after a two hour resting period beginning after the final bull was catheterized. Plasma and serum sample tubes were refrigerated on ice and subsequently centrifuged at 4ºC at 2000 x g for 30 min and stored at -20ºC until assays could be performed.

Table 4.1 Santa Gertrudis Bulls Diet

| Dietary Composition, (as fed) | % |
|---|---|
| Chopped Alfalfa | 35.00 |
| Cottonseed Hulls | 21.50 |
| Dry Rolled Corn | 20.95 |
| Pelleted Alfalfa | 15.00 |
| Molasses | 7.00 |
| Salt | 0.40 |
| Vitamin E | 0.14 |
| COOP Beef TM | 0.02 |

*RFI Determination*

RFI was calculated as described in Chapter III. Initial BW and ADG were computed from linear regression of BW on day of test using the PROC REG function of SAS (2002). Mid-test BW was estimated using initial BW and ADG and adjusting for a 3% shrink. Considering all males as cohorts, RFI was calculated as the residual from the linear regression of average daily dry matter intake (DMI) on mid-test $BW^{0.75}$ and ADG using the GLM procedure of SAS (2002).

*Glucose Colorimetric Assay*

Plasma glucose concentrations were determined by the manual protocol of the commercially available enzymatic Autokit Glucose (*(#439-90901;* Wako Chemical USA, Inc., Richmond, VA). Resulting inter-assay and intra-assay coefficients of variation were 1.62 and 2.29% respectively.

*Insulin RIA*

Serum insulin concentrations were determined in a single assay using a commercially available radioimmunoassay Coat-A-Count kit (Siemens Healthcare Diagnostic, Los Angeles, California). The concentration of insulin was 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). The intra-assay coefficient of variation was 8.76%.

*Cortisol RIA*

Serum cortisol concentrations were determined using a commercially available radioimmunoassay Coat-A-Count kit (Siemens Healthcare Diagnostic, Los Angeles, California). The concentration of cortisol was calculated using Assay Zap software (Biosoft, Cambridge, UK) using CPM obtained from a Cobra II auto-gamma-counter (Perkin Elmer, Waltham, MA). The inter-assay and intra-assay coefficients of variation were 13.44% and 9.38%, respectively.

*Exit Velocity*

Exit velocity (EV) was calculated on individual animals. Exit velocity is a rate reported as meters per second (m/s) and is calculated from the time it takes an animal to travel 1.83 m. after being released from a working chute. Animals were evaluated for initial exit velocity (IEV) after the 28d adaptation period prior to start of RFI feeding period prior to the start of the RFI feeding period.

*Statistical Analysis*

A repeated measures ANOVA was conducted using the MIXED model procedure of SAS (2002) for analysis of RFI group, time, and the RFI group x time interaction on insulin concentration and glucose concentration. Insulinogenic index was calculated by dividing the concentration of insulin by the concentration of glucose (I/G) for each collection time for each experiment individually. A repeated measure ANOVA was conducted using the MIXED model procedures of SAS (2002) for analysis of

insulinogenic index for each experiment individually. Time to peak concentration of insulin and half-life concentration of glucose were determined using the GLM procedure of SAS (2002) for each experiment. Area under the curve (AUC) was calculated using the trapezoidal rule for glucose, insulin, and cortisol concentrations and IIND. Exit velocity of each RFI group was determined using the GLM procedure of SAS (2002).

**Results**

Residual feed intake groups did not differ ($P > 0.05$) in EV. Negative RFI and positive RFI bulls had mean EV (m/s) of 2.99 ± 0.17 and 2.95 ± 0.17, respectively (Table 4.2). Residual feed intake groups did not differ ($P > 0.05$) in cortisol concentrations (Figure 4.1). There was no RFI group x time interaction affecting ($P > 0.05$) cortisol concentrations. Time relative to glucose infusion, had a significant affect ($P < 0.0001$) on cortisol concentrations; this would be expected as cortisol is known to decline during a period of restraint as the animal becomes more adjusted to the surroundings and is assumed to be experiencing less stress throughout the 3 h period. Any extraneous outside variables during the GTT were similar and experienced by both RFI groups. Data reported from cortisol concentrations, coupled with EV data suggest temperament did not likely play a substantial role in glucoregulatory mechanisms between low and high RFI groups for this experiment; consequently, EV was not included as a covariate for any model for statistical analysis.

Time, relative to glucose infusion, had a significant effect on glucose ($P < 0.0001$) and insulin concentrations ($P < 0.05$). There were no RFI x time interaction affecting ($P$

> 0.05) insulin or glucose concentrations. Residual feed intake group did not affect ($P$ > 0.05) glucose (Figure 4.2) or insulin (Figure 4.3) concentrations. RFI group did not affect ($P$ > 0.05) the insulin peak concentration or time to insulin peak. Negative RFI and positive RFI bulls had insulin peak concentrations (mIU/mL) of 50.6 ±13.3 and 67.7 ±13.3, respectively and insulin peak concentration times (min) were 46.2 ±23.1 and 81.2 ±23.1, respectively. RFI group did not affect ($P$ > 0.05) glucose half life concentrations. Negative RFI and positive RFI bulls had glucose half life concentrations (mg/dL) of 60.8 ± 3.2 and 59.9 ± 3.2, respectively and glucose half life times (minutes) were 43.0 ± 3.0 and 42.3 ± 3.0, respectively. Mean peak insulin concentration and glucose half life concentration, and their respective times, are summarized in Table 4.3. Insulinogenic index (Figure 4.4) was affected by RFI Group ($P$ < 0.05), but was not affected ($P$ > 0.05) by time or an RFI group x time interaction. Negative RFI and positive RFI bulls IIND (I/G) were 0.17 ± 0.02 and 0.26 ± 0.02, respectively. Insulinogenic index was analyzed from 10 to 60 min for both RFI groups. Area under the curve was calculated using the trapezoidal rule for glucose, insulin, and cortisol concentrations and IIND; no variables were significantly different ($P$ > 0.05) in AUC between RFI groups. Table 4.4 outlines the AUC least square means, standard errors and correlated P values for each variable.

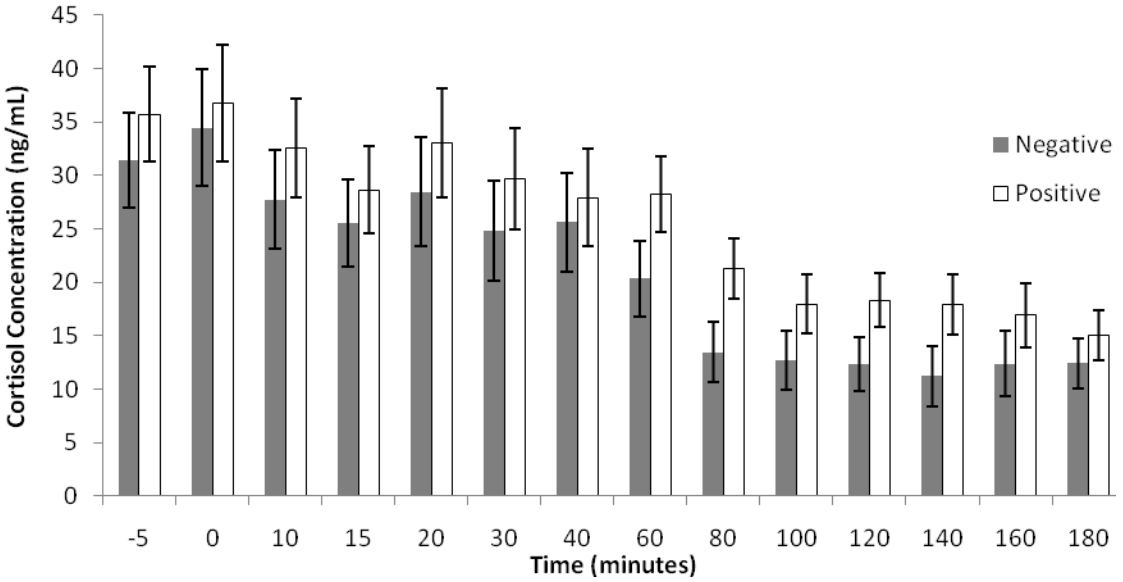


Figure 4.1 Santa Gertrudis Bulls. Mean Cortisol Concentration Over Time by RFI Group.

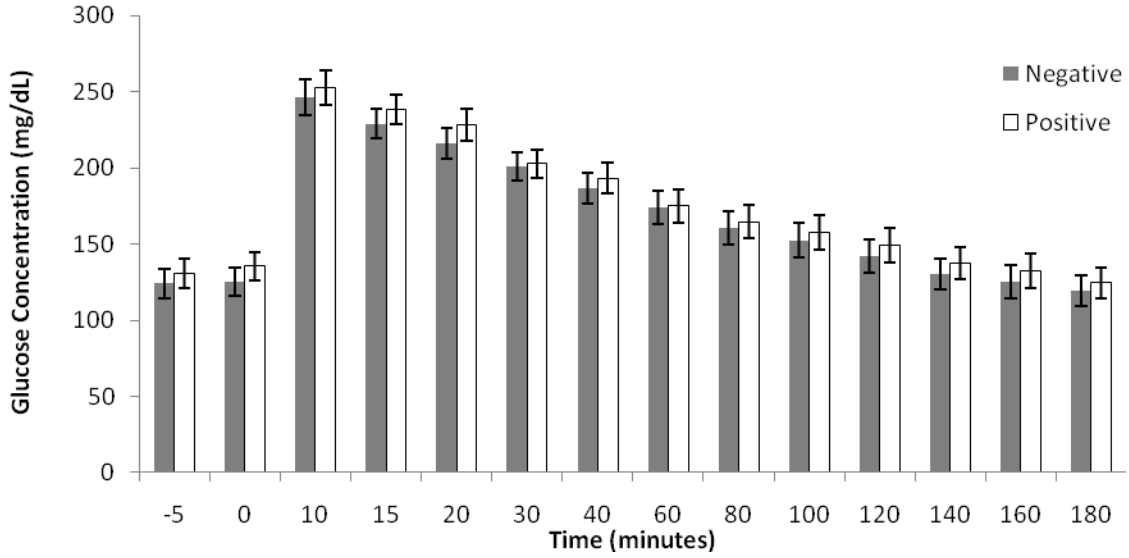


Figure 4.2 Santa Gertrudis Bulls. Mean Glucose Concentration Over Time by RFI Group.

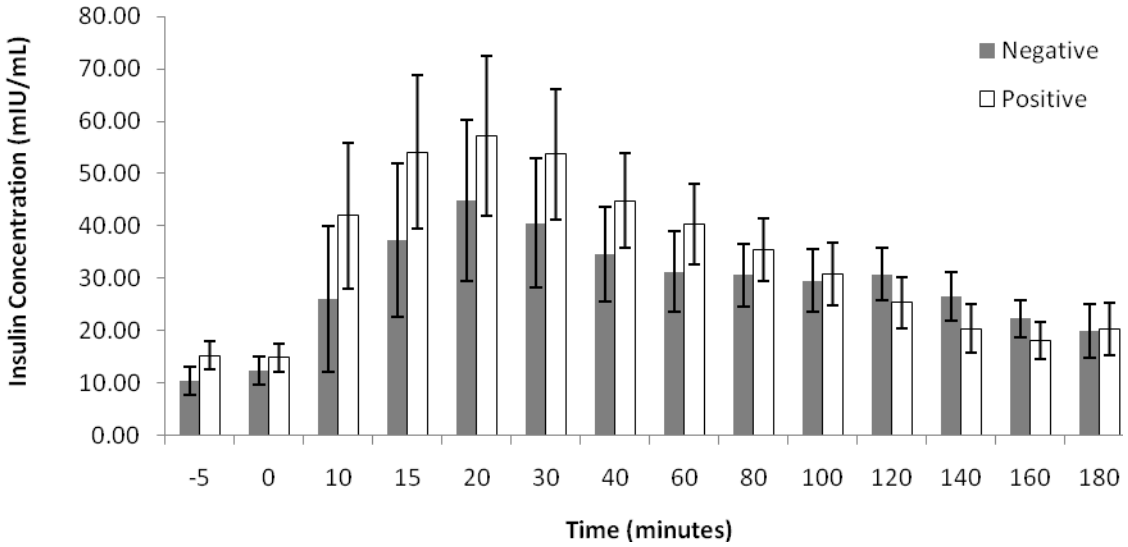


Figure 4.3 Santa Gertrudis Bulls. Mean Insulin Concentration Over Time by RFI Group.

