EVALUATION OF POSTPARTUM REPRODUCTIVE PERFORMANCE IN BRAHMAN FEMALES WITH DIVERGENT RESIDUAL FEED INTAKE

A Thesis

by

ANNA KATHRYN POOVEY

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

MASTER OF SCIENCE

August 2010

Major Subject: Physiology of Reproduction

Evaluation of Postpartum Reproductive Performance in Brahman Females with

Divergent Residual Feed Intake

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Approved by:

Co-Chairs of Committee,	Ronald D. Randel
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ABSTRACT

Evaluation of Postpartum Reproductive Performance in Brahman Females with Divergent Residual Feed Intake. (August 2010)

Anna Kathryn Poovey, B.S., Oklahoma State University

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Dr. Thomas H. Welsh

These studies were designed to evaluate the relationships that exist between residual feed intake, parity, rate of return to estrous cyclicity and nonesterified fatty acid (NEFA) concentrations, as well as changes in both body weight (BW) and body condition score (BCS) during the prepartum and postpartum time periods in Brahman females. Residual feed intake classification was evaluated for all females during the course of 70-d trials conducted prior to these experiments. Heifers (n = 30) and cows (n = 63) were evaluated for BW and BCS, as well as by collection of weekly blood samples beginning five weeks prior to calving. Blood serum samples were utilized to assay for NEFA concentrations by enzymatic colorimetry both pre- and postpartum. Multiparous females (n = 44) were sampled weekly for five weeks following parturition. Beginning 28d postpartum, weekly blood samples were collected and assayed for progesterone concentrations by radioimmunoassay to determine return to estrous cyclicity. Following calving, females were exposed to epididymectomized bulls fitted with chin-ball markers

to aid in estrus detection. After detection, estrus females were evaluated for presence of a corpus luteum by trans-rectal ultrasonography.

Prepartum, it was found that inefficient females had a greater BCS than efficient females ($P < 0.05$), significant BW changes occurred during the sampling period ($P < 0.05$) and moderate to low correlations existed between BW and BCS. Additionally, it was found that the interaction between RFI x parity had a significant affect upon NEFA concentrations, BW and BCS ($P < 0.05$). During the postpartum period it was found that efficient females were lower in both BW and BCS ($P < 0.05$), no change occurred over time in NEFA concentrations ($P > 0.1$) and a greater pregnancy rate was achieved in efficient females, as well as in females that returned to estrous cyclicity rapidly (< 90d) following calving.

DEDICATION

This thesis is dedicated to Pedro and Howie, my two best friends.

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First, I would like to thank the members of my committee for their help, support and patience for the past two years. Dr. Forbes for demonstrating the proper fecal collecting technique, Dr. Welsh for his silent pat on the arm that said it all when words would not suffice, and Dr. Randal for his endless supply of birds – not fowl.

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Thank you to my partner in crime, my dad. I don't think we'll ever have a time when we actually have to finish a thought without the other already answering the unasked question. Thanks to my mother as well. If I turn out to be half the mother she is, I'll have been a great success. The habits she instilled in me are priceless. I never realized I had a perfect childhood until I left home.

To the girls that make me complete, my sisters; I can't ever fully thank you. Like my brother-in-law Mitch says, we may fight like crazy, but try to pick on one of us and we'll circle the wagons and defend to the death. We are rough, tough, and unique; we are the Poovey girls.

Finally, to my best friend who is soon to be my husband, thank you for the miles of driving, the phone calls, and most of all just listening to me the past two years. I love the fact that we decided to take the plunge and I'm sorry we didn't think of it sooner. I am very excited to start our new life together and someday soon to be the best wife and mother I possibly can.

NOMENCLATURE

BCS	Body Condition Score
BW	Body Weight
Kg	Kilogram
mEq/L	Milliequivalent per Liter
mL	Milliliter
NEFA	Nonesterified Fatty Acid

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CHAPTER I

INTRODUCTION AND

LITERATURE REVIEW

Introduction

Beef cattle production represents the largest sector of meat animal production in the United States with the most pounds of meat product produced annually. In 2007, U.S. cattle and calf production added more than $36 billion to the United States economy (http://www.ers.usda.gov/news/BSECoverage.htm) and therefore is recognized as a viable industry that deserves further scrutiny into its advancement and refinement.

Feed expenses typically represent the largest cost of any cattle production budget, accounting for at least 60-65% of the total costs (Montaño-Bermudez and Nielsen, 1990; Parnell et al., 1994; and Arthur et al., 2005). This number is steadily increasing as the demand for land for crop production and the costs of technology are also rising. Therefore, it has now become more crucial than ever to identify and produce cattle that are efficient in their utilization of feeds. In order to identify cattle that are more efficient in feed utilization, producers have often relied on the feed to gain (F:G) ratio that takes into account how many pounds of feed are offered to an animal per pound of weight gain. This method; however, is flawed and confusing. Not only does a higher ratio equate to a lower efficiency, but it has also been found that two animals

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This thesis follows the style of Journal of Animal Science.

could have the same F:G ratio yet be different in terms of body size and intake. Additionally, one animal may have several different ratios depending upon the stage of growth, although the genetics of the animal remain constant (Sainz and Paulino, 2004). Due to the large number of problems that occur when implementing the F:G system, an improved system to identify efficient animals is needed.

Residual feed intake (RFI) has been offered as an alternative to F:G that is independent of stage of growth or maturity. Instead, RFI bases all calculations upon the difference between actual feed intake and the expected feed requirements for maintenance of body weight and weight gain (Koch et al., 1963). This measurement remains unbiased regardless of pre-testing rearing treatments (Herd and Bishop, 2000). Although this measurement tool has not yet gained as widespread popularity among producers in the U.S. as it has elsewhere, researchers in the U.S. have instead chosen to scrutinize the biological factors associated with it (Sainz and Paulino, 2004). According to research conducted by Herd et al. (2003), the correlation between post-weaning and post-calving RFI is compelling (0.98) and moderately to highly heritable between generations. As well, Castro Bulle et al. (2007) demonstrated that low RFI cattle consumed less dry matter daily, yet had no significant difference in final body weight or average daily gain versus their high (inefficient) RFI cohorts. Therefore, by consistently selecting cattle that are more efficient in their RFI it is possible to create progeny that are genetically predisposed to eat less feed and yet not sacrifice growth and performance traits (Herd et al., 1997; Richardson et al., 1998).

Recently, significant progress has been made and new research areas explored concerning residual feed intake and its subsequent impact upon future generations of cattle. According to research by Hagger (1994) and Hughes and Pitchford (2004), positive correlations exist between egg number and RFI in poultry and litter size in mice, respectively. Methane emissions have been shown to be reduced in low RFI cattle (Nkrumah et al., 2005, 2006; Hegarty et al., 2007). Additionally, research by Arthur et al. (2005) suggests a trend in low RFI Angus first calf heifers to calve approximately five days later than their high RFI contemporaries. Basarab et al. (2007) concluded that low RFI cows produced the same number of pounds of calf weaned per cow exposed and also had a lower rate of twins and therefore lower calf death loss percentage. While selection for low RFI in *Bos taurus* cattle might lead to later calving in 1st calf heifers and a lower calf death loss, researchers and producers should be cautious in accepting that this is also true for *Bos indicus* cattle.

Literature linking feed efficiency with reproductive parameters in cattle, much less a correlation between them, is limited, although the association between the two has been hypothesized for nearly 50 years. Researchers, including: Reid, 1960; Wiltbank et al., 1965; Baker, 1969; Lamond, 1970; Topps, 1977; Bowden et al., 1979; Dunn and Kaltenbach, 1980; Dziuk and Bellows, 1983; Entwistle, 1983; and Hanzen, 1986; were among the first to construe a positive association between optimal nutrition and its subsequent affect upon reproductive status. Additionally, only in more recent years has there been an expressed interest solely in the measurement of input traits related to reproduction (Crews et al. 2005) rather than the conventional output traits. Some of the

hesitation regarding research in these areas is due in part to lack of estimates of covariances of RFI with other economically important traits (Archer et al., 1999a) as well as the high costs associated with obtaining individual intake data for RFI. Most of the research which has been conducted has been done with *Bos taurus* cattle.

In view of these previous studies, it is important to explore the relationship between feed efficiency (RFI) and reproductive success in *Bos indicus* cattle. Because *Bos indicus* cattle are tropically adapted, they are well suited for the climates found in the southern and southeastern regions of the U.S. (Bailey, et al. 1990). The economic impact, whether positive or negative, that may be discovered by exploring the correlation between feed efficiency (RFI) and reproductive success will be important to producers and consumers alike.

Residual Feed Intake

The costs of feedstuffs in any livestock enterprise today are increasing at a rapid rate, especially in cattle due to their size and slower maturity rate as compared to other farm animal species. Great strides have already been taken within the poultry and swine industries as they have undergone vertical integration and essentially become limited to confinement settings (Luiting et al., 1991; Herd et al., 2003). Since vertical integration is not likely to become practical for the beef industry it has becoming increasingly important for producers to implement and utilize a system that identifies more efficient animals in their utilization of nutrients and feedstuffs.

The first such system that attempted to identify animals which might be more efficient in nutrient utilization was the Feed:Gain ratio (F:G). This system was first reported upon in 1945 by Samuel Brody (Johnson et al., 2003). This ratio is defined as the amount of feed required to produce one unit of weight gain in an animal as an indication of efficiency (Brody, 1945). While the simplicity of the equation is attractive to users of this system, many inconsistencies and anomalies occur as a result of its implementation in a production setting. The F:G ratio is positively correlated with traits such as rate of growth and body size (Herd and Bishop, 2000). Repeated selection for these traits is not always beneficial. For example, as mature cow size increases, the nutrient and energy maintenance requirements may become too extreme and the productivity of the entire herd may be compromised (Barlow, 1984; Basarab et al., 2003).

An alternative to F:G has been proposed that is able to resolve many of the dilemmas created by this earlier system. Residual feed intake (RFI) takes into account the difference in individual animal feed intake either above or below the predicted amount from published feeding standards or in comparison to cohorts (Herd and Arthur, 2009), and is based upon both size and growth rate of the animal (Archer et al., 1999). To be more specific, this is a derivation of actual feed intake compared to expected feed intake for body weight maintenance and average daily gain of an animal over a defined period of time (Herd et al., 2003). Therefore, animals that consume less feed than predicted have a negative RFI value and are identified as efficient in comparison to their more inefficient cohorts who have a positive RFI. Since RFI is independent of other

production parameters, it represents inherent variations that exist among different animals' basic metabolic processes (Herd and Arthur, 2009). Because feed efficiency is not a directly measurable trait, Koch et al. (1963) suggested that it be comprised as a function of feed consumed, body weight gain and average weight throughout the course of the trial.

This particular system is gaining popularity and legitimacy in both the scientific and production communities for a number of reasons. Compared to F:G, selection for negative RFI (more efficient) animals will increase subsequent feed efficiency in a herd without detrimental impact upon mature body size (Koch et al., 1963). Additionally, the genetic correlations between post-weaning F:G with feed intake and F:G are weak (0.15 and 0.20, respectively; Archer et al., 2002), although the same is not the case for RFI. A direct comparison finds RFI genetic correlations, both phenotypic and genetic, to be stronger (0.64 and 0.98, Archer et al., 2002). This evidence leads to the conclusion that selection for post-weaning RFI (more efficient) animals has the potential to reduce feed intake (Basarab et al., 2003; Kolath et al., 2006; Golden et al., 2008), maintain the same body size (Basarab et al., 2002) and positively improve the efficiency of the entire cowherd (Herd et al., 2003) with no detrimental effects upon measured growth variables (Arthur et al., 2001; Richardson et al., 2001; Basarab et al., 2001; Golden et al., 2008).

The economic and environmental impact of selection of more efficient animals is substantial, especially when considering that 75% of the total feed costs of producing a beef carcass are attributed to maintenance of the breeding herd (Moore et al., 2009). Crews (2005) reported that during feeding trials lasting 150 days, efficient animals cost

$38 per head less to feed. After testing, when ideally more efficient individuals are consistently placed into the breeding herd, it is estimated that there would be a 9-10% reduction in cowherd maintenance costs, 10-12% reduction in feed intake, 25-30% reduction in methane emission (Nkrumah et al., 2006; Hegarty et al., 2007) and 15-20% reduction in manure production (Basarab et al., 2002).

Variables concerning residual feed intake

Five major components have been identified as contributing substantially to the variation in RFI (Herd et al., 2004; Herd and Arthur, 2009). Physiological components include: intake of feed, digestion of feed, body composition regarding anabolic and catabolic metabolism, physical activity and thermoregulation.

Robinson and Oddy (2004) reported three distinct feeding behavioral patterns in feedlot steers. Increased feed consumption times and number of eating sessions were shown to be positively correlated with RFI (Golden et al., 2008), while an increased rate of feed consumption was shown to be inversely correlated (Robinson and Oddy, 2004). Additionally, it has been observed that inefficient animals have a greater amount of variation in patterns of feed intake (Golden et al., 2008).

The total digestibility of feed tends to decrease as the maintenance energy requirements of the animal increase (Herd and Arthur, 2009). However, it has been indicated that genetic variations exist and allow for greater digestive properties. Richardson et al. (1996) observed a moderately negative correlation ($r = -0.44$) between RFI and digestibility of a high concentrate diet, and suggested that the differences in

digestibility accounted for 19% of the phenotypic variation in RFI. Therefore, greater feed efficiency and a lower RFI are positively correlated with increased digestive capabilities (Richardson and Herd, 2004). Nevertheless, precise measurements in digestive ability are difficult to accurately obtain with high accuracy. Thus, it is recommended that utilizing variation in digestibility as a means of explaining variation in of RFI be used with caution (Herd and Arthur, 2009).

