THE EFFECT OF GLUCOSE UTILIZATION AND FEED EFFICIENCY ON BEEF CATTLE PRODUCTION

A Thesis

by

BROOK LYN BRADBURY

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

December 2011

Major Subject: Physiology of Reproduction

The Effect of Glucose Utilization and Feed Efficiency on Beef Cattle Production Copyright 2011 Brook Lyn Bradbury

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ABSTRACT

The Effect of Glucose Utilization and Feed Efficiency on Beef Cattle Production.

(December 2011)

Brook Lyn Bradbury, B.S., Kansas State University

Co-Chairs of Advisory Committee: Dr. Ronald D. Randel Dr. Thomas H. Welsh, Jr.

Feed efficiency and metabolism affect profitability of the various components of the beef industry by modulating distribution and use of nutrients within cattle. Separate studies were conducted to determine the 1) repeatability of feed efficiency measurements over time as beef heifers mature into cows, and 2) whether the production and regulation of glucose in heifers is affected by temperament.

The influence of temperament on glucoregulatory hormones was studied in Angus crossbred heifers and Brahman heifers whose temperament was determined at weaning. The 6 most calm and 6 most temperamental heifers of each breed were fitted with jugular cannulas. Blood was collected at cannulation and then via the cannula during a 90-min rest period. Following 90 min, dextrose was infused (0.5 mg/kg BW) and blood samples were collected at specific intervals for 3 h total. In the crossbred heifers cortisol (P = 0.0560) and glucose (P = 0.0485) concentrations during the challenge were higher in temperamental relative to calm crossbred heifers. Insulin concentrations tended (P = 0.0737) to be higher in temperamental crossbred heifers. Cortisol (P = 0.0282) and glucose (P = 0.0011) concentrations were significantly higher in temperamental Brahman heifers. Insulin concentrations tended (P = 0.0793) to be greater for calm Brahman heifers. Temperamental cattle had a greater HPA axis response, which led to greater concentrations of cortisol and glucose, possibly because the glucose was being utilized differently by the temperamental cattle.

Mature Brahman cow feed efficiency data was collected over two years, on two different cohorts of cows that had previous residual feed intake data as post-weaning heifers. In 2009 and 2010, 37 and 41 cows, respectively, in their first trimester of gestation were evaluated for RFI via the Calan gate system. Cows were fed 2.6% BW for 70 d with BW recorded weekly. Cows were classified according to their RFI values as either efficient or inefficient. Heifer RFI was not correlated to mature cow RFI based on assessment of the Pearson's correlation coefficient (r = -0.06, P = 0.57). This study indicates that establishment of RFI in heifers may not accurately predict their feed efficiency as mature cows.

DEDICATION

To the late Clyde Hale and Vyrel Nitcher. The two hardest working, selfless people I have ever met. Their strong dependence on the Lord's love and guidance got them through good and bad. They knew the true meaning of strength, perseverance, and love. Their example and guidance instilled in me a drive to work hard, pursue my dreams, and never give up.

For I know the plans I have for you," says the Lord. "They are plans for good and not for disaster, to give you a future and a hope - Jeremiah 29:11

ACKNOWLEDGEMENTS

First, I would like to thank God for giving me the ability to strive for my goals and for the opportunities that have been granted to me along the way. Faith in his reason for everything and leaning on his strength has allowed me to persevere through each obstacle and meet my goals.

The continued support and love of my family has made everything I've done possible. They are my rock and no matter how far away they are, I always know that I can count on them. My dad taught me the true meaning of hard work, faith in God, a firm handshake, and a love for agriculture. He has a strong love for his family and the simple life of farming that I am forever grateful for being a part of. My mom has a huge heart full of unconditional love. She is always there with a cheery voice and a good sense of humor to remind me to not sweat the small stuff, keep working hard, and appreciate everything. Bub has loved and put up with me, his little sister, for so many years and even still he never misses a chance to tell me how proud he is. His persistence and passion in his own life has always inspired me. My grandma, Violet, has the ability to make me laugh about everything. She always knows how to lift my spirits, whether it's a friendly phone call, card, or warm hug. Thanks to all of my friends, especially Katie and Paige that have persistently called when I forget to. Thank you for sticking with me from so far away and for being so encouraging over the last couple of years.

I want to express my heartfelt appreciation to my committee members: Dr. Randel, Dr. Welsh, Dr. Forbes, Dr. Vann, Dr. Riley, and Dr. Lawhon for making my time at Texas A&M a truly rewarding experience. I want to thank Dr. Randel, for taking a chance on a fellow Wildcat and Kansas kid. He is never short of patience, knowledge, or a good laugh, he is a truly great mentor. Thank you Dr. Welsh for providing guidance, inspiring curiosity, and always having an open office. He is always there to lend a helping hand, to tell a good story (or fact), and to provide a candy bar when you need it the most. Thank you Dr. Forbes for being there to answer my questions and for having a great sense of humor. I want to thank Dr. Vann for the good times spent together burning up and down the highway and her ability to explain everything in a practical manner. Thank you Dr. Riley for your kindness and patience, but most of all for taking the time to teach me. I want to thank Dr. Lawhon and her lab group for giving me the opportunity to learn and experience outside of my discipline.

I cannot thank everyone enough at Overton for making the time I spent at the research center truly unforgettable. Special thanks go to Don Neuendorff for being an inspiration and a friend. Thank you for taking me under your wing and for the countless good times, stories, and laughs. I would like to thank Dr. Jason Banta for his assistance with my projects. I also thank Jennifer, Joel, Gary, Allen, Dr. Higginbotham, Dave, Andy, the farm crew, and all of those at the center that went out of their way to make me feel at home.

Thanks to Dr.Forrest and Dr. Amstalden for the opportunity to teach, which I have found to be extremely rewarding. Dr. Harms, Dr. Sterle, Dr. Wickersham, all deserve a big thanks, as well as everyone else I interacted with in Kleberg, for making my time at TAMU a great experience. The past two years just wouldn't have been the

same without my fellow graduate students. I would like to thank Steven and Lisa Mapel for taking me in. They are incredible people, who have become my life- long friends. There are not enough words to thank Lisa for her patience, knowledge, and help not only in the lab, but with life itself. Thanks to Andrea Loyd for being an amazing friend (we mid-west girls had to stick together) and traveling pal; we spent many hours blazing down the road together. She is a genuine, kind friend who is never too busy to help or listen. To Gentrie Shafer, thanks for the great laughs, lunch time chats, and late night study sessions. Her zest for life and faith are inspiring and I'm fortunate to have her as a friend. Thanks to Shane Morgan and Anna Poovey for all the fun and laughs along the way. Thanks also to my second floor friends for their support and humor through the good and bad days.

Last, but not least I would like to thank Josh Gaskamp. His constant love and support has not only pushed me to finish, but to be a better person. Even on the worst days, he can make me smile and laugh. I could not have made it through the last two years without his endless patience, kind words, and weekend excursions. I would also like to thank Josh's family for their continual help and love.

I am exceptionally thankful for this once in a lifetime opportunity that has enabled to me grow not only as a scientist, but as a person. The friends, experiences, and knowledge that I will take away are irreplaceable, and for that, I am fortunate and forever grateful.

NOMENCLATURE

АСТН	Adrenocorticotrophic hormone
ADFI	Average daily feed intake
ADG	Average daily gain
ANOVA	Analysis of variance
BW	Body weight
СР	Crude protein
СРМ	Counts per minute
CRH	Corticotrophin releasing hormone
CV	Coefficient of variation
DMI	Dry matter intake
EBV	Estimated breeding value
EDTA	Ethylenediaminetetraacetic acid
EV	Exit velocity
F:G	Feed to gain ratio
FIG	Fasting insulin glucose ratio
GAS	General adaptation syndrome
GLM	General linear model
GTT	Glucose tolerance test
HPA	Hypothalamic pituitary adrenal axis
IACUC	Institutional Animal Care and Use Commitee

I.D.	Inner diameter
IGF-1	Insulin-Like Growth Factor-1
IIND	Insulinogenic index
MBWT	Mid-test metabolic weight
O.D.	Outer diameter
PVN	Paraventricular nucleus
RFI	Residual feed intake
SD	Standard deviation
SEM	Standard error of the mean
SNP	Single-nucleotide polymorphism
SRIF	Somatotrophin release inhibiting factor
TDN	Total digestible nutrients
VP	Vasopressin

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

In the beef cattle industry, efficient production is imperative. Cattle production is a multifaceted industry composed of many crucial elements that must be optimized in order to have an effective, successful business. Some factors that beef producers have no control over are weather, market, and government control; however, producers can play an active role in cattle health, nutrition, and breeding. When inputs exceed outputs, and human population growth increases as land availability decreases, the average farmer comes closer to business failure. In 2009, 30% of the family farms with beef cattle operations grossed negative farm incomes and in turn had to resort to off farm alternatives to supplement their income (USDA ERS, 2011a). On a much larger scale, United States beef production in 2009 added \$73 billion dollars and cow/calf production added another \$31.8 billion dollars to the economy (USDA ERS, 2011a). With an impact this large, the industry is recognized as noteworthy and well worth the time put into improvement and advancement of beef cattle production and improved profitability for beef producers.

One way to make a significant difference in efficiency and profitability is to reduce feed costs. Feed costs generally represent the largest segment of expenses accounting for 68% to 71% of the total costs associated with beef cattle production from

This thesis follows the format of the Journal of Animal Science.

2008 to 2010 (USDA ERS, 2011b). Recently feed costs have increased due to widespread drought and reallocation of crops formerly available for use as cattle feed to ethanol production. With increased feed costs, it is essential to identify cattle that will utilize feed efficiently. Typically, feed to gain ratio has been the standard measure of feed efficiency, which was introduced by Brody (1945), because of its ease of calculation. However, F:G has been found to have deep-seated errors that lead to undesirable traits. This led to the introduction of residual feed intake by Koch et al. (1963) as an alternative method.

RFI experiments have generally targeted weaned calves that would typically be back grounded or finished in a feedlot (Herd and Bishop, 2000; Basarab et al., 2003; Nkrumah et al., 2004, 2007). While important, these experiments offer little guidance for cow/calf producers retaining heifers or even for producers looking to preserve genetics in breeding lines. If RFI does not give a producer insight into how the next generation will perform then the question becomes: Is it really worth the time and money to determine their RFI?

Many aspects of beef production such as reproduction, feed efficiency, immune function, and carcass traits may be altered by high stress responsiveness or poor temperament. Cattle exhibiting excitable temperament have been reported to have lower ADG (Voisinet et al., 1997a; Fell et al., 1999), higher occurrence of dark cutters (Voisinet et al., 1997b), lower dressing percentages and body condition scores (Petherick et al., 2002), and reduced immunity (Fell et al., 1999). Temperament has been described as a fear or avoidance in response to human interactions (Fordyce et al., 1988b; Murphy

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et al., 1994). Certain interactions with humans can produce a stress response in cattle. Stress response increases the rate of metabolism, caused by the activation of the hypothalamic-pituitary-adrenal axis or stress axis.

Temperamental animals have been found to have higher circulating concentrations of cortisol (Curley et al., 2006; 2008). Cortisol is a glucocorticoid and plays a major role in metabolism due to its ability to influence glucose synthesis and use. Glucose tolerance tests, originally developed for humans, assess response of insulin to an infusion of an exogenous source of glucose. This test could be exploited to help understand the utilization of glucose in temperamental versus calm cattle, giving important insight into the allocation of energy, and possibly partially explaining why temperamental animals do not perform as well as calm animals.

Areas such as feed efficiency and temperament play vital roles in the close knit network that regulates performance of beef cattle. These experiments examine different components separately, but with the same goal of gaining a more complete understanding of factors affecting beef production. Improvements in efficiency of beef production will ultimately lead to advantages for both consumers and producers.

Temperament

Characterizing Animal Temperament

Temperament in cattle is generally defined as the response of an animal to being handled by a human (Fordyce et al., 1988a). From conception to slaughter, cattle are exposed to many forms of human handling. Livestock handling has generally been found to induce a fear response, great or small, to being handled by humans (Hemsworth and Coleman, 1998). However, Petherick et al. (2009) reported that fear can be reduced with proper human handling and association with positive events. The question then becomes: if fear is lessened does temperament change? In the same study by Petherick et al. (2009) temperament scores were reevaluated over a 12-month period (starting at 4 to 6 months) and no change was found. Studies have shown that animals handled at early ages for long periods of time have no difference in temperament, only some improvement in their ability to adapt to their surroundings (Boivin et al., 1992). Thus, animals are going to have a fear response to new surroundings or animals, sudden stimuli, and social interactions; it is not specific to just human interactions. The term "temperament" is used in science to distinguish an animal's response to humans, but in reality it represents the ease of excitability of an animal.

Evaluating for Temperament

Multiple methods have been developed and tested to measure temperament. These tests range from complex behavioral tests to simple, more subjective measures and assess cattle behavior in both restrained and non-restrained conditions. Restrained tests focus on animal response to a squeeze chute which is a staple tool used in cattle management. The objective of a restrained test is to subjectively evaluate the animals' response when confined in a squeeze chute or in some countries, what is referred to as a crush. Examples of restrained tests include crush score (Grandin, 1997), temperament test (Hearnshaw et al., 1979), and bail test (Fordyce et al., 1982). Non-restrained testing is aimed at assessing the amount of movement and haste in reaction to a variety of stressors. Flight speed (Burrow et al., 1988) and open field tests (Kilgour, 1975), are both examples of temperament testing that do not require restraining the animal. By combining a restrained and non-restrained test it may more closely relate to the animals "fight or flight" reaction. To successfully test for temperament the test should be easy, reliable, and relatively simple to incorporate into a beef cattle management system.

In Australia, the crush score (Tulloh, 1961) is used extensively in the beef cattle industry to select for calmer cattle. The crush score evaluates the degree of agitation an animal demonstrates while being confined and restrained in a crush (Tulloh, 1961). It is valued for its subjective assessment and ease of application. Crush scores are set on a scale of 1-6 with a calmer animal having a low crush score as compared to a high crush score for a temperamental animal. This is similar to the chute score as adapted by Grandin, (1993). Chute score is different in that the animal is in the squeeze chute, but not restrained and only assessed on a scale from 1-5 with lower numbers indicating calmer animals.

Two common methods used in our research are flight speed (Burrow et al., 1988) and pen score (Hammond et al., 1996). Flight speed, otherwise termed, exit velocity is the rate (m/s) at which the animal transverses 1.83 m after being released from a working chute (Curley et al., 2006). As the animal is released from the chute it crosses an infrared beam that starts the timer. After the animal has transversed 1.83 m the second plane of the infrared beam is broken and the timer stops. Exit velocity can be measured at any age, but has been found to be most accurate when observed at weaning (Burdick et al., 2009; 2011). Temperamental animals are those with higher exit velocities, while their calm counterparts will be slower coming out of the chute. Faster exit velocity has been found to be correlated with higher concentrations of serum cortisol in cattle (Fell et al., 1999; Curley et al., 2006). Cattle with slower measurements of flight speed (exit velocity) gain weight faster than those with faster flight speeds (Burrow and Dillon, 1997; Müller and von Keyserlingk, 2006). Pen score (Table 1.1.) is a subjective measurement using a scale of 1 to 5 to rank the animal's responsiveness to a human observer (Hammond et al., 1996). Low values of pen score indicate animals with calmer or more docile temperaments, while higher values indicate aggressive and unpredictable animals.