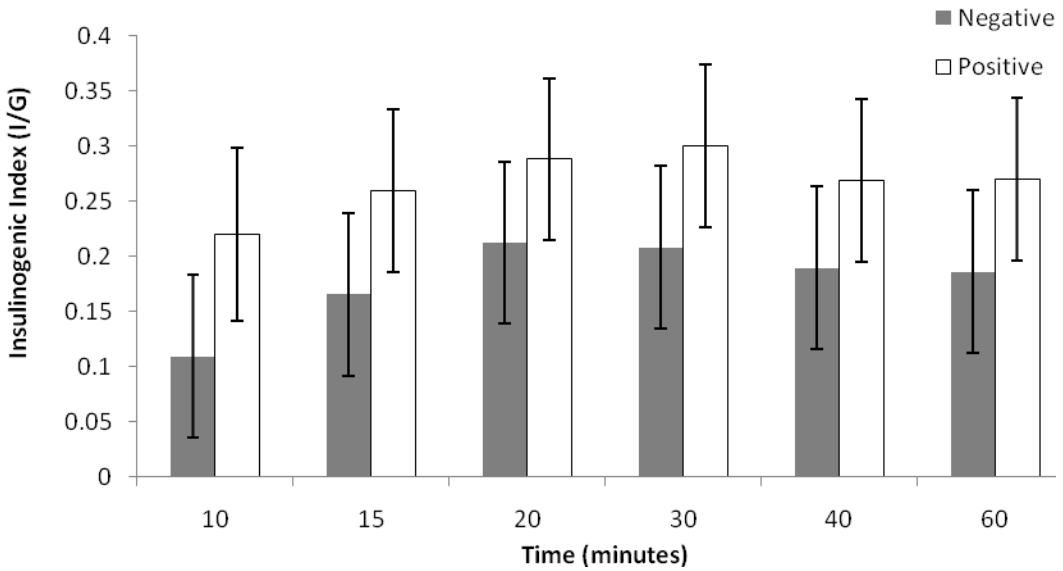


Figure 4.4 Santa Gertrudis Bulls. Insulinogenic Index Over Time by RFI Group.

Table 4.2 Santa Gertrudis Bulls Exit Velocity

| Variable | RFI GROUP | | P Value |
|---|---|---|---|
| | Negative | Positive | |
| Exit Velocity (Initial) | 2.99 ± 0.17 | 2.95 ± 0.17 | 0.8632 |

Table 4.3 Santa Gertrudis Bulls Insulin Peak and Glucose Half Life Data

| Variable | RFI GROUP | | P Value |
|---|---|---|---|
| | Negative | Positive | |
| Insulin Peak Concentration | 50.6± 13.3 | 67.7 ± 13.3 | 0.3776 |
| Insulin Peak Time | 46.2 ± 23.1 | 81.2 ± 23.1 | 0.3039 |
| Glucose Half Life Concentration | 60.8 ± 3.2 | 59.9 ± 3.2 | 0.8290 |
| Glucose Half Life Time | 43.0 ± 3.0 | 42.3 ± 3.0 | 0.8674 |

Table 4.4 Santa Gertrudis Bulls Area Under Curve: Glucose, Insulin, Cortisol, IIND

| Variable | RFI GROUP | | P Value |
|---|---|---|---|
| | Negative | Positive | |
| Glucose | 97.6 ± 10.5 | 89.9 ± 10.5 | 0.6124 |
| Insulin | 52.9 ± 9.1 | 50.9 ± 9.1 | 0.8810 |
| Cortisol | 46.8 ± 8.0 | 62.4 ± 8.0 | 0.1900 |
| IIND | 0.16 ± 0.06 | 0.23 ± 0.06 | 0.4188 |

**Discussion**

Residual feed intake groups did not differ ($P > .05$) in EV or cortisol concentration throughout the GTT. This suggests temperament did not likely play a substantial role in glucoregulatory mechanisms between low and high RFI groups for this experiment; consequently, EV was not included as a covariate for any model for statistical

analysis. Cortisol concentrations were affected ($P > .05$) by time; however, this would be expected as the animals are getting acclimated to their surroundings and the situation as the GTT progresses. This response is typical for a period of restraint. Animals representing both RFI groups were subjected to a GTT at the same time; therefore, any extraneous variables during the challenge were similar for both RFI groups.

Negative RFI and positive RFI bulls had similar glucose response over time; demonstrating that the ability to clear glucose from circulation did not differ between RFI groups. However, there were some numerical differences in insulin response between RFI groups. Positive RFI bulls (inefficient) appear to have a numerically elevated insulin response in comparison to negative RFI bulls (more efficient). Although this difference in insulin response between RFI groups is not significantly different, it did have an effect on IIND values. RFI group did affect IIND, whereas positive RFI-classified bulls showed a higher insulinogenic index compared to negative RFI-classified bulls. This suggests that inefficient bulls are releasing an increased concentration of insulin to clear the same concentration per body weight of glucose from their blood circulation in comparison to more efficient animals.

**Conclusion**

In this study, negative RFI animals had a lesser response of insulin to glucose; suggesting that inefficient Santa Gertrudis bulls have a greater insulin sensitivity to an influx of glucose and must produce higher concentrations of insulin to clear glucose from the circulation. The lower amount of insulin required for clearance of the glucose from

the circulation of the low RFI Santa Gertrudis cattle fits with our hypothesis that more efficient cattle would require less insulin than the less efficient cattle. Although RFI group did not significantly affect ($P > 0.05$) insulin response, the ratio of insulin to glucose may provide more information and be a better indicator of feed efficiency in comparison to actual concentrations of insulin response to a GTT. Due to the differences ($P < 0.05$) observed between RFI groups in IIND, it may be implied that IIND after a glucose tolerance test may be a useful indicator of RFI in Santa Gertrudis bulls. More specifically, these data suggest that IIND may be a useful predictor 10-30 minutes after a glucose tolerance test and an IIND value of 0.20 (I/G) or greater would equate to a positive RFI (less efficient) Santa Gertrudis bull.

# CHAPTER V

# INSULIN SENSITIVITY IN BRAHMAN CATTLE WITH DIVERGENT RESIDUAL FEED INTAKE

## Introduction

The Brahman breed was the first beef breed developed in the United States and has become a staple for beef cattle production in the tropics around the world. Since their development Brahman cattle have gained respect for their unique environmental adaptation, longevity, mothering ability and efficient beef production throughout the world. Brahman cattle have the ability to regulate their body temperature in areas of tropical and subtropical climates, allowing them to spend less energy dissipating heat and instead utilizing that energy to put on efficient gains. Incorporating this breed into a crossbreeding program has allowed producers of diverse climates and terrains to enhance profitability by matching their cattle to the environment. As feed costs continue to rise, producers are realizing that more steps must be taken to minimize production costs; identifying feed efficient animals is one such method. Early detection of efficient animals would benefit producers by potentially minimizing feed costs. As stated in Chapter II, insulin sensitivity can be an indicator of metabolic efficiency in many species. If this is true, low RFI animals would be expected to have reduced insulin sensitivity in response to an increase in glucose concentration, thus being more efficient in the uptake of glucose. The objective of this study was to investigate the glucoregulatory mechanisms of Brahman cattle previously selected for divergent RFI to investigate a potential indirect

method of determining feed efficiency, thus optimizing profitability for producers located in tropical or subtropical regions. Two experiments were conducted to evaluate the glucoregulatory mechanisms of Brahman bulls and heifers. Animals were first evaluated for RFI. A glucose tolerance test was then performed on the most efficient and most inefficient animals of each cohort. All animal procedures were approved by the Texas A&M University System Institutional Animal Care and Use Committee (IACUC).

## Materials and Methods

### *Animals and Experimental Design: Experiment 1*

Brahman heifers (n=37) from the Texas AgriLife Research-Overton herd were fed a commercially available growing ration (Table 5.1) in a feedlot environment at Overton using Calan gates in order to determine their relative feed efficiency ranking. Individual BW were collected and animals were assigned to pens according to BW (n=5, one pen of n=2) containing Calan Gate Feeders. Heifers were fed 2.65% of BW daily over a 70 d period with half of the daily ration being fed at 0800 and the other half being fed at 1600. Individual BW was collected weekly and the amount of ration fed was recalculated each week accordingly. Orts, if any, were collected and weighed weekly.

Following the feeding trial, RFI was calculated and a glucose tolerance test was conducted on the most efficient (n=6) and least efficient (n=6) heifers in the cohort. A glucose tolerance test was performed over two days (May 12-13$^{th}$, 2010) with 3 high and 3 low RFI-classified animals being tested each day. Negative RFI and positive RFI heifers had average weights of 322 kg and 323 kg, respectively. On dates of GTT,

negative RFI and positive RFI heifers had a mean age of test of 420 d and 422 d, respectively. Six heifers were placed in chutes in the surgery building at the Texas AgriLife Research-Overton North Farm after being allowed access to water but not feed for a period of 10 h prior to the challenge. An area over the jugular vein was clipped and scrubbed. After donning sterile gloves, a 14-gauge needle was inserted in the jugular vein. Approximately 15-20 cm of a 1.0 m length of tygon tubing (0.10 cm i.d., 0.18 cm o.d.) was threaded through the needle and into the jugular vein. The remaining tubing was then secured to the heifer's neck using adhesive tape. The end of the tubing was plugged using an 18-gauge needle and a 10 mL syringe. All cannula materials were sterilized by gas sterilization. The tubing was flushed with heparin solution prior to capping. After the last heifer was catheterized, the animals were allowed a rest period of 2 h; then a 50% dextrose solution was infused at 0.5 mL/kg BW via the indwelling jugular catheter. Two blood samples (10 mL each) were collected at -5, 0, 10, 15, 25, 30, 40, 60, 80, 100, 120, 140, 160 and 180 min relative to dextrose infusion. Blood for plasma was collected into 10 mL EDTA coated Vacutainer® tubes (366643, BD Biosciences; Franklin Lakes, NJ) and blood for serum was collected into 10 mL Vacutainer® tubes (366430, BD Biosciences; Franklin Lakes, NJ). A total of 280 mL blood was drawn per animal. Following collection of blood at each sampling time, 10 mL of sterile saline followed with 4 mL of heparinized saline was delivered via the catheter. Following collection of the last blood sample, the catheters were removed and the animals were returned to their original pens. The remaining group (comprised of 3 high and 3 low RFI animals each) was catheterized in the same manner the following day and blood samples

were collected after a 2 h resting period beginning after the final heifer was catheterized. Plasma and Serum sample tubes were refrigerated on ice and subsequently centrifuged at 4ºC at 2000 x g for 30 min and stored at -20ºC until assays could be performed.

Table 5.1 Brahman Experiment 1 Diet

| Dietary Composition, (as fed) | % |
|---|---|
| Cottonseed Hulls (pelleted) | 33.08 |
| Cottonseed Hulls (loose) | 25.00 |
| Distillers Dried Grains | 15.80 |
| Soybean Meal 48% | 9.74 |
| Rice Hulls | 5.00 |
| Car-Mil-Glo | 5.00 |
| Rice Bran-High Fat | 2.04 |
| Corn (Crimped) | 2.00 |
| Calcium Carbonate | 1.29 |
| Supplement | 1.05 |

*Animals and Experimental Design: Experiment 2*

Brahman bulls (n=37) from the Texas AgriLife Research-Overton herd were fed a commercially available growing ration in a feedlot environment at Overton using Calan gates in order to determine their relative feed efficiency ranking. Individual BW were collected and animals were assigned to pens according to BW (n=5, one pen of n=2) containing Calan Gate Feeders. Bulls were fed 2.65% of BW (Table 5.2) daily over a 70-day period with half of the daily ration being fed at 0800 and the other half being fed at

1600. Individual BW was collected weekly and the amount of ration fed was recalculated each week based on body weights. Orts, if any, were collected and weighed weekly.

Following the feeding trial, RFI was calculated and a glucose tolerance test was conducted on the most efficient (n=6) and least efficient (n=6) bulls in the cohort. Glucose tolerance tests were performed over two days (August 5-6$^{th}$, 2010) with 3 high and 3 low RFI-classified animals being tested each day. Negative RFI and positive RFI bulls had average weights of 407 kg and 468 kg, respectively. On dates of GTT, negative RFI and positive RFI bulls had a mean age of test of 482.5 d and 520.5 d, respectively. Six bulls were placed in chutes in the surgery building at the Texas AgriLife Research-Overton North Farm after being allowed access to water but not feed the prior evening. An area over the jugular vein was clipped and scrubbed. Animals were fitted with a jugular cannula using the same method described in Experiment 1 of this chapter. After the last bull was catheterized, the animals were allowed a rest period of 2 h then a 50% dextrose solution was infused at 0.5 mL/kg BW via the indwelling jugular catheter. Two blood samples (10 mL each) were collected at -5, 0, 10, 15, 20, 30, 40, 60, 80, 100, 120, 140, 160 and 180 min relative to dextrose infusion. Blood for plasma was collected into 10 mL EDTA coated Vacutainer® tubes (366643, BD Biosciences; Franklin Lakes, NJ) and blood for serum was collected into a 10 mL Vacutainer® tubes(366430, BD Biosciences; Franklin Lakes, NJ). A total of 280 mL blood was collected per animal. Following collection of blood at each sampling time, 10 mL of sterile saline followed with 4 mL of heparinized saline was delivered via the catheter. Following collection of the last blood sample, the catheters were removed and the animals were returned to their

original pens. The remaining group (comprised of 3 high and 3 low RFI-classified animals each) was catheterized in the same manner the following day and blood samples were collected after a 2 h resting period beginning after the final bull was catheterized. Plasma and serum sample tubes were refrigerated on ice and subsequently centrifuged at 4ºC at 2000 x g for 30 min and stored at -20ºC until assays could be performed.