It has been observed that variations in body composition play a less crucial role for determination of feed efficiency, although they still contribute to significant differences between animals (Herd et al., 2004). The percentage of fat versus protein in the beef animal differs greatly in both accretion and turnover (Herd and Arthur, 2009). Although the nutrient efficiency of fat deposition is much higher (70-95%) than protein deposition (40-50%), total body protein content is greater. Richardson et al. (2001) demonstrated that feedlot steers from low RFI parents contained a greater percentage of whole-body protein and less whole-body fat than did male progeny of high RFI parents as well as less protein turnover in low RFI progeny (Richardson and Herd, 2004). Lower protein turnover was observed in low RFI progeny as well. This is beneficial considering that a decreased rate of protein degradation results in improved efficiency in protein accretion and lean muscle mass (Herd and Arthur, 2009). The difference in energy retained in the body due to differing chemical composition in those individuals was rather minor as it accounted for only 5% of the difference in feed intake while heat production accounted for 95% of the difference (Richardson and Herd, 2004).

Although the energetic efficiency of cell mitochondrial function has been suggested to explain the differences in feed efficiency in some species (McDonald and Nielson, 2008), this has yet to be elucidated in beef cattle. It has been suggested that low RFI animals have an increased rate of mitochondrial respiration as well as increased flux of electrons through the electron transport chain (Kolath et al., 2006). This theory is further supported by the research of Richardson et al. (2004), who reported that more efficient RFI steers contained lower concentrations of several metabolites. Leptin, which is commonly associated with increased cattle fatness (Minton et al., 1998), urea, which is negatively associated with leanness and protein accretion in cattle (Cameron, 1992; Robinson et al., 1992; Clarke et al., 1996) and creatinine, negatively associated with adipose tissue deposition in sheep (Cameron, 1992; Clarke et al., 1996) all supported the theory that more efficient animals would have greater amounts of protein accretion and less adipose deposition (Richardson et al., 2001, 2004).

Suggestions have been made alluding to a correlation between RFI and stress susceptibility in an individual (Herd and Arthur, 2009). Richardson et al., (2004) observed a positive correlation between inefficient steers and elevated plasma cortisol concentrations, typically indicative of stress in an individual. However, Knott et al., (2008) argue that animals in a stressful situation would also experience elevated metabolic rates, increased energy consumption and increased lypolysis. Therefore, additional research into this area is warranted before conclusions are formed.

Physical activity of any kind produces heat from energy breakdown and diverts energy from other demanding functions such as maintenance and growth (Herd and

Arthur, 2009). Extensive research has been conducted in other species such as: swine, mice and poultry, and data indicate a positive correlation in an individual's level of physical exertion to their RFI (Luiting et al., 1991; de Haur et al., 1993; Mousel et al., 2001). Recent work in cattle suggests similar conclusions (Richardson et al., 1999; Herd et al., 2004, Nkrumah et al., 2006), and demonstrates that variation in the level of physical activity is associated with a difference in RFI in cattle. Although activities such as feeding and ruminating account for some of the variation, locomotion plays a substantial role (Herd and Arthur, 2009). Pedometers are becoming a more valuable tool utilized to measure the level of physical exertion in an experimental animal (Richardson et al., 1999).

Blaxter (1962) concluded that the primary source of energy loss within ruminants was evaporative heat loss. This is mainly due to heat exchange that occurs in the lungs and nasal passages during respiration. Only in extreme situations and climates is rate of heat loss significantly affected (Herd and Arthur, 2009). Studies have been conducted investigating thermoregulation in smaller species such as chickens (Luiting et al., 1991); however, dramatic differences in surface area and body size make comparison between chickens and cattle improbable. Recent studies in beef cattle conducted concerning the relationship between RFI, respiration rates and methane production are rather imprecise and limited in number (Hegarty et al., 2007).

Measuring residual feed intake

Pioneering researchers in the field of RFI originally suggested trial lengths of 168 days (Koch et al.; 1963). Later trials used shortened studies of 140 days and 112 days; each time without reporting negative impacts (McPeake and Buchanan, 1986; Franklin et al., 1987). Each trial was also preceded by an adjustment period of 21 to 28 days (BIF, 1986) and during the trials cattle were weighed every 28 days (Kemp, 1990). Archer et al. (1997) were more extreme in their approach as they compared trials varying in length from seven to 119 days and concluded that a 70-day trial with weighing at two-week intervals was sufficient for a reasonable level of confidence in estimating growth rate, feed conversion and residual feed intake in British breeds of cattle. Therefore, 70-day trials have become the most common practice.

Robinson et al. (1997) observed distinct differences between *Bos taurus* and *Bos indicus* cattle in feeding patterns when maintained in the same feedlot environment. Therefore, Archer and Bergh (2000), investigated the possible differences in test lengths that might impact results among breeds by utilizing a wide array of cattle that included both temperate and tropically adapted breeds. Observations indicated that there was little evidence to support the claim that different breeds of cattle require testing periods of varying durations. Additionally, they suggest that the testing period may even be shortened when evaluating cattle to be used in replacement breeding programs. Archer and Bergh (2000) argued that the economic impact of shortening the trial period far outweighs any minor discrepancies that might occur in the data, especially when information gathered from closely related individuals are considered.

Reproductive Performance

Trenkle and Wilham (1977) stated that improving the reproductive performance of cattle can initiate a plethora of positive responses, including: reducing the cost of production by spreading expenses over an increased number of animals, increase the potential from possible crossbreeding, and increase the response to selection as a result of greater numbers of animals from which to choose. By improving the reproductive success of cattle only one percent, from 86% to 87%, producers would be able to wean 300,000 more calves from 30 million cows. The economic impact of this example is further compounded when coupled with the fact that reproductive success within the cow herd is five times more crucial in commercial operations than growth rate or milk production (Trenkle and Wilham, 1977). Simply put, there is no reason to focus upon carcass merit or other terminal traits if reproduction of the superior animal is not adequate.

The positive correlation that exists between proper nutrient intake and reproductive success has been recognized for a number of years (Reid, 1960; Wiltbank et al., 1965; Baker, 1969; Lamond, 1970; Bowden, 1977; Topps, 1977; Dunn and Kaltenbach, 1980; Echternkamp et al., 1982; Oyedipe et al., 1982; Dziuk and Bellows, 1983; Entwistle, 1983; Doornbos et al., 1984; Hanzen, 1986; Selk et al., 1988; Warren et al., 1988; Richards et al., 1989a; Randel, 1990; Rasby et al., 1991; Rhodes et al., 1995; Armstrong and Benoit, 1996; Keisler and Lucy, 1996; Hawkins et al., 2000; Ciccioli et al., 2003; Wettemann et al., 2003). With increased costs for inputs, it has become necessary for cattle to fully utilize all available feeds and forages. Additionally, because

of the rather low reproduction rate of cattle, 0.86 calf weaned per cow (Trenkle and Wilham, 1977), it is crucial that every effort be made to maximize available opportunities to reproduce in the shortest amount of time possible.

Relationship between nutrition and reproduction

Dunn and Kaltenbach (1980) developed regression equations that defined the relationship between energy status, as expressed by change in body weight, against subsequent reproductive performance. Careful planning should be taken into consideration when preparing for multiple, consecutive seasons of calving as pre-partum nutrition may be more crucial than postpartum nutrition in regard to determining the length of postpartum anestrus (Wiltbank et al., 1962; Dunn and Kaltenbach, 1980; Dziuk and Bellows, 1983; Randel, 1990).

Basic life processes such as growth and maintenance have priority over reproductive measures such as estrous cyclicity and subsequent establishment and maintenance of pregnancy (Short et al., 1990; Grimard et al., 1997; Guedon et al., 1999). Reproductive processes are controlled through the hypothalamic-pituitary-gonadal axis are sensitive to the availability of metabolic fuels (Schneider, 2004). Therefore, a negative energy balance during lactation has been shown to affect reproductive factors such as: follicular growth (Beam and Butler, 1999; Butler, 2000), oocyte development, competence and morphology (McEvoy et al., 1995; O'Callaghan and Boland, 1999; Boland et al., 2001) and size of the ovulatory follicle (Bergfeld et al., 1994; Rhodes et al., 1995; Mackey et al., 1999; Bossis et al., 2000; Armstrong et al., 2001). Additionally,

inadequate nutrient intake retards the actions of the hypothalamic-pituitary axis (Echternkamp et al., 1982; McCann and Hansel, 1986; Imakawa et al., 1987; Richards et al., 1989a) and decreases pituitary LH content (Beal et al., 1978; Moss et al., 1982). Conversely, maintenance of adequate body condition has been shown to enhance pituitary function in females (Rutter and Randel, 1984) therefore negating many of these deleterious events.

Loss of luteal function and cessation of estrous cyclicity due to losses in body weight and body condition from nutrient deprivation is difficult to overcome. Richards et al. (1989a) found that mature cows ceased luteal activity when body condition score declined to 3.5 ± 0.1% and 24 ± 9% of initial body weight was lost. These females regained estrous cyclicity, but only after dramatically increasing nutrient intake. This is similar to findings by Imakawa et al. (1986) who reported that heifers were heavier upon resumption of estrous cyclicity versus their weights before a nutritionally induced anestrous. Greater body fat and body energy reserves are required to reinitiate estrous cyclicity rather than to maintain estrous cycles in females that are losing weight (Imakawa et al., 1986; Louw et al., 1988; Richards et al., 1989a).

Some differences of opinion still exist concerning the roles of body weight and body condition score (1 = severely emaciated, 9 = very obese; Wagner et al., 1985) and their influence upon rebreeding capabilities. Somerville et al. (1979) suggested that body weight loss during the postpartum period was more crucial than absolute body weight. Rutter and Randel (1984) accounted for the greatest differences in return to estrus when classifying cattle according to their body condition scores as defined by

Whitman (1975) instead of only body weights. This theory is supported by many (Wiltbank et al., 1962; Richards et al., 1986; Wagner et al., 1988; McNamara et al., 1995) due to the growth of the conceptus during pregnancy and its role in variation of cow body weight.

Rakestraw et al. (1986) reported that regardless of body condition score at calving, cows that received inadequate nutrition after calving were not guaranteed a timely return to estrus. This is further supported by Lucy et al. (1991) who claimed that both predicted energy balance and dietary treatments influenced the number of follicles after calving, as well as follicular size (Rhodes et al., 1995). This later work does not support the findings of Richardson (1976) who differs in his belief that rebreeding performance is related to actual body weight at breeding rather than the rate of change in body weight from calving to rebreeding, with no mention of body condition scores. Although estimation of reproductive efficiency through measurements such as body weight or body condition scoring is rather subjective, it is also one of the most cost effective and simplistic means by which both researchers and producers alike can easily evaluate beef females.

Puberty

In the same manner that successful reproduction must be achieved prior to concentration on other, more terminal traits; puberty must be attained before reproduction can occur. In that regard, puberty is the most important step in a female's life as it signifies her readiness to enter the breeding herd. Heifers that calve early

during their first season tend to calve earlier throughout the remainder of their productive years versus their later calving cohorts (Lesmeister et al., 1973). Additionally, earlier calving heifers tend to not only wean heavier calves but also have a higher lifetime average calf production (Lesmeister et al., 1973). First calf heifers require extra time to return to estrous cycles following calving because they resume ovarian function 20 to 40 days later than mature cows (Wiltbank, 1970; Ciccioli et al., 2003).

Puberty has been defined in a multitude of ways and the definition is rather subjective. Kinder et al. (1987) described the process as the coexistence of multiple factors. They stated that photoperiodic cues and dietary intake act upon the hypothalamus to modulate gonadotrophin secretion during sexual maturation and, in turn, influence the time when puberty occurs. Simply put, there must be observation of a behavioral standing estrus followed immediately by development of a functional corpus luteum (Short, 1984; Kinder et al., 1987). After this point, reproduction can occur and puberty is achieved (Robinson, 1977). Placement of heifers in an environment of limited dietary intake or on a low plane of nutrition during the pre-pubertal period will delay puberty by inhibiting the development of a mature reproductive endocrine system (Day et al., 1986). Plasse et al. (1968) further affirmed this fact, demonstrating the positive correlation between heavy weaning weights, age at first corpus luteum, and thus earlier puberty. Advancement of this maturation system may also be achieved through pasturing of sexually mature bulls with prepuberal heifers (Roberson et al., 1991; Bastidas et al., 1997) rather than isolating them, although some conflicting data has been

reported (Berardinelli et al., 1978; MacMillan et al., 1979; Roberson et al., 1987). Additionally, research conducted suggests that pheromones from bull urine, when injected into the nasal passages of heifers, are more successful in initiating puberty than a water placebo (Izard and Vandenberg, 1982).

Just as every effort should be made to ensure that heifers reach puberty in a timely fashion in order to calve early enough to join the cowherd for subsequent years; attention should be paid to the mature cows within the herd as well. Failure to conceive or early embryonic deaths are the most negative factors affecting reproductive efficiency of the cow herd (Wiltbank et al., 1961). In order to maximize the possible number of services per cow, increase her chances of conceiving, and therefore increase the calf crop it is necessary to reduce the length of the postpartum interval (Wiltbank et al., 1961).