Table 1.1. Observations associated with the individual categories of pen scores (Hammond et al., 1996).

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Pen Score	Description
1	Walks slowly, can be approached slowly, not excited by humans
2	Runs along fences, stands in corner if humans stay away
3	Runs along fences, head up and will run if humans come closer, stops before hitting gates and fences, avoids humans
4	Runs, stays in back of group, head high and very aware of humans, may run into fences and gates
5	Excited, runs into fences, runs over anything in its path

Those two scores (exit velocity and pen score) can then be averaged together to form a temperament score that has been used to assign animals to calm, intermediate, or temperamental categories (Curley et al., 2006, 2008; King et al., 2006). Temperament score is an average of an objective and subjective measurement of temperament. It has been observed that objective measurements are stronger alone than subjective observation, but a temperament score using both exit velocity and pen score provides information on more than one facet of cattle behavior making it a more inclusive assessment (Vann et al., 2011).

Temperament is predominantly innate and found to be heritable in *Bos taurus* cattle breeds; for example: German Angus (0.61) and Simmental (0.53) (Gauly et al., 2001). Loyd et al. (2011) has recently reported that pen score, exit velocity, and temperament score are moderately to highly heritable, 0.44, 0.28, and 0.41, respectively, in Brahman cattle. Hoppe et al. (2010) observed heritability differences for flight speed (exit velocity) between breeds of German Angus (0.20), Charolais (0.25), Hereford (0.36), Limousine (0.11), and German Simmental (0.28). These studies suggest that temperament can be included effectively as a selection tool for beef cattle producers.

Temperament and Beef Production

Temperamental animals pose a threat to themselves, other animals, facilities, and their handlers. The impact that temperamental animals may have goes beyond immediate destruction and injury as it also affects production efficiency. Producers and researchers have considered cattle temperament an important trait for years. Early work found that nervousness could be related to elevated energy requirements (Hafez and Lindsay, 1965) and decreased conception rates (Pounden and Firebaugh, 1956).

Recent findings indicate that excitable cattle have decreased average daily gains (ADG) (Voisinet et al., 1997b; Petherick et al., 2002) and body condition scores relative to calmer cattle (Petherick et al., 2003). Café et al. (2011) observed significant decreases in time spent eating, feed intake, and feedlot and back-grounding performance. Sires that are more excitable tend to have progeny with lower yearling body weights (Burrow and Dillion, 1997) and excitable dams have inhibited milk production (Drugociu et al., 1977; Breuer et al., 2000). Temperament has also been linked to decreases in immune function (Fell et al., 1999; Oliphint, 2006), allowing cattle to be more susceptible to disease-causing pathogens (Oliphint, 2006).

The effects of poor performance have also been found to alter meat quality. Cattle that are more excitable have less fat (Café et al., 2011), lighter carcass weights, and less tender meat (King et al., 2006); more specifically they have been found to have a higher Warner-Bratzler shear force value (as indication of tenderness) than calm animals (del Campo et al., 2010). Excitable temperament in cattle also leads to a greater bruise score (Fordyce et al., 1985; Fordyce et al., 1988b), darker meat (Voisinet et al., 1997a), increase carcass pH and abnormal meat flavor (Fordyce et al., 1988b; King et al., 2006). As concluded by Vann et al. 2008, temperamental cattle have higher treatment costs and lower net profits than their calm counterparts, due to the fact that temperament not only affects ease of handling, but also feedlot performance and carcass quality (Busby, 2005). The big picture is that temperamental cattle cost producers more inputs, provide less output, and have a greater chance of harming themselves, other animals, facilities, and the producers.

Temperament and the Hypothalamic-Pituitary-Adrenal Axis

Stress

Stress has many facets, is widely studied, and has been found to profoundly affect the health and productivity of all living animals. Stress can be categorized as physical, psychological, or interoceptive, but generally contains a combination of the three categories (von Borell, 2011). The concept of stress was first recognized by Walter Cannon (1914). Cannon (1914) detected the short- term stress response known as the fight or flight syndrome, in which the adrenal medulla serves as an emergency function to quickly trigger the release of epinephrine. Selye (1936) observed that when rats underwent a non-specific, acute potentially harmful event, a syndrome would appear that causes swelling of adrenal glands and shrinkage of the thymus, lymph nodes, spleen, and liver. These symptoms are labeled the "alarm stage", and if the stress continues will lead to a resistance stage, and then exhaustion stage (death). This syndrome now known as General Adaptation Syndrome (Selye, 1973) is how the body copes with stress. The amount of damage that the organism sustains will depend on its ability to adapt to the stress. Further research demonstrates that the majority of the stress response is mediated by the hypothalamus, anterior pituitary, and adrenal glands working in synchrony to elicit a response in reaction to a multitude of events.

To the general public stress has a negative connotation. Stress to most people implies worry, fear, anxiety or mental strain. Outside of the scientific world, stress has become a broadly used term and can be confusing to the general population when actually talking about stress biology. Stress can be characterized as good stress (eustress), bad stress (distress) (Selye, 1975) and even further categorized as acute or chronic stress. Eustress is termed a good stress, with the idea that it results in a beneficial adaption reaction. The outcome of distress would be an unsuccessful adaptation reaction (Selye, 1975). The degree of the stress affects the severity and duration of effects on homeostasis. The longer the stress reaction, the more detrimental effects it can have. It is important to realize that all stress is not bad and that events that trigger stress vary by species.

Role of HPA in Stress Response

The HPA axis (Figure 1.1) is involved in the reaction to stress and its role is to adapt the organism to the physical, biological, and psychosocial environment. This role is actually quite large, as the HPA axis must facilitate adaption of the organism in its entirety to everyday life, and also individually affect single responses of the body (tissues, organs, cells) and as well as more complex systems (immune and brain). As stress levels build a cascade of reactions in the HPA axis occur. The medial parvocellular and magnocellular divisions of the lateral paraventricular nucleus (PVN) of the hypothalamus will be triggered to synthesize corticotrophin-releasing- hormone (CRH) (Vale et al., 1981) and vasopressin (VP) (Martini and Morpurgo, 1955) and store them to the median eminence (Guillemin and Rosenburg, 1955; Saffran et al., 1955). CRH and VP are then secreted from the axon terminal and act upon the corticotropic cells to stimulate the secretion of adrenocorticotrophic hormone (ACTH) from the corticotropic cells of the anterior pituitary (Liu et al., 1983). This release of ACTH triggers the secretion of glucocorticoids from the adrenal cortex.



Figure 1.1. Regulation of glucocorticoid secretion (modified from Axelrod and Reisine, 1984). ACTH = Adrenocorticotrophic hormone; CRH = corticotrophin-releasing hormone; SRIF = somatotropin releasing inhibitor factor (somatostatin); VP = vasopressin.

Relationship of Temperament and Glucoregulatory Mechanisms

Relationship of Glucose and Insulin

Glucose is a carbohydrate used by cells for energy and is the most important cellular energy source. It is vital to life as it is the primary source of metabolic energy for the central nervous system because there is no oxidation of ketones in the brain. Glucose is also required for the turnover and synthesis of fat, as a precursor of muscle glycogen and as an essential metabolite for lactating and gestating ruminants (McDowell, 1983). Ruminants are far more efficient in digesting complex carbohydrates than monogastrics. This is possible because the rumen contains microorganisms that assist in the breakdown of fibrous material such as cellulose and hemicelluloses (Hocquette and Abe, 2000). The fermentation of carbohydrates produces volatile fatty acids, mainly acetate, propionate, and butyrate. Volatile fatty acids derived from rumen fermentation provide up to 70 % of the energy requirements of a ruminant (Bergman, 1973). Low levels of glucose are absorbed from the diet in ruminants and therefore glucose must be synthesized from the liver. Eighty five to ninety percent of glucose production occurs in the liver and the rest is produced in the kidney (Bergman, 1973, Lindsay, 1978). Propionate accounts for the largest amount of glucose synthesis, accounting for up to 76% of liver glucose synthesis (Reynolds et al., 1994). Almost all of the propionate absorbed into the portal vein is removed by the liver and used for glucose synthesis. Other precursors for glucose synthesis include glycogenic amino acids, lactate, glycerol, i-butyrate and n-valerate (Leng, 1970). When animals are fasted, glycerol and amino acids from adipose and muscle tissue, respectively, are precursors to glucose synthesis,

as a result of propionate quantities being greatly reduced (Bergman, 1973). Intake is important to provide the substrate to support glucose synthesis and provide enough glucose available for the animal. If glucose concentrations become too high then insulin will be secreted to decrease glucose concentrations.

Insulin, a metabolic hormone that is synthesized and secreted from the beta cells of the islets of Langerhans of the pancreas, primarily regulates the concentration of glucose in the blood by lowering the concentration (Banting et al., 1923). Insulin is in control of intermediary metabolism, organizing what fuels are stored or oxidized. The rising and falling of insulin is regulated by the amount of glucose present (Porte and Puppo, 1969). When blood concentrations of glucose are elevated, insulin is secreted and serves to affect the liver and peripheral tissues to return the blood concentrations to homeostatic levels (Meglasson and Matschinsky, 1986). This insulin release inhibits gluconeogenesis and glycogenolysis and promotes glucose uptake by the liver as well as fat and muscle tissue (Hocquette and Abe, 2000).

As insulin begins to bind to its' receptors, the receptors will fuse to the plasma membrane and insert glucose transporters (GLUT 1-4). These transporters allow glucose to enter the tissue (muscle, liver, adipose, central nervous system, etc). GLUT 2 specifically works in the gut, liver, and pancreatic cells, while GLUT 4 is present in insulin-sensitive tissue, skeletal tissue, adipose tissue, and the heart. These transporters facilitate diffusion of the glucose into beta cells, which elevates the glucose concentration in the extracellular fluid. This allows high concentrations of glucose to enter the cell and subsequently depolarize the membrane, which stimulates the influx of

calcium (Hocquette and Abe, 2000). The influx of calcium is thought to activate the exocytosis of insulin containing secretory granules. Humans tend to be less sensitive to insulin than cattle. Humans need a concentration of 30 uIU/mL of insulin to reduce glucose production (Rizza et al., 1982), where cattle need 50-60 uIU/mL to reduce glucose production by 50% (Brockman, 1983). As blood concentrations of insulin decline the receptors will no longer be bound and the transporters will be recycled back into the cytoplasm. Some tissues, such as the brain and liver are not insulin dependent in their regulation of glucose uptake. The second important effect of insulin is to stimulate the liver to store glucose as glycogen. When blood glucose concentrations are too high, hepatocytes will immediately uptake the glucose absorbed from the circulation and store it as glycogen. As glucose concentrations become too low, insulin will signal release of glucagon (Samols et al., 1972), which will in turn stimulate the breakdown of glycogen into glucose. Insulin recognizes the concentration of glucose present (low or high) and works to store or utilize glucose in an effort to maintain homeostasis.

Glucocorticoids and Glucoregulatory Mechanisms

Cortisol is the predominate glucocorticoid in cattle and is an influential component in adjusting blood glucose concentrations. Glucose homeostasis is imperative during a stress response since additional energy will be needed. As a result of altered blood glucose concentrations during a stress response, exaggerated insulin concentrations may be released (Munck et al., 1984). The first to respond are the catecholamines and glucagon by inhibiting insulin-mediated glucose uptake as well as increasing substrates for gluconeogenesis. Within minutes glucagon can increase glucose production by activating gluconeogenesis or glycogenolysis (Unger et al., 1962). Epinephrine's roll is more intricate as it can stimulate glucose production and limit its utilization. Actions of epinephrine are mediated by alpha and beta adrenergic mechanisms and act in minutes (Rizza et al, 1980). If stress is drawn out then glucocorticoids will assist the first responders in the regulation of other glucose mechanisms (Sapolsky et al., 2000). Since insulin is the primary facilitator of cellular uptake of glucose, glucocorticoids reduce the number of insulin receptors in an effort to counteract the role of insulin. It is thought this reduction in the uptake of glucose into adipose, lymphoid, and skin tissues, caused by the glucocorticoids, leads to catabolism in those tissues (Munck, 1971). Increases in blood concentrations would therefore result from glucocorticoid induced gluconeogenesis.

Glucocorticoids have two roles: activate enzymes needed to induce gluconeogenesis (Pilkis and Granner, 1992) and to increase availability of the substrates needed for gluconeogenesis through lipolysis and proteolysis (Exton, 1987). With the assistance of glucocorticoids, catecholamines can induce triglyceride hydrolysis, increasing the concentrations of nonesterified fatty acids (Dallman et al., 1993). Glucocorticoids have also been discovered to increase utilization of amino acids for carbohydrate production, causing increased urea production (Long et al., 1940).

As an outcome of these actions a stressed animal is likely to not perform as well due to compromised growth. When an animal is subjected to stress, maintenance for survival becomes a priority, outweighing the need for growth or development. By blocking the absorption of glucose into certain cell types it ensures that energy is available and not being used for lower priority processes. The length of the stress period dictates how much trauma the animal will sustain. Significant losses of lipid and protein stores can occur during long periods of distress (Sapolsky et al., 2000). This can be detrimental to growth, in addition to the fact that the mechanisms of growth will be inhibited during periods of stress. Periods of stress are not favorable to maintain growth, reproduction, lactation, or development, as a result of the lack of energy and physiological mechanisms necessary to maintain homeostasis.

Glucose Tolerance Test

Glucose tolerance tests are designed to monitor the insulin response after administration of exogenous glucose. An increase in insulin secretion should cause an influx of glucose into the animal's tissues (Abdelmannan et al., 2010). Insulin secretion rate is a sigmoidal function of glucose in plasma (Lemosquet and Faverdin, 2001). To conduct a glucose tolerance test, glucose is infused and blood samples are taken at distinct time points over a specific period of time. The results of the test will determine the relationships between glucose and insulin that are produced and the time that insulin takes to clear glucose from the system. Glucose and insulin concentrations make it possible to calculate glucose half life, the time to glucose half life, the peak insulin concentration, and time to peak insulin. This information proves valuable for understanding the differences in insulin sensitivity through metabolic pathways. In order to observe the ratio of insulin to glucose at a given time point, a baseline sample must be taken after 12 hours of fasting and prior to the glucose tolerance test.

Glucose tolerance testing is most commonly used in humans to detect type-2 diabetes, but has also made its way into the dairy cattle industy. Insulin has a direct effect on the partitioning of nutrients that are vital for the synthesis of milk constituents between the mammary glands and other tissues. Therefore, understanding the differences that nutrion and physiological states can play in altering milk quality and production are imperative to the industry (Lemosquet and Faverdin, 2001). Having a test of glucoregulatory mechanisms has led dairy scientists to a better understanding of the role of glucose in many other areas as well, such as illness, dietary changes, medication, and exercise. More specifically glucose tolerance testing has led to discoveries of metabolic disorders (Bossaert et al., 2008) during early lactation (Terao et al., 2010), and a better understanding of nutritional effects on milk production (Lohrenz et al., 2010). As of today there are no studies using glucose tolerance tests to examine the direct link between temperament and glucose tolerance. Temperamental animals have greater concentrations of basal cortisol, which remain greater than calmer cattle when stressed (Café et al., 2011). With this in mind it would be expected that more excitable cattle experience a higher peak in insulin concentration as cortisol rises. Cortisol is a glucocorticoid and therefore blocks the absorption of glucose into tissues such as adipose and muscle, increasing the amount of glucose to be cleared from the circulation. From this we hypothesize that temperamental cattle will have greater concentrations of insulin in response to infusion of an exogenous glucose source. If true, this may lead to insight
into how feed is utilized in the calm or temperamental animal and explain why temperamental cattle have lower average daily gains.