Table 5.2 Brahman Experiment 2 Diet

| Dietary Composition, (as fed) | % |
|---|---|
| Cottonseed hulls (pelleted) | 45.00 |
| Corn | 40.00 |
| Premix (Protein, Vitamin, Mineral) | 15.00 |

*RFI Determination*

The two groups of Brahman cattle were fed during two separate feeding trials and were treated as two separate cohorts as they were of different genders. RFI was calculated as described in Chapter III. Initial BW and ADG were computed from linear regression of BW on day of test using the PROC REG function of SAS (2002). Mid-test BW was estimated using initial BW and ADG and adjusting for a 3% shrink. Considering all animals as cohorts given their respective sexes, RFI was calculated as the residual from the linear regression of average daily dry matter intake (DMI) on mid-test $BW^{0.75}$ and ADG using the GLM procedure of SAS (2002).

*Glucose Colorimetric Assay*

Plasma glucose concentrations were determined by the manual protocol of the commercially available enzymatic Autokit Glucose (#439-90901; Wako Chemical USA, Inc., Richmond, VA). For Experiment 1, the intra-assay coefficient of variation was 1.17%. For Experiment 2, the intra-assay coefficient of variation was 1.64%.

*Insulin RIA*

Serum insulin concentrations were determined in a single assay (per experiment) using a commercially available radioimmunoassay Coat-A-Count kit (Siemens Healthcare Diagnostic, Los Angeles, California). The concentration of insulin was 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). The intra-assay coefficients of variation for Experiment 1 and Experiment 2 were 12.46% and 32.89%, respectively.

*Cortisol RIA*

Serum cortisol concentrations were determined using a commercially available radioimmunoassay Coat-A-Count kit (Siemens Healthcare Diagnostic, Los Angeles, California). The concentration of cortisol was calculated using Assay Zap software (Biosoft, Cambridge, UK) using CPM obtained from a Cobra II auto-gamma-counter (Perkin Elmer, Waltham, MA). The inter-assay and intra-assay coefficients of variation were 13.44% and 9.38%, respectively.

*Exit Velocity*

Exit velocity (EV) was calculated on each individual animal. Exit velocity is a rate reported as meters per second (m/s) and is calculated from the time it takes an animal to travel 1.83 m. after being released from a working chute. Exit velocity (EV) was collected on Day 0 relative to weaning.

*Statistical Analysis*

A repeated measure ANOVA was conducted using the MIXED model procedure of SAS (2002) for analysis of RFI group, time, and the RFI gropu x time interaction on insulin concentration and glucose concentration for each experiment individually. Insulinogenic index was calculated by dividing the concentration of insulin by the concentration of glucose (I/G) for each collection time for each experiment individually. A repeated measures ANOVA was conducted using the MIXED model procedures of SAS (2002) for analysis of insulinogenic index for each experiment individually. Time to peak concentration of insulin and half-life concentration of glucose were determined using the GLM procedure of SAS (2002) for each experiment. Area under the curve (AUC) was calculated using the trapezoidal rule for glucose, insulin, and cortisol concentrations and IIND. Exit velocity of each RFI group was determined using the GLM procedure of SAS (2002) for each experiment. Because EV was significantly different between RFI groups in experiment one, all data analyses were accomplished with EV as a covariate in the model.

## Results

*Experiment 1*

Residual feed intake groups did not differ ($P > 0.05$) in EV. Negative RFI and positive RFI heifers had mean EV (m/s) of 3.21 ± 0.62 and 3.23 ± 0.34, respectively (Table 5.3). Residual feed intake groups did not differ ($P > 0.05$) in cortisol concentrations (Figure 5.1). There was no RFI group x time interaction affecting ($P > 0.05$) cortisol concentrations. Time relative to glucose infusion, had a significant affect ($P < 0.05$) on cortisol concentrations; this would be expected as cortisol is known to decline during a period of restraint as the animal becomes more adjusted to the surroundings and is assumed to be experiencing less stress throughout the 3 h period. Any extraneous outside variables during the GTT were similar and experienced by both RFI groups. Data reported from cortisol concentrations, coupled with EV data suggest temperament did not likely play a substantial role in glucoregulatory mechanisms between low and high RFI groups for this experiment; consequently, EV was not included as a covariate for any model for statistical analysis.

Time relative to glucose infusion, had a significant effect ($P < 0.0001$) on glucose (Figure 5.2) and insulin concentrations (Figure 5.3). There was no RFI group x time interaction ($P > 0.05$) on insulin or glucose concentrations. Residual feed intake group alone had no affect ($P < 0.05$) affecting insulin or glucose concentrations. Residual feed intake group did not affect ($P > 0.05$) peak insulin concentrations or time of insulin peak. Negative RFI and positive RFI heifers had insulin peak concentrations (mIU/mL) of 62.3 ±13.4 and 89.2 ± 13.4, respectively and insulin peak concentration times (min) were 22.5

± 2.6 and 23.3 ±2.6, respectively. Residual feed intake group did not affect ($P > 0.05$) glucose half life concentrations or time that half life was achieved. Negative RFI and positive RFI heifers had glucose half life concentrations (mg/dL) of 66.1 ± 3.5 and 66.1 ± 3.5, respectively and glucose half life times (min) were 39.2 ± 4.4 and 34.0 ± 4.4, respectively. Mean peak insulin concentration and glucose half life concentrations, and their respective times, are summarized in Table 5.4. Insulinogenic index was affected by RFI Group ($P < 0.05$), but was not affected ($P > 0.05$) by time or the RFI group x time interaction (Figure 5.4). Negative RFI and positive RFI heifers IIND (I/G) were 0.27 ± 0.03 and 0.36 ± 0.03, respectively. Insulinogenic index was analyzed from 10 to 60 minutes for both RFI groups. Area under the curve was calculated using the trapezoidal rule for glucose, insulin, and cortisol concentrations and IIND; no variables were significantly different ($P > 0.05$) in AUC between RFI groups. Table 5.5 outlines the AUC least square means, standard errors and correlated P values for each variable.

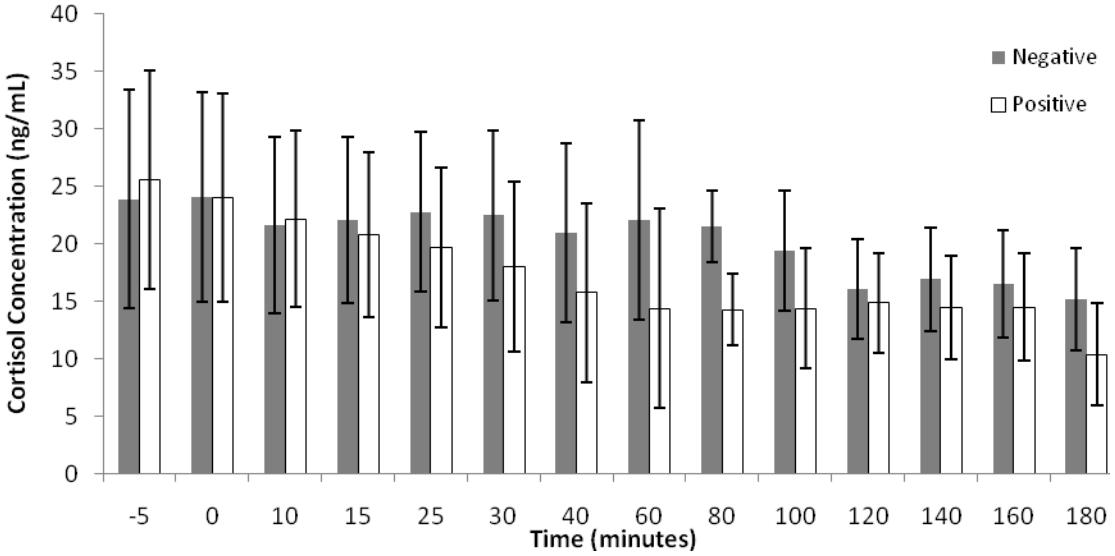


Figure 5.1 Brahman Experiment 1. Mean Cortisol Concentration Over Time by RFI Group.

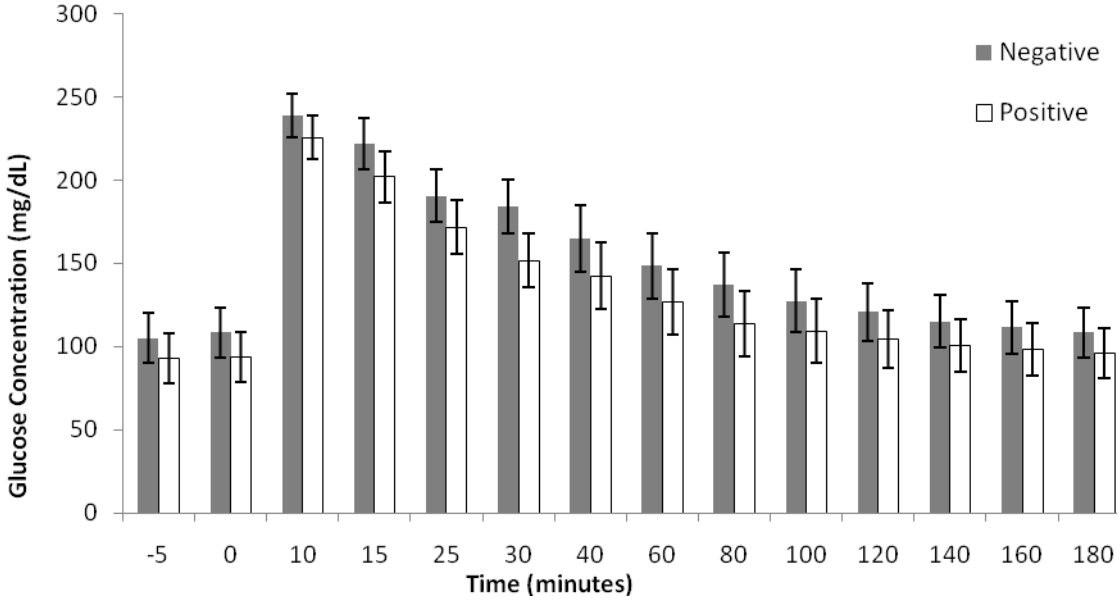


Figure 5.2 Brahman Experiment 1. Mean Glucose Concentration Over Time by RFI Group.

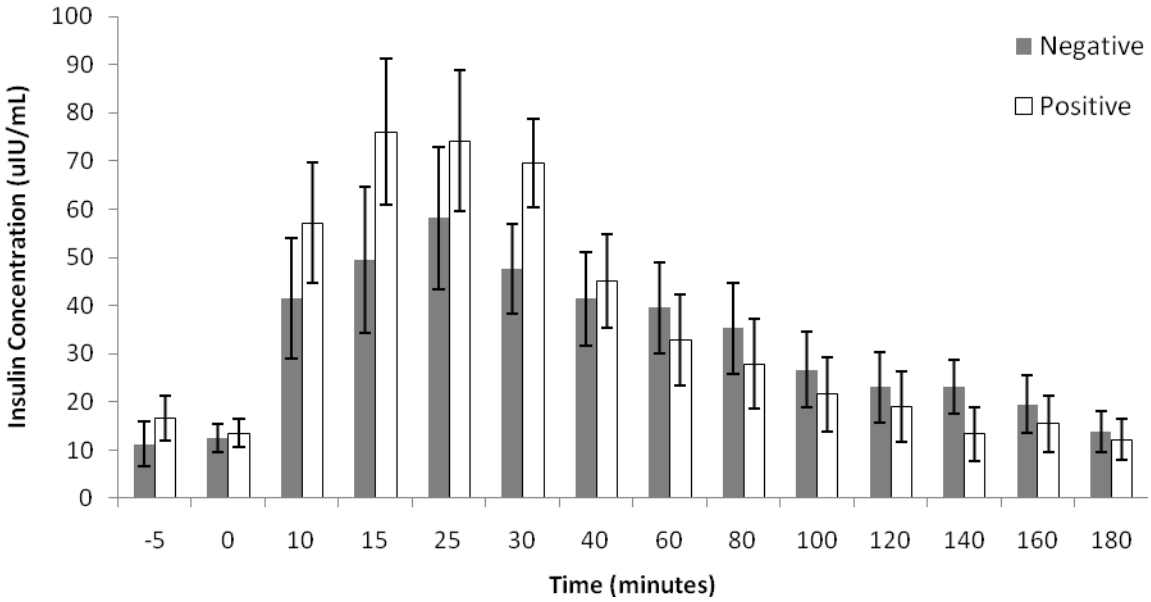


Figure 5.3 Brahman Experiment 1. Mean Insulin Concentration Over Time by RFI Group.