Factors affecting the postpartum interval

Reproduction is the main limiting factor regarding beef cattle production efficiency (Dickerson, 1970; Dzuik and Bellows, 1983; Koch and Algeo, 1983). The failure of females to become pregnant is the largest contributor to potential calf crop loss within the herd (Wiltbank et al., 1961; Bellows et al., 1978). The length of the postpartum interval following calving plays a role in the ability of a female to rebreed during an allotted breeding season (Symington, 1969; Wiltbank, 1970).

Randel (1990) defines the average gestation length of *Bos indicus* cattle as 290 days, as compared to 282 days for *Bos taurus* females (Lush, 1945) although some reports observe gestation lengths as long as 293 days in *Bos indicus* females (Plasse et

al., 1968). This places *Bos indicus* females at an immediate disadvantage when considering the ideal calving interval is 365 days or less and therefore they must conceive within 75 days after calving to maintain this production schedule. Heifers are penalized even further as the length of their postpartum interval is extended for an additional period of time due to higher incidence of dystocia, first lactation and increased nutritional demands (Filley et al., 1999).

Long intervals from calving to rebreeding are a major cause of reproductive inefficiency (Casida, 1971; Edgerton, 1980) because cows that initially calve late create a pattern that eventually prohibits rebreeding during the subsequent year (Burris and Priode, 1958; Wiltbank, 1970; Burrell, 1972; Lesmeister et al., 1973). Additionally, calves born later in the calving season have substantially lighter weaning weights (Williams, 1990) and create a less uniform calf crop which becomes a major concern for producers who market calves at weaning. Four main causes have been identified relating to the length of the postpartum interval in beef cattle, including: lack of uterine involution, short estrous cycles, anestrus and general infertility (Short et al., 1990). Knowing this, it becomes imperative that management protocols are adjusted accordingly in order to maximize reproductive potential.

Uterine involution

Uterine involution involves the return of the uterus to a state capable of supporting another conceptus after parturition (Kiracofe, 1980) when the two uterine horns are again similar in size, tone and diameter (Casida et al., 1968). Although the

behavioral and physiological signals and resulting endocrine and histological changes that must occur in order for involution to take place are nebulous (Landaeta-Hernandez et al., 2004), it includes three primary causes: a reduction in size of the uterus, loss of tissue and repair (Gier and Marion, 1968) as well as bacterial elimination and endometrial regeneration (Edqvist et al., 1978; Bondurant, 1999). This process typically takes place between 26 days (Casida and Venzke, 1936) and 50 days (Gier and Marion, 1968) following parturition.

Typically, the length of time for uterine involution is not the limiting issue regarding reproduction in beef cattle and is not related to anestrus. Other issues such as existing postpartum hormonal imbalances play a much larger role (Short et al., 1990). The length of time required for uterine involution; however, has been demonstrated to have a strong positive association with postpartum fertility (Archbald et al., 1998) and the first postpartum estrus (Landaeta-Hernandez et al., 2004). Similarly, a negative correlation exists between secretion of the eicosanoid PGF2α and length of time for uterine involution as well as postpartum interval (Madej et al., 1984) and thus must not be disregarded entirely. Factors that influence the length of uterine involution are many of the same that control the length of the postpartum interval and include: parity (Bastidas et al., 1984), breed (Rao and Rao, 1980), and dystocia (Landaeta-Hernandez et al., 2004).

Although measuring the changes in uterine size are difficult to obtain and often imprecise (Landaeta-Hernandez et al., 2004), a pattern regarding uterine regression has been identified. The greatest changes in uterine tone and size occur during the first few

days following parturition and are a consequence of peristaltic contractions happening at three to four minute intervals (Jordan, 1952; Venable and McDonald, 1958; Gier and Marion, 1968) due to the combined actions of PGF2α, estrogen and oxytocin (Edqvist et al., 1978; Bondurant, 1999). Gier and Marion (1968) reported upon the substantial changes in postgravid uterine size in the following days following calving. After five days, uterine size had regressed to half the maximum gestational diameter and after fifteen days the authors reported that uterine length had decreased by 50% as well. Aided in part by the constricting of the caruncular blood vessels which begins near day two after calving, caruncular tissue masses begin to slough and are nearly completely removed by day 15 (Gier and Marion, 1968). Suckling by the calf also plays a crucial role in reduction of uterine size (Yavas and Walton, 2000). Although both oxytocin and PGF2α increase following parturition (Yavas and Walton, 2000), oxytocin further stimulates additional release of PGF2α by the uterine endometrium (Guilbault et al., 1984).

Short estrous cycles

When cows enter the breeding season it is imperative that they are physiologically prepared to rebreed and have resumed normal estrous activity following calving. Numerous studies have demonstrated that conception rates are lower in females bred during their first estrus following parturition versus those that have resumed normal estrous cyclicity and experienced several consecutive estrous cycles (Perkins and Kidder, 1963; Casida et al., 1968; Short et al., 1972; Whitmore et al., 1974). First

ovulation following parturition is commonly associated with short-lived corpus lutea in the female that last less than 14 days (Ramirez-Godinez et al., 1981, 1982a,b; Pratt et al., 1982) while a typical estrous cycle is expected to last 18-24 days in length (Landaeta-Hernandez et al., 2004).

Short estrous cycles within the female are a major cause of concern because although the ova released are normal and can be fertilized, corpus luteum regression happens too quickly for the ovary to receive a signal from the uterus that a pregnancy exists (Graves et al., 1968; Short et al., 1972, 1974; Odde et al., 1980; Ramirez-Godinez et al., 1982a,b). Kesler et al. (1981) theorized that this was due to three major factors, including: insufficient presence of LH, failure of the luteal tissue to recognize the presence of LH, and the presence of either a luteolytic or antiluteotropic factor such as prostaglandin F2α being released from the uterine endometrium (Garverick et al., 1992; Yavas and Walton, 2000). However; several researchers have found discrepancies in this theory and provide evidence to refute it.

Ramirez-Godinez et al. (1982b) found that FSH concentrations as well as estradiol-17β (Garverick et al., 1988) were lower during the pre-ovulatory period of a short luteal phase but LH concentrations remained similar in both normal and subnormal luteal phases (Ramirez-Godinez et al., 1982b; Rutter et al., 1985; Garcia-Winder et al., 1986; Copelin et al., 1987). Additionally, 30 days following parturition, pituitary concentrations of LH, FSH and GnRH receptors were not deficient in anestrous females (Moss et al., 1985; Parfet et al., 1986). Failure of luteal tissue to recognize the presence of LH is also disputed to have an effect upon short estrous cycles (Garverick et al., 1988)

because LH receptor numbers found on CL's of both normal and short estrous cycling females were found to be similar and therefore receptor numbers were found not to be the underlying cause (Smith et al., 1986).

Garverick et al. (1988) differ from previous research in that they believe a wide range of possibilities exist regarding the causes of subnormal luteal function. Because development of the CL is simply a continuation of follicular development, they believe that there are a wide array of problems that may occur either pre- or post-ovulation. Issues of concern include: inadequate pre-ovulatory follicular development, a decrease in concentrations of luteotropic stimuli, untimely release of a luteolysin or increased sensitivity to a luteolysin (Garverick and Smith, 1986). Earlier work of Ramirez-Godinez et al. (1982b) provides a basis for this theory. During short estrous cycles in females they observed a pre-estrus rise in serum progesterone concentrations, indicative of ovulation in absence of an observable estrus during a short luteal phase.

Several studies have observed the crucial changes that occur in the CL between days 5 and 7 of the estrous cycle regarding a 2.5-fold increase in CL weight and progesterone concentration (Donaldson et al., 1965; Erb et al., 1971; Garverick et al., 1971). In females anticipated to have a short luteal phase, Zoller et al. (1993) observed an undesirable decrease in numbers of endometrial progesterone receptors and increased numbers of endometrial oxytocin receptors on day 5. Butcher et al. (1992) were able to compensate for short lived CL through administration of exogenous progesterone and maintain pregnancy. They surmise that in addition to short-lived CL's, an undesirable

environment in both the oviduct and uterus may be responsible although additional research into this area is necessary.

Kesler et al. (1981) noticed the relationship between the CL and its responsiveness to LH during this time frame and surmised that both CL weight and progesterone concentrations were due to the increased responsiveness of the secretory cells to LH. Additionally, growth of the CL between days four and seven of the estrous cycle (Donaldson et al., 1965) has been related to an increase in the mitotic activity of theca cells rather than granulosa cells (Donaldson and Hansel, 1965). Therefore, they deduced that cells that had the greatest responsiveness to LH were primarily theca rather than granulosa cells. Garverick et al. (1988) who reported that proper follicular maturation occurs in response to coordinated efforts of LH and FSH upon the thecal and granulosal cells, respectively, supports these earlier findings.

Anestrus

Major regulators of the postpartum interval are suckling (Williams, 1990; Stagg et al., 1998) and nutrition (Selk et al., 1988; Randel, 1990; Wettemann et al., 2003). Also identified as minor supporting factors within the postpartum anestrus are season, breed, age, dystocia, bull presence, and uterine palpation.

Calf suckling

Suckling is an exteroceptive stimulus that plays a crucial role regarding the length of the postpartum interval in the cow (Short et al., 1990; Williams, 1990). The

suppression of the LH pulse generator is caused primarily by neural connections in the mammary gland that are initiated by the suckling calf (Short et al., 1972; Williams et al., 1987). This stimulus is able to suppress pulsatile GnRH secretion from the hypothalamus (Carruthers et al., 1980; Wettemann, 1980; Walters et al., 1982b; Schallenberger and Peterson, 1982) which causes a decrease in both amplitude (Troxel et al., 1980; Peters et al., 1981) and frequency of LH secretion (Lu et al., 1976; Carruthers and Hafs, 1980; Walters et al., 1982a,b; Sirinathsinghji and Martini, 1984; Ben-Jonathan, 1985; Edwards, 1985). In addition to a decrease in GnRH secretion from the hypothalamus, Carruthers et al. (1978) and Smith et al. (1981) suggest that suckling causes the pituitary gland to also have a decreased sensitivity to GnRH.

Extended suppression of LH concentration also prolongs the inhibited period directly following calving due to the chronic effects of gestational steroids (Moss et al., 1981). Even after the decline of gestational steroids, ovarian hormones such as estradiol are able to negatively influence gonadotropin release through suckling (Acosta et al., 1983; Garcia-Winder et al., 1984; Hinshelwood et al., 1985; Chang and Reeves, 1987) because during late gestation increased concentrations of placental estrogen inhibit synthesis of LH and LH stored in the pituitary gland is depleted at parturition (Williams, 1990). This is also due to the fact that the body has an inability to respond to positive estradiol feedback during the first three weeks after calving and furthermore has an increased sensitivity to negative feedback from estradiol (Short et al., 1979). Typically in suckled cows, the requisite pattern of LH secretion is able to recommence due to the hypothalamic center's ability to once again respond to positive feedback from estradiol

concentrations (Williams, 1990). As the postpartum period progresses, the pituitary gland eventually regains receptivity to GnRH secretions (Kesler et al., 1977; Webb et al., 1977; Fernandes et al., 1978; Schallenberg et al., 1978).

Differences have been detected in the effect of suckling between heifer and bull calves. Custer et al. (1990) observed that cows suckling heifer calves returned to estrus an average of 15 days later than their bull calf suckling cohorts. This is opposite to the findings of Bellows et al. (1982), who surmised that cows suckling bull calves returned to estrus "more slowly" than those suckling heifer calves. While Custer et al. (1990) are unable to provide an answer for why this occurs; Bellows et al. (1982) hypothesize that bull calves nurse more aggressively and therefore are able to enhance the inhibitory effect of suckling upon the postpartum interval.

Early weaning has been proposed by many as a means to reinitiate estrus in a timelier manner following calving (Smith and Vincent, 1972; Bellows et al., 1974) as well as increase conception rates (Laster et al., 1973; Ray et al., 1973). However, due to the decreased economic gain and increased labor input of early weaning calves this type of scenario is only recommended under the most adverse of conditions (Williams, 1990). A more practicable alternative to early weaning is temporary calf removal from the cow.

Limited suckling through calf removal has proven to be beneficial at increasing conception rates (Baud and Cummins, 1977; Stuedemann et al., 1981; Montgomery, 1982) and decreasing the interval from parturition to first estrus (Randel, 1981; Reeves and Gaskins, 1981). In scenarios where this occurs for less than 45 days, there has not been shown to be any negative consequences regarding long-term growth rates or

weaning weights of the calves. Only in studies occurring for longer than 45 days have there been any detrimental effects associated with limited suckling (Stuedemann et al., 1981; Montgomery, 1982). However, this type of scenario is best suited for intensively managed operations with a rather limited number of cattle (Williams, 1990).

Nutrition

Numerous studies have consistently linked adequate nutrient intake and energy balance with decreased postpartum interval in the beef cow (Dunn and Kaltenbach, 1980; Rutter and Randel, 1984; Lucy, 2000). Early research was conducted to elucidate the relationship between calculated nutrient intake and reproductive performance (Wiltbank et al., 1962, 1964; Beal et al., 1978; Jordan and Swanson, 1979; Lishman et al., 1979; Moss et al., 1982). This method of research, however, does not take into account varying body energy reserves of the females and thus females within the same treatment group may be actually receiving above or below what is necessary. Therefore, Rutter and Randel, (1984) determined that it is more beneficial to recognize whether or not a female must mobilize body energy reserves in order to meet production and lactational demands during the postpartum period, irregardless of the calculated nutritional requirements.