Insulinogenic Index

Glucose tolerance testing was developed to test humans for risk of type-two diabetes. The test however is useless without a quantitative way to assess the results. Therefore, the insulin and glucose concentrations collected throughout the glucose tolerance test are typically subjected to a ratio or index in order to determine the insulin sensitivity of the patient being tested. There are multiple ratios and indexes that could be utilized, but the focus for this thesis will be on insulinogenic index.

In 2001, Guerrero–Romero and Rodriguez-Moran tested the fasting insulin to glucose ratio to determine whether it was correlated with impaired glucose tolerance. This study was completed over a three yr time period utilizing adult humans. Humans were fasted over night and then administered 75 grams of glucose orally, with blood samples collected at time 30 min and 130 min post ingestion. For this study IIND was simply the ratio of insulin to glucose concentration present at the time of sample, numbers were not adjusted for baseline concentrations. From this index we can determine the insulin sensitivity of the sampled patient. This study demonstrated that high fasting insulin to glucose ratio is highly correlated to impaired glucose tolerance, which may lead to decreased glucose metabolism or type-2 diabetes (Guerrero-Romero and Rodriguez-Moran, 2001).

The other approach to insulinogenic index, as demonstrated by Abdelmannan et al. (2010) would be to use the insulin to glucose ratio at each sampling point, but only after removing the baseline concentrations of insulin and glucose. Utilizing fasted, adult humans, a baseline sample was collected and then 75 grams of glucose was administered orally. Blood samples were then collected at 30, 60, 90, and 120 min post ingestion. The baseline sample is taken prior to the glucose tolerance test and represents the basal concentrations for the specific patient. The goal of this study was to determine the proper dosing and timing of dexamethasome as a stress test to indicate possible development of type-2 diabetes. Due to small numbers and lack of testing within patients actually prone to type-2 diabetes further testing is needed (Abdelmannan et al., 2010).

The insulinogenic index is utilized to determine the sensitivity of insulin to an influx of glucose. From this index you can characterize how likely a patient may be to the development of glucose metabolism disorders. For the work in this thesis we decided to utilize the IIND that does not remove baseline concentrations. This index will capture the whole response profile and take into account the differences between the cattle temperaments. By removing baseline concentrations the IIND would have only showed the specific response of the animals and not accounted for basal differences.

Feed:Gain Ratio

Traditionally, efficiency has been evaluated using the feed to gain ratio as presented by Brody (1945), which represents the amount of feed consumed relative to the

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body weight gained. An animal with a high F:G ratio requires more feed to put on a unit of weight as compared to an animal with a low F:G ratio. It has been preferred over other methods because of its simplicity and minimal costs. Over the years it has been used as a selection tool to improve feed efficiency, but further research has found that F:G ratio has flaws that could have a profound impact on beef cattle production. Animals with different intake and of different sizes can have the same F: G ratio and one animal may have several different F:G ratios depending on its stage of growth (Sainz and Paulino, 2004). Composition of gain, growth rate and body size in growing cattle have all been found to be negatively correlated with F:G (Mrode et al., 1990; Koots et al., 1994; Herd and Bishop, 2000; and Arthur et al., 2001b). Over time F:G ratio has resulted in selecting for high growth rate and larger body size (Arthur et al., 2001a). This inevitably leads to larger framed cattle at maturity (Herd and Bishop, 2000). Large framed cattle are undesirable in beef production systems, as they are far less efficient, requiring greater amounts of nutrients and increased energy maintenance requirements (Barlow, 1984).

Residual Feed Intake

Residual feed intake, introduced by Koch et al. (1963), has been proposed as an alternative to F:G ratio. To calculate RFI, linear regression is used to estimate feed intake from BW and average daily gain (Koch et al., 1963). The predicted daily feed intake value is obtained by regressing daily dry matter intake on ADG and mid-test-metabolic body weight. RFI is then calculated as the difference in the actual feed intake

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for an individual animal, compared to the predicted feed intake (may be above or below), which is based on the animal's size and growth rate (Archer et al., 1999). Unlike F:G ratio, RFI is phenotypically independent of growth rate and body weight (Kennedy et al., 1993; Herd and Bishop, 2000; Arthur and Herd, 2005), leading to less alterations in mature body size and feed consumption (Koch et al., 1963; Nkrumah, 2004; Arthur, 2001b, 2004). Animals that eat more than predicted will have a positive RFI and are said to be more inefficient than other animals in their cohort. Animals that eat less than predicted are identified as more efficient animals, with a negative RFI value. Lancaster et al. (2005) observed that high RFI calves consumed 15 % more feed than their low RFI counterparts when calves were separated by ± 0.5 standard deviation from the mean. Similarly, Bingham et al. (2009) reported that high RFI heifers (Brangus) consumed 22.5% more feed than the lower RFI heifers when separated based on ± 1 standard deviation from the mean. RFI is not a direct measurement of feed efficiency. It does imply that it is a function of feed consumed, body weight gain, and average weight within a cohort, throughout the course of a trial.

There are a number of reasons why RFI has become favored over F:G. One major point is that selecting for negative RFI (efficient animals) will increase feed efficiency in successive progeny without an impact on mature body size or feed consumption (Koch et al., 1963). RFI has a strong phenotypic and genotypic genetic correlations at 0.40 and 0.98, respectively (Archer et al., 2002). Other research has shown that heritability estimates RFI range from 0.16 (Herd and Bishop, 2000) to 0.47 (Lancaster et al., 2009). This means producers may be able to reduce feed intake and

sustain the same body size, while still improving the efficiency of a herd by selecting for negative RFI (Herd et al., 2003). The key to RFI becoming a selection tool lies in determining whether it is heritable and repeatable. If neither of these is true than there will be little genetic progress and RFI will not be a tool to predict feed efficiency. As of now RFI is not widely used in cattle production due to the large cost and time needed to complete the feeding period.

Evaluating Cattle for RFI

Breedtypes, Sex, Age

The concept of residual feed intake has been in existence for almost 60 years now but as of yet there is not a standard calculation (Knott et al., 2008). There has been progress in determining the proper variables to consider when forming a cohort of animals for determining RFI. In cattle varying in sex, age, and breed type there will be differing total energy requirements for maintenance (NRC, 2000). Significant differences in RFI for divergent breedtypes were reported (Schenkel et al., 2004; Riley et al., 2007) demonstrating that comparing across breeds is not appropriate. *Bos indicus* and *Bos indicus* x *Bos taurus* breeds will have 10% and 5%, respectively, lower maintenance requirements than British breeds (Carstens et al., 1989). Brahman influenced cattle that were compared to Angus influenced cattle in a sub-tropical environment had a tendency to be more efficient than the Angus influenced cattle (Elzo et al., 2009). Multiple studies conducted over many years established that cattle of different gender will perform unequally (Brinks et al., 1961; Bogart et al., 1963; Wilson et al., 1969). As concluded by NRC (2000), bulls will typically have maintenance requirements that are 15% greater than those steers or heifers that are of the same genotype. Elzo et al. (2009) detected that heifers are less efficient than steers and that steers are less efficient than bulls (Nkrumah et al., 2004).

When comparing animals of different ages it is evident that maturity patterns may produce variation between animals. Carstens et al. (1989) reported that cattle from age 9 -20 months of age had an 8% decrease in required metabolizable energy required for maintenance. Calves studied in the same cohorts, at two different ages had only moderate correlations (r = 0.55) (Crews et al., 2003) and (r = 0.59) (Johnston, 2007) from post-weaning to feedlot. Originally it was thought that the incorporation of metabolic BW in the RFI model would account for differences in age and breedtypes (Arthur, 2001b), but after further analysis multiple studies have proved the theory wrong. In order to collect the most precise RFI value, cattle of the same breedtype, age, and sex should be used when evaluating RFI (Herd and Arthur, 2009).

Physiological Status and Production and Production Level

As beef cattle physiological status changes, then also their energy requirements should change due to the energy constraints at that time. Maturing cattle go through multiple states such as: growth, maintenance, gestation, lactation. In order to be productive at all of these stages it is crucial to have positive energy maintenance. Lactating cows have maintenance requirements that are 20 % greater than a nonlactating, mature cow (NRC, 2000). Crossbred beef cattle of the same body size and growth rate were categorized into low, medium, and high milk production levels, those cattle characterized as low required 12 % less energy per unit of metabolic weight than the other cattle to maintain their weight through gestation and lactation (Montano-Bermudez and Nielsen, 1990). Additionally, they found that milk production differences accounted for 23 % of the variation in maintenance requirements and on average an 18% increase in maintenance requirements from gestation to lactation. It is apparent that even in beef cattle, lactation status can play a large role in the maintenance requirements of a mature female.

For cattle transitioning between feeding phases, the net energy required for an animal to gain weight is conditional to the proportion of protein and fat deposited within the tissue. Lean protein and adipose tissue deposition comes at different energy costs due to their diverse chemical composition (NRC, 2000). Phases such as growing and finishing differ in protein and fat deposition and so the energy requirements therefore differ between the two phases.

Diet

A concern with using RFI evaluation is the lack of a standard protocol, particularly lack of a standard diet. Prior research demonstrated that the type and amount of feed may impact the results of an RFI test. Cattle can either have *ad libitum* availability to feed, which allows them to express appetite or be limit-fed, basically

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eradicating the influence of appetite. The majority of RFI studies conducted allow *ad libitum* access to the diet; however, the amount fed should coincide with the production goals of the animals being fed. It seems appropriate to put animals in a feedlot setting on *ad libitum* feed availability, but this does not reflect the true feed efficiency of heifers being retained as breeding females. The argument has been made that RFI determined in a feedlot setting should be relevant to a cowherd (Arthur et al., 2001a; Arthur and Herd, 2005); but minimal studies have tested this theory. Herd et al. (1998) and Meyer et al. (2008) found similar results when comparing females evaluated for RFI on a high concentrate diet and then evaluated again as cows on pasture. In both studies no difference was found between the dry matter intake of cows previously determined efficient.

When testing the effect of type of feed, Fan et al. (1995) observed a significant difference in RFI values calculated for bulls on two differing diets (concentrate vs. roughage). Angus and Hereford bulls that were fed a high roughage diet had a negative RFI relative to bulls fed a high concentrate diet (-1.67 ± 0.12 vs. 0.36 ± 0.12 kg/d). Conversely, Goonewardene et al. (2004) concluded in crossbred steers that as the proportion of roughage increased RFI became more positive and as the proportion of grain was increased, RFI decreased. Durunna et al. (2011) used crossbred steers to study the effect of diet type. Treatment group one was only fed a finishing diet, treatment two only a growing diet and treatment three was fed the growing diet followed by the finishing diet. The only calves to maintain their RFI were the calves on the finishing diet. The other two treatment groups did not maintain the same RFI with calves on the

diet changing treatment having the greatest number of calves switching RFI (efficient or inefficient). These results suggest that animals may perform differently depending on the diet provided.

Estimated Feed Intake

There are currently two methods used to estimate feed intake in cattle. The original method as described by Koch et al. (1963) which uses linear regression of actual feed intake on growth rate and mid-test BW. Later, this model was modified to use metabolic BW instead of actual BW (Arthur et al., 1996; Knott et al, 2008). This modification accounts for the wide discrepancy of maintenance requirements that have been reported between animals, even when they are at similar production levels (Montano-Bermudez and Nielsen, 1990).

The second method of determining expected feed intake for RFI utilizes equations to calculate expected feed intake rather than using the actual data. BW and growth rate using NRC requirements are utilized to estimate the net energy requirements for maintenance and growth (Fan et al., 1995). Nutrient content of the feed provided is used to determine the expected feed intake for each individual animal. After further scrutiny, Fan et al. (1995) observed correlations between RFI and ADG of (r = -0.58) in Angus bulls and (r = -0.50) in Hereford bulls. In the same study, negative correlations were found between RFI and yearling weight of Angus (r = -0.53) and Hereford (r = -0.44) bulls. After examining this model, Knott et al. (2008) concluded that this model overestimated feed consumption in 6 month old sheep and underestimated intake in 13 month old sheep. From these conclusions it appears to be more appropriate to use the linear regression model to estimate feed intake for RFI determination.

Test Duration

In order to determine RFI, animals must be fed for a period of days. This feeding period requires a large expense for feed and as a result would be most ideal if the duration was as short as possible. Initially, it was suggested that a 168 day feeding trial was needed to accurately assess RFI in cattle (Koch et al., 1963). From there the feeding period was reduced to 140 days (McPeake et al., 1986) and then 112 days (Kemp, 1990; Brown et al., 1991). In order to find the optimal number of days to feed, Archer et al. (1997) did extensive testing in Angus, Hereford, and Shorthorn heifers and bulls. The number of days was gradually increased in each feeding trial from 7 to 119 days. At the conclusion of the trial it was determined that the variation between RFI was minute after day 70. For that reason, a 70 day feeding period was deemed an adequate amount of time to accurately assess RFI. The downfall to this study is that only British breeds were incorporated and Robinson et al. (1997) documented that Bos indicus and Bos taurus cattle managed in the same feedlot setting had diverse feeding behaviors. This influenced Archer and Bergh, (2000) to examine what are sufficient days on feed for Angus, Hereford, Simmental, Afrikaner, and Bonsmara young bulls. It was concluded that a 70 day feeding period was also ample for breeds of cattle other than British. These studies suggest that a 70 day feeding trial is the shortest and most accurate duration to determine RFI.

Season

There have been few studies investigating the effect of seasonality on feed efficiency in beef cattle. Mujibi et al. (2010) examined the differences in performance and feed efficiency in crossbred steers tested in either fall and winter or winter and spring for three consecutive years. Correlations between feed intake and air temperature, relative humidity, solar radiation, and wind speed observed in the fall/winter were: -0.26, 0.23, 0.30, -0.14 and 0.31, 0.04, 0.14, and 0.16 for the winter/ spring, respectively (Mujibi et al., 2010). The nature and magnitude of seasonality were significantly (P <0.05) different. The authors still suggested that season possibly affects feed intake and feed efficiency and noted that more data was needed to make a conclusion.

As season and temperature changes, beef cattle will have altered performance and energy expenditures (NRC, 2000). When cattle begin to reach critical thresholds they will no longer maintain their thermoneutral zone and both feed intake and production will decline with the upper threshold or increase when the upper threshold is met. Animals exposed to extreme heat will have to work harder to dissipate heat and animals at critically low temperatures will increase metabolism to increase body temperature, in either case maintenance energy requirements will increase (NRC, 2000). Therefore it seems reasonable that the season in which cattle are tested could alter feed efficiency results.

Sources of Variation of RFI

In order for RFI to be used economically as a tool to determine feed efficiency an indirect marker is needed that will eliminate the need for costly and lengthy trials. By understanding the biological mechanisms that influence feed efficiency, it may be possible to decipher why feed consumption differs among cattle, accounting for maintenance and production requirements. Discovering the traits that are responsible for phenotypic expression of feed efficiency could lead to the identification of indirect markers of feed efficiency. Historically, study of other feed efficiency traits has suggested that there is not a single mechanism controlling the phenotypic feed efficiency (Oddy, 1999). This has led many scientists to investigate numerous biological mechanisms for their role in the expression of feed efficiency.