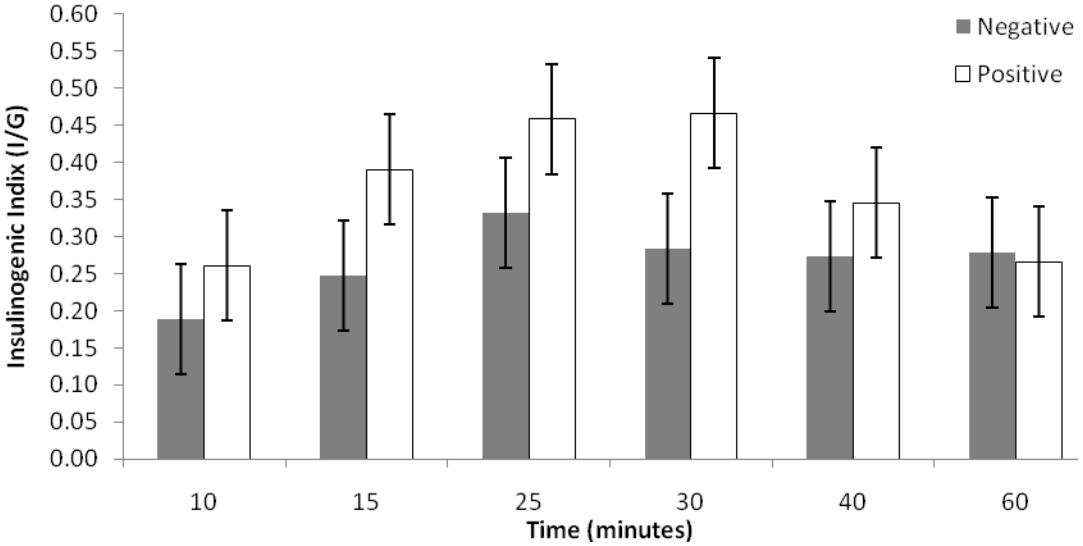


Figure 5.4 Brahman Experiment 1. Insulinogenic Index Over Time by RFI Group.

Table 5.3 Brahman Experiment 1 Exit Velocity

| Variable | RFI GROUP | | P Value |
|---|---|---|---|
| | Negative | Positive | |
| Exit Velocity (Day 0) | 3.22 ± 0.62 | 3.23 ± 0.62 | 0.9881 |

Table 5.4 Brahman Experiment 1 Insulin Peak and Glucose Half Life Data

| Variable | RFI GROUP | | P Value |
|---|---|---|---|
| | Negative | Positive | |
| Insulin Peak Concentration | 62.3± 13.4 | 89.2 ± 13.4 | 0.1863 |
| Insulin Peak Time | 22.5 ± 2.6 | 23.3 ± 2.6 | 0.8284 |
| Glucose Half Life Concentration | 66.1 ± 3.5 | 39.2 ± 4.4 | 0.9903 |
| Glucose Half Life Time | 39.2 ± 4.4 | 34.0 ± 4.4 | 0.4235 |

Table 5.5 Brahman Experiment 1 Area Under Curve: Glucose, Insulin, Cortisol, IIND

| Variable | RFI GROUP | | P Value |
|---|---|---|---|
| | Negative | Positive | |
| Glucose | 94.6 ± 8.9 | 79.9 ± 8.9 | 0.2728 |
| Insulin | 55.8 ± 11.4 | 43.8 ± 11.4 | 0.4726 |
| Cortisol | 54.3 ± 16.1 | 42.7 ± 16.1 | 0.6205 |
| IIND | 5.1 ± 1.2 | 4.9 ± 1.2 | 0.8881 |

*Experiment 2*

Residual feed intake group did differ significantly ($P > 0.05$) in EV; suggesting, there may be a temperament factor involved in this experiment. . Negative RFI and positive RFI bulls had EV (m/s) of 3.44 ± 0.31 and 2.33 ± 0.31, respectively Table 5.6. Residual feed intake groups did not differ ($P > 0.05$) in cortisol concentrations (Figure

5.5); suggesting cortisol did not likely play a role in glucoregulatory mechanisms of this experiment. There was no RFI group x time interaction affecting ($P > 0.05$) cortisol concentrations. Time relative to glucose infusion, had a significant affect ($P < 0.0001$) on cortisol concentrations; this would be expected as cortisol is known to decline during a period of restraint as the animal becomes more adjusted to the surroundings and is assumed to be experiencing less stress throughout the 3 h period. Any extraneous outside variables during the GTT were similar and experienced by both RFI groups. In efforts to account for any differences in temperament that may exist between RFI groups, EV was included as a covariate in all models for statistical analysis in this experiment.

Time relative to glucose infusion had a significant effect ($P < 0.0001$) on glucose (Figure 5.6) and on insulin (Figure 5.7) concentrations. The interaction of RFI group x time affected ($P < 0.01$) insulin concentrations; however there was no RFI group x time interaction ($P > 0.05$) affecting glucose concentrations. Residual feed intake group affected ($P < 0.05$) the insulin response; whereby negative RFI bulls had a greater insulin response than positive RFI bulls. Residual feed intake group did not affect ($P > 0.05$) glucose concentrations. Exit velocity did not affect ($P > 0.05$) glucose or insulin concentrations. (Residual feed intake group did not affect ($P > 0.05$) peak insulin concentration or time of insulin peak. Negative RFI and positive RFI bulls had peak insulin concentrations (mIU/mL) of 44.3 ± 16.9 and 74.4 ± 16.9, respectively and insulin peak concentration times (min) were 49.3 ± 14.5 and 21.6 ± 14.5, respectively. RFI group did not affect ($P > .05$) glucose half life concentrations or the time half life was achieved. Negative RFI and positive RFI bulls had glucose half life concentrations (mg/dL) of 62.0

± 2.6 and 58.9 ± 2.6, respectively and glucose half life times (min) were 43.5 ± 4.0 and 41.6 ± 4.0, respectively. Mean peak insulin concentration and glucose half life concentrations, and their respective times, are summarized in Table 5.7. Insulinogenic index (Figure 5.8) was affected by RFI group ($P < 0.01$), but was not affected ($P > 0.05$) by time or EV. Negative RFI and positive RFI bulls IIND (I/G) were 0.16 ± 0.02 and 0.31 ± 0.02, respectively. Insulinogenic index was analyzed from 10 to 60 min for both RFI groups. Area under the curve was calculated using the trapezoidal rule for glucose, insulin, and cortisol concentrations and IIND; no variables were significantly different ($P > 0.05$) in AUC between RFI groups. Table 5.8 outlines the AUC least square means, standard errors and correlated P values for each variable.

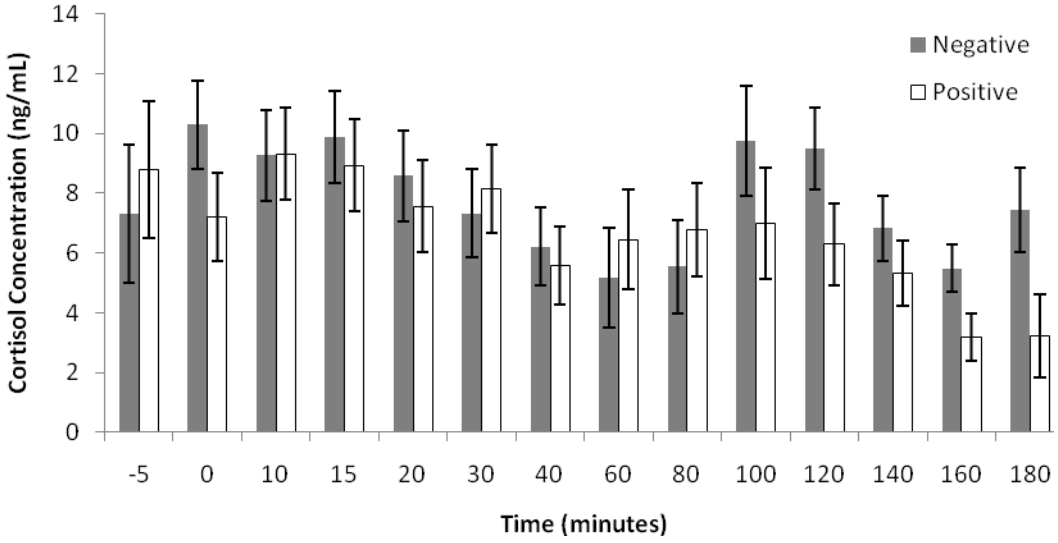


Figure 5.5 Brahman Experiment 2. Mean Cortisol Concentration Over Time by RFI Group.

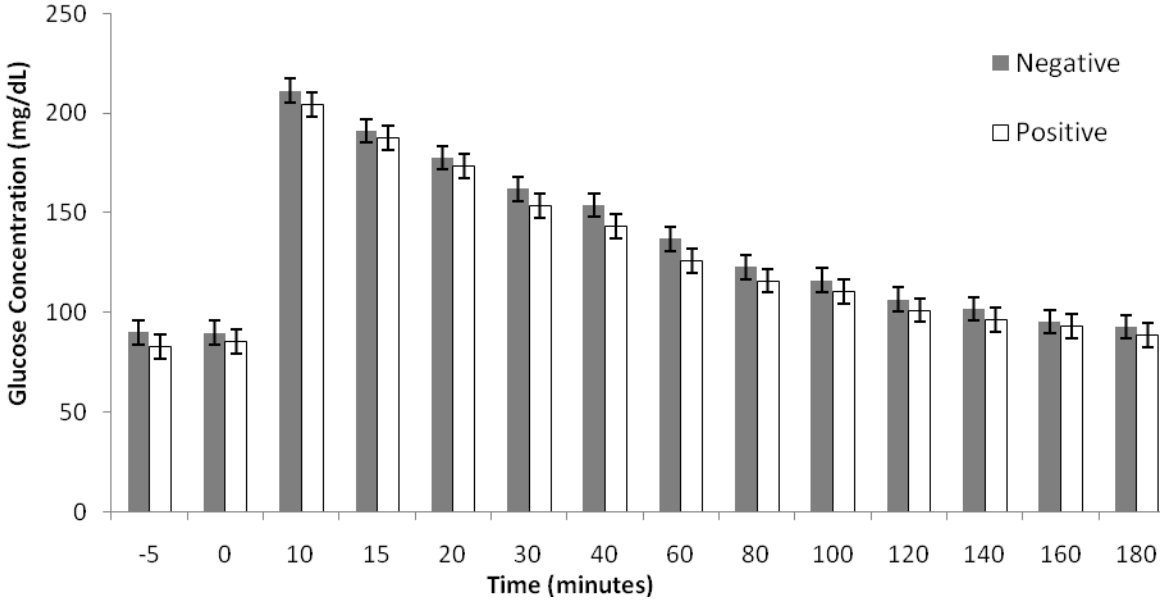


Figure 5.6 Brahman Experiment 2. Mean Glucose Concentration Over Time by RFI Group.