Reproduction is influenced both directly and indirectly through nutritional mediators such as the thyroid (De Moraes et al., 1998). Indirectly, decreased levels of thyroid activity lead to decreased rumen motility and passage rate and increased digestibility (Miller et al., 1974; Kennedy et al., 1977). The major metabolically active

thyroid hormone, triiodothyronine (T3) is found in greater concentrations in cows with moderate versus thin body condition scores (Flores et al., 2008). Similarly, concentrations of thyroxine (T4), a precursor to T3, were decreased in nutrient deficient cattle (Richards et al., 1995; Capuco et al., 2001) and elevated in cattle with higher levels of nutrient intake (Lents et al., 2005).

Acting directly upon reproduction, both T3 and T4 are shown to stimulate thecal cell steroidogenesis in vitro, therefore increasing estrogen production by the follicle (Spicer et al., 2001). Furthermore, Flores et al. (2008) found that anestrous cattle in low body condition scores possessed lower concentrations of T4, therefore resulting in a smaller dominant follicle.

Rasby et al. (1991) surmised that the effects of inadequate nutrition upon pituitary and ovarian characteristics are minimized to a degree because thin cows are able to compensate through a more efficient utilization of nutrients. This theory is supported by other researchers (Wagner et al., 1988; Lake et al., 2004; Lake et al., 2006) who have noted similar compensatory responses in bodily function as a coping and survival mechanism.

Seasonality

Due to their superior performance in the less temperate regions of the southern United States *Bos indicus* cattle have been utilized extensively. It has been well documented that the greatest single detriment to the breed is the relatively low fertility rate (Kincaid, 1957; Warnick, 1963; Plasse, 1973). Rhodes et al. (1982) surmised that

this is due to the vastly different endocrinology patterns between *Bos taurus* and *Bos indicus* cattle and their seasonality concerning reproductive patterns. The hormonal events are influenced by three primary factors, including: minimum temperature, plane of nutrition and bull exposure during the breeding season (Plasse et al., 1968).

Brahman heifers observed by Plasse et al. (1968) were found to exhibit a seasonal pattern regarding uterine size and tone. The majority of females in January had small uteri without tone. As the seasons progressed and the ambient temperature rose so did the number of estrous cycling females. The number of small uteri females steadily increased beginning in September, peaked in January, and decreased until April indicating seasonal cyclicity. A similar pattern was observed by Plasse et al., (1970) regarding the incidence of quiet ovulations and those lasting abnormally longer in length (> 24 days) during the winter months. Rhodes et al. (1982) observed a similar seasonal pattern in corpus luteum weights. Heifer CL weights and progesterone concentrations were greater in the winter versus summer months; however, gonadotropin sensitivity was opposite and peaked during the summer months.

Seasonality in many species of mammals is known to be controlled by the functions of the thyroid gland where estrous cyclicity, ovulation and hormone secretion are all altered due to seasonal changes(Cabell and Esbenshade, 1990; Jahn et al., 1995; Mattheij et al., 1995). Rhodes et al. (1982) and Stahringer et al. (1990) both observed a potential link between thyroid gland activities in *Bos indicus* cattle and a subsequent change in estrous cyclicity and ovarian function. However; because it has also been suggested that because the thyroid gland mediates nutritional factors such as rumen

motility, passage rate, and digestibility (Miller et al., 1974; Kennedy et al., 1977) and nutrition influences reproductive success (Randel, 1990) the thyroid gland therefore has a greater influence upon reproduction in this indirect manner (De Moraes et al., 1998).

Breed

The endocrine relationships experienced by *Bos indicus* females are different from *Bos taurus* females or even crossbreeds between the two. This is caused by a multitude of factors, including: decreased responsiveness to estrogen, decreased weight of the CL, lower progesterone concentrations and a smaller preovulatory LH surge (Irvin et al., 1978; Rhodes et al., 1982; Randel, 1984).

Age

Challenges may exist for breeding females due to either their lack of maturity or advanced age. Primiparous heifers have a greater incidence of dystocia at calving (Nelson and Beavers, 1982; Doornbos et al., 1984; Gregory et al., 1991), 17% versus 4% for multiparous cows (Nix et al., 1997). This is contributable primarily to the smaller body frame and thus decreased pelvic diameter of the heifer (Doornbos et al., 1984). Dystocia at calving in heifers tends to delay uterine involution and thus extend the length of the postpartum interval (Renquist et al., 2006). Additionally, although all cows tend to lose one body condition score from calving to subsequent breeding the next season, heifers are the most affected and have the lowest BCS of any age group (Renquist et al.,

2006). However; not all reproductive challenges are faced by younger, primiparous females.

Cows greater than nine years of age tend to birth lighter weight calves that are significantly lighter at weaning as well (Renquist et al., 2006). This is in agreement with findings by Trail et al. (1982) and Nadarajah et al. (1984) working with 9 and 11 year old females. As a female begins to age the interval between consecutive calvings tends to decrease (Plasse et al., 1968) although some researchers find very little significant difference in this trend (Renquist et al., 2006). The general trend in the literature suggests that most females begin a decline in their reproductive potential after nine years of age and thus should be culled from the herd to forego any future losses due to age.

Dystocia

Dystocia is a major concern in the beef industry as it is the major cause of perinatal calf losses (Anderson and Bellows, 1967). It also causes females to conceive later in the breeding season or fail to rebreed entirely (Rutter et al., 1983; Doornbos et al., 1984). Primiparous and younger females typically have the highest incidence of calving difficulty, attributable to their continuing growth and smaller pelvic diameter (Doornbos et al., 1984; Basarab et al., 1993a,b). In addition to parity (Laster, 1974; Rutter et al., 1983) and decreased pelvic size, dystocia factors include excessive calf size (Berglund and Philipsson, 1987; Johnson et al., 1988) and malpresentation (Basarab et al., 1993a).

Bull exposure

Bull exposure to females, or biostimulation (Chenoweth, 1983), has been conducted throughout the years with varying success and results. Some research conducted regarding bull exposure hastening the onset of estrus in females failed to achieve any positive results (Berardinelli et al., 1978; MacMillan et al., 1979; Roberson et al., 1987). However, bull presence after calving in both primiparous and multiparous females has been shown to reduce the length of postpartum anestrus in several other studies (Zalesky et al., 1984; Alberio et al., 1987; Naasz and Miller, 1987; Gifford et al., 1989; Custer et al., 1990; Burns and Spitzer, 1992; Landaeta-Hernandez et al., 2004). Strong evidence supports this theory in both mice (Bronson and Desjardins, 1974) and sheep (Martin et al., 1980; Poindron et al., 1980).

Cupp et al. (1993) found that bulls as young as one year old are just as capable of shortening the postpartum anestrus in cows through biostimulation. Zalensky et al. (1984) reported that in two groups of females exposed to a bull beginning either three days following calving or fifty three days later, the females that received early exposure returned to estrus an average of 21 days earlier. Burns and Spitzer (1992); however, did not observe any effects of biostimulation beyond 60 days following parturition, suggesting that other mechanisms begin to play a more crucial role so late after calving. It is hypothesized that bull presence is able to stimulate the central nervous system causing an increase in LH release in the female immediately following exposure (Custer et al., 1990). Research by Fernandez et al. (1996) supports this claim. They found a rise in both LH and FSH pulses after intermittent exposure of bulls to postpartum cows.

However, this temporary rise in hormone pulsatility was not sufficient to induce early ovarian activity and cyclicity.

Some researchers have even gone so far as to suggest that pheromones from excretory products are sufficient in stimulating estrus behavior in females. Barauh and Kanchev (1993) observed elevated concentrations of both LH and FSH in dairy cattle 70-80 minutes after oronasal administration of bull urine. Similarly, resumption of ovarian activity was found to be shorter for females exposed to bull excretory products than those that were not exposed (Berardinelli and Joshi, 2005a). Interestingly, females exposed to other females' excretory products were found to have a decreased postpartum interval as well. This is finding supported by Burns and Spitzer (1992), who observed that androgenized females elicited the same response as both penile deviated and epididymectomized bulls. They hypothesize that androgens found in the urine act as pheromones. Again; however, studies conducted have disputed these findings and reported dissimilar results.

Tauck et al. (2006) found no difference in the length of the postpartum interval in females exposed to either bull urine or steer urine. They admit that these findings may be due to experimental design. It is possible that the females in their study were subjected to overexposure as they were in the presence of a stimulus 24 hours a day while in other studies (Berardinelli and Joshi, 2005a,b) presence was limited to 12 hours a day maximum. While research through the years has unearthed several interesting phenomena, it is evident that further studies must be conducted in order to gain more

definitive conclusions regarding practicality and utilization of biostimulation and excretory products.

Reproductive hormones and blood metabolites

Reproductive hormones and some blood metabolites have been identified that may play a role in the resumption of normal follicular activity including progesterone and nonesterified fatty acids. While neither one is solely responsible for recommencement of follicular development following the postpartum period, they are crucial in both the endocrine and nutritional pathways.

Although the precise mechanism by which progesterone is able to hasten the onset of ovarian cyclicity in females is not entirely known, it has been identified as an effective means to stimulate follicular growth (Patterson et al., 1992). It has been hypothesized that a correlation exists between proper nutritional status and positive feedback to the hypothalamus necessary to initiate GnRH secretion; therefore, allowing pulsatile LH secretions to begin and cause the maturation and subsequent ovulation of the dominant follicle (Wettemann et al., 2003).

Prior to parturition in the multiparous beef cow, estradiol concentrations remain elevated and inhibit pulsatile release of LH (Arije et al., 1974). After parturition, not only are estrogen concentrations significantly reduced (Arije et al., 1974), but adequate nutrient reserves allow for the resumption of normal, pulsatile release of GnRH from the hypothalamus (Wettemann et al., 2003). The combined effect of these two occurrences leads to the subsequent ovulation of the dominant follicle.

Lucy et al. (1991) demonstrated that the number of more desirable, class three follicles (10 to 15 mm diameter) increased with a positive energy balance. This is further supported by the idea of progesterone "priming" (Gonzalez-Padilla et al., 1975a,b; Williams and Ray, 1980; Ramirez-Godinez et al., 1981) where the feeding of a high density lipoprotein diet promotes the earlier onset of transitional changes such as higher concentrations of progesterone that lead to uterine PGF2α secretion (Lamming and Mann, 1995; Mann and Lamming, 2001) and estrous cyclicity (Williams, 1989; Landaeta-Hernandez et al., 2004). Leung et al. (1986) suggests that LH concentration changes have the greatest affect upon reestablishment of estrous cycles. Pituitary and circulating content of LH following parturition is quite low (Humphrey et al., 1983). Although the pituitary content immediately begins to increase during the first 30 days following parturition in suckled females (Saiduddin and Foote, 1964; Graves et al., 1968; Wagner et al., 1969), circulating concentrations fluctuate very little during the first 18 days following parturition (Carruthers et al., 1980; Rawlings et al., 1980; Williams and Ray, 1980; Riley et al., 1981).

Spicer et al. (1986) surmise the increased number of large and medium follicles containing high concentrations of estradiol positively influence LH secretion in order to promote further folliculogenesis. While it is clear that further investigation into the role that metabolic hormones play in follicular development is necessary there is also a considerable amount of literature on the subject readily available.

Measurement of blood metabolites such as nonesterified fatty acids (NEFA) may be useful in indicating both nutritional status and subsequent rebreeding performance

potential (Randel, 1990). Hart et al. (1978), Vasilatos and Wangness (1981), and Kunz et al. (1985) all found evidence to conclude that blood metabolites, such as NEFA, are crucial in the understanding of mobilization of adipose tissue reserves in order to meet energy demands.

Several studies in dairy cattle have observed parallels between elevated NEFA concentrations and undesirable reproductive consequences (Reist et al., 2000; Landaeta-Hernandez et al., 2004; Hayhurst et al., 2007; Walsh et al., 2007; Wathes et al., 2007; Oikonomou et al., 2008). Additionally, nonesterified fatty acid concentrations are negatively correlated with the energy balance of an animal, body weight of the cow (McCann and Hansel, 1986; Richards et al., 1989b) and conception rate following first AI (Oikonomou et al., 2008) as they are the byproducts of the metabolism of adipose tissue (Lucy et al., 1991). Vizcarra et al. (1998) observed similar results in the relationship between NEFA concentrations and the presence or absence of luteal activity in females.

During gestation, the female is expected to accumulate stores of lipids (McNamara, 1991) until approximately one month (McNamara and Hillers, 1986) to15 days prepartum (McNamara et al., 1995). During times of either negative energy balance or insufficient concentrations of insulin, lipase secretion is stimulated thus causing lypolysis and the release of NEFA concentrations into the bloodstream (Nelson and Cox, 2000; Melendez et al., 2009). Lactating cattle typically experience an energy deficit early after calving when maximum milk production is attained prior to maximum feed consumption (Lucy et al., 1991; McNamara, 1991; McNamara et al., 1995) and the

nutrient demands of the mammary gland exceed those of the rest of the body (Barber et al., 1997). Thus, as energy demands are not adequately met within the diet, an animal's own adipose reserves must be mobilized and there is a subsequent increase in NEFA concentrations (McNamara, 1991). Because nutrient mobilization and lipid deposition is repartitioned towards support of mammary function (Bauman and Currie, 1980; McNamara et al., 1987; Lake et al., 2006; McNamara et al., 1995) body condition of the females suffers as a result (Smith and Walsh, 1988; Lake et al., 2006) and negatively influences reproductive function (Hess et al., 2005) although over the course of the postpartum interval the rate of lipogenesis and esterification gradually increases (McNamara et al., 1995).