Composition of Gain

As cattle mature the amount of fat deposition increases, while long bone growth and protein accrual decrease. This means that slower maturing cattle will deposit less fat by a given age than the faster maturing animals. In the long run those faster maturing cattle will require more energy to deposit fat (Trenkle and Willham, 1977) and will have decreased efficiency as they fatten due to the higher energetic costs of depositing adipose tissue (Gregory et al., 1962). RFI has been positively correlated with gain in 12th rib back fat thickness (r = 0.30); P < 0.05) and final 12th rib back fat thickness (r = 0.20; P < 0.05; Lancaster et al., 2009) and gain in empty body fat (r = 0.22; P < 0.01; Basarab et al., 2003). Moderate, negative correlations between RFI and lean carcass content (r = - 0.22; P < 0.05) and lean growth rate (r = -0.33; P < 0.05) suggests that efficient (low RFI) cattle have a larger percentage of lean muscle than their inefficient counterparts (Herd and Bishop, 2000). Low RFI steers have more bone and protein and less fat content than the high RFI steers, this could imply a difference in maturity patterns of cattle with divergent RFI (Richardson et al., 2001). Despite these observations body compositions has only been estimated to account for 5 (Richardson and Herd, 2004) to 9 (Lancaster et al., 2009) percent of the total variation in RFI.

Feeding Behavior

Animals in a healthy state generally maintain the same feeding behavior (Nkrumah et al., 2007), however the behavior of cattle fed in the same environment has been shown to be extremely inconsistent (Robinson et al., 1997; Gibb et al., 1998). For that reason it is assumed that the deviation in feeding behavior between animals may potentially be a source of variation in observed RFI. Animals that are more inefficient have been reported to have more frequent eating bouts per day (Golden et al., 2008), head-down time, feed duration, and increased daily pedometer counts (Richardson et al., 2001; Nkrumah et al., 2007; Lancaster et al., 2009). Growing and finishing steers (with the same ADG) that had low RFI consumed 19-22% less feed than growing and finishing steers with high RFI (Brown, 2005). Hafla (2011) found that heifers with low RFI had lower DMI (9.00 vs. $11.6 \pm 0.54 \text{ kg/d}$; P < 0.01) when compared to heifers with high RFI; however, initial BW and ADG were similar during the trial. This would suggest that high RFI cattle likely spend more time eating and more time walking to and from the

bunk. The increased physical activities in high RFI cattle require an energetic cost and therefore may partially explain reduced efficiency. Cohorts should be given the same basic feeding conditions in order to reduce energy loss due to fighting for bunk space or locomotion. This could also be a major factor in *ad libitum* feeding versus limit-fed diets. With limit-fed diets animals are more likely to eat in one setting, decreasing the effect of eating behavior on energy expenditure.

Feed Digestibility

Not all animals have the same abilities to digest and absorb nutrients efficiently, a large impetus for the establishment of RFI to select for those that are more efficient. Multiple studies have reported that high RFI cattle have increased daily feed intake, as compared to low RFI cattle. Increased daily feed consumption, increases ruminal passage rate, and decreases the amount of time feed remains in the rumen for digestion (Grovum and Hecker, 1973). This led researchers to believe that the increased feed intake of high RFI cattle may actually be result in reduced digestion and nutrient absorption. High RFI steers recovered 10% less metabolizable energy than low RFI steers and were also found to have a negative correlation with metabolizable energy (r = -0.44; P < 0.05; Nkrumah et al., 2006). Low RFI heifers had a higher dry matter (731 vs. 705 ± 12 g/kg dry matter; P < 0.05) and crude protein (691 vs. 657 ± 13 g/kg dry matter digestibility than high RFI heifers. This coincides with the trend (P < 0.10) that high RFI cattle have decreased digestibility compared to low RFI cattle (Richardson et al., 1996). The study only reported a 1% difference in digestibility between RFI classes, but the

authors thought that this small difference could account for as much as 14% of the detected differences in feed efficiency.

Indirect Measures of RFI

The testing process associated with RFI is extremely costly and time consuming due to the fact that measuring individual feed intake is necessary to calculate RFI accurately. This testing process is estimated to cost between \$150 and \$450 per head on a 70-d test period. Multiple groups have tried to group-feed cattle and derive pen estimates of feed efficiency (Guiroy et al., 2001; Tedeschi et al., 2006; Williams et al., 2006). These estimates are then subjected to a mathematical model that weakens the inherent differences in intake between the pen mates (Moore et al., 2009). As a result researchers have refocused on trying to find an indirect measure of RFI that would reduce the costs associated with RFI assessment.

Insulin Like Growth Factor-1

Insulin like growth factor-I, is a peptide hormone related to growth and development that is produced by the liver in response to growth hormone released from the anterior pituitary. Its primary role is to travel to various tissues and stimulate glucose metabolism, protein synthesis and growth (Baxter, 1986). IGF-I is also produced in the lungs, kidneys, heart, stomach, gonads, muscle, and bone (Daftary and Gore, 2005). Circulating concentrations of IGF-I are easily quantifiable and heritable (Herd et al., 1995), as well as correlated with growth traits in cattle (Bishop et al., 1989; Davis and Simmen, 1997). Due to its role in growth and development and ease of measuring, it has been proposed that IGF-I could possibly be an indirect measure of feed efficiency in cattle.

IGF-I concentrations in *Bos taurus* cattle were found to be positively correlated with RFI (Johnston et al., 2002). In growing young animals, plasma IGF-I concentrations were reported to be phenotypically positively correlated and positively genetically correlated (r = 0.56) (Moore et al., 2005). However, further work in Brangus heifers, found no correlation between RFI and IGF-I (Lancaster, 2007). Similarly, Kelly et al. (2010) found no correlation between RFI and plasma IGF-I in yearling beef heifers. From this study they hypothesized that the inconsistency may be due to the differences in age and diets between the studies. Younger animals may have a greater rate of lean tissue gain and reduced carcass fattening, hindering the IGF-I concentrations in circulation. Caldwell (2009) also reported no correlation between IGF-I and RFI in various purebred and crossbred cattle. From these studies there is no apparent correlation between ages and breeds to warrant their use in feed efficiency detection.

Genetic Markers

Genetic indicators have been found that are correlated between RFI and feed efficiency traits. In a whole-genome study of feedlot cattle of varying breedtypes and RFI values it was discovered that 161 single nucleotide polymorphisms are significantly related to RFI (Barendse et al., 2007). It may be possible that genetic markers are more accurate than circulating analytes; 20 of the most significant SNPs accounted for 76% of the genetic variation in RFI (Moore et al., 2009). IGENITY® (Merial Limited, Duluth, GA) and GeneSTAR® (Pfizer Animal Genetics, 2009), are genetic tests that are now available to identify feed efficiency in individual animals. According to their reports genetic correlations between the markers and RFI existed, but only 15% of the variation in feed intake is accounted for by these tests. An outside party (National Beef Cattle Evaluation Consortium, 2009) tested GeneSTAR® and only found a phenotypic RFI correlation of ($\mathbf{r} = 0.40$; P = 0.02) in *Bos taurus* cattle and no correlation (P = 0.55) in *Bos indicus* cattle. With these varying results, caution should be exercised when deciding how to utilize these tests and results.

Estimated Breeding Values

Estimated breeding values are statistical predictions of the relative genetic value of a particular animal of a specific trait. EBVs are used by producers to more accurately select animals for their breeding herds. For nearly ten years, EBVs have been published for RFI. They were developed based on within and across herd comparisons from individual RFI feeding trials (Sherman et al., 2009). The accuracy of these tests looks promising as Richardson et al. (2004) reported that Angus steers had a positive correlation with their respective sire's RFI EBV (r = 0.35; P < 0.05). More work is needed and producers should be sure to always make breeding decisions based on a multiple trait selection index, never a single trait selection (Crews et al., 2005).

Repeatability of RFI from Post-Weaning Heifer to Mature Cows

Despite the fact that there are substantial costs with cow maintenance related to overall costs of the production system (Montano-Bermudez and Nielsen, 1990), few studies have assessed the repeatability of RFI from the post weaning heifer to mature cow. An essential issue is whether RFI measured early in an animal's life represents the same RFI as the animal matures. The answer to this question could alter the significance of RFI and its' power to determine feed efficiency accurately in cattle.

Of the minimal studies focusing on RFI repeatability, most have been focused on younger animals, at two different feeding periods. Durunna et al. (2011) used three groups of steers to examine repeatability over age and diet type. The calves were fed in two 70 day trials with RFI calculated at the completion of each trial. The first treatment group was fed only a finisher diet, the second only a grower diet, and the third was fed the grower diet first and then the finisher diet. Calves on the finisher diet were the only group that maintained their RFI; the other two treatments did not maintain the same RFI classifications, with the calves on two different diets having the greatest number of calves changing efficiency classifications (Durunna et al., 2011). Crossbred heifers with RFI determined post-weaning were reevaluated as mature cows and had a moderate correlation (r = 0.53) of RFI phenotypes between evaluations, although no correlation of the RFI values from the two evaluations was detected (Minton, 2010). A very recent study by Loyd, (2011) found similar conclusions between heifers of divergent breeds, with RFI values recorded post-weaning, and then re-evaluated as lactating cows. Second parity cow/calf pairs were moved into a Growsafe system and fed ad libitum for 70 d for

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RFI evaluation. The cow RFI values were then compared to their heifer RFI values and ranks (low, medium, high). The relationship between RFI of the heifer and as a lactating cow was very lowly correlated (r = 0.19) and there was also no relationship between heifer RFI rank and cow RFI rank (r = 0.02). There was minimal correlation between heifer RFI rank and cow RFI (r = 0.23) and a low correlation between heifer RFI and cow RFI rank (r = 0.15). From these observations it was concluded that selecting for the most feed efficient heifers, may not result in the same level of efficiency when they become lactating females. This follows Archer et al. (1998) who stated that cattle efficiency during post-weaning may be altered later in maturity due to physiological states, such as gestation and lactation that would require more energy.

Alternatively, Herd et al. (1998) noted that it was possible to have a phenotypic connection between RFI determined in confinement as post-weaned heifers and their performance on pasture as mature *Bos taurus* cows. Pre-pubertal crossbred heifers re-evaluated post-pubertally were found to have correlation between measurements (r = 0.48) with 32.5 % of the heifers changing their RFI phenotype (Loyd, 2009). Crews et al. (2003) reported that one cohort of steers had a correlation (r = 0.55) between RFI determinations during growing and finishing phases. A similar correlation (r = 0.59) was found between post-weaning RFI and feedlot RFI in a single group of calves (Johnston, 2007). More recently, heifers evaluated first during a growing phase and then during a finishing phase were found to have a greater association of RFI than F:G between phases (Kelly et al., 2010). Just over half (54%) of the heifer's RFI values re-ranked varied by 0.5 a standard deviation and just 24% changed by a full standard deviation (Kelly et al.,

2010). From these conclusions we proposed to examine the repeatability of RFI from post-weaning heifers to mature cows, in an effort to determine if RFI can remain unchanged throughout the process of maturation.

CHAPTER II

EFFECT OF TEMPERAMENT ON RESPONSE TO CANNULATION AND GLUCOSE CHALLENGE IN CROSSBRED BEEF HEIFERS

Introduction

The term "temperament" is used to characterize an animal's response to being handled by a human (Burrow, 1997). Cattle that demonstrate more excitable temperaments have been found to have a lower ADG (Voisinet et al., 1997a; Fell et al., 1999), lower dressing percentage, body condition scores (Petherick et al., 2002), and a higher incidence of dark cutters (Voisinet et al., 1997b). The lower performance of these animals is complex, but the role of cortisol in energy metabolism may give insight into differences between temperamental and calm animals.

Human-animal interaction can produce fear and as a result the animal becomes stressed. This stress will in turn activate the HPA axis triggering a cascade of endocrine mediated events that will eventually lead to the release of a glucocorticoid. In humans and domestic livestock, the glucocorticoid released in response to stress is cortisol. As a glucocorticoid, cortisol plays a key part in metabolism due to its ability to influence glucose synthesis and use. HPA axis functional characteristics are different between cattle of diverse temperaments (Curley et al., 2008). Cattle that are more excitable have greater concentrations of stress hormones (such as cortisol and epinephrine) which are correlated with temperament (King et al., 2006; Curley et al., 2006; 2008, Burdick et al., 2010). Through the use of glucose tolerance testing it is possible to assess response of insulin to an infusion of an exogenous source of glucose. This test could be exploited to help understand the utilization of glucose in temperamental versus calm cattle, giving insight into the allocation of energy, and may partially explain why temperamental animals do not perform as well as calm animals. The objective of this study was to determine the affect of temperament on blood glucose and insulin following a stressor and a subsequent glucose challenge.

Materials and Methods

Animals and Experimental Design

Angus crossbred heifers (n = 37) at the Brown Loam Experiment Station in Raymond, MS were weighed (mean weight = 244.33 kg), pen scored, and exit velocity recorded at weaning (mean age = 10 mo), June 8, 2010. All processes required to complete this project were approved by the Texas A&M University IACUC. Pen scores were assessed by an experienced observer. Three to five animals were placed in a pen and assigned pen scores from 1 to 5 according to their reaction to the observer. Exit velocity was obtained as they were released from the chute. Exit velocity is the rate (m/s) that the calf travels 1.83 m (Burrow et al., 1988). Infrared beams and timers were utilized to record the time to travel this distance. The exit velocity and pen score were then averaged for each animal to generate their temperament score. The 6 most temperamental and 6 most calm of the weaning group were utilized for the glucose tolerance test. The mean temperament score of the 6 most calm and 6 most temperamental were (1.77 ± 0.17) $(4.37 \pm 0.17; P < 0.0001)$ (Table 2.1), respectively.

Variable	Temperament		P- Value
	Calm	Temperamental	
Weaning Weight (kg)	263.84 ± 12.14	248.57 ± 12.14	0.3946
Exit Velocity (m/s)	1.36 ± 0.25	4.56 ± 0.25	< 0.0001
Pen Score	2.17 ± 0.17	4.17 ± 0.17	< 0.0001
Temperament Score	1.77 ± 0.17	4.37 ± 0.17	< 0.0001

Table 2.1. Weaning characteristics of crossbred heifers (n = 12) utilized for GTT.

In order to incorporate all heifers (n = 12) the glucose challenge took place over the span of two days, July 28 & 29, 2010, with six animals each day. Animals were randomly assigned to a day, with three calm and three temperamental calves on each of the two days. Each night the calves to be glucose tolerance tested the next morning had access to water, but were fasted for 12 h prior to cannulation.