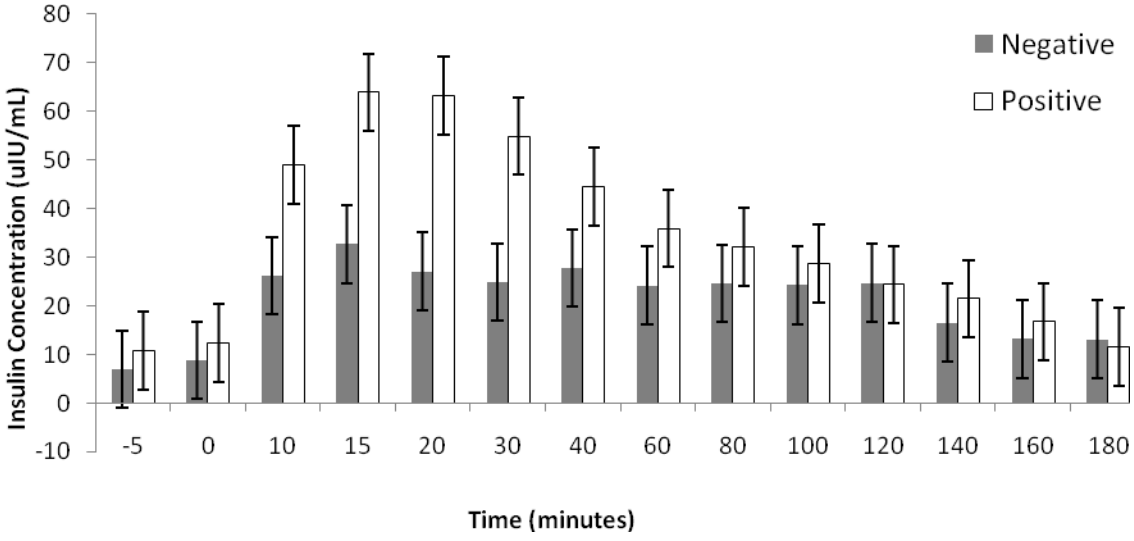


Figure 5.7 Brahman Experiment 2. Mean Insulin Concentration Over Time by RFI Group.

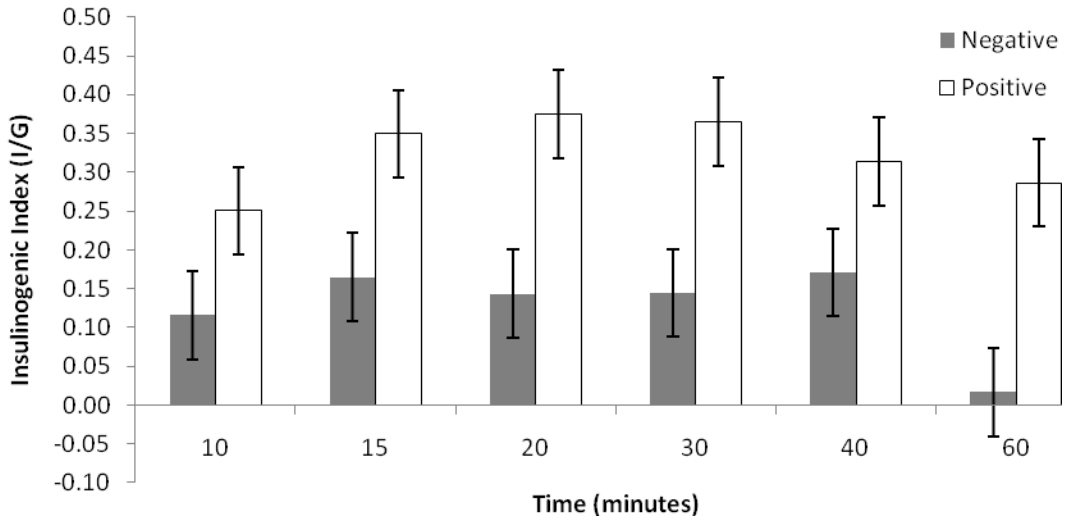


Figure 5.8 Brahman Experiment 2. Mean Insulinogenic Index Over Time by RFI Group.

Table 5.6 Brahman Experiment 2 Exit Velocity

| Variable | RFI GROUP | | P Value |
|---|---|---|---|
| | Negative | Positive | |
| Exit Velocity (Day 0) | 3.44 ± 0.31 | 2.33 ± 0.31 | 0.0287 |

Table 5.7 Brahman Experiment 2 Insulin Peak and Glucose Half Life Data with EV as Covariate

| Variable | RFI GROUP | | P Value |
|---|---|---|---|
| | Negative | Positive | |
| Insulin Peak Concentration | 44.3 ± 16.9 | 74.4 ± 16.9 | 0.2876 |
| Insulin Peak Time | 49.3 ± 14.5 | 21.6 ± 14.5 | 0.2558 |
| Glucose Half Life Concentration | 62.0 ± 2.6 | 58.9 ± 2.6 | 0.4581 |
| Glucose Half Life Time | 43.5 ± 4.0 | 41.6 ± 4.0 | 0.7808 |

Table 5.8 Brahman Experiment 2 Area Under Curve: Glucose, Insulin, Cortisol, IIND

| Variable | RFI GROUP | | P Value |
|---|---|---|---|
| | Negative | Positive | |
| Glucose | 99.2 ± 12.3 | 97.9 ± 12.3 | 0.9167 |
| Insulin | 40.5 ± 19.2 | 57.8 ± 19.2 | 0.3899 |
| Cortisol | 20.4 ± 2.8 | 17.3 ± 2.8 | 0.4457 |
| IIND | 1.7 ± 0.7 | 1.5 ± 0.7 | 0.8442 |

**Discussion**

In Experiment 1 RFI group did not differ ($P > .05$) in EV or cortisol concentration throughout the GTT. This suggests temperament did not likely play a substantial role in glucoregulatory mechanisms between low and high RFI groups for this experiment; consequently, EV was not included as a covariate for any model for statistical analysis. . However, in Experiment 2, EV differed significantly ($P < 0.05$) between RFI groups, suggesting there may be a temperament factor involved in this experiment. Similar to Experiment 1, cortisol concentrations did not differ between RFI groups. This suggests that cortisol did not likely play a role in glucoregulatory mechanisms of this experiment. Yet, in efforts to account for any differences in temperament that may exist between RFI groups, EV was included as a covariate in all models for statistical analysis in Experiment 2. In both experiments, animals representing both RFI groups were subjected to a GTT at the same time; therefore, any extraneous variables during the challenge were similar to both RFI groups.

In Experiment 1 and 2, negative RFI-classified and positive RFI-classified animals had similar glucose responses over time, demonstrating that the ability to clear

glucose from circulation did not differ between RFI groups. Experiment 1 and 2 RFI groups showed similar insulin responses; however, Experiment 2 was significantly different ($P > 0.05$) while Experiment 1 was not. In Experiment 1 there were some numerical differences in insulin response between RFI groups. Positive RFI heifers (inefficient) appear to have a numerically elevated insulin response in comparison to negative RFI heifers (more efficient). In Experiment 2, RFI group and the interaction of RFI x Time both significantly affected insulin response; whereas positive RFI bulls (inefficient) had an elevated insulin response in comparison to negative RFI bulls (efficient). As expected from the pattern observed with insulin's response, in both Experiment 1 and 2, RFI group did affect IIND; whereas positive RFI-classified animals showed a higher insulinogenic index compared to negative RFI-classified animals. This suggests that inefficient Brahman cattle are releasing an increased concentration of insulin in response to glucose and could be categorized as more sensitive to an influx of glucose. These animals are releasing an increased concentration of insulin to clear the same concentration per body weight of glucose from their blood circulation in comparison to more efficient animals. Although differences were observed in insulin response, RFI group had no affect on insulin peak concentration or concentration of glucose at half life in either Experiment 1 or 2. These data suggest that efficient cattle clear glucose from circulation with less insulin in comparison to inefficient cattle.

**Conclusion**

In Experiment 1 and Experiment 2 alike, negative RFI animals had a lesser response of insulin to glucose; suggesting that inefficient Brahman cattle have a greater insulin sensitivity to an influx of glucose and must produce higher concentrations of insulin to clear glucose from the circulation. The lower amount of insulin required for clearance of the glucose from the circulation of the low RFI Brahman cattle fits with our hypothesis that more efficient cattle would require less insulin than the less efficient cattle. Although RFI group did not significantly affect ($P > 0.05$) insulin response in Experiment 1, the ratio of insulin to glucose may provide more information and be a better indicator of feed efficiency in comparison to actual concentrations of insulin response to a GTT. Due to the differences ($P < 0.05$) observed between RFI groups in IIND, it may be implied that IIND after a glucose tolerance test may be a useful indicator of RFI in Brahman cattle. More specifically, these data suggests that IIND may be a useful predictor 15-30 minutes after a glucose tolerance test and an IIND value of 0.25 (I/G) or greater would equate to a positive RFI (less efficient) in Brahman cattle.

# CHAPTER VI

# CONCLUSION

Providing feed for the cow herd is a major cost input associated with cattle production as it has been found to account for 68-71% (USDA, 2011) of the total cost of cow calf production in the United States. Efficient feed utilization has become an essential component of beef production systems as we are in an era of dwindling resources and escalating costs of production. Residual feed intake is a method utilized in the beef cattle industry to identify feed efficient animals in a cohort. Evaluating potential herd sires for residual feed intake is becoming a popular selection tool in the beef industry as RFI has been found to be moderately heritable; therefore, identifying efficient herd sires has the potential for making genetic gains in feed efficiency. Despite the proposed accuracy associated with RFI, it is costly and labor intensive; thus, preventing it from being widely utilized by beef cattle producers.

Beef cattle feed efficiency is affected by many different physiological processes; these processes create variance in feed efficiency across a given herd. Richardson and Herd (2004) suggested that feed intake, digestion of feed, metabolism, activity of the animal and thermoregulation may all be factors affecting variance in feed efficiency. Yet, 70% of the variation in feed efficiency remains unexplained (Richardson and Herd, 2004). There has been strong interest in the research community to evaluate indirect measures of feed efficiency; however, none have proven to be effective. In efforts to improve feed efficiency in the beef industry, Johnston et al. (2002) proposed that “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." Insulin sensitivity can be an indicator of metabolic efficiency in many species; therefore, evaluating glucoregulatory mechanisms in cattle selected for RFI may provide an indirect measure of feed efficiency. Evaluating glucoregulatory mechanisms in other species has led to a better understanding of nutrition, lactation, immunology, and energy expenditure. Previous research has attempted to evaluate glucoregulatory mechanisms with regard to RFI; however these studies failed to evaluate differences in insulin sensitivity in RFI identified cattle. By utilizing a GTT, we are able to evaluate differences among insulin sensitivities in response to an influx of exogenous glucose; IIND allows us to put a numerical value to the relationship between glucose and insulin after a GTT. Results from studies within this thesis imply that IIND after a glucose tolerance test may be a useful predictor of RFI in tropically adapted cattle. Although breed differences between insulin responses among RFI groups were observed, notable trends existed between cohorts of animals evaluated. As a point of reference, Table 6.1 lists basal concentrations of insulin and glucose across multiple breeds; this table was compiled from previous studies and includes the experiments in this thesis as well.

Table 6.1 Baseline Concentrations of Insulin and Glucose in Divergent Breeds Compiled from Previous Studies

| Breed | Gender | RFI | n= | Age (mo) | Weight (kg) | Insulin BL (uIU/mL) unless noted | Glucose BL (mg/dL) | Reference |
|---|---|---|---|---|---|---|---|---|
| Angus | Male (Steer) | Low | 9 | ------ | 566 | 9.19 ng/mL | 86.44 | Kolath et al., 2006 |
| Angus | Male (Steer) | High | 8 | ------ | 563 | 11.1 ng/mL | 101.12 | Kolath et al., 2006 |
| Angus | Male (Steer) | Low | 16 | 14 | 423 | 2.48 ng/mL | 81.36 | Richardson et al., 2004 |
| Angus | Male (Steer) | High | 17 | 14 | 428 | 3.38 ng/mL | 85.68 | Richardson et al., 2004 |
| Angus Cross | Female | ------ | 12 | 9-10 | 266 | 14.16 | 119.16 | Bradbury et al., 2011 |
| Bonsmara | Female | Low | 18 | 12-14 | 368 | 11.09 | 93.55 | Shafer, 2011 |
| Bonsmara | Female | High | 18 | 12-14 | 362 | 10.82 | 88.77 | Shafer, 2011 |
| Brahman | Female | Low | 6 | 14 | 322 | 11.87 | 106.81 | Shafer, 2011 |
| Brahman | Female | High | 6 | 14 | 323 | 15.06 | 93.33 | Shafer, 2011 |
| Brahman | Male | Low | 6 | 16 | 407 | 7.90 | 89.95 | Shafer, 2011 |
| Brahman | Male | High | 6 | 17 | 468 | 11.56 | 84.04 | Shafer, 2011 |
| Brahman | Female | ------ | 12 | 6-10 | 192 | 6.23 | 140.26 | Bradbury et al., 2011 |
| Gir x Holstein | Female | ------ | 10 | Mature | 587 | 17.86 | 69.65 | Vieira et al., 2010 |
| Limousin x Holstein Friesian | Female | Low | 21 | 8 | 312 | 15.28 | 92.34 | Kelly et al., 2010 |
| Limousin x Holstein Friesian | Female | High | 23 | 8 | 314 | 14.67 | 91.80 | Kelly et al., 2010 |
| Santa Gertrudis | Male | Low | 8 | 14-17 | 383 | 11.50 | 124.75 | Shafer, 2011 |
| Santa Gertrudis | Male | High | 8 | 14-17 | 373 | 15.08 | 133.08 | Shafer, 2011 |
| Santa Gertrudis | Male (Steer) | Low | 85 | ------- | 395 | 4.72 ng/mL | 83.28 | Brown et al., 2005 |
| Santa Gertrudis | Male (Steer) | High | 87 | ------- | 395 | 5.33 ng/mL | 82.45 | Brown et al., 2005 |

Both experiments of Bonsmara heifers used in this study had a different insulin response to the exogenous glucose than the Brahman and Santa Gertrudis cattle. With Bonsmara heifers the negative RFI group, more efficient animals, had a greater insulin response than positive RFI animals. Conversely, Santa Gertrudis bulls and Brahman cattle (both bulls and heifers) exhibited a greater insulin response in positive RFI groups, more ineffcient animals, than the negative RFI groups. Due to the differences in insulin response observed in IIND. between negative and positive RFI-classified animals, there were also differences observed in IIND. With Bonsmara heifers the negative RFI group, more efficient animals, had a greater IIND than positive RFI animals. Conversely, Santa Gertrudis bulls and Brahman cattle (both bulls and heifers) exhibited a greater IIND in positive RFI groups, more ineffcient animals, than the negative RFI group. In all cohorts evaluated, insulinogenic index was affected ($P < 0.05$) by RFI group; suggesting that glucoregulatory differences do exist between efficient and inefficient animals.