Westwood et al. (2002) observed that dairy females with greater NEFA concentrations had a lower probability of conceiving by day 150 of lactation. Similar results were observed by Lake et al. (2006), who found that cows in a BCS 4 had greater concentrations of lipoprotein lipase compared to cows with a BCS 6. Because lipoprotein lipase is needed to catabolize hydrolysis of fatty acids from circulating triacylglycerols (Gauster et al., 2005; Lake et al., 2006) they hypothesize that an increase in lipoprotein lipase is indicative of subsequent increases in NEFA concentrations available to the adipocyte surface for storage. Lake et al. (2004) observed decreased NEFA concentrations in BSC 4 cows versus BCS 6 and attributed this to the fact that individuals in an undesirable BCS have a greater need to increase body energy and lipid stores (Wagner et al., 1988).

While this might lead one to believe that increased NEFA concentrations act as a signal to the hypothalamus, and regulate recrudescence of follicular activity, such is not always the case. Vizcarra et al., (1998) found that cows in a moderate body condition fed on an increased plane of postpartum nutrition compared with their contemporaries actually had higher NEFA concentrations. This may be occurring in part because the cows on a higher plane of nutrition were able to wean heavier calves and therefore the increased NEFA concentrations may be more closely associated with an increase in milk production (Spitzer et al., 1995). These factors led researchers to believe that while NEFA concentrations are not absolute indicators of energy balance or predictive of probable resumption of ovarian activity (Vizcarra et al., 1998), they may serve as useful indicators (Reist et al., 2002; Clark et al., 2005; Oikonomou et al., 2008).

It is obvious that nutrition and reproduction are intertwined in their roles within any beef cattle operation. While no one factor is absolutely indicative of either nutritional status or guaranteed reproductive success, taken as a whole they are able to create a broader understanding of profitable animal husbandry. Residual feed intake has been identified as a means to identify animals that are more efficient in metabolic nutrient utilization, and energy efficiency is one of the key components needed to minimize the length of postpartum interval. As a result, the following experiment was designed primarily to evaluate the relationship that exists between RFI, cow productivity and subsequent calf performance within the *Bos indicus* breeding herd.

CHAPTER II

RELATIONSHIPS OF RESIDUAL FEED INTAKE SELECTION AND PARITY UPON NEFA CONCENTRATIONS, CHANGES IN BODY WEIGHT AND CHANGES IN BODY CONDITION SCORE

Introduction

As a female experiences different phases during the year related to producing a calf (i.e., gestation, calving and lactation), her body energy reserves change to reflect her nutritional state. This becomes crucial when considering the multiple studies (Dunn and Kaltenbach, 1980; Rutter and Randel, 1984; Lucy et al., 1991; Rhodes et al., 1995) that demonstrate how body energy reserves influence reproductive performance. Adipose tissue metabolism in the female occurs cyclically as stores are accumulated during mid-gestation and then are subsequently released prior to parturition in order to maintain homeorhesis (McNamara and Hillers, 1986).

Body condition scoring is a useful visual predictor of body energy reserves of adipose tissue stored within the female at a given time. Wiltbank et al., 1962; Richards et al., 1986; Wagner et al., 1988; McNamara et al., 1995 conclude that use of BCS measurements are more indicative of available energy reserves during the prepartum period than sole use of body weights that are unable to account for conceptus growth during late gestation. Stores of adipose tissue are mobilized during times of nutrient restriction or periods of negative energy balance when a female is unable to ingest as much energy through feedstuffs as is being utilized for processes such as lactation. Also

during this time, there is a dramatic decrease in hepatic lipogenesis (Mayes and Topping, 1974). Ookhtens et al. (1987) demonstrated the rapid mobilization of fat reserves and an increase in lypolysis in 48 hour fasted mice which directly contributed to the sustenance of the individual.

Lipogenesis is regulated by and inversely correlated with plasma concentrations of nonesterified fatty acids (NEFA) (Mayes and Topping, 1974). Beginning approximately one month prior to parturition, lipogenesis rates consistently decline and remain this way for a period of approximately three months. These rates experience dramatic rebounds during mid-lactation (between two and six months) following calving. Conversely, lypolysis rates peak in order to sustain lactational demands early in the postpartum period and subsequently begin to return to their basal levels over time (McNamara and Hillers, 1986).

As previously reviewed in Chapter I, Herd and Arthur (2009) demonstrated that individuals divergently selected for RFI had underlying physiological mechanisms that accounted for variation in their RFI values. Among these variables were differences in: protein turnover, tissue metabolism, stress response, digestibility and body composition. Therefore, one may assume that these variations may play a role in the rate of lypolysis in an individual as demonstrated by NEFA concentrations. Therefore, the following study was conducted in order to elucidate any possible relationships that may exist among residual feed intake status, NEFA concentrations, and changes in both body weight and body condition scores during the five-week period immediately prior to parturition.

Materials and Methods

Animals and experimental design

Ninety-three pregnant Brahman females, both primiparous (n = 30) and multiparous (n = 63) ranging from two to seven years of age were previously evaluated in feeding trials to characterize positive or negative residual feed intake efficiency at the Texas *Agri*Life Research facility in Overton, Texas. The earliest that females were ever subjected to RFI evaluation was one week after weaning, while the oldest females ever subjected were two year old pregnant females. The age of the females when evaluated is irrelevant because Residual Feed Intake (RFI) characterization is independent of body size, unlike feed:gain ratio, and each cohort tested was of a similar age.

Beginning approximately five weeks prior to each individual's expected calving date during the 2009 calving season, females grazing ryegrass pasture were assessed weekly for body condition scores as well as body weight. In addition, a 15-mL blood sample was taken at the same time via caudal venipuncture and later assayed for nonesterified fatty acid concentrations.

Analysis of blood metabolites

As previously indicated, serum samples were collected from the females weekly beginning approximately five weeks prior to calving. The samples were collected in 15-mL Vacutainer tubes and held in a refrigerator for 24 hours before being centrifuged at 3200 x g for 40 minutes. After removal from the centrifuge, serum samples were stored at -20° until the time of analysis for nonesterified fatty acid concentrations. Non-

esterified fatty acid serum samples were analyzed utilizing a commercially available enzymatic colormetric analysis kit (NEFA-C, Wako Chemicals USA, Inc., Richmond, Virginia) at Texas A&M University in College Station, Texas.

Cow performance evaluation

Multiple parameters were included in order to evaluate cow performance, including:

1. Residual feed intake status
2. Parity
3. Body condition score
4. Change in BCS
5. Body weight
6. Change in BW
7. Nonesterified fatty acid concentrations

Statistical analysis

Females were first classified based upon their respective feed efficiency status. The females with a negative RFI were deemed “efficient” and conversely the females with a positive RFI were identified as “inefficient” based upon numerical RFI values as using numerical RFI values across multiple cohort groups is impossible. Additionally, females were also stratified by parity, either primiparous or multiparous. NEFA concentrations, BW, BCS, and changes in both BW and BCS were subjected to ANOVA

specific for repeated measures utilizing the PROC MIXED function of SAS (2002) with RFI and parity as class variables. In the event that no interactions existed between RFI and parity, the interaction was then omitted from the final model. In order to reveal relationships among RFI, parity, NEFA concentrations, and pre-calving changes in BW and BCS, Pearson correlations were obtained using PROC CORR (SAS 2002).

Results

During the five week sampling period that occurred prior to calving, mean BW for females based upon RFI status was not significantly different. Mean BW for efficient females was 543.77 ± 6.14 kg and 546.36 ± 5.57 for inefficient females (Table 2.1). Mean BCS did differ; however, as mean BCS was 6.2 ± 0.06 and 6.6 ± 0.06 for efficient and inefficient females; respectively. Nonesterified fatty acid concentrations for efficient and inefficient females were 0.328 ± 0.0170 mEq/L and 0.341 ± 0.0154 mEq/L; respectively, and were not significantly different. Body weights based upon

parity were significantly different from one another as mean BW for multiparous females was 582.07 ± 4.28 kgs and 482.53 ± 5.67 kgs for primiparous females (Table 2.2). Similarly, a difference was observed in BCS as mean BCS for multiparous females was 6.6 ± 0.05 and 6.1 ± 0.07 for primiparous females. Nonesterified fatty acid concentrations for multiparous and primiparous females did not differ and were 0.334 ± 0.0142 mEq/L and 0.330 ± 0.0196 mEq/L; respectively.

Effects of time

Utilizing repeated measures analysis over time for all females, no significant effects regarding changes over time prepartum were observed for absolute BW, BCS, or NEFA concentrations. It was observed; however, that time prepartum created a significant effect upon change in BW (P = 0.0026, Figure 2.1), although not upon change in BCS. Additionally, the interaction between RFI status and sampling day during the prepartum period had a significant effect upon the change in BW (P = 0.0376, Figure 2.2).

Table 2.1. Summary statistics for Brahman females based upon RFI status.

	Efficient	Inefficient	P-Value
BW, kgs	543.77	546.36	0.7549
BCS	6.2	6.7	< 0.0001
NEFA, mEq/L	0.328	0.341	0.5601
Change BW, kgs	2.51	2.87	0.7953
Change BCS	-0.017	0.002	0.6702

[a] BW = body weight, BCS = body condition score, NEFA = nonesterified fatty acid.

Table 2.2. Summary statistics for Brahman females based upon parity.

	Multiparous	Primiparous	P-Value
BW, kgs	582.07	482.53	< 0.0001
BCS	6.6	6.1	< 0.0001
NEFA, mEq/L	0.334	0.330	0.848
Change BW, kgs	2.34	-0.46	0.1284
Change BCS	-0.01	-0.09	0.1475

[a] BW = body weight, BCS = body condition score, NEFA = nonesterified fatty acid.

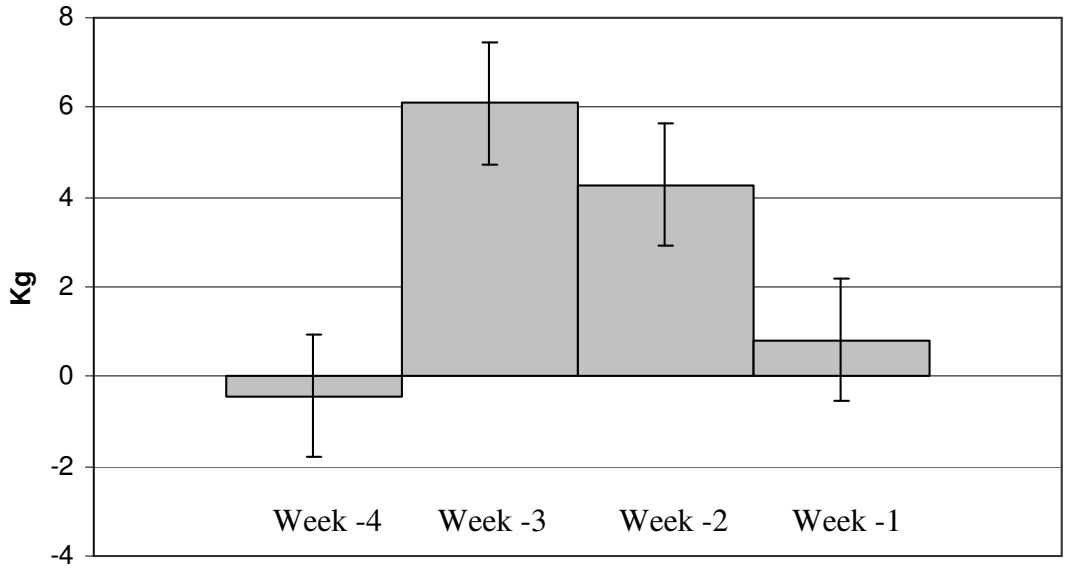

Figure 2.1. Prepartum changes in body weight over time in Brahman females (P = 0.0026).

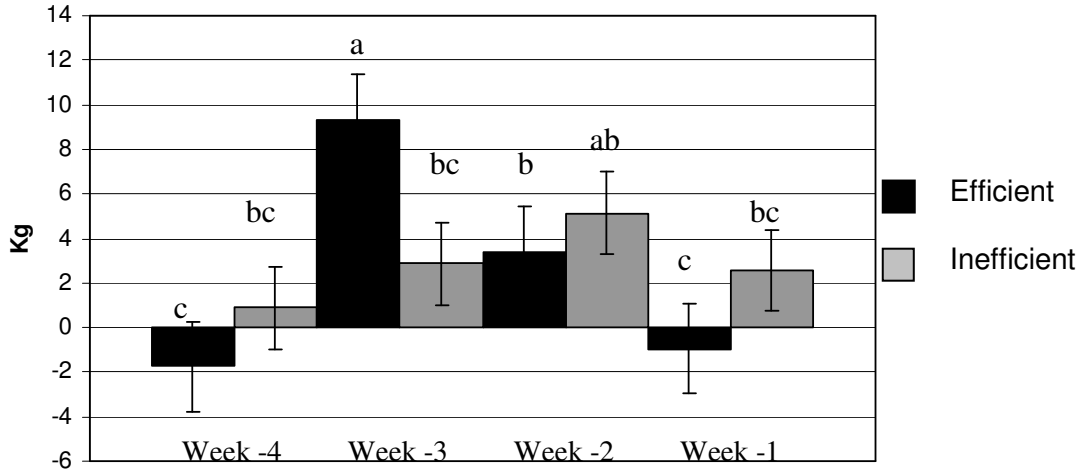

Figure 2.2. Effect of RFI status x time prepartum interaction upon change in BW in Brahman females (P = 0.0376).