Glucose Tolerance Testing

Day one, July 28, 2010, (n = 6) heifers were fitted with jugular cannulas to allow for blood collection. At each sampling one 10 mL EDTA coated Vacutainer® tube (366643, BD Biosciences; Franklin Lakes, NJ) and one 10 mL no additive Vacutainer® tube (366430, BD Biosciences; Franklin Lakes, NJ) for serum was collected for each animal. Pre-challenge blood samples that were taken: initial (as soon as they were caught in the chute), jugular (when the jugular was punctured), and test (as the cannula was checked for functionality). The average time elapsed from the initial sample to the test sample was approximately 10 minutes. To insert the cannula for blood collection an area over the jugular vein was clipped and prepped. All cannula materials were sterilized prior to use by gas sterilization. After donning sterile gloves, a sterile 14-gauge needle was inserted into the jugular vein. Approximately 15 to 20 cm of a 1.0 m length of tygon tubing (0.10 cm i.d., 0.18 cm o.d.) was passed through the needle and into the jugular vein. The spare tubing was secured to the heifer's neck using glue, adhesive tape, and vet wrap. An 18-gauge needle with a 10 mL syringe was used to plug the end of the tubing. Prior to capping, the line was flushed with a heparin solution (1 IU/mL) to maintain patency of the cannula. After cannulation each animal was placed in an individual stall. At the completion of the 6th calf, the heifers were allowed a 1.5 h rest period. Blood samples were collected at 30, 60, 90 min relative to the completion of cannulation. After the rest period of 2 h, a blood sample was collected at -5 and 0 min relative to glucose infusion. After the sample was collected at time 0 min, a 50% dextrose solution was infused at 0.5 mL/kg BW via the jugular cannula. Time 0 was used as a baseline concentration of cortisol, glucose, and insulin. Following infusion blood samples were collected at 10, 15, 20, 30, 40, 60, 80, 100, 120, 140, 160 and 180 min relative to glucose infusion. Following collection at each time point an equivalent volume (10 mL) of sterile saline was replaced via the cannula, followed by heparinized saline (5 mL) to keep the cannula patent. At completion of the glucose challenge cannulas were removed and heifers were returned to their original pens. The next day,

July 29, 2010, the remaining six heifers were cannulated, rested, challenged, and sampled following the same protocol as the day before.

Blood Samples and Analysis

Blood samples were centrifuged at 2000 X g for 25 min at 4° C to harvest plasma or serum. Tubes coated with EDTA were centrifuged within 30 min of collection to yield plasma and serum tubes were allowed to clot @ 4° C overnight before centrifugation. Plasma and serum samples were aliquoted into 12 X 75 mm plastic culture tubes and stored at -20° C. Plasma samples were removed from storage and assayed for concentrations of glucose and insulin. Serum samples were removed from storage and assayed for concentrations of cortisol.

Cortisol RIA

Concentrations of cortisol were determined by radioimmunoassay Coat-A-Count kit which is commercially available (Siemens Healthcare Diagnostic, Los Angeles, California). Unknown concentrations of cortisol were calculated using Assay Zap software (Biosoft, Cambridge, UK) using counts per minute obtained from a Cobra II auto-gamma-counter (Perkin Elmer, Waltham, MA). All cortisol samples were analyzed in a single assay and the inter-assay and intra-assay CV was 13.11% and 6.44%, respectively.

Glucose Colorimetric Assay

Concentrations of glucose were determined by the manual protocol of the commercially available Autokit Glucose (Wako Chemical USA, Inc., Richmond, VA). All glucose samples were analyzed using a single assay and the intra-assay CV was 3.00 %.

Insulin RIA

Concentrations of insulin were determined by radioimmunoassay Coat-A-Count kit that is commercially available (Siemens Healthcare Diagnostic, Los Angeles, California). Unknown concentrations of insulin were calculated using Assay Zap software (Biosoft, Cambridge, UK) using counts per minute obtained from a Cobra II auto-gamma-counter (Perkin Elmer, Waltham, MA). Inter-assay and intra-assay CV were 8.68% and 8.49%, respectively.

Statistical Analysis

Repeated measures ANOVA were conducted using the MIXED procedures of SAS (2002) for analysis of cortisol, insulin, and glucose concentrations. Fixed effects of interest were temperament group, time, and their interaction. Animal was the random effect. Insulinogenic index was calculated by dividing the concentration of insulin by the concentration of glucose at each time point a sample was collected. Insulinogenic index

was analyzed as repeated measures using the MIXED procedure of SAS (2002) using the same fixed and random effects. Time to peak concentration of insulin, peak concentration of insulin, half-life concentration, and time to glucose half-life concentration were evaluated using the GLM procedures of SAS (2002).

Results

Pre-Challenge Period

Initial cortisol samples were higher in temperamental heifers than calm heifers 52.44 ± 7.42 versus 41.67 ± 8.13 , respectively. During the pre-challenge (cannulation) period temperamental heifers had numerically higher concentrations of cortisol, which remained elevated over the course of the cannulation period (Figure 2.1). Temperamental heifers had greater (P = 0.0496) concentrations of insulin (Figure 2.2) and a strong tendency to have greater concentrations of glucose (P = 0.0517) (Figure 2.3). Time of sample was significant for cortisol (P < 0.0001), glucose (P = 0.0001), and insulin (P = 0.0123). There was no significant time by temperament interaction for cortisol or insulin, but there was for glucose (P = 0.0324) during the pre-challenge period.



Figure 2.1. Cortisol concentrations over the course of the pre-challenge period (cannulation) in calm (grey) or temperamental (black) crossbred heifers. Temperament effect (P = 0.1675), time effect (P < 0.0001), and temperament x time effect (P = 0.2112). Mean SEM (calm) = 7.691; (temperamental) = 7.02.



Figure 2.2. Insulin concentrations over the course of the pre-challenge period (cannulation) in calm (grey) or temperamental (black) crossbred heifers. Temperament effect (P = 0.0496), time effect (P = 0.0123), temperament by time effect (P = 0.2153). Mean SEM (calm) = 1.790; (temperamental) = 1.64.



Figure 2.3. Glucose concentration over the course of the pre-challenge period (cannulation) in calm (grey) or temperamental (black) crossbred heifers. Temperament effect (P = 0.0517), time effect (P < 0.0001), temperament x time effect (P = 0.0324). Mean SEM (calm) = 16.915; (temperamental) = 15.94.

Challenge Period

Heifers that were more temperamental tended to have higher concentrations of cortisol (P = 0.0560) throughout the glucose challenge (Figure 2.4). There was no difference in glucose concentrations between temperaments (Figure 2.5); however, temperamental heifers had greater (P = 0.0485) glucose half-life concentrations. Glucose half-life concentrations (mg/dL) for calm and temperamental heifers were 108.09 ± 5.29 and 124.91 ± 5.29 , respectively (Table 2.2). Calm heifers (88.17 ± 13.07) reached glucose half-life sooner (min) than temperamental heifers (93.50 ± 13.07) (Table 2.2). Insulin concentrations had a tendency (P = 0.0737) to be greater in temperamental heifers (Figure 2.6). Time was significant for cortisol (P < 0.0001), glucose (P < 0.0001), gluco (0.0001), and insulin (P = 0.0001) concentrations. There was a significant time by temperament interaction for glucose (P = 0.004) and insulin (P = 0.0112), but not cortisol during the glucose challenge. Peak insulin concentrations had a tendency to be greater in temperamental heifers (P = 0.0851), but there was no difference in the time (min) to peak insulin concentration between temperamental (30.00 ± 5.82) and calm (23.33 ± 5.82) heifers (Table 2.2). Peak insulin concentrations (uIU/mL) for the calm and temperamental heifers were 27.52 ± 12.96 and 62.54 ± 12.96 , respectively (Table 2.2). There was no statistical difference in insulinogenic index between temperaments, although, numerically, temperamental heifers had a higher insulinogenic index as shown in Figure 2.7. Time was significant for insulinogenic index (P < 0.0001), but there was no significant time by temperament interactions throughout the glucose challenge.



Figure 2.4. Cortisol concentrations for the duration of the glucose challenge (3 h) in calm (grey) or temperamental (black) crossbred heifers. Temperament effect (P = 0.0560), time effect (P < 0.0001), temperament x time effect (P = 0.2595). Mean SEM = 6.37.



Figure 2.5. Glucose concentrations for the duration of the glucose challenge (3 h) in calm (grey) or temperamental (black) crossbred heifers. Exogenous glucose (0.5 mL/kg BW) infused at 0 min. Temperament effect (P = 0.1229), time effect (P < 0.0001), temperament x time effect (P = 0.0004). Mean SEM = 12.17.

Variable	Temperament		P -Value
	Calm	Temperamental	
Insulin Peak Concentration (uIU/mL)	27.52 ± 13	62.54 ± 13	0.0851
Insulin Peak Time (min) Glucose Half Life Concentration	23.33 ± 5.80	30.0 ± 5.80	0.4369
(mg/dL)	108.09 ± 5.30	124.91 ± 5.30	< 0.0001
Glucose Half Life Time (min)	88.17 ± 13.10	93.5 ± 13.10	< 0.0001

Table 2.2 Crossbred heifer peak insulin and glucose half-life concentrations.



Figure 2.6. Insulin concentrations for the duration of the glucose challenge (3h) in calm (grey) or temperamental (black) crossbred heifers. Temperament effect (P = 0.0737), time effect (P < 0.0001), temperament x time effect (P = 0.112). Mean SEM = 6.16.



Figure 2.7. Insulinogenic index values for calm (grey) and temperamental (black) crossbred heifers. Temperament effect (P = 0.1169), time effect (P < 0.0001), temperament x time effect (P = 0.0620). Mean SEM = 0.04.

Discussion

Pre-Challenge

By exposing the heifers in this study to an acute stressor (cannulation period), it elicited a stress response and allowed their reaction to be observed. The cannulation period served as a profile and verification that the cattle of each temperament responded to stress with elevated concentrations of cortisol as observed by King et al., (2006), Curley et al., (2006, 2008), Burdick et al., (2010). The temperamental heifers began the study with greater concentrations of cortisol, which remained elevated throughout the study. As for the calm heifers, they did have an increase in cortisol when exposed to stress but dropped to normal concentrations during the rest period. This correlation between temperament and cortisol concentration has been observed in previous studies (Curley et al., 2006, 2008; Café et al., 2011).

Glucose and insulin concentrations for the calm heifers remained at a steady state throughout the pre-challenge period. The temperamental heifers remained steady throughout cannulation, but had increased glucose and insulin concentrations during the rest period. The heifers were all fasted 12 h prior to the study to remove the interference of postprandial glucose concentrations (Evans et al., 1975). Therefore, the increase in glucose concentrations in temperamental heifers may be due to the stimulation of gluconeogenesis. Café et al. (2011) found similar results of increased cortisol and glucose during their pre-challenge period for steers that were more temperamental. As the temperamental animals continued to stay excited, gluconeogenesis may have occurred to supply the body with needed energy. From the pre-challenge period we can
conclude that temperamental heifers had greater concentrations of cortisol that remained higher than the calm heifers during the pre-challenge period.

Challenge Period

Very little glucose tolerance testing has been used in animal work. The test was developed for humans to assist with type-2 diabetes testing. Glucose tolerance testing is utilized most in the dairy industry to characterize metabolic physiology in various facets of milk production and disease (Lohrenz et al., 2010, Teroa et al., 2010). For this study the goal was to determine if differing cortisol concentrations in animals of divergent temperaments could be playing a role in glucose utilization. Therefore, we utilized the glucose tolerance test to observe the response of glucose and insulin to an infusion of exogenous glucose in calm and temperamental heifers.

Temperamental heifers started the glucose challenge with greater concentrations of cortisol, which remained elevated over the calm heifers throughout the course of the glucose challenge. Baseline samples for the calm heifers are slightly elevated and tend to decrease as the glucose challenge continues. Cortisol concentrations for the temperamental heifers seem to remain highest until 60 minutes into the challenge, where there appears to be some decrease in concentrations. Even if an animal is producing less cortisol, it will take around 30 minutes to see any difference in the collected samples. On average about 30 minutes into the glucose challenge temperamental animals started releasing less cortisol. At 0 min glucose was infused through the cannula and a spike in glucose is observed at the 10 min sample, representing the exogenous source. Heifers that are temperamental have greater concentrations of glucose than the calm heifers out to the 60 min sample. As the glucose challenge continues out past 60 minutes, the heifer's glucose concentrations steadily drop and come closer together. Cortisol concentrations also begin to drop at the 60 min time sample, decreasing the inhibitory effect on glucose and possibly allowing for similar glucose concentrations to be achieved to the calm heifers. Glucose half-life is achieved about five minutes sooner in the calm heifers than the temperamental heifers. However, temperamental heifers had significantly greater concentrations of glucose at half-life. Glucose was infused by body weight, and therefore greater concentrations of glucose should be due to greater concentrations of glucose prior to infusion. This appears to be the case as temperamental heifers had greater glucose concentrations prior to glucose infusion and continued to have higher concentrations of glucose after the exogenous source was infused.

As for insulin, temperamental heifers had a large insulin response, especially from 20 to 60 min. It may be assumed that the large increase in insulin was to help lower blood glucose concentrations, by signaling glucose to be taken up by adipose and muscle tissue (Hocquette and Abe, 2000). Calm heifers had a much smaller increase in insulin concentrations. Peak insulin concentration was reached about 7 minutes sooner in the calm heifers than the temperamental, but temperamental heifers had almost 2.5 times the peak insulin concentration than the calm heifers. Glucose half-life was reached at about the same time; therefore glucose appeared to be removed from circulation at about the same rate between temperaments. Therefore, it took greater concentrations of insulin to remove the greater concentrations of glucose that were circulating in the temperamental heifers.

The IIND, as used in human studies, represents the sensitivity of insulin to the concentration of glucose present. It is calculated by dividing the concentration of insulin by the concentration of glucose at a certain time point (Guerrero-Romero and Rodriguez-Moran and, 2001; Abdelmannan et al., 2010). Statistically there was no difference between index values between temperaments. There was a tendency for an interaction between temperament and time (P = 0.0625). Numerically, the temperamental heifers had higher IIND values that peaked 30 to 40 min after glucose infusion. A higher index value for the temperamental heifers implies that they had a greater response of insulin to the influx of glucose than the calm heifers. Temperamental heifers were releasing a greater concentration of insulin to clear the greater concentration of glucose from circulation. Utilizing the GTT and IIND it was possible to capture the response of insulin to an influx of glucose and determined that female crossbred heifers that are temperamental need greater concentrations of insulin to clear glucose from circulation.

Conclusion

Temperamental heifers in this study had greater concentrations of cortisol throughout cannulation and the challenge. This in turn led to greater concentrations of glucose, which stimulated greater concentrations of insulin to help clear glucose from circulation to return to homeostasis. Therefore, throughout this stressful period less

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glucose is being stored and more is being utilized by the body for immediate energy. In conclusion, this may suggest that temperamental cattle do not utilize glucose as efficiently as calm animals and this may partially explain the lower performance of temperamental cattle. As with most systems of the body, metabolism is complex, and further research should be done to discover the other possibilities effecting performance of temperamental cattle.

CHAPTER III

EFFECT OF TEMPERAMENT ON RESPONSE TO CANNULATION AND GLUCOSE CHALLENGE IN BRAHMAN HEIFERS

Introduction

Cattle that exhibit more excitable behavior are more complicated to work with and create a safety hazard for the handlers, themselves, facilities, and other animals. Temperament has not only been found to be hazardous, but also has an impact on production, efficiency, and performance in cattle. Cattle that have more excitable temperaments have been found to have a lower ADG (Voisinet et al., 1997a; Fell et al., 1999), lower dressing percentages, body condition scores (Petherick et al., 2002), a higher incidence of dark cutters (Voisinet et al., 1997b) and decreased meat tenderness (del Campo et al., 2010) when compared to their calmer counterparts. However, how temperament biologically alters performance is not well understood.

Fear elicited by human interaction, a sudden stimulus in nature, or unfamiliar species, may stimulate a stress response in cattle. The stress response will then activate the HPA axis triggering a cascade of endocrine mediated events that will lead to the release of cortisol in cattle. Cortisol is a glucocorticoid, and as a result, plays a role in mediating metabolism by influencing the synthesis and use of glucose. Concentrations of cortisol and epinephrine, which are correlated to temperament, have been found to be greater in temperamental cattle when compared to less excitable cattle (King et al., 2006; 2008, Burdick et al., 2010). Elevated concentrations of cortisol in

temperamental cattle have been shown to increase losses due to dark cutters (Lacourt and Tarrant, 1985), decreased carcass lean tissue content (Trenkle and Topel, 1978), and reduced growth rates (Obst, 1974; Purchas et al., 1980).