In Bonsmara cattle, RFI group affected ($P < .05$) peak insulin concentration in Experiment 1 and a trend ($P < .10$) for RFI group to affect peak insulin concentration was observed in Experiment 2. Peak insulin concentration was not effected by RFI group in the Santa Gertrudis or Brahman cattle evaluated. A summary of peak insulin concentrations and IIND by RFI groups for each experiment can be found in Table 6.2. Glucose half life concentration or time half life was achieved was not significanly different between RFI groups in any of the groups evaluated. There were notable trends and differences in concentrations and times between the breeds observed. Bonsmara heifers had a higher insulin peak concentration and shorter glucose half life than the

Brahman and Santa Gertrudis cattle evaluated, whereas the latter two breeds were producing lower overall concentrations of insulin at a slower rate in comparison to the Bonsmara heifers. Experiment 1 Bonsmara heifers had a mean insulin peak that was 2x higher in concentration and a glucose half life that was one half shorter in time (min) than the Santa Gertrudis bulls. Although differences in insulin response were observed, we are not able to explain how efficient and ineffecient cattle differ in clearing glucose from the circulation. This implies that the actual concentration of insulin or glucose may not be as important as the ratio between the two concentrations measured.

The reason for the differences observed in insulin response for Bonsmara heifers compared to the other breeds evaluated in this study is unknown. Bonsmara cattle are a unique breed, developed to be very efficient in producing quality cuts of meat in tropical and subtropical environments with low feed and forage availability. Bonsmara heifers fed a roughage based diet of 2.07 mcal/kg DE (Table 3.1) have been observed to have better ADG than Brangus heifers of similar age fed the same diet. Bonsmara heifers had an ADG of 1.21 ± 0.082 (low RFI) and 1.22 ± 0.065 (high RFI; Wiley et al., 2011) compared to Brangus heifers which had an ADG of 1.07 ± 0.05 (low RFI) and 1.08 ± 0.04 (high RFI; Bingham et al., 2009). There may be differences in the Bonsmara's glucoregulatory mechanisms, compared to Brahman influenced breeds of cattle due to the Bonsmara breed's development and function.

Table 6.2 Mean Insulin Peak Concentrations (uIU/mL) and Insulinogenic Index Values Across Experiments

| | Mean Insulin Peak Concentrations (uIU/mL) | | | IIND (I/G) | | |
|---|---|---|---|---|---|---|
| | RFI Group | | | RFI Group | | |
| Experiment | Low RFI | High RFI | P Value | Low RFI | High RFI | P Value |
| Bonsmara Heifers-Experiment 1 | 108.1 ± 12.1 | 75.5 ± 12.1 | 0.0761 | 0.44 ± .03 | 0.29 ± .03 | 0.0026 |
| Bonsmara Heifers-Experiment 2 | 93.6 ± 13.4 | 50.3 ± 13.4 | 0.0455 | 0.35 ± .02 | 0.17 ± .02 | 0.0010 |
| Santa Gertrudis Bulls | 50.6 ± 13.3 | 67.7 ± 13.3 | 0.3776 | 0.18 ± .03 | 0.27 ± .03 | 0.0443 |
| Brahman Heifers | 62.3 ± 13.4 | 89.2 ± 13.4 | 0.1863 | 0.27 ± .03 | 0.36 ± .03 | 0.0453 |
| Brahman Bulls | 44.3 ± 16.9 | 74.4 ± 16.9 | 0.2876 | 0.16 ± .02 | 0.31 ± .02 | 0.0008 |

It is important to keep the cohorts used in this thesis as separate studies and not make cross study comparisons as there is no control among experiments. Animals that were evaluated are of different genders, breed-types and ages and diverse environmental conditions and settings occurred during each GTT. Herd et al., (2004) suggested that feed efficiency is relative to the type and amount of feed consumed, the sex and breed of that animal, and environmental conditions in which that animal is managed. Additionally, differences in HPA and endocrine function among gender and breedtype of cattle evaluated were reported by Welsh et al. (2009). Also impeding our ability to evaluate all cohorts as one experiment are the differences observed in RFI among varying feeding experiments; such as providing feed *ad libitum* or in restricted amounts. Bonsmara and Santa Gertrudis breeds were evaluated for RFI in systems providing feed *ad libitum* using either the GrowSafe system or Calan gates during the feeding trials, where the Brahman cattle evaluated were provided limited feed using a Calan gate system during the feeding trials. Providing feed *ad libitum* results in a wider range of RFI values for the cohort evaluated meaning a wider numerical range in differences between efficient and inefficient animals. Providing feed *ad libitum* simulates a feed lot setting; however, a feed lot setting is sometimes undesirable when selecting heifers for RFI that are to be retained in the breeding herd, as was the case with the Brahman heifers evaluated.

Previous literature reports temperament to be a factor when evaluating glucoregulatory mechansisms (Bradbury, 2011). By evaluating animals for EV prior to the RFI feeding period and measuring cortisol concentrations during the glucose

tolerance test, we attempted to account for any diversity in temperament between RFI groups of each cohort. If any experiment showed differences between EV among RFI groups, EV was added as a covariate to the statistical model Out of the 5 experiments within this study, the Brahman Experiment 2 (bulls) were the only cohort that varied in EV for RFI groups; however EV did not have a significant affect on insulin or glucose concentrations or IIND. Furthermore, cortisol concentrations did not vary ($P > 0.05$) between RFI groups during the glucose tolerance test. Previous research reports cortisol to be a major distruptor of glucoregulatory mechanisms; however, with the lack of differences seen in this study temperament did not likely play a substantial role in differences in glucoregulatory mechanisms between RFI groups.

Insulinogenic index after a glucose tolerance test was affected ($P < 0.05$) by RFI in all experiments within this thesis. This implies that differences in glucoregulatory mechanisms do exist between efficient and inefficient cattle. The different insulin responses observed among breeds and RFI groups suggests definite differences exist in glucoregulatory mechanisms exist between breeds of cattle evaluated. Due to the differences ($P < 0.05$) observed between RFI groups in IIND, it may be implied that IIND after a glucose tolerance test may be a useful indicator of RFI in tropically adapted cattle. In Bonsmara heifers, the IIND may be a useful predictor of RFI 15-30 minutes after a GTT; whereas an IIND value of 0.30 (I/G) or greater would equate to a negative RFI (more efficient) Bonsmara heifer. In Brahman or Santa Gertrudis cattle, the IIND may be a useful predictor of RFI 15-30 minutes after a GTT; whereas an IIND value of 0.30 (I/G) or less would equate to a negative RFI (more efficient) animal.

## LITERATURE CITED

Abdelmannan, D., R. Tahboub, S. Genuth, and F. Ismail-Beigi. 2010. Effect of Dexamethasone on oral glucose tolerance in healthy humans. Endo. Prac. 16:770-776.

Archer, J. A., P. F. Arthur, R. M. Herd, and W. S. Pitchford. 1997. Optimum postweaning test for measurement of growth rate, feed intake, and feed efficiency in British breed cattle. J. Anim. Sci. 75:2024-2032.

Archer, J. A., P. F. Arthur, R. M. Herd, and E. C. Richardson. 1998. Genetic variation in feed efficiency and its component traits. Proc. 6th Wld. Congr. Genet. Appl. Livest. Prod. Armidale, Australia. 25:81-84.

Archer, J. A. and L. Bergh. 2000. Duration of performance tests for growth rate, feed intake and feed efficiency in four biological types of beef cattle. Livest. Prod. Sci. 65:47-55.

Archer, J. A., E. C. Richardson, R. M. Herd, and P. F. Arthur. 1999. Potential for selection to improve efficiency of feed use in beef cattle: a review. Aust. J. Agric. Res. 50:147-161

Arthur, P. F., J. A. Archer, D. J. Johnson, R. M. Herd, E. C. Richardson, and P. F. Parnell. 2001a. Genetic and phenotypic variance and covariance components for fed intake, feed efficiency, and other postweaning traits in Angus cattle. J. Anim. Sci. 79:2805-2811.

Arthur, P. F., J. A. Archer, A. Reverter, D. J. Johnston, and R. M. Herd. 2004. Genetic correlations between postweaning feed efficiency and cow traits. J. Anim. Sci. 82(Suppl 1):449.

Arthur, P. F. and R. M. Herd. 2005. Efficiency of feed utilization by livestock; Implications and benefits of genetic improvement. Can. J. Anim. Sci. 85:281-290.

Arthur, P. F., R. M. Herd, J. Wright, G. Xu, K. Dibley, and E. C. Richardson. 1996. Net feed conversion efficiency and its relationship with other traits in beef cattle. Proc. Aust. Soc. Anim. Prod. 21:107-110.

Arthur, P. F., G. Renand, and D. Krauss. 2001b. Genetic and phenotypic relationships among different measures of growth and feed efficiency in young Charolais bulls. Livest. Prod. Sci. 68:131-139.

Barandse, W., A. Reverter, R. J. Bunch, B. E. Harrison, W. Barris, and M. B. Thomas. 2007. A validated whole genome association study of efficient food conversion in cattle. Genetics 176:1893-1905.

Barlow, R. 1984. Selection for growth and size in ruminants: Is it time for a moratorium. In: Hofmeyr, J. H., Meyer, E. H. H. (Eds.), Proc. 2nd World Congr. Sheep Beef Cattle Breed, Pretoria, pp. 421-432.

Basarab, J. A., M. A. Price, J. L. Aalhus, E. K. Okine, W. M. Snelling, and K. L. Lyle. 2003. Residual feed intake and body composition in young growing cattle. Can. J. Anim. Sci. 83:189-204.

Basarab, J. A., M. A. Price, and E. K. Okine. 2002. Commercialization of net feed efficiency. Proc. 23rd West. Nut. Conf., Sep. 19-20. Edmonton, Alberta, Canada. 183-194.

Bingham, G. M., T. H. Friend, P. A. Lancaster, and G. E. Carstens. 2009. Relationship between feeding behavior and residual feed intake in growing Brangus heifers. J. Anim. Sci. 87:2685-2689.

Bogart, R., R. F. R. Ampy, A. F. Anglemier, and W. K. Johnston, Jr. 1963. Some physiological studies on growth and feed efficiency of beef cattle. J. Anim. Sci. 22:993-1000.

Bonsma, J. C. 1949. Ecological animal husbandry. Review. Government printer, Pretoria, Union of South Africa.

Bradbury, B. L., S. L. Morgan, A. N. Loyd, D. A. Neuendorff, A. W. Lewis, J. P. Banta, D. G. Riley, T. D. A. Forbes, T. H. Welsh, Jr., and R. D. Randel. 2011. Comparison of Brahman females evaluated for residual feed intake (RFI) as heifers and reevaluated for RFI as gestating cows. Accepted for presentation at American Society of Animal Science Meetings, New Orleans, LA. July 10-14, 2011.

Bradbury, B. L., L. C. Caldwell, A. W. Lewis, R. C. Vann, T. H. Welsh, Jr., R. D. Randel. 2011. Effect of temperament on response to cannulation and glucose challenge in Brahman heifers. Accepted for presentation at American Society of Animal Science Meetings, New Orleans, LA. July 10-14, 2011.