Effects of RFI status

Residual feed intake status did significantly affect BCS ($P < 0.0001$, Figure 2.3) as efficient females had a considerably lower BCS than their inefficient counterparts (6.2 BCS vs. 6.6 BCS); however, it had no significant influence upon either BW or NEFA concentrations during the prepartum period.

Effects of parity

Parity had a significant effect upon BW ($P < 0.0001$, Figure 2.4) and BCS ($P < 0.0001$, Figure 2.5) during the prepartum period. Multiparous females were not only heavier than their primiparous counterparts (582.07 ± 4.28 kg vs. 482.53 ± 5.67 kg, respectively); they also possessed a greater BCS as well (6.6 ± 0.1 BCS vs. 6.1 ± 0.1 BCS). Although parity did not have an effect upon NEFA concentrations, a tendency for an interaction between parity and RFI status did exist with regard to NEFA concentrations ($P = 0.0745$, Figure 2.6). Similarly, the interaction between RFI status and parity was significant for BW ($P = 0.0256$, Figure 2.7) and BCS ($P = 0.0003$, Figure 2.8).

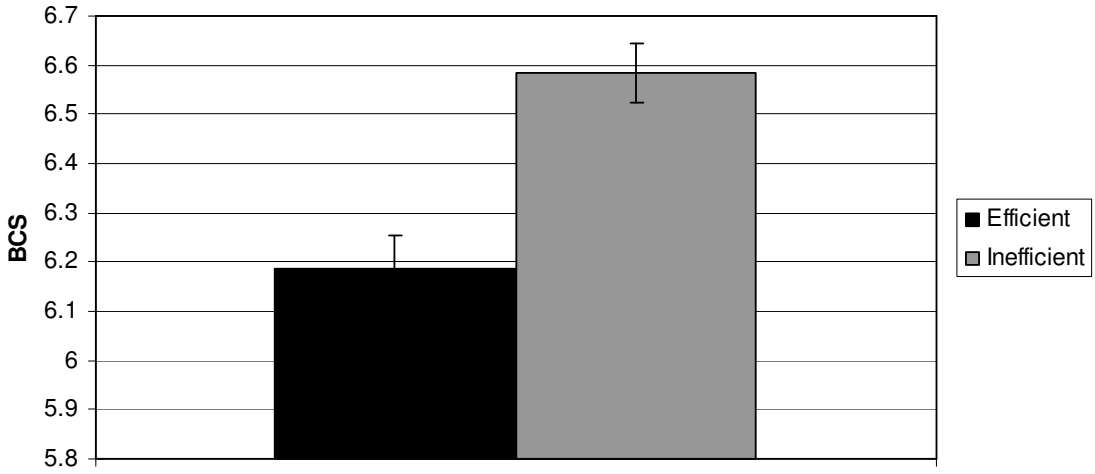

Figure 2.3. Effect of RFI status upon BCS during the prepartum period in Brahman females ($P < 0.0001$).

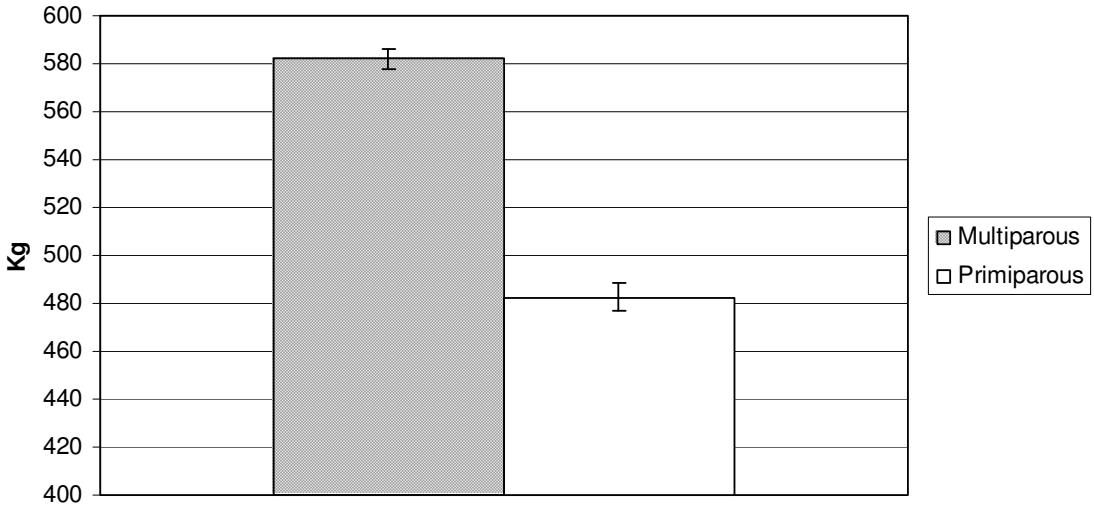

Figure 2.4. Effect of parity status upon BW of females during the prepartum period in Brahman females ($P < 0.0001$).

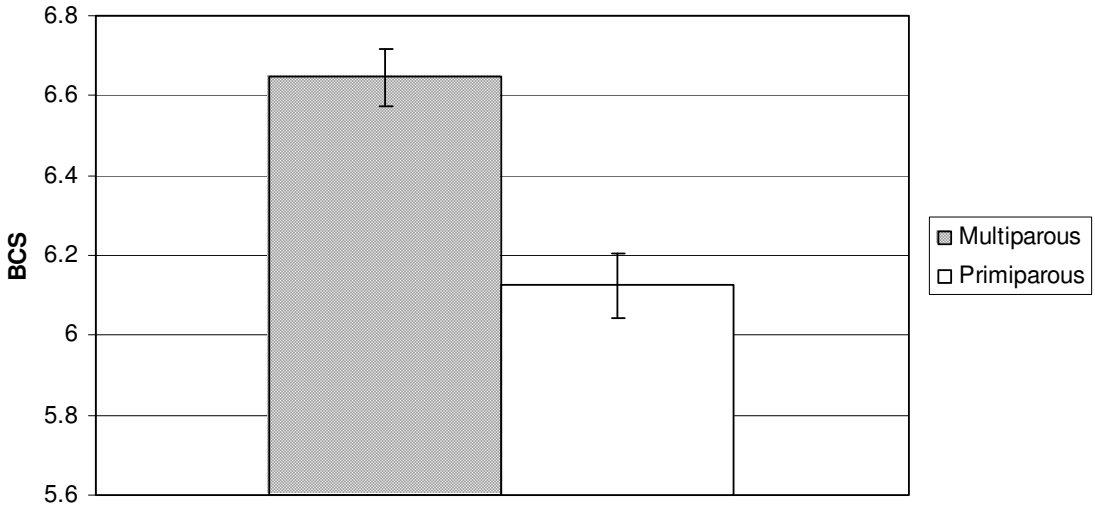

Figure 2.5. Effect of parity status upon BCS in Brahman females during the prepartum period ($P < 0.0001$).

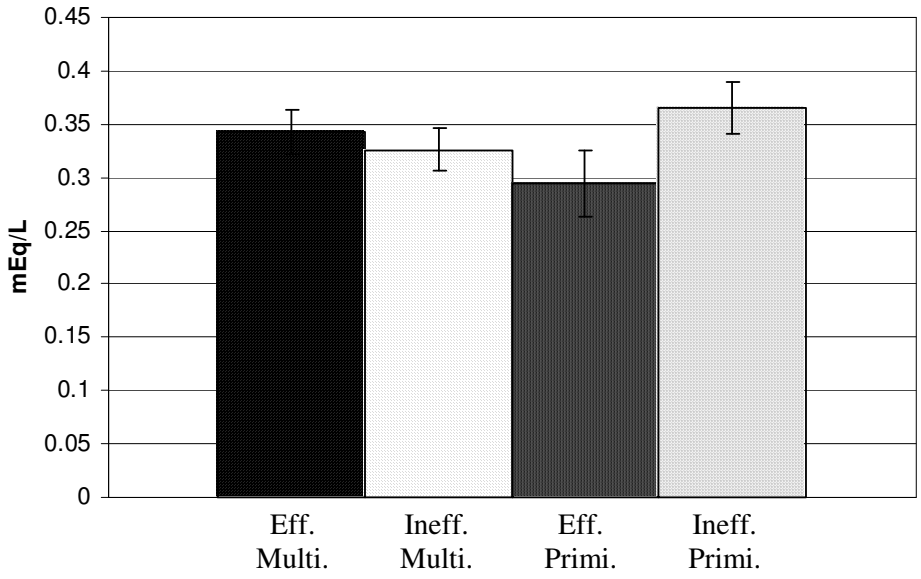

Figure 2.6. Effect of the parity x RFI status interaction upon NEFA concentrations in Brahman females during the prepartum period (P = .0745).

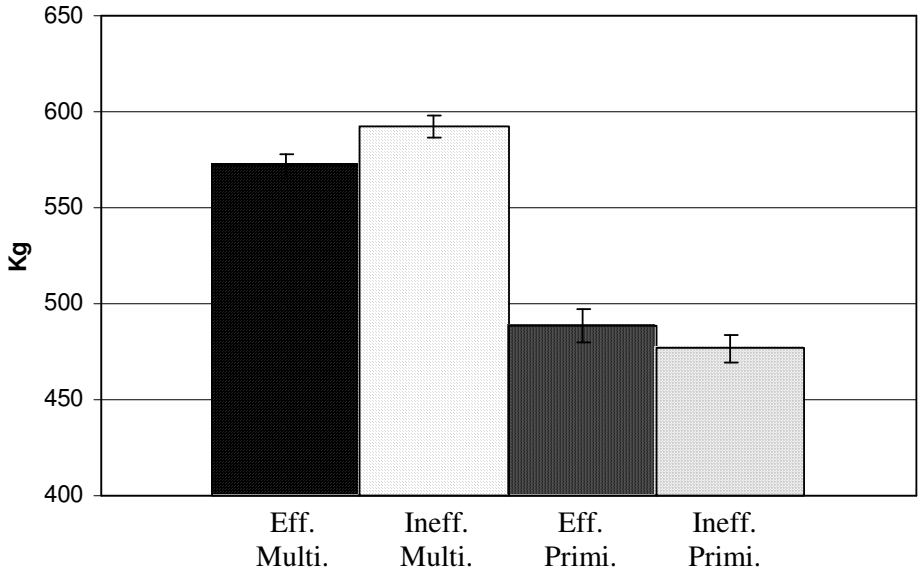

Figure 2.7. Effect of the parity x RFI status interaction upon BW during the prepartum period in Brahman females (P = 0.0256).

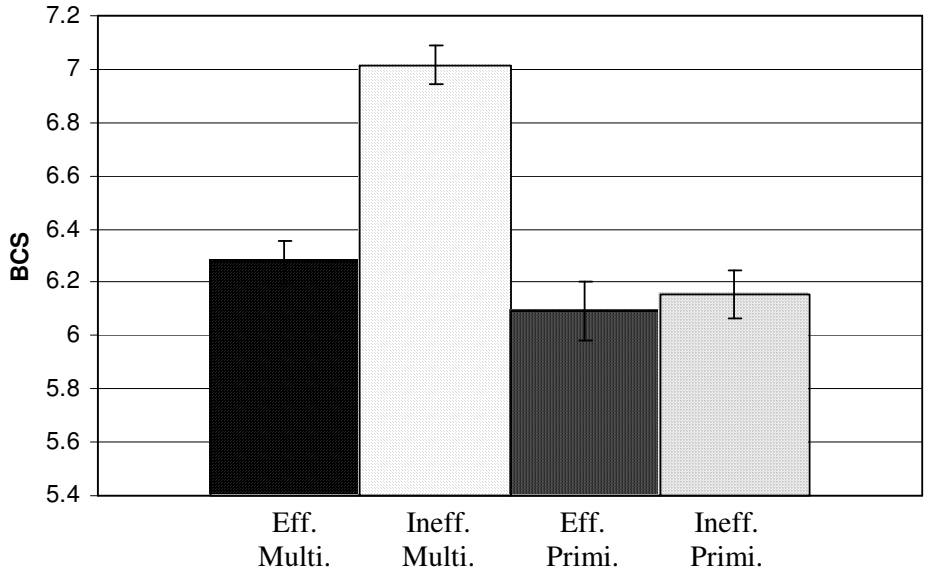

Figure 2.8. Effect of the parity x RFI status interaction upon BCS in Brahman females during the prepartum period (P = 0.0003).

Correlations among NEFA concentrations, BW and BCS

When considering all sampling days combined during the prepartum period, strong correlations between the changes in BW and BCS were observed in multiple classifications of females (Table 2.3). When separated according to parity or according to RFI status, all classes of females showed significant, moderate- to low-, positive, correlations ($P < 0.05$). Upon combining the two classifications, it was found that the majority of the groups retained their significance. Both efficient and inefficient multiparous females, as well as inefficient, primiparous females all had significant correlations between the change in BW and change in BCS during the prepartum period ($P < 0.05$). Efficient, primiparous females were the only group that did not follow this trend.