It is apparent that cortisol and temperament have a substantial effect on performance; however, it is crucial to know why calm and temperamental cattle perform differently. Therefore, our objective was to determine the effects of temperament on blood glucose and insulin following a stressor and a subsequent glucose challenge. The utilization of glucose tolerance testing allowed us to assess the response of insulin to an infusion of an exogenous glucose source to test differences in utilization of glucose between temperamental and calm cattle. This could give insight into the allocation of energy, and partially explain why temperamental animals do not perform as well as calm animals.

Materials and Methods

Animals and Experimental Design

Brahman heifers (n = 36) at the Texas Agrilife Research Center in Overton, TX were weighed (mean weight = 180.30 kg), pen scored, and recorded for exit velocity at weaning (mean age = 7.1 mo). All processes required to complete this project were approved by the Texas A&M University IACUC. Pen scores were assessed by an experienced observer. Three to five animals were placed in a pen and assigned pen scores from 1 to 5 according to their reaction to the observer. Exit velocity was obtained as they were released from the chute. Exit velocity is the rate (m/s) that it takes the calf

to travel 1.83 m (Burrow et al., 1998). Infrared beams and timers were utilized to record the time as they left the chute. The exit velocity and pen score were then added and averaged for each animal to generate their temperament score. From these observations a temperament score was assigned and the 6 most temperamental and the 6 most calm of the weaning group were utilized for the glucose tolerance test. The mean temperament score of the 6 most calm and 6 most temperamental were (1.59 ± 13.33) and $(4.21 \pm$ 13.33; P < 0.0001), respectively (Table 3.1).

In order to incorporate all heifers (n = 12) the glucose challenge took place over the span of two days November 3 & 4, 2010, with six animals each day. Animals were randomly assigned to a day, with three calm and three temperamental calves on each of the two days. Each night the calves to be glucose tolerance tested the next morning had access to water, but were fasted for 12 h prior to cannulation.

Variable	Temperament		P - Value
	Calm	Temperamental	
Weaning Weight (kg)	195.30 ± 13.33	188.11 ± 13.33	0.7106
Exit Velocity (m/s)	1.51 ± 0.17	3.59 ± 0.17	< 0.0001
Pen Score	1.67 ± 0.19	4.83 ± 0.19	< 0.0001
Temperament Score	1.59 ± 0.09	4.21 ± 0.09	< 0.0001

Table 3.1. Weaning characteristics of Brahman heifers (n = 12) utilized for GTT.

Glucose Tolerance Testing

Day one, November 3, 2010, (n = 6) heifers were fitted with jugular cannulas to allow for blood collection. At each sampling one 10 mL EDTA coated Vacutainer® tube (366643, BD Biosciences; Franklin Lakes, NJ) and one 10 mL no additive Vacutainer® tube (366430, BD Biosciences; Franklin Lakes, NJ) for serum was collected for each animal. Pre-challenge blood samples that were taken: initial (as soon as they were caught in the chute), jugular (when the jugular was punctured), and test (as the cannula was checked for functionality). The average time elapsed from the initial sample to the test sample was approximately 10 minutes. To insert the cannula for blood collection, an area over the jugular vein was clipped and prepped. All cannula materials were sterilized prior to use by gas sterilization. After donning sterile gloves, a sterile 14-gauge needle was inserted into the jugular vein. Approximately 15 to 20 cm of a 1.0 m length of tygon tubing (0.10 cm i.d., 0.18 cm o.d.) was passed through the needle and into the jugular vein. The spare tubing was secured to the heifer's neck using glue, adhesive tape, and vet wrap. An 18-gauge needle with a 10 mL syringe was used to plug the end of the tubing. Prior to capping, the line was flushed with a heparin solution (1 IU/mL) to maintain patency of the cannula. After cannulation each animal was placed in an individual stall and a sample was collected (chute). At the completion of the 6th calf. the heifers were allowed a 1.5 h rest period. Blood samples were collected at 30, 60, 90 min relative to the completion of cannulation. After the rest period of 2 h, a blood sample was collected at -5 and 0 min relative to glucose infusion. After the sample was collected at 0 min, a 50% dextrose solution was infused at 0.5 mL/kg BW via the jugular

cannula. Time 0 was used as a baseline concentration for cortisol, glucose, and insulin. Following infusion blood samples were collected at 10, 15, 20, 30, 40, 60, 80, 100, 120, 140, 160 and 180 min relative to glucose infusion. Following collection at each time point an equivalent volume (10 mL) of sterile saline was replaced via the cannula, followed by heparinized saline (5 mL) to keep the cannula patent. At completion of the glucose challenge, cannulas were removed and heifers were returned to their original pens. The next day, November 4, 2010, the remaining six heifers were cannulated, rested, challenged, and sampled following the same protocol.

Blood Samples and Analysis

Blood samples were centrifuged at 2000 X g for 25 min at 4° C to harvest plasma and serum. EDTA coated tubes were centrifuged within 30 min of collection to yield plasma and serum tubes were allowed to clot over night at 4° C before centrifugation. After centrifugation plasma and serum samples were aliquoted into 12 X 75 mm plastic culture tubes and stored at -20° C. Plasma samples were removed from storage and assayed for concentrations of glucose and insulin. Serum samples were removed from storage and assayed for concentrations of cortisol.

Cortisol RIA

Concentrations of cortisol were determined by radioimmunoassay Coat-A-Count kit which is commercially available (Siemens Healthcare Diagnostic, Los Angeles, California). Unknown concentrations of cortisol were calculated using Assay Zap software (Biosoft, Cambridge, UK) using counts per minute obtained from a Cobra II auto-gamma-counter (Perkin Elmer, Waltham, MA). All cortisol samples were analyzed in a single assay and the inter-assay and intra-assay CV was 13.11% and 6.35%, respectively.

Insulin RIA

Concentrations of insulin were determined by radioimmunoassay Coat-A-Count kit that is commercially available (Siemens Healthcare Diagnostic, Los Angeles, California). Unknown concentrations of insulin were calculated using Assay Zap software (Biosoft, Cambridge, UK) using counts per minute obtained from a Cobra II auto-gamma-counter (Perkin Elmer, Waltham, MA). Intra- and inter-assay CV were 9.80% and 9.66%, respectively.

Glucose Colorimetric Assay

Concentrations of glucose were determined by the manual protocol of the commercially available Autokit Glucose (Wako Chemical USA, Inc., Richmond, VA). All glucose samples were analyzed using a single assay and the intra-assay CV was 3.00 %.

Statistical Analysis

Repeated measures ANOVA were conducted using the MIXED procedures of SAS (SAS Inst., Inc., Cary, NC) for analysis of cortisol, insulin, and glucose concentrations. Fixed effects of interest were temperament group, time, and their interaction. Animal was the random effect. Insulinogenic index was calculated by dividing the concentration of insulin by the concentration of glucose at each time point a sample was collected. Insulinogenic index was analyzed as repeated measures using the MIXED procedure of SAS (2002) using the same fixed and random effects. Time to peak concentration of insulin, peak concentration of insulin, half-life concentration, and time to glucose half-life concentration were evaluated using the GLM procedures of SAS (2002).

Results

Pre-Challenge Period

Initially during the pre-challenge period temperamental heifers had greater cortisol concentrations (ng/mL) 54.2 ± 8.6 than the calm heifers 19.9 ± 8.6 . During the cannulation period the serum concentration of cortisol was greater (P = 0.0238) in the temperamental heifers relative to that of the calm heifers, and remained elevated over the course of the pre-challenge period (Figure 3.1). There was a significant difference by time (P < 0.0001), but not a significant time by temperament interaction (P > 0.05). Temperamental heifers had greater (P = 0.0005) concentrations of glucose throughout the cannulation period (Figure 3.2). Differences by time and the time by temperament interaction for glucose were not significant. There was no significant (P > 0.05) difference in insulin concentration between temperaments or by time, but there was a significant (P = 0.0078) time by temperament interaction (Figure 3.3).



Figure 3.1. Cortisol concentrations over the course of the pre-challenge period (cannulation) in calm (grey) or temperamental (black) Brahman heifers. Temperament effect (P = 0.0238), time effect (P < 0.0001), temperament by time effect (P = 0.1886). Mean SEM = 8.48.



Figure 3.2. Glucose concentration over the course of the pre-challenge period (cannulation) in calm (grey) or temperamental (black) Brahman heifers. Temperament effect (P = 0.0005), time effect (P = 0.0555), temperament by time effect (P = 0.0821). Mean SEM = 17.94.



Figure 3.3. Insulin concentrations over the course of the pre-challenge period (cannulation) in calm (grey) or temperamental (black) Brahman heifers. Temperament effect (P = 0.1560), time effect (P = 0.4715), temperament by time effect (P = 0.0078). Mean SEM = 2.15.

Challenge Period

Heifers that were more temperamental had greater concentrations of cortisol (P =0.0282) throughout the glucose challenge (Figure 3.4) than their calm counterparts. There was a difference in time (P = 0.0026) and the time by temperament (P = 0.0007) interaction. Glucose concentrations were significantly greater (P = 0.0011) for temperamental heifers (Figure 3.5). There was also a significant time ($P \le 0.0001$) and time by temperament (P = 0.0006) interaction for glucose concentrations. Temperamental heifers had greater (P = 0.0092) concentrations of glucose at half-life and took longer (P = 0.0001) to reach half-life than calm heifers. Calm heifers had a glucose half-life concentration (mg/dL) of 113.39 ± 8.16 at 75.65 ± 7.94 min after glucose infusion, while temperamental heifers reached a glucose half-life concentration (mg/dL) of 153.35 ± 8.94 at 151.39 ± 8.70 min after glucose infusion (Table 3.2). Insulin concentrations had a tendency (P = 0.0793) to be greater in calm heifers (Figure 3.6), with significant differences over time (P < 0.0001) and an interaction of time by temperament (P < 0.0001). Calm heifers reached greater (P = 0.0350) peak insulin concentrations $(53.49 \pm 7.64 \text{ uIU/mL})$ than temperamental heifers (27.16 ± 7.46) uIU/mL). Time to peak insulin concentration (min) was faster (P = 0.0007) for calm heifers (12.50 ± 15.58) than temperamental heifers (118.33 ± 15.58) (Table 3.2). Insulinogenic index (Figure 3.7) was significantly different by temperament (P =0.0173), time (P < 0.0001), and the interaction of time and temperament (P < 0.0001).



Figure 3.4. Cortisol concentrations for the duration of the glucose challenge (3 h) in calm (grey) or temperamental (black) Brahman heifers. Temperament effect (P = 0.0282), time effect (P = 0.0026), temperament by time effect (P = 0.0007). Mean SEM = 6.93.



Figure 3.5. Glucose concentrations for the duration of the glucose challenge (3 h) in calm (grey) or temperamental (black) Brahman heifers. Exogenous glucose (0.5 mL/kg BW) infused at time 0. Temperament effect (P = 0.0011), time effect (P < 0.0001), temperament by time effect (P = 0.0006). Mean SEM = 14.64.

Variable	Temperament		P -Value
	Calm	Temperamental	
Insulin Peak Concentration (uIU/mL)	53.49 ± 7.64	27.16 ± 7.64	0.0350
Insulin Peak Time (min) Glucose Half Life Concentration	12.50 ± 15.58	118.33 ± 15.58	0.0007
(mg/dL)	113.39 ± 8.16	153.35 ± 8.94	0.0092
Glucose Half Life Time (min)	74.65 ± 7.94	151.39 ± 8.70	0.0001

Table 3.2 Brahman heifer peak insulin and glucose half-life concentrations.



Figure 3.6. Insulin concentrations for the duration of the glucose challenge (3h) in calm (grey) or temperamental (black) Brahman heifers. Temperament effect (P = 0.0793), time effect (P < 0.0001), temperament by time effect (P < 0.0001). Mean SEM = 4.82.



Figure 3.7. Insulinogenic index values for calm (grey) and temperamental (black) Brahman heifers. Temperament effect (P = 0.0173), time effect (P < 0.0001), temperament by time effect (P < 0.0001). Mean SEM = 0.03.

Discussion

Pre-Challenge Period

The initial cannulation stressor generated an observable stress response in the heifers, as planned. This allowed the endocrine reactions to be contrasted between the temperamental and calm heifers. Temperamental heifers had greater basal concentrations of cortisol, which were elevated and remained elevated above the concentration of cortisol in the calm heifers throughout the cannulation stressor. Many studies have also found that more excitable cattle have greater concentrations of cortisol (King et al., 2006, Curley et al., 2006; 2008, Burdick et al., 2010). Cortisol concentrations for calm heifers did increase during the cannulation, but decreased to normal concentrations during the rest period. This coincides with the results of previous studies that cortisol concentrations are correlated with temperament (Curley et al., 2006; 2008; Café et al., 2011).

Glucose concentrations remained relatively the same throughout the prechallenge period for both temperaments. However, the temperamental heifers had greater concentrations of glucose than the calm heifers. There was no significant difference in insulin concentrations between temperaments, but calm heifers had numerically greater concentrations of insulin throughout much of the pre-challenge period. Temperamental heifers had lower concentrations of insulin through the prechallenge period, until one hour into the rest period. The heifers were all fasted 12-hr prior to the study to remove the interference of postprandial glucose concentrations (Evans et al., 1975). Therefore, the increase in glucose concentrations in temperamental heifers may be due to the stimulation of gluconeogenesis. Café et al. (2011) found similar results of increased cortisol and glucose during their pre-challenge period for steers that were more temperamental. As the temperamental animals continued to stay stressed, gluconeogenesis may have occurred to supply the body with required energy. Increased concentrations of glucose and decreased concentrations of insulin in the temperamental heifers may be due to insulin resistance. From the pre-challenge period we can conclude that temperamental heifers had greater concentrations of cortisol that remained higher than the calm heifers during the pre-challenge period.

Challenge Period

Glucose tolerance testing was originally developed to help diagnose type-2 diabetes in humans. Minimal glucose tolerance testing has been used in beef cattle research, but GTT has been heavily utilized in the dairy industry. From the exploitation of the GTT, the dairy industry has gained insight into metabolic disorders (Bossaert et al., 2008), issues with transition dairy cows (Teroa et al., 2010), and to understand nutritional effects on lactation (Lohrenz et al., 2010). The goal of this trial was to use GTT to determine if there is a difference in glucose utilization between Brahman heifers of differing temperaments, based on the prior knowledge of varying cortisol concentrations in calm versus temperamental cattle.

The temperamental heifers had greater concentrations of cortisol than the calm heifers throughout the glucose challenge period. Calm heifers had minimal fluctuation in their cortisol concentrations, and had baseline concentrations that were much lower (approximately 75%) than the temperamental heifers. This would imply that the calm heifers were not as stressed during the glucose challenge as they were during the prechallenge period. As for the temperamental heifers, they seemed to have remained at a steady state throughout the glucose challenge.