Brinks, J. S., R. T. Clark, F. J. Rice and N. M. Kieffer. 1961. Adjusting birth weight, weaning weight and preweaning gain for sex of calf in range Hereford cattle. J. Animal Sci. 20:363-364.

.

Brockman, R. P. 1983. Effects of insulin and glucose on the production and utlilization of glucose in sheep. Comp. Biochem. Physiol. 74A:681-685

Brockman, R. P. 1993. Glucose and short-chain fatty acid metabolism. Page 249-265 in Quantaitative Aspects of Ruminant Digestion and Metabolism, JM, Forbes, J, France, ccl. Cambridge:CAB International, Wallingford.

Brody, S. 1945. Bioenergetics and growth, with special reference to the efficiency complex in domestic animals. Reinhold Publishing Corp., New York.

Brown, E. G. 2005. Sources of biological variation in residual feed intake in growing and finishing steers and relationships with other feed efficiency traits. Ph.D. Dissertation Texas A&M Univ., College Station.

Burdick, N. C. 2007. Calf temperament: effect on passive immunity and secretion of cortisol and catecholamines. M.S. Thesis. Texas A&M Univ., Kingsville, TX.

Caldwell, L. C. 2009. 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. M.S. Thesis. Texas A&M Univ., College Station, TX.

Cartwright, T. C. 1955. Responses of beef cattle to high ambient temperatures. J. Anim. Sci. 14:350-362.

Carvalho, F. A., M. A. Lammoglia, M. J. Simoes, and R. D. Randel. 1995. Breed affects thermoregulation and epithelial morphology in imported and native cattle subjected to heat stress. J. Anim. Sci. 73:3570-3573.

Crews, Jr. D. H., G. E. Carstens, and P. A. Lancaster. 2005. Development of a multiple trait selection index for feedlot traits in beef cattle including feed efficiency. Proc. West. Sec. Amer. Soc. Anim. Sci. 56:71-74.

Crews, Jr. D. H., N. H. Shannon, B. M. A. Genswein, R. E. Crews, C. M. Johnson, and B. A. Kendrick. 2003. Genetic parameters for net feed efficiency of beef cattle measured during postweaning growing versus finishing periods. Proc. West. Sec. Amer. Soc. Anim. Sci. 54:125-128.

Curley Jr., K.O., Neuendorff, D.A., Lewis, A.W., Cleere, J.J., Welsh Jr., and T.H., Randel, R.D., 2008. Functional characteristics of the bovine hypothalamic–pituitary–adrenal axis vary with temperament. Horm. Behav. 53:20–27.

Curley Jr. K. O, Jr., J. C. Paschal, T. H. Welsh, Jr, and R. D. Randel. 2006. Technical note: Exit velocity as a measure of cattle temperament is repeatable and associated with serum concentration of cortisol in Brahman bulls. J. Anim. Sci. 84:3100-3103.

Daftary, S. S. and A. C. Gore. 2005. IGF-I in the brain as a regulator of reproductive neuroendocrine function. Exp. Biol. Med. 230:292-306.

Davis, M. E. and R. C. M. Simmen. 1997. Genetic parameter estimates for serum insulin-like growth factor-I concentration and performance traits in Angus beef cattle. J. Anim. Sci. 75:317-324.

Fan, L. Q., D. R. C. Bailey, and N. H. Shannon. 1995. Genetic parameter estimation of postweaning gain, feed intake and feed efficiency for Hereford and Angus bulls fed two different diets. J. Anim. Sci. 73:365-372.

Fell, L.R., Colditz, I.G., Walker, K.H., Watson, D.L., 1999. Associations between temperament, performance and immune function in cattle entering a commercial feedlot. Aust. J. Exp. Agric. 39:795–802.

Finch, V. A. 1986. Body temperature in beef cattle: Its control and relevance to production in the tropics. J. Anim. Sci. 62:531-539.

Fordyce, G., M. E Goddard, and G. W. Seifert. 1982. The measurement of temperatment in cattle and the effect of experience and genotype. Proc. Aust. Sot. Anim. Prod. 14:329-332.

Fordyce, G., J. R. Wythes, W. R. Shorthose, D. W. Underwood, and R. K. Shepherd. 1988. Cattle temperaments in extensive beef herds in northern Queensland. 2: Effect of temperament on carcass and meat quality. Aust. J. Exp. Agr. 28:689-693.

Golden, J. W., M. S. Kerley, and W. H. Kolath. 2008. The relationship of feeding behavior to residual feed intake in crossbred Angus steers fed traditional and no roughage diets. J. Anim. Sci. 86:180-186.

Goonewardene, L. A., E. Okine, Z. Wang, D. Spaner, P. S. Mir, and T. Marx. 2004. Residual metabolizable energy intake and its association with diet and test duration. Can. J. Anim. Sci. 84:291-295.

Grovum, W. L. and J. F. Hecker. 1973. Rate of passage of digesta in sheep. 2. The effect of level of food intake on digesta retention times and on water and electrolyte absorption in the large intestine. Br. J. Nutr. 30:221-230.

Guerrero-Romero, F., M. Rodriguez-Moran. 2001. Glucose intolerance is predicted by the high fasting insulin-to-glucose ratio. Diabetes Metab. (Paris) 27: 117-121.

Herbein, J. H., R. J. Aiello, L. I. Eckler, R. E. Pearson, and R. M. Akers. 1985. Glucagon, insulin, growth hormone, and glucose concentrations in blood plasma of lactating dairy cows. J. Dairy Sci. 68:320-325.

Herd, R. M. and P. F. Arthur. 2009. Physiological basis for residual feed intake. J. Anim. Sci. 87:E64-E71.

Herd, R. M. and S. C. Bishop. 2000. Genetic variation in residual feed intake and its association with other production traits in British Hereford cattle. Liv. Prod. Sci. 63:111-119.

Herd, R. M., V. H. Oddy, and E. C. Richardson. 2004. Biological basis for variation in residual feed intake in beef cattle. 1. Review of potential mechanisms. Aust. J. Exp. Agric. 44:423-430.

Herd, R. M., E. C. Richardson, R. S. Hegarty, R. Woodgate, J. A. Archer, and P. F. Arthur. 1998. Pasture intake by high versus low net feed efficient Angus cows. Animal Production in Australia. 22:137-140.

Hergarty, R. S., J. P. Goopy, R. M. Herd, and B. McCorkell. 2007. Cattle selected for lower residual feed intake have reduced daily methane production. J. Anim. Sci. 85:1479-1486.

Hocquette, J. F. and H. Abe. 2000. Facilitative glucose transporters in livestock species. Reprod. Nutr. Dev. 40:517-533.

Hocquette, J. F. and D. Bauchart. 1999. Intestinal absorption, blood transport and hepatic and muscle metabolism of fatty acids in preruminant and ruminant animals. J. Reprod. Nutr. Dev. 39:27-48.

Hocquette, J.-F., B. Graulet, and T. Olivecrona. 1998. Lipoprotein lipase activity and mRNA levels in bovine tissues. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 121:201-212.

Johnston, D. J. 2007. Technical update NFI & IGF-I. Beef Tech. Note March 2007. Anim. Breed. Genet. Unit, Iniv. New England, Armidale, Australia. http://agbu.une.edu.au/cattle/beef9.pdf Accessed Jan, 5, 2011.

Johnston, D. J., R. M. Herd, M. J. Kadel, H. U. Graser, P. F. Arthur, and J. A. Archer. 2002. Evidence of IGF-I as a genetic predictor of feed efficiency traits in beef cattle. Proc. 7th World Cong. Genet. Appl. Livest. Prod. Montpellier, France. INRA, Montpellier, France., Communications No. 10-16 p. 257-260.

Johnston, D. J., R. M. Herd, A. Reverter, and V. H. Oddy. 2001. Heritability of IGF-I in beef cattle and its association with growth and carcass traits. Proc. Assoc. Adv. Anim. Breed. Genet. 14:163-166.

Kelly, A. K., M. McGee, D. H. Crews, Jr., A. G. Fahey, A. R. Wylie, and D. A. Kenny. 2010. Effect of divergence in residual feed intake on feeding behavior, blood metabolic variables, and body composition traits in growing beef heifers. J. Anim. Sci. 88:109-123.

Kennedy, B. W., J. H. J. van der Werf, and T. H. E. Meuwissent. 1993. Genetic and statistical properties of residual feed intake. J. Anim. Sci. 71:3239-3250.

King, D.A., C.E. Schuehle Pfeiffer, R. D. Randel, T. H. Welsh Jr., R. A. Oliphint, B.E. Baird, K. O. Curley Jr., R.C. Vann, D. S. Hale, and J. W. Savell, 2006. Influence of animal temperament and stress responsiveness on the carcass quality and beef tenderness of feedlot cattle. Meat Sci. 74:546–556.

Knott, S. A., L. J. Cummins, F. R. Dunshea, and B. J. Leury. 2008. The use of different models for the estimation of residual feed intake (RFI) as a measure of feed efficiency in meat sheep. Animal Feed Science and Technology. 143:242-255.

Knut, H. 1978. Insulin secretion in lactating cows: responses to glucose infused intravenously in normal, ketonemic, and starved animals. J. of Dairy Sci. 61:1407-1413.

Koch, R. M., L. A. Swiger, D. Chambers, and K. E. Gregory. 1963. Efficiency of feed use in beef cattle. J. Anim. Sci. 22:486-494.

Kolath, W. H., M. S. Kerley, J. W. Golden, and D. H. Keisler. 2006. The relationship between mitochondrial function and residual feed intake in Angus steers. J. Anim Sci. 84: 861-865.

Koots, K. R., J. P. Gibson, and J. W. Wilton. 1994. Analysis of published genetic parameter estimates for beef production traits. 2. Phenotypic and genetic correlations. Anim. Breed. Abstr. 62:826-853.

Krueger, W.K., G. E. Carstens, P. A. Lancaster, L. J. Slay, J. C. Miller and T. D. A. Forbes. 2008. Relationships between residual feed intake and apparent nutrient digestibility in growing calves. J. Anim. Sci. 86(E. Suppl 3):25.

Kuneko, I. J., and E. A. Rhodes. 1964. Diabetes mellitus in a cow. J. Amer. Vet. Med. Assn. 144:367–373.

Lancaster, P. A., G. E. Carstens, E. G. Brown, R. D. Randel, T. H. Welsh Jr., T. D. A. Forbes, D. T. Dean, and A. D. Herring. 2005. Relationships between residual feed intake, ultrasound, and temperament traits in Brangus heifers. J. Anim. Sci. 83(Suppl. 1):325. (Abstr.)

Lancaster, P. A., G. E. Carstens, J. G. Lyons, T. H. Welsh, Jr., R. D. Randel, and T. D. A. Forbes. 2007. Characterization of residual feed intake and relationships with serum insulin-like growth factor-I in growing Brangus heifers. J. Anim. Sci. 85(Suppl. 1):667.

Lancaster, P. A., G. E. Carstens, F. R. B. Ribeiro, L. O. Tedeschi, and D. H. Crews, Jr. 2009. Characterization of feed efficiency traits and relationships with feeding behavior and ultrasound carcass traits in growing bulls. J. Anim. Sci. 87:1528-1539.

Liu, J.-G., P. Cui-Ling, L. Yong-Wang, S. Wei-Dong, Z. Hong-Jin, L. Yan-Juan, H. Cheng-Hua, and W. Xiao-Long. 2004. The intravenous glucose tolerance test in water buffalo. Research in Veterinary Science 77: 23-27.

McDowell, G. H. 1983. Hormonal control of glucose homeostasis in ruminants. Proc. Nutr. Soc. 42:149-167.

McPeak, C. A., Buchanan, D. S., 1986. A comparison of 140 day provided by Meat and Livestock Australia vs shorter test periods for evaluating average daily gain in beef bulls at central test stations (abstract). J. Anim. Sci. (Suppl. 1): 209.

Minton, J. E. 1994. Function of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system in models of acute stress in domestic farm animals. J. Anim. Sci. 72:1891-1898.

Montaño-Bermudez, M., M. K. Nielsen, and G. H. Deutscher. 1990. Energy requirements for maintenance of crossbred beef cattle with different genetic potential for milk. J. Anim. Sci. 68:2279-2288.

Moore, K. L., D. J. Johnson, H-U. Graser, and R. Herd. 2005. Genetic and phenotypic relationships between insulin-like growth factor-I (IGF-I) and net feed intake, fat, and growth traits in Angus beef cattle. Aust. J. Exp. Agric. 56:211-218.