Discussion

As shown in Table 2.1, inefficient females had greater BCS during the prepartum period than did their more efficient counterparts. This difference may be due in part to increased feed consumption by the inefficient females. Golden et al. (2008) demonstrated that inefficient RFI steers had an increased amount of feed consumption per day when fed either no-roughage or roughage-containing diets although this study was conducted in a feedlot setting and not a pasture situation. In addition to consuming more throughout the day, these crossbred Angus steers also had an increased number of eating bouts during the course of a 24 hr period. These facts help support the findings of this study and suggest how inefficient females were able to have increased BCS values.

Table 2.3. Correlations among NEFA concentrations, BW and BCS in Brahman females.[1]

	NEFA Concentrations, BW	NEFA Concentrations, BCS	BW, BCS
	Correlation	Correlation	Correlation
Efficient Multiparous	- 0.03371	0.05902	0.5058*
Inefficient Multiparous	- 0.01476	0.04293	0.66363*
Efficient Primiparous	0.04635	- 0.06691	0.0253
Inefficient Primiparous	- 0.11922	- 0.1262	0.47176*
Efficient	0.01074	0.08265	0.38022*
Inefficient	- 0.02291	0.03476	0.64635*
Multiparous	- 0.01862	0.05562	0.61028*
Primiparous	- 0.05792	- 0.09245	0.25422*

[1] Females were weighed once weekly beginning approximately five weeks prior to parturition. A 15-mL blood sample was taken in addition in order to determine NEFA concentrations in addition to BW and BCS measurements.
* indicates $P < 0.05$.

Body weight was shown to significantly change over the course of the prepartum period. Caution should be exercised; however, when considering the validity of this finding. Multiple studies (Wiltbank et al., 1962; Richards et al., 1986; Wagner et al., 1988; McNamara et al., 1995) indicate that utilizing BW measurements might not be an accurate representation of actual growth or change in the female over time. This is especially true during the prepartum period when BW measurements cannot account for the weight of the developing fetus and thus may be misleading. These studies suggest that utilization of body condition scores as a more useful indicator of change in growth or body composition. Selk et al. (1988) draws an elegant conclusion that helps to combine the results in both BW and BCS from the current study to results regarding the significance of BW in previous literature. Body condition scores are modulated by variations in BW over time and therefore females with similar BCS may differ in actual BW. This relationship suggested by Selk et al. (1988) is in agreement with the moderate to low correlations between changes in BW and changes in BCS reported in the current study.

Upon further scrutiny, when considering the addition of RFI status to the change in BW during the prepartum period, it was observed that efficient females were more extreme in their fluctuations in weight, experiencing both gains and losses. Inefficient females experience a steady increase in weight gain until one week prior to parturition. Perhaps the differences between the two groups of females are due to inefficient females increased amount of feed consumption and number of eating sessions daily as previously demonstrated by Golden et al. (2008), or perhaps a rapid increase in fetal growth during

Week -3 for efficient females, although accurate measurement of fetal weight is nearly impossible. Residual feed intake status did affect BCS of females, as inefficient females were found to be in better body condition than their efficient counterparts. This is in agreement with Loyd (2009) who found similar results during the prepartum period.

Differences in parity were found to affect BW as multiparous females were considerably heavier than their primiparous counterparts. This is to be expected, as not only have the primiparous females not yet reached their mature size, they are also in competition with the growing fetus for available nutrients (Spitzer et al., 1995). Similarly, primiparous females had lower body condition scores than their mature counterparts. These findings support those of Renquist et al. (2006). Over the course of five years among cattle ranging in age from 3-10 years of age, primiparous females consistently had lower BCS values. Loyd (2009) found similar conclusions regarding differences in both BW and BCS in regards to parity status of the female.

Although neither RFI status, parity nor sampling time prepartum had an effect upon NEFA concentrations when analyzed individually, when combined the interaction between RFI and parity tended to produce differences between efficient and inefficient primiparous females. Although it is evident that inefficient females are mobilizing an increased amount of energy reserves, the exact mechanisms remain unclear. This may be in part due to the adaptation of enzymatic systems (McNamara, 1989) in anticipation of lactation and increased energy demands following parturition. Lipogenesis is regulated by and inversely correlated with plasma concentrations of nonesterified fatty acids (NEFA) (Mayes and Topping, 1974). Beginning approximately one month prior to

parturition, lipogenesis rates consistently decline and remain this way until approximately two weeks following parturition. After minute increases in lipogenesis rates for approximately two weeks, a drastic increase occurs until roughly two months following calving (McNamara and Hillers, 1986). Conversely, lypolysis rates peak in order to sustain lactational demands early in the postpartum period and subsequently begin to return to their basal levels (McNamara and Hillers, 1986).

Body weight was affected by the interaction between RFI status and parity, although the role of parity is much more evident than RFI in this interaction. This difference in BW can be attributed to aforementioned reasons, including continued growth of the females in addition to support of a pregnancy. Inefficient, multiparous females were found to have the greatest BCS when considering the interaction between RFI status and parity and their affect upon BCS. This finding is in agreement with both Robinson and Oddy (2004) and Golden et al. (2008) and suggests that the greater BCS in the inefficient females may be attributable in part due to an increased number of both eating sessions and increased feed intake.

Conclusions

The results of this study suggest that BW and BCS are strongly influencing each other, and in addition are influenced by RFI status and parity. Efficient females are lower in BCS, suggesting that there are underlying mechanisms controlling utilization of feedstuffs. The lower BCS of efficient females is not a cause for concern as Hess et al. (2005) reported that optimal reproductive performance is achieved when BCS is at or

above 5, as females in this study were. Our findings support those of Lake et al. (2005; 2006) and Houghton et al. (1990) who surmise that cows in suboptimal body condition (BCS < 5) are able to utilize nutrients more efficiently to maintain homeostasis. Inefficient, primiparous females had the highest NEFA concentrations, suggesting that they must mobilize more of their nutrient stores in order to sustain life processes, maintenance of gestation and growth of the fetus as well as to prepare for upcoming lactation demands.

CHAPTER III

RELATIONSHIPS BETWEEN RESIDUAL FEED INTAKE SELECTION, AND RATE OF RETURN TO ESTROUS CYCLICITY UPON NEFA CONCENTRATIONS, CHANGES IN BODY WEIGHT, CHANGES IN BODY CONDITION SCORE AND REPRODUCTIVE PERFORMANCE IN MULTIPAROUS BRAHMAN COWS

Introduction

It has been previously stated in Chapters I and II that body energy reserves influence reproductive performance in the female (Dunn and Kaltenbach, 1980; Rutter and Randel, 1984; Lucy et al., 1991; Rhodes et al., 1995). These energy reserves are cyclic in nature and fluctuate with each calf produced during the course of a female's productive years. Lipogenesis rates are higher during the majority of gestation, while catabolic events such as lipolysis increase during the periparturient period and slowly decline throughout the lactational phase (McNamara and Hillers, 1986). Between one month (McNamara and Hillers, 1986) and 15 days prior to parturition body energy reserves from adipose tissue are mobilized in order to establish and maintain lactation (McNamara et al., 1995). Although both BCS and BW are useful indicators of energy balance over time, Russel and Wright (1983) determined that utilizing NEFA concentrations was beneficial in determining energy balance in both non-pregnant and pregnant females in a more immediate fashion. It is crucial that a female have adequate body energy stores and be able to readily metabolize them because it can affect not only

the establishment of lactation but also the amount of milk produced (McNamara et al., 1995).

Although not as important in the beef industry as compared to dairy where much higher milk production rates are achieved, rates of lipolysis, lipogenesis and esterification of fatty acids fluctuate depending upon genetic potential for milk production and stage of lactation (McNamara et al., 1995). This becomes significant when the goal is to resume estrous cyclicity in a timely fashion. When triacylglycerols (TAG) are able to be maintained and stored instead of degraded into nonesterified fatty acids, homeostasis is achieved. During this time of homeostasis the body is also much more sensitive and receptive to hormonal signals and thus able to respond more quickly (Ookhtens et al., 1987). Brooks et al. (1982) theorized that it was easier for the body to maintain life processes at the expense of wasted energy, yet be able to readily respond to endocrine signals and changes, rather than to be operating at an energy deficit and then be asked to become highly active.

Progesterone serves as a primary regulator of not only estrous cyclicity, but also establishment and maintenance of pregnancy in the female (Mann et al., 1999) and thus warrants attention. Progesterone is necessary to reinitiate estrous cyclicity in females following parturition, as it is the negative feedback control for LH pulsatile secretion through a cascade of endocrine events (Kinder et al., 1996). Progesterone regulates the release of GnRH from the hypothalamus in the brain. Lessened secretion of GnRH, in turn, regulates the pulsatile secretion of LH from the anterior pituitary (Inskeep, 2004) which then stimulates growth and maturation of the dominant, ovulatory follicle (Taft et

al., 1996). The body is sensitive to endocrine changes and therefore shifts in progesterone concentrations have a dramatic negative correlation with corresponding LH concentrations (Bergfeld et al., 1996).

It is evident that the nutritional status of the individual as indicated by NEFA concentrations plays a permissive role in the regulation of the HPA axis and subsequent reproductive performance. Therefore, the following study was designed in order to elucidate the possible relationships that may exist between RFI status, NEFA concentrations, progesterone concentrations, BW, BCS, and changes in the BW and BCS during the early postpartum period and in reproductive performance in Brahman females.

Materials and Methods

Post-calving procedures undertaken were similar to those earlier reported in the prepartum period described in detail in Chapter II. However, because this study dealt only with lactating females postpartum, some individuals included earlier were omitted due to calf loss. Additionally, only multiparous females (n = 44) that returned to estrous cyclicity before the cessation of the finite breeding season were included and separated into two categories: returning to estrus cyclicity in 90 days or less, and those returning to estrus cyclicity in greater than 90 days.

Following calving, females were measured for BCS and BW at intervals, including: 24 hrs, 7, 14, 21, 28 and 35 d post-calving. Blood samples were also taken during the first five weeks postpartum in order to determine NEFA concentrations while

sampling for progesterone analysis commenced on d 28 after calving. During the first five weeks postpartum the cows and calves were retained in a smaller pasture near the working facilities where they could be more closely monitored. After the completion of five weeks of individual sampling the females and their calves entered into a larger group where data collection continued once a week on a fixed schedule for these specific females. In this larger group, BCS, BW, and serum samples for determination of progesterone concentrations were collected. The females were observed twice daily (approximately 0730 and 1800h) and retained within this group until such time as they were found to be in standing estrus. During this time females were exposed to epididymectomized bulls fitted with chin-ball markers. Females were considered to be in estrus when observed standing to be mounted by a bull or other females as well as the presence of ink marks on their back indicating a prior mount.

A controlled breeding season began May 13 and lasted until July 7. Females were maintained with five epididymectomized bulls fitted with chin-ball markers and observed twice daily for signs of estrus. After a female was found to be in estrus, she was artificially inseminated with frozen, thawed bull semen approximately 12 hours after observation of standing estrus by one of three trained technicians. Following insemination, the technician would massage the clitoris for a period of three to ten seconds in order to increase pregnancy rate (Lunstra et al., 1983). This is a successful technique proven to increase conception rates in cows through activation of a stimulatory pathway that initiates ovulatory events (Randel et al., 1973).

On days eight, nine and ten following estrus, females were subjected to both rectal palpation and ultrasonography (Sonovet Universal SA600) in order to determine the presence of a corpus luteum on the ovary. Additionally, a 15-mL blood sample was taken each day and later evaluated by progesterone assay to confirm resumption of estrous cyclicity. Estrous cyclicity was determined to have resumed when the female had experienced three consecutive days of progesterone concentrations above 1ng/mL. Ultrasonography was performed by inserting the transducer rectally and moving along the dorsal surface of the reproductive tract. The transducer was then directed laterally in both directions to examine each ovary (Pierson and Ginther, 1987).

When a corpus luteum was found to be present for three consecutive days the female was considered to have resumed estrous cyclicity and the weekly procedures then ceased for that individual. If not, the weekly proceedings including: blood sampling, BW measuring and body condition scoring; continued on a fixed schedule until standing estrus followed by development of a corpus luteum was observed.

Beginning July 8, the artificial breeding season ceased and females were exposed to three intact males that were subsequently removed on August 8. During this time when a female was found to be in estrus the same procedures regarding palpation and ultrasonography on days eight, nine and ten were still observed. Approximately 45 d following the completion of the natural breeding season, females underwent rectal palpation in order to determine pregnancy status.

Analysis of hormones and blood metabolites

As previously indicated, blood samples were collected from females following calving. Progesterone sampling continued until a female was found to be in standing estrus followed by detection of a corpus luteum, while NEFA sampling was conducted only during the five weeks immediately following parturition. Samples were collected in 15-mL Vacutainer tubes, refrigerated and allowed to clot for approximately 24 hours and then centrifuged at 3200 x g for 40 minutes. After removal from the centrifuge, samples were stored in two aliquots at -20° until time of analysis for progesterone and NEFA concentrations.

Progesterone serum samples were analyzed at the Texas *Agri*Life Research Center in Overton, Texas using a radioimmunoassay adapted from Williams (1989). Intra- and interassay CV were 8.33 and 9.07%, respectively. Non-esterified fatty acid serum samples were analyzed utilizing a commercially available enzymatic colorimetric analysis kit (NEFA-C, Wako Chemicals USA, Inc., Richmond, Virginia) at Texas A&M University in College Station, Texas.