The spike in glucose concentrations at 10 min is a result of the exogenous glucose that was infused through the cannula at 0 min. Temperamental heifers have greater concentrations of glucose than the calm heifers during the glucose challenge. The temperamental heifers had greater concentrations of glucose that steadily dropped over the course of the challenge. Calm heifers followed the same pattern, but had much lower concentrations of glucose present. Calm heifers reached glucose half-life in half the time it took the temperamental heifers to reach glucose half-life, with a glucose half-life concentration difference of 40 mg/dL less than the temperamental heifers. Overall, the calm heifers were able to clear the glucose much quicker than the temperamental heifers. Glucose infusion rate was based on body weight and therefore any differences after 0 min should be due to concentration differences before infusion. Temperamental heifers had greater concentrations of glucose before infusion and therefore had greater concentrations after infusion.

Contrary to the crossbred heifers described in Chapter II of this thesis, insulin concentrations were greater in the calm Brahman heifers. Baseline insulin concentrations were similar between temperament groups, but at 10 min relative to glucose infusion insulin concentrations were much greater in calm heifers. At 80 min the insulin concentrations were the same between temperaments and by 100 min temperamental heifers had greater insulin concentrations, which remained greater throughout the remainder of the glucose challenge. It would be assumed that the large increase of insulin in the calm heifers was to help lower blood glucose concentrations by causing glucose to be taken up by adipose and muscle tissue (Hocquette and Abe, 2000). Calm heifers reached peak insulin concentrations almost 10 times faster than the temperamental heifers and had a peak insulin concentration that was almost double the peak insulin concentration of the temperamental heifers. By the end of the challenge both temperaments were back to relatively their baseline glucose concentration, however temperamental Brahman heifers ended with greater concentrations of glucose at the end of the challenge. The temperamental heifer's final concentrations of glucose were double the final glucose concentration for the calm heifers, even though they had both returned to concentrations similar to basal. It appears that the temperamental heifers are more resistant to insulin, and as a result, do not clear as much glucose from circulation. This inconsistency between Chapter II and III follows the results of Shafer, (2011) that observed varying conclusions of insulin's response to glucose between cattle breeds.

The idea behind the IIND was to provide a quantitative scale to represent the sensitivity of insulin to the concentration of glucose present. First developed for use in humans studies, the IIND is calculated by dividing the concentrations of insulin by the concentration of glucose at a certain time point (Guerrer-Romero and Rodriguez-Moran, 2001; Abdelmannan et al., 2010). IIND was greater in calm heifers from 10- 80 min, for the rest of the sample periods there were little to no difference between temperaments. From this we can conclude that the calm heifers were more sensitive to the

concentrations of glucose present from 10-80 min. After this point the concentration of insulin for the calm heifers becomes low, which means the index becomes low. The temperamental heifers maintained a considerably lower index value throughout the glucose challenge as a result of the low insulin response to glucose concentrations. Utilizing the GTT and IIND it was possible to capture the response of insulin to an influx of glucose and determined that Brahman heifers that are temperamental may exhibit insulin resistance due to greater cortisol concentrations, which causes them to store less glucose and have greater circulating concentrations of glucose.

Conclusion

Temperamental Brahman heifers had greater blood concentrations of cortisol, which led to greater concentrations of glucose. We hypothesized that the temperamental Brahman heifers would in turn need greater concentrations of insulin to remove the glucose as we found in the crossbred heifers. It seems that in the case of the temperamental heifers that insulin didn't respond as well, possibly due to the greater concentrations of cortisol that may have generated insulin resistance. This in turn causes less glucose to be stored and more glucose to be utilized by the body for an immediate energy source. The insulin response of this trial is opposite to that of the crossbred heifers. It seems the calm heifers were more sensitive to the concentration of glucose present. However, the insulin resistance in the temperamental cattle may suggest that temperamental cattle do not utilize glucose as well as calm cattle and may partially explain the lower performance of temperamental cattle.

CHAPTER IV

COMPARISON OF BRAHMAN FEMALES EVALUATED FOR RFI AS HEIFERS AND RE-EVALUATED FOR RFI AS GESTATING COWS

Introduction

One way to make a positive difference in profitability is to reduce feed costs. Feed costs generally represent the largest segment of expenses, accounting for 68 % to 71 % of the total costs associated with beef cattle production from 2008 to 2010 (USDA ERS, 2011b). Selecting for efficient cattle, which consume less feed per unit of gain, may possibly be a way to decrease feed costs. Traditionally, feed to gain ratio was used to assess feed efficiency. However, after further investigation it was found to have unfavorable underlying flaws. Feed: gain ratio has a negative correlation with body weight and growth rate (Mrode et al., 1990; Koots et al., 1994; Arthur et al., 2001a), which leads to selection of cattle that are larger at maturity (Herd and Bishop, 2000). Residual feed intake was introduced in 1963 as an alternative method to determine feed efficiency (Koch et al., 1963). RFI has generally been applied to experiments targeting weaned calves, that would typically be back grounded or finished in a feedlot (Herd and Bishop, 2000; Basarab et al., 2003; Nkrumah et al., 2004, 2007). While important, this is not as helpful for cow/calf producers retaining heifers for use in their cow herd as dams of future progeny. Herd et al. (1998) documented that there is a possibility of a phenotypic association between RFI determined in confinement as post-weaned heifers and their performance on pasture as mature Bos taurus cows. Others have found a

moderate correlation between animals evaluated during specific growing and finishing phases of their lives, but not from youth to maturity (Crews et al., 2003; Johnston, 2007; Kelly et al., 2010). Conversely, Loyd (2011) found that there was no correlation between post-weaning and mature cow RFI values in various breeds and Minton, (2010) found a low (r = 0.07) correlation between post-weaning and mature cow RFI categories. As there are relatively few published studies on this topic and lack of consensus among the published works, the objective of this study was to assess the relationship between post-weaning RFI and mature RFI in the same Brahman (*Bos indicus*) females.

Materials and Methods

Animals and Experimental Design

Heifers

Post-weaning heifer data utilized in this trial were recorded using the Calan Gate system at the Texas Agrilife Research Center in Overton, TX. All heifers were originally evaluated in large cohorts, in their respective year and season. Their RFI values calculated in their respective years were not used, given that we only wanted to compare the animals that had been re-evaluated as mature cows. RFI is highly dependent on the cohort that is being analyzed, so recalculating post-weaning RFI for the 78 animals utilized increased the accuracy of our results. Heifers were fed twice daily (0800 h and 1600 h) for 70 d with body weight recorded weekly. Heifers were fed a balanced ration at a designated percent of their body weight, which differed according to year and season. Orts, if any, were collected and recorded weekly. Details of number of head utilized from each year, year fed, and percent of body weight fed are included in Table (4.1). Diet formulations were not the same between years, they are referenced as following: heifers fed 2004 and 2005 (Table 4.2), 2006 (Table 4.3), and 2007 and 2009 (Table 4.4).

Year fed	Season	N =	Percent of BW fed
2004	Fall	7	2.5%
2005	Winter	7	2.5%
2005	Fall	6	2.5%
2006	Winter	7	2.5%
2006	Fall	10	2.5%
2007	Winter	29	2.65%
2009	Winter	12	2.65%

Table 4.1. Feeding details for heifers fed 2004 to 2009

Ingredients (as fed basis):	%
Cottonseed hulls	37.5
Corn, ground	6.37
Alfalfa dehydrated (20%)	12.5
Wheat middling	5.53
Rice bran	8.5
Cottonseed meal (41%)	4.3
Soybean meal	4.75
Corn gluten feed	5
Corn, cracked	5
Nutrients (dry matter basis):	%
СР	13.4
TDN	60.45

 Table 4.2. Diet formulation for heifers fed 2004 and 2005

Ingredients (as fed basis):	%
Cottonseed hulls	25
Soy hulls	20
Corn, ground	10
Alfalfa dehydrated (20%)	8.73
Wheat middling	7.35
Rice bran	6.25
Cottonseed meal (41%)	6.01
Soybean meal	0.58
Corn gluten feed	5
Corn, cracked	5
Nutrients (dry matter basis):	%
СР	13.4
TDN	69.04

 Table 4.3. Diet formulation for heifers fed 2006

Ingredients (as fed basis):	%
Cottonseed hulls	25
Soy hulls	7
Corn, crimped	2
Alfalfa dehydrated (20%)	15
Salt	0.83
Rice bran	9
Soybean meal (48%)	10
Cottonseed hull pellet	30
Premix	0.0275
Nutrients (dry matter basis):	%
СР	12
TDN	55

 Table 4.4.
 Diet formulation for heifers fed 2007 and 2009

Cows-year 1

Year one of this study was designed to determine the correlation between RFI in *Bos indicus* females post weaning and as mature cows. Brahman cows from the Texas Agrilife Research Center at Overton, TX with previous RFI data (post-weaning) were palpated and confirmed to be in their first trimester of pregnancy. Of those cows, 37 Brahman cows (age 3 to 7) were ultimately chosen to be weighed, assigned to pens, and retrained to eat from the same Calan Gate system that they ate from as heifers. Cows were fed twice daily (0800 h and 1600 h) at 2.2 % of their individual BW for 70 d

starting on October 5, 2009. Body condition score and body weights were recorded weekly. Cows were fed a balanced ration high in cotton seed hulls (Table 4.5). Orts, if any, were collected and recorded weekly. At the conclusion of feeding, cows were classified as either positive RFI = inefficient or negative RFI= efficient, according to their RFI values.

Dietary composition (as fed)%Corn Gluten Feed21Cottonseed Hull Pellets56.8Cottonseed Hulls8.8Chemical composition (DM basis)9.1CP%9.1TDN%57.7Crude Fat2.5

Table 4.5. Experiment Year 1 Summary of dietary and chemical composition of diets fed during trial

Cows-year 2

This trial was a repeat of the previous year's trial and used a new cohort of cows. Brahman cows from the Texas A&M Agrilife Research Center-Overton station with previous RFI data (post-weaning) were palpated to confirm that they were in their first trimester of pregnancy. Of those cows, 41 Brahman cows (age 2 to 3) were ultimately picked to be weighed, assigned to pens, and retrained to eat from the same Calan Gate system they ate from as heifers. Cows were fed twice daily (0800 h and 1600 h) at 2.6 % of their individual BW for 70 d starting on October 4, 2010. Body weight was recorded weekly. Cows were fed a balanced ration high in cotton seed hull pellets (Table 4.6). Orts, if any, were collected and weighed weekly. Body condition scores were collected at d 0 and d 70. At the conclusion of feeding, females were classified with a negative RFI = efficient and a positive RFI = inefficient according to their RFI data.

Dietary composition (as fed)	%
Corn Gluten Feed	25
Cottonseed Hull Pellet	66.2
Premix	8.8
Chemical composition (DM basis)	
CP%	11.7
TDN%	55
Crude Fat	1.9

Table 4.6. Experiment Year 2 Summary of dietary andchemical composition of diet fed during trial

Statistical Analysis

Data were analyzed considering heifers and cows (n = 78) as distinct groups. Initial BW and average daily gain (ADG) were computed using linear regression of BW on test day using the REG procedure of SAS (2002). Mid-test body weight (MBWT) was estimated using the initial BW and ADG and then adjusted for 3% shrink. Mid-test metabolic body weight (MBWT^{0.75}) was computed as MBWT^{0.75}. Average daily feed intake (ADFI) residuals were produced for each animal for each period by subtracting the animal's expected feed intake (estimated from MIXED model, SAS, 2002) from its actual feed intake. Both heifer and cow models included ADG and MBWT^{0.75} as covariates (Table 4.7 and Table 4.8) and sire as a random effect (Table 4.9 and Table 4.10). Fixed effects for heifers were ADG, and MBWT^{0.75}. The heifer model also included the fixed effect of group, which categorized the season (fall or winter) in which heifers were fed (Table 4.11). The cow model also included year of record (corresponding to mature cow feeding trials), cow age (2 to 7 yrs), lactation status prior to evaluation (weaned a calf or not; "prior" because the evaluation occurred after weaning in a given year), and pen (n = 11), within year (Table 4.12). All variables included in the model were significant (P < 0.05) (Table 4.11 and Table 4.12), except for calf weaned or not prior to study, which was actually approaching significance (P = 0.0694) (Table 4.12). As this was a strong tendency it was kept in the model. Residuals were submitted to CORR procedures of SAS (2002) to determine the correlation between post-weaning and mature RFI.

Effect	Estimate with standard error	P -value
MBWT	0.11 ± 0.004	< 0.0001
ADG	0.12 ± 0.20	0.5583

 Table 4.7. Regression coefficients for heifer model

 Table 4.8.
 Regression coefficients for cow model

Effect	Estimate with standard error	<i>P-</i> value
MBWT	0.09 ± 0.02	< 0.0001
ADG	0.76 ± 0.28	0.0117

 Table 4.9.
 Random effect for heifer model

Effect	Estimate with standard error	P -value
Sire	0.04 ± 0.02	0.0494

 Table 4.10.
 Random effect for cow model

Effect	Estimate with standard error	<i>P</i> -value
Sire	0.27 ± 0.23	0.1172

Table 4.11. ANOVA table for heifer model
 Effect Degrees of freedom F value **P** value 1 0.5583 ADG 0.35 MBWT 730.18 < 0.0001 1 < 0.0001 Group 1 29.80

Effect	Degrees of freedom	F value	<i>P-</i> value
Age (year)	5	7.98	< 0.0001
Year evaluated	1	3.36	< 0.0001
Calf weaned or not prior to study (year)	1	3.51	0.0694
Pen (year)	18	4.58	< 0.0001
Days pregnant when entered Calan gates	1	8.40	0.0650
MBWT	1	39.41	< 0.0001
ADG	1	7.1	0.0117

 Table 4.12.
 ANOVA table for cow model

Results

Using the residuals from data analysis, heifers and cows were ranked within their respective cohorts. Rank (1-4) was used to categorize efficiency. Ranks were determined by calculating the mean and standard deviation and then 0.5 standard deviation of each cohort. A rank of 1 would have a residual value less than 0.5 standard deviation subtracted from the mean and represents the most efficient animals in the cohort. A rank of 2 is within 0.5 standard deviation subtracted from below the mean and is an efficient animal. A rank of 3 is within 0.5 standard deviation above the mean and is an inefficient animal. A rank of 4 is greater than 0.5 standard deviation added to the mean and represents the most inefficient animal. When comparing the post-weaning rankings (Figure 4.1.) to the mature cow rankings 21 cows did not change rank, 26 changed one rank, 20 changed two ranks, and 11 changed three ranks. In terms
of changing efficiency as categorized as efficient (negative residual value) or inefficient (positive residual value), 36 cows did not change rank from post-weaning to maturity, while 19 changed from efficient to inefficient and 23 changed from inefficient to efficient (Figure 4.2.). Nearly 54 % of the cows evaluated had reversed feed efficiency rankings from post-weaning to maturity. The low magnitude of the observed Pearson's correlation coefficient (r = -0.06, P = 0.57) indicates that heifer RFI may not be an accurate predictor of mature cow RFI in Brahman females.

Rank	Equation	Efficiency
1	$RFI < \mu$ - 0.5 SD	Most efficient
2	$RFI \ge \mu \text{ - } 0.5 \text{ SD}$	Efficient
3	$RFI \leq \mu + 0.5 ~SD$	Inefficient
4	$RFI > \mu + 0.5 SD$	Most inefficient

Table 4.13. Equations depicting how rank was computed



Figure 4.1. Change in rank from post-weaning heifer to mature cow.