Moore, S. S., F. D. Mujibi, and E. L. Sherman. 2009. Molecular basis for residual feed intake in beef cattle. J. Anim. Sci. 87:E41-E47.

Mrode, R. A., C. Smith, R. Thompson, 1990. Selection for rate and efficiency of lean gain in Hereford cattle. 2. Evaluation of correlated responses. Anim. Prod. 51:35-46

Munck, A., P. M. Guyre, and N. J. Holbrook. 1984. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev. 5:25-44.

Nkrumah, J. D., J. A. Basarab, M. A. Price, E. K. Okine, A. Ammoura, S. Guercio, C. Hansen, C. Li, B. Benkel, B. Murdoch, and S. S. Moore. 2004. Different measures of energetic efficienty and their phenotypic relationships with growth, feed intake, and ultrasound and carcass merit in hybrid cattle. J. Anim. Sci. 82:2451-2459.

Nkrumah, J. D., E. K. Okine, G. W. Mathison, K. Schmid, C. Li, J. A. Basarab, M. A. Price, Z. Wang, and S. S. Moore. 2006. Relationships of feedlot feed efficiency performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. J. Anim. Sci. 84:145-153.

Oddy, V. H., P. A. Speck, H. M. Warren, and P. C. Wynn. 1995. Protein metabolism in lambs from lines divergently selected for weaning weight. J. Ag. Sci. 124:129-137.

Richardson, E. C. and R. M, Herd. 2004. Biological basis for variation in residual feed intake in beef cattle. 2. Synthesis of results following divergent selection. Aust. J. Exp. Agric. 44:431-440.

Richardson, E. C., R. M. Herd, J. A. Archer, and P. F. Arthur. 2004. Metabolic differences in Angus steers divergently selected for residual feed intake. Aust. J. Exp. Agric. 44:441-452.

Richardson, E. C., R. M. Herd, P. F. Arthur, J. Wright, G. Xu, K. Dibley, and V. H. Oddy. 1996. Possible physiological indicators of net feed conversion efficiency. Proc. Aust. Soc. Anim. Prod. 21:901-908.

Richardson, E. C., R. M. Herd, V. H. Oddy, J. M. Thompson, J. A. Archer, and P. F. Arthur. 2001. Body composition and implications for heat production of Angus steer progeny of parents selected for and against residual feed intake. Aust. J. Exp. Agric. 41:1065-1072.

Riley, D. G., G. R. Hansen, S. W. Coleman, and C. C. Chase. 2007. Residual feed intake and temperament breed differences among Florida heifers. J. Anim. Sci. 85(Suppl. 1):263.

Rizza, R. A., L. J. Mandarino, and J. E. Gerich. 1982. Effects of growth hormone on insulin action in man. Mechanisms of insulin resistance, impaired suppression of glucose production, and impaired stimulation of glucose utilization. Diabetes. 31:663-339.

Robinson, D. L. and V. H. Oddy. 2004. Genetic parameters for feed efficiency, fatness, muscle area and feeding behavior of feedlot finished beef cattle. Livest. Prod. Sci. 90:255-270.

Robinson, D. L., W. Skerrit, and V. H. Oddy. 1997. Measurement of feed intake and feed efficiency in beef cattle. Proc. Assoc. Advmt. Anim. Breed. Genet. 12:287-291.

SAS. 2002. SAS 8.2 user's guide: statistics. SAS Inst. Inc., Cary, NC.

Schenk, S., S. Maziyar, M. Olefsky. 2008. Insulin sensitivity: modulation by nutrients and inflammation. J. Clin. Invest. 118(9):2992-3002.

Schenkel, F. S., S. P. Miller, and J. W. Wilton. 2004. Genetic parameters and breed differences for feed efficiency, growth, and body composition traits of young beef bulls. Can. J. Anim. Sci. 84:177-185.

Schuehle, C., C. Adams, D. King, L. Lucia, E. Cabrera-Diaz, T. Welsh Jr., R. Randel, K. Curley Jr., R. Oliphint, R. Vann, G. Acuff, J. Savell, 2005. Relationship between stress responsiveness, animal temperament, and fecal shedding of Escherichia coli 0157:H7 in feedlot cattle. Meat Science 81:300-306.

Sherman, E. L., J. D. Nkrumah, B. M. Murdoch, and S. S. Moore. 2008. Identification of polymorphisms influencing feed intake and efficiency in beef cattle. Anim. Genet. 29:225-231.

Shrode, R. R., F. R. Quui, I. W. Rupel, and R. E. Leighton. 1960. Variation in rectal temperature, respiration rate, and pulse rate of cattle as related to variation in four environmental variables. J. Dairy Sci. 43:1235.

Turnbow, M. A., S. R. Keller, K. M. Rice, and C. W. Garner. 1994. Dexamethasone down-regulation of insulin-receptor substrate-1 in 3T3-L1 adipocytes. J. Biol. Chem. 269:2516-2520.

USDA, E. 2011. Commodity costs and returns: Data. http://www.ers.usda.gov/Data/CostsAndReturns/testpick.htm

Vieira, F. V. R., C. N. Lopes, B. I. Cappellozza, A. B. Scarpa, R. F. Cooke, and J. L. M. Vasconcelos. 2010. Effects of intravenous glucose infusion and nutritional

balance on serum concentrations of nonesterified fatty acids, glucose, insulin, and progesterone in nonlactating dairy cows. J. Dairy Sci. 93(7):3047-3055.

Welsh, T.H., Jr., N.C. Burdick, K.O. Curley, Jr., B.J. Agado, S.T. Willard, J.A. Carroll, R.C. Vann, and R.D. Randel. 2009. Influence of gender and breedtype on basal and induced secretion of cortisol in cattle. Presented at the 91st Annual Meeting of the Endocrine Society, Washington, D.C., June 2009.

Wiley, L. M., T. D. A. Forbes, A. N. Hafla, C. M Hensarling, B. G. Warrington, G. E. Carstens. Relationships between performance and feed efficiency in Bonsmara heifers when confinement fed or on pasture. Accepted for presentation at Southern Section American Society of Animal Science Meetings February 7-9, 2011.

Wilson, L. L, J. H. Ziegler, J. L. Watkins, C. E. Thompson, and H. R. Purdy. 1969. Influence of sex and sire upon growth and carcass traits of beef cattle. J. Anim. Sci. 28:607-611.

Yeates, N. T. M., T. N. Edey, and M. K. Hill. 1975. Animal science, reproduction, climate, meat, wool. P. 226-227. Pergamon Press, Headingdon Hill Hall, Oxford, United Kingdom.

# APPENDIX A

## INSULIN RADIOIMMUNOASSAY FOR BOVINE SERUM

Intended for use with Coat-A-Count Cortisol Radioimmunoassay (Siemens, PITKIN-8)

**Materials Supplied:**

1. Insulin Ab-Coated Tubes
   Protect from moisture by resealing storage bags after use, store at 4ºC.
2. $^{125}$I Insulin
   Stable at 4ºC for 30 days after opening.
3. Insulin Calibrators (Standards)
   Processed in nonhuman serum. Seven vials, labeled A through G, of lyophilized processed in nonhuman serum. At least 30 minutes before use, reconstitute the zero calibrator A with 6.0 mL of distilled or deionized water, and each of the remaining calibrators B through G with 3.0 mL of distilled or deionized water. Stable at -20ºC 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 Provided**

1. Gamma counter: Compatible with standard 12x75 mm tubes
2. Vortex mixer
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
7. Distilled or deionized water
8. Graduated cylinder: 100 mL
9. Volumetric pipettes: 3.0 mL and 6.0 mL

**Sample Collection**

1. Collect plasma via venipuncture into EDTA coated 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.
4. Volume Required: 200 µL of serum per tube.
5. Storage: 2-8ºC for 7 days, or for up to 3 months frozen at -20ºC.

**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 fourteen Insulin Ab-Coated Tubes A (maximum binding) and B through G in duplicate for standards.

| Calibrator | Approximate µIU/mL 1st IRP [66/304] |
|---|---|
| A (MB) | 0 |
| B* | 5 |
| C | 15 |
| D | 50 |
| E | 100 |
| F | 200 |
| G | 350 |

* Omit calibrator b if the alternate, 3-hour alternate, 3-hour incubation at room temperature (15-28ºC) will be used at step 4

4. Prepare extra standards:
   0.125ug/dL: Add 50ul of 0ug/dL standard to 50ul of 0.25ug/dL standard.
   0.25ug/dL: Add 50ul of 0ug/dL standard to 50ul of 0.5ug/dL standard.
   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.
5. Label pooled control and unknown sample Ab-coated tubes in duplicate.
   Pipette 200 µL of the 0ug/dL into the NSB and A tubes. Pipette 200 µL of each remaining standard, pooled control or unknown sample into the labeled tubes.
   **PIPETTE DIRECTLY TO BOTTOM OF TUBE.**
6. Add 1.0 mL of $^{125}$I Insulin to every tube and vortex. (Minimum of 10min from start to finish).
7. Following sample addition to tracer addition should be completed with minimal delay, with no more than 40 minutes elapsing between the addition of the first sample and the completion of tracer addition.
8. Incubate for 18-24 hours at room temperature
9. Decant thoroughly. Remove all visible moisture by patting inverted tubes.
10. Count for 1min on gamma counter.
11. Use Assay Zap (Biosoft, Cambridge, UK) to calculate unknown concentrations against standard curve.

# APPENDIX B

## 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:
   0.125ug/dL: Add 50ul of 0ug/dL standard to 50ul of 0.25ug/dL standard.
   0.25ug/dL: Add 50ul of 0ug/dL standard to 50ul of 0.5ug/dL standard.
   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 C

## GLUCOSE FOR PROTOCOL BOVINE SERUM

Intended for use with WAKO Autokit Glucose Series Enzymatic Method 439090901F. The following modifications were made to the protocol: The cuvettes used were 1.0 mL, therefore 6.7 uL of sample and 1.0 mL of color reagent was used.

**Materials Supplied**

1. Buffer Solution 2 x 150 mL
   60 mmol/L Phosphate buffer (pH 7.1) containing 5.3 mmol/L Phenol.
   Store at 2-10ºC
2. Color Reagent (When reconstituted) 2 x for 150 mL
   Contain 0.13 U/mL Mutarotase, 9.0 U/mL Glucose oxidase, 0.65 U/mL Peroxidase, 0.50 mmol/L 4-Aminoantipyrine, 2.7 Ascorbate oxidase.
   Store at 2-10ºC
3. Standard Solution I 1 x 10 mL
   Containing 200 mg/dL Glucose.
   Store at 2-10ºC
4. Standard Solution II 1 x 10 mL
   Containing 500 mg/dL Glucose.
   Store at 2-10ºC

Reagent Preparation

Working Solution:
Dissolve the whole contents of one bottle (for 150 mL) of Color Reagent in one bottle 150 mL of Buffer Solution. This solution is stable for one month at 2-10ºC.

**Materials Required But Not Supplied**

1. Pipettes
5. Waterbath that can hold constant 37ºC
6. Spectrophotometer

**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.

**Test Procedure**

Wavelength: 505*[1] Light path: 1 cm
Temperature: 37ºC

| | | Sample (S) | Standard (Std) | Blank (BL) |
|---|---|---|---|---|
| Pipette into a cuvette | | | | |
| Sample | (uL) | 6.7[B] | -- | *2 |
| Standard 1 or 2 | (mL) | -- | 0.02 | -- |
| Working Solution | (mL) | 3 | 3 | 3 |
| Mix well, incubate for 5 minutes and measure the absorbance of S ($A_s$) and Std ($A_{std}$) against Bl ($A_{bl}$) at 505 nm | | | | |

1. Accurately pipette 0.02 mL of sample or standard into the cuvettes[A] (test tubes)
2. Add 3.0 mL of Working solution.
3. Mix, incubate for 5 minutes and measure the absorbance of Sample ($A_s$) and Standard ($A_{std}$) against Blank ($A_{bl}$) at 505 nm.

*1 When measure with two wavelengths λ1/ λ2 = 505/600 nm
*2 The omission of 0.2 mL of water does not significantly affect the absorbance measured.

Concentration in the test

60 mmol/L Phospate buffer, 5.3 mmol/L Phenol, 0.13 U/mL Mutarotase, 9.0 U/mL GOD, 0.65 U/mL POD, 0.50 mmol/L 4-Aminoantipyrine and 2.7 U/mL AOD.

**Results**
Calculation

Glucose (mg/dL) = $A_S/A_{Std}$ x $C_{Std}$
$A_S$ = Absorbance of sample
$A_{Std}$ = Absorbance of Standard I or II
$C_{Std}$ = Concentration of Standard I or II in mg/dL

# VITA


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