Figure 4.2. Change in RFI from post-weaning heifer to mature cow

Discussion

When choosing our cow model we found it imperative to include variables that could have a significant effect on the outcome. The cows utilized in this study were only chosen if early in their first trimester of gestation in hope of minimizing any changes in feed intake or weight change due to the growth of the fetus. Therefore, we found it essential to include the effects of age, the lactation status prior to evaluation, pen, year evaluated, and days pregnant when entering the gates. It seemed relevant that all of these factors could possibly influence MBWT, ADFI, ADG, and consequently impact feed efficiency.

The results of our study suggest that post-weaning RFI in heifers does not accurately predict mature cows RFI during gestation. Of the work done on repeatability of RFI it is mostly focused on young cattle. Pre-pubertal crossbred heifers re-evaluated post-pubertal were found to be correlated (r = 0.48) with 32.5 % of the heifers changing their RFI phenotype (Loyd, 2009). In a study by Kelly et al. (2010) just over half (54 %) of the heifer's RFI values re-ranked varied by 0.5 standard deviation and just 24 % changed by a full standard deviation. Crews et al. (2003) reported that one cohort of steers had a correlation (r = 0.55) between RFI determinations during finishing and growing phases. A comparable correlation (r = 0.59) was found between post-weaning RFI and feedlot RFI in a single group of calves (Johnston et al., 2007). There seems to be sufficient evidence that calves re-evaluated at a young age can to some extent maintain feed efficiency. This seems likely as none of the calves in these studies have reached maturity and have yet to enter a different production setting such as: gestating or lactating which may alter their feed efficiency.

Studies that do encompass the change from youth to maturity have opposing outcomes. Non-pregnant, non-lactating cows with previous RFI data were re-evaluated 10 days after weaning their second calf. The cows were fed the same *ad libitum* diet as they received as heifers. Phenotypic correlation was (r = 0.40) and genetic correlation was (r = 0.98) when comparing RFI post-weaning to mature *Bos taurus* cows (Archer et al., 2002). The Archer study (2002), while different from our study, indicates that it is the production cycle (breeding, gestating, and lactating) that alters feed efficiency. Since the cows were able to maintain their RFI values from post-weaning to mature cow (non-lactating or gestating), it suggests that the animals may partially maintain the same efficiency, but efficiency may be altered during different periods of production.

Nearly 54 % of the heifers re-evaluated as cows in the present study reversed their efficiency classification. This is supported by the low correlation (r = -0.06; P = 0.57), suggesting that there was practically no correlation between the Brahman postweaning heifer RFI and mature gestating cow RFI. Loyd (2011) found very similar conclusions between heifers of divergent breeds, with RFI values recorded post-weaning, and then re-evaluated as lactating cows. Second parity cow/calf pairs were moved into a Growsafe system and fed *ad libitum* for 70d to assess RFI. They were then compared to their heifer RFI values and ranks (low, medium, high). The relationship between RFI of the heifer and lactating cows was very lowly correlated (r = 0.19; P = 0.12), there was also no relationship between heifer RFI rank and cow RFI rank (r = 0.0175; P = 0.148). There was minimal correlation between heifer RFI rank and cow RFI (r = 0.227; P =0.0585) and a low correlation between heifer RFI and cow RFI rank (r = 0.151; P =0.213). From these observations it was also concluded that selecting for the most efficient heifers may not result in the same level of efficiency when they become lactating females. Work done by Minton (2010) also found results that suggested RFI may not be repeatable in mature cows. In Minton (2010) crossbred cows with prior RFI values were re-evaluated for RFI as mature lactating cows. Cows were fed with their calves for 70d in a Growsafe system, *ad libitum*. Pearson's correlations between heifer and cow RFI were not significant with a low correlation (r = 0.17; P > 0.10) and also for heifer and cow categories (low, medium, high) (r = 0.18; P > 0.10). They did however find a moderate correlation between heifer and cow phenotype (r = 0.54; P < 0.0001). Cattle efficiency during post-weaning may be altered later in maturity due to physiological states such as gestation and lactation that would require more energy (Archer et al., 1998). When cattle were subjected to gestation or lactation, RFI values did not remain similar, whereas in the cattle evaluated while not in a physiological state of energy consumption, tended to maintain the same feed efficiency.

There are many differences in the way that the cited studies were conducted. Multiple studies have examined repeatability at young ages, but deliver no information as to how the animals efficiency may change as they mature. Another study actually looked into the change over time from post-weaning to maturity, but tested the cows in a non-lactating, non-pregnant state. This seems unrealistic as a productive, fertile cow will spend the majority of its life as a pregnant or lactating cow. Surprisingly, only a few studies have actually examined whether feed efficiency of a heifer varies from postweaning to maturity as a cow in a production setting (lactating or gestating). Although the studies were similar, they all were unique in their experimental design and statistical analysis. There was a lack of uniformity among studies with respect to cattle breed, age, production status, feeds, how they were fed, etc. For this reason, is seems that more research is needed to understand the relationship between growing calves and mature cows before using RFI as a tool to select replacement females.

Conclusion

Inconsistency in RFI results to date may be due to the absence of a standard set of rules or regulations for evaluation or analysis of feed efficiency of mature cows from an RFI standpoint. Residual feed intake is very cohort driven. Recent research has proven that comparisons are legitimate only when comparison is within a cohort group, not between groups of cohorts. When forming a cohort they should be within the same sex, breed type, age and fed the same diet to ensure the most accurate results. Our findings suggest that RFI evaluated as a heifer is not an accurate predictor of mature cow RFI. The model clearly demonstrated the need to include more variables in statistical analysis, once the animal has reached maturity. A mature female goes through stages of production: breeding, gestating, lactating, and weaning, that may affect her feed efficiency. As we hypothesized, it seems very unlikely that an animal, especially a female, could maintain the same feed efficiency from post-weaning to maturity. Therefore, RFI may be repeatable at an early age, in a feedlot setting, but post-weaning

RFI probably should not be used as an indicator for feed efficiency in retained females that will be used in a breeding program. Further research is warranted to examine the repeatability between post-weaning RFI and mature cow RFI before being implemented into production practices.

CHAPTER V CONCLUSION

Temperament and feed efficiency have both been found to play key roles in beef cattle production. Rising inputs and falling availability of resources for producers has catapulted them into a time where raising efficient beef cattle is more important than ever. Temperamental cattle are more harmful to producers and facilities and there are a substantial number of studies suggesting that they do not perform as well as calmer cattle. One suggestion as to the difference in performance is the contrasting reactions to stress. Temperamental cattle have greater HPA axis responses, which lead to greater concentrations of cortisol and epinephrine released when stressed. As a glucocorticoid, cortisol has a role in metabolism and with elevated concentrations inhibits the uptake of glucose into adipose and muscle tissue. In these studies temperamental cattle had increased concentrations of cortisol that led to greater concentrations of glucose. This suggests that the temperamental animals do not utilize glucose in the same way as calmer counterparts and this difference may account for observed reductions in performance of temperamental cattle. By choosing cattle that are less temperamental, it may help increase performance and feed efficiency in a herd.

The obvious variable to adjust in an effort to improve feed efficiency would be feed intake, as nearly 68%-71% of the costs of raising cattle is in feed (USDA,ERS, 2011b). Initially F:G ratio was used to categorize efficiency in cattle, but a short time later it was found to be detrimentally flawed and counteracting the goals of producers.

Residual feed intake was then proposed as an alternative calculation to F:G. The lack of correlation between RFI and BW and growth rate deemed it a more appropriate measurement of feed efficiency, which may, more closely follow the goals of beef cattle producers. RFI has also been found to be moderately heritable and is beginning to be used as a selection tool for herd sires that have been evaluated for RFI. However, producers should take caution when selecting for RFI as there is a paucity of research done comparing RFI to other economic traits and performance in cattle retained in breeding herds.

When evaluating for RFI careful consideration should be taken when choosing a cohort. RFI is a cohort driven calculation and multiple research studies have published work on the inappropriateness of comparing cattle of divergent sex, age, breed, and also in differences between environment, feed type, physiological state, etc. Studies suggest that animals will have differing maintenance energy requirements at different stages of production. This follows suit to the results found in this study that RFI was not correlated between post-weaning and mature cows. The study suggests that RFI cannot overcome the differences in maintenance energy requirements between physiological ages and states and therefore may not be an accurate predictor of feed efficiency in mature cattle. Other studies have found RFI repeatable in young cattle; however, this does not accurately depict the feed efficiency of cattle that will be retained for breeding herds. More research is warranted to know if RFI can be used as a selection tool for feed efficiency.

As for RFI, contradicting results between studies coupled with the large time commitment and cost associated with testing cattle leaves more work to be done. It can be concluded that the effect that temperament plays on glucose utilization is part of a more intricate system. Further research is needed to discover other pathways that could be affecting the performance of temperamental cattle. Further research is needed to build a better understanding of feed efficiency and to detect possible markers of feed efficiency in an effort to decrease the cost and time required for testing.

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APPENDIX A

INSULIN RADIOIMMUNOASSAY PROCEDURES

(Siemens, TKIN2)

Materials Supplied In the Kit:

- 1. Insulin Ab-Coated Tubes (Protect from moisture by resealing storage bags after use, store at 4° C.)
- 2. ¹²⁵I Insulin (Stable at 4° C for 30 days after iodination- check date on vial)
- 3. Insulin 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 -20° C for 30 days after opening. Can extend stability by freezing. Aliquot to avoid freeze/thaw.

4. Use bovine serum pool for quality control.

Materials Required But Supplied By Kit:

- 1. Gamma counter: Compatible with standard 12x75 mm tubes
- 2. Vortex mixer
- 3. 12 x75 mm assay tubes
- 4. Micropipettes and compatible disposable tips: Rainin P200 and P1000
- 5. Water bath that can hold constant 37° C
- 6. Foam decanting racks and reservoir and radioactive work space
- 7. Distilled or deionized water
- 8. Graduated cylinder: 100 mL
- 9. Volumetric pipets: 3.0 mL and 6.0 mL

Radioimmunoassay Procedure:

- 1. Allow all components to warm to room temperature.
- 2. Label four uncoated 12 x 75 mm polypropylene tubes as follows: NSB (nonspecific binding) and T (total counts) in duplicate.
- 3. Label fourteen Insulin Ab-Coated Tubes as A (maximum binding) and B through G in duplicate for standards.

	Approximate µIU/mL	
Standard	1st IRP [66/304]	
A (MB)	0	
B*	5	
С	15	
D	50	
Е	100	
F	200	
G	350	

- 4. Preparation of extra standards:
 - 0.125 ug/dL: Add 50 ul of 0 ug/dL standard to 50 ul of 0.25 ug/dL standard. 0.25 ug/dL: Add 50 ul of 0 ug/dL standard to 50 ul of 0.5 ug/dL standard.
 - 0.5 ug/dL: Add 50 ul of 0 ug/dL standard to 50 ul of 1 ug/dL standard.
 - 2. 5 ug/dL: Add 50 ul of 0 ug/dL standard to 50 ul of 5 ug/dL standard.
- 5. Label pooled control and unknown sample Ab-coated tubes in duplicate.
- Pipette 200 μL of the 0 ug/dL standard 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.
- Add 1.0 mL of ¹²⁵I Insulin to every tube and vortex. (Addition of samples and tracer should be completed with minimal delay, with no more than 40 minutes elapsing between the addition of the first sample and the completion of the 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 PROCEDURES

(Siemens, TKCO2)

Materials Supplied

- 1. Cortisol Ab-Coated Tubes (Protect from moisture by resealing storage bags after use, store at 4° C.)
- 2. ¹²⁵I Cortisol (Stable at 4° C for 30 days after ionization- check date on vial)
- 3. Cortisol Standards

Processed in human serum. Stable for 30 days after opening. Can extend stability by freezing. Aliquot to avoid freeze/thaw.

4. Pooled bovine serum pool for quality control sample.

Materials Required But Not Supplied

- 1. Gamma counter compatible with 12 x 75 mm tubes
- 2. Vortex
- 3. 12 x 75mm assay tubes
- 4. Micropipettes and compatible disposable tips: Rainin P200 and P1000
- 5. Water bath that can hold constant 37° C
- 6. Foam decanting racks and reservoir and radioactive work space

Radioimmunoassay Procedure

- 1. Allow all components to warm to room temperature.
- 2. Label four uncoated 12 X 75 mm polypropylene tubes as follows: NSB (nonspecific binding) and T (total counts) in duplicate.
- 3. Label 12 Ab-coated tubes as A-H (2 extra standards) in duplicate for standards.
- 5. Preparation of extra standards:
 - 0.125 ug/dL: Add 50 ul of 0 ug/dL standard to 50 ul of 0.25 ug/dL standard.
 - 0.25 ug/dL: Add 50 ul of 0 ug/dL standard to 50 ul of 0.5 ug/dL standard.
 - 0.5 ug/dL: Add 50 ul of 0 ug/dL standard to 50 ul of 1 ug/dL standard.
 - 2. 5 ug/dL: Add 50 ul of 0 ug/dL standard to 50 ul of 5 ug/dL standard.
- 6. Label pooled control and unknown sample Ab-coated tubes in duplicate.
- Pipette 25 ul of the 0 ug/dL standard into the NSB and A tubes. Pipette 25 ul of each remaining standard, pooled control or unknown sample into the labeled tubes.
 PIPETTE DIRECTLY TO BOTTOM OF TUBE.
- 8. Add 1mL of ¹²⁵I Cortisol to every tube and vortex.
- 9. Cover tubes with foil and incubate for 45min at 37° C.
- 10. Decant thoroughly. Remove all visible moisture by patting inverted tubes.
- 11. Count for 1 min on gamma counter.
- 12. Use Assay Zap (Biosoft, Cambridge, UK) to calculate unknown concentrations against standard curve.

APPENDIX C

GLUCOSE COLORIMETRY PROCEDURES

(WAKO Autokit Glucose, 439-90901)

Materials Supplied:		
1. Buffer Solution		2 x
150 mL		
60 mmol/L Phosphate bu	ffer (pH 7.1) containg 5.3 mmol/L Phen	ıol.
Store at 2-10°C		
2. Color Reagent (When rec	constituted)	2 x for
150 mL		
Contain 0.13 U/mL Muta	rotase, 9.0 U/mL Glucose oxidase, 0.65	U/mL Peroxidase,
0.50 mmol/L 4-Aminoan	tipyrine, 2.7 Ascorbate oxidase.	
Store at 2-10°C		
3. Standard Solution I		1 x 10 mL
Containing 200 mg/dL G	lucose.	
Store at 2-10°C		
4. Standard Solution II		1 x
10 mL		
Containing 500 mg/dL G	lucose.	
Store at 2-10°C		

Working Solution:

Dissolve the entire 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 2-10° C.

Materials Required But Not Supplied:

- 1. Pippettes
- 2. Water bath that can hold constant 37° C
- 3. Spectrophotometer

Test Procedure:

Wavelength: 505* ¹	Light path: 1 cm
Temperature: 37° C	

		Sample (S)	Standard (Std)	Blank (BL)
Pipette into a cuvett	e			
Sample	(uL)	6.7		*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 the1.0 mL cuvettes (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 $\lambda 1/\lambda 2 = 505/600$ nm
- *2 The omission of 0.2 mL of water does not significantly affect the absorbance measured.

Results:

Concentrations are determined using the following equation as supplied by the kit protocol:

Glucose (mg/dL) = As/Astd x Cstd

As = Absorbance of sample

 $A_{Std} = Absorbance of Standard I or II$

Cstd = Concentration of Standard I or II in mg/dL

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