Cow performance evaluation

Multiple parameters were included in order to evaluate cow performance, including:

1. Residual feed intake status
2. BCS
3. Change in BCS

4. BW
5. Change in BW
6. Nonesterified fatty acid concentrations
7. Progesterone concentrations
8. Number of days from calving to first observed estrus
9. Number of days from calving to formation of a functional corpus luteum (confirmed by progesterone assay)
10. Number of days from calving to first observed estrus followed by subsequent formation of a functional CL (confirmed by progesterone assay)
11. Rate of return to estrus cyclicity
12. Julian date of calving
13. Pregnancy rate

Statistical analysis

In order to effectively analyze the dataset of postpartum females, several were disqualified from the data due to different reasons. Females that lost their calves at any point during the sampling period were removed. Additionally, females that did not return to estrous cyclicity during the defined breeding season were excluded. This group of females not used was comprised primarily of both two and three year old heifers, the majority of which calved later in the calving season. Due to the exclusion of the

majority of primiparous females, only multiparous females were included in the post-calving data.

Comparison of numerical RFI values across six different contemporary groups in our dataset was impossible and thus females were only categorized as being either efficient (negative RFI) or inefficient (positive RFI). NEFA concentrations, BW, BCS, and changes in BW and BCS were analyzed with RFI as the class variable utilizing the GLM procedure specific for repeated measures function of SAS (2002). In order to elucidate relationships among RFI, NEFA concentrations, and post-calving changes in body weights and body condition scores, Pearson correlations were utilized using SAS (2002). Chi-square analysis (SAS, 2002) was also utilized to discern any differences that existed between efficient and inefficient females, as well as between females which were either rapid or slow in their return to estrous cyclicity, with regard to pregnancy rate.

Results

Results reported during the postpartum period that included BW, BCS and NEFA concentrations occurred only during the five weeks of sampling immediately following parturition. During this immediate postpartum period, mean BW was not significantly different between efficient and inefficient females as mean BW was 545.57 ± 7.07 kg among seventeen efficient individuals and 557.83 ± 6.30 kg among twenty-three inefficient individuals; respectively (Table 3.1). A significant difference ($P < 0.05$) was observed as mean BCS for efficient and inefficient females was 6.2 ± 0.09 and 6.7 ±

0.08; respectively. Significant differences were not observed in NEFA concentrations as efficient females had a mean NEFA concentration of 0.497 ± 0.0300 mEq/L and inefficient females a mean of 0.446 ± 0.0267 mEq/L; respectively. Mean Julian date of calving for efficient females was April 5 and April 1 for inefficient females. No significant differences were observed in days from calving to: first observed behavioral estrus, first formation of a functional CL, and first observed behavioral estrus followed by subsequent formation of a functional CL between efficient and inefficient cows.

Effects of time

The effect of the sampling day after calving had no significant influence upon any parameter, including: BW, BCS, NEFA concentration, change in BW or change in BCS.

Effect of RFI status

Residual feed intake status did not significantly affect BW, NEFA concentrations, change in BW or change in BCS; however, it did have a significant effect upon BCS (P = 0.0002, Figure 3.1) as inefficient females had a substantially greater BCS than efficient females (6.7 ± 0.1 vs. 6.2 ± 0.1). Additionally, RFI status had no significant effect upon: days from calving to first observed behavioral estrus, days from calving to formation of a functional CL, or days from calving to first behavioral estrus followed by subsequent formation of a functional CL.

Effect of rate of return to estrous cyclicity

Females were classified into two groups according to their rate of return to estrous cyclicity. Females that returned in ninety days or less following calving were referred to as returning "rapidly" and those resuming estrous cyclicity in greater than ninety days were deemed "slow" in returning. Rate of return to estrous cyclicity had no significant influence upon BW, BCS, or NEFA concentrations. The interaction between rate of return and day of sampling during the postpartum period was significant with regard to change in BCS ($P = 0.0076$, Figure 3.2) as well as change in BW ($P = 0.0184$, Figure 3.3).

Correlations among NEFA concentrations, BW and BCS

When considering all sample days combined during the course of the five week postpartum period immediately following parturition, strong, moderate, correlations

Table 3.1. Summary statistics for postpartum multiparous Brahman cows by RFI status.

	Efficient	Inefficient	P-Value
BW, kgs	545.57	557.83	0.2025
BCS	6.2	6.7	0.0002
NEFA, mEq/L	0.497	0.446	0.2061
Change BW, kgs	2.40	2.31	0.9702
Change BCS	0.004	-0.014	0.7874
Days from calving to 1st estrus (PPI to E)	69	55	0.1851
Days from calving to 1st CL (PPI to CL)	68	54	0.1990
Days from calving to 1st estrus followed by CL (PPI total)	68	54	0.1990

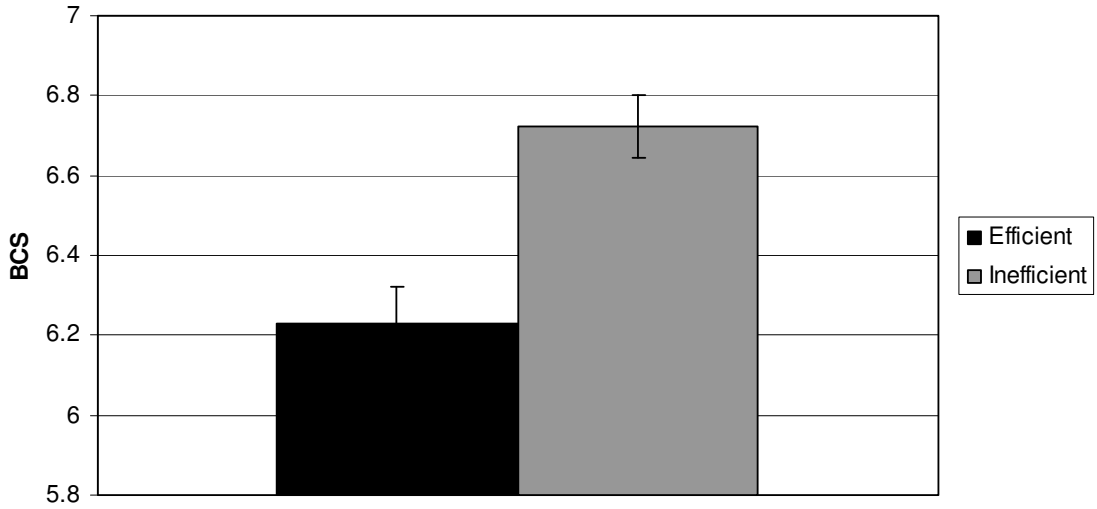

Figure 3.1. Effect of RFI status upon BCS in multiparous Brahman cows during the postpartum period (P = 0.0002).

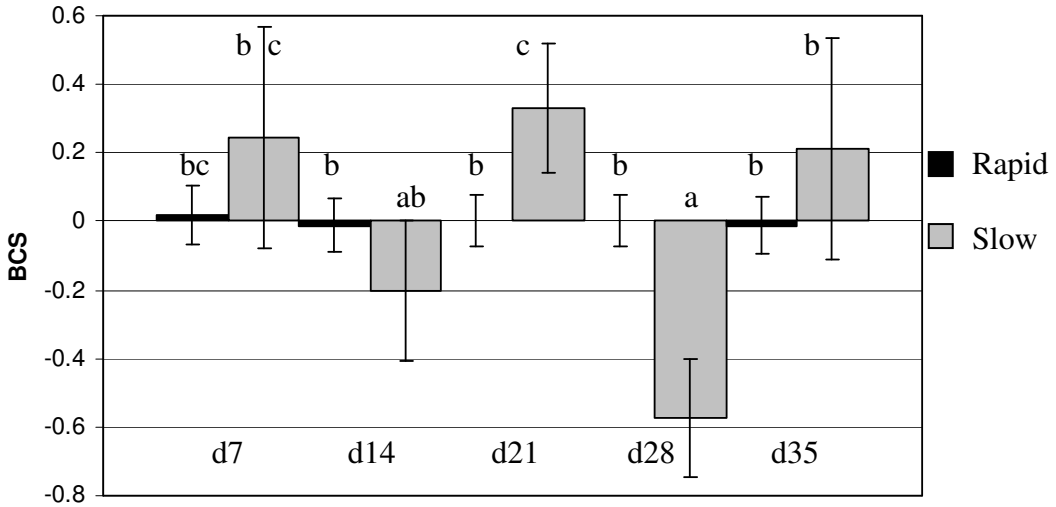

Figure 3.2. Effect of the day of sampling postpartum x rate of return interaction upon change in BCS in multiparous Brahman cows (P = 0.0076).

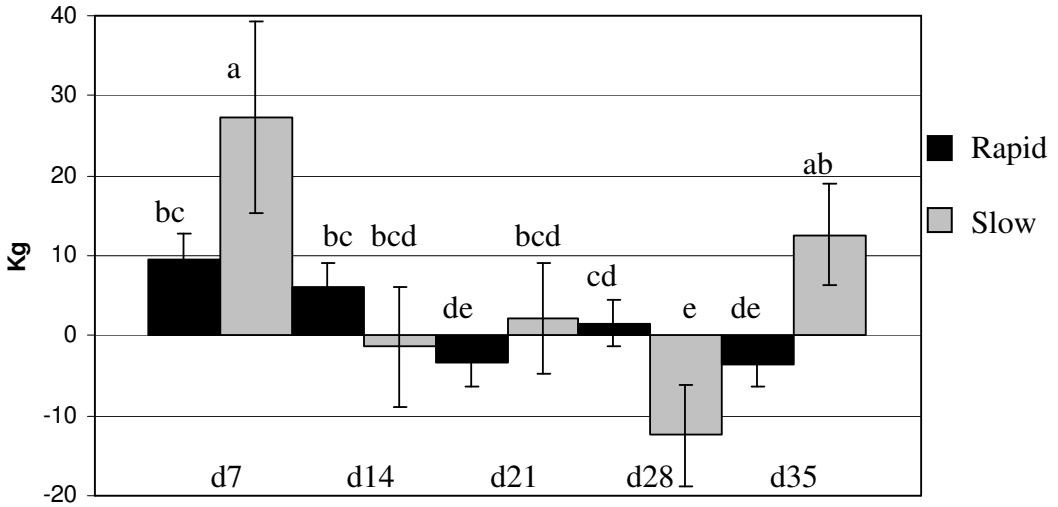

Figure 3.3. Effect of the day of sampling postpartum x rate of return interaction upon change in BW in multiparous Brahman cows (P = 0.0184).

Table 3.2. Correlations among NEFA concentrations, BW and BCS in multiparous Brahman females during the postpartum period.[1]

	NEFA Concentrations, BW	NEFA Concentrations, BCS	BW, BCS
Efficient	0.00077	0.0737	0.4805*
Inefficient	0.12989	0.17596**	0.55705*

[1] Females were weighed once weekly following parturition and five weeks thereafter. A * indicates P < .05, ** indicates P = .051-0.1

between BW and BCS were observed (Table 3.2), as well as a tendency for a correlation between NEFA concentrations and BW in inefficient females.

Reproductive performance

Due to the low number of primiparous females that returned to estrous cyclicity in a timely manner, Chi-square analysis of pregnancy rate by parity was impossible. Adequate numbers of multiparous females were available for analysis of pregnancy rate by RFI status (Figure 3.4) and rate of return to estrous cyclicity (Figure 3.5). Efficient females obtained a significantly greater end of breeding season pregnancy rate than did their inefficient cohorts (64.71% vs. 20.83%, respectively; P = 0.0046). Additionally, females rapid to return to estrous cyclicity following calving also achieved a greater end of season pregnancy rate than those that returned to estrous cyclicity slowly although it was not statistically significant (76.47% vs. 57.14%, respectively; P = 0.2933).

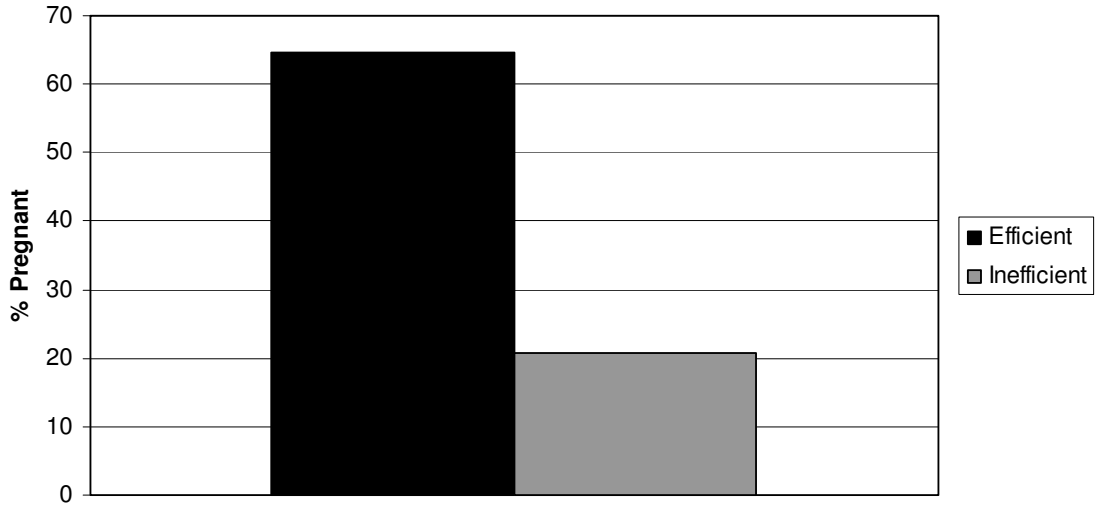

Figure 3.4. End of breeding season pregnancy rate for efficient (n = 17) and inefficient (n = 24) Brahman females (P = 0.0046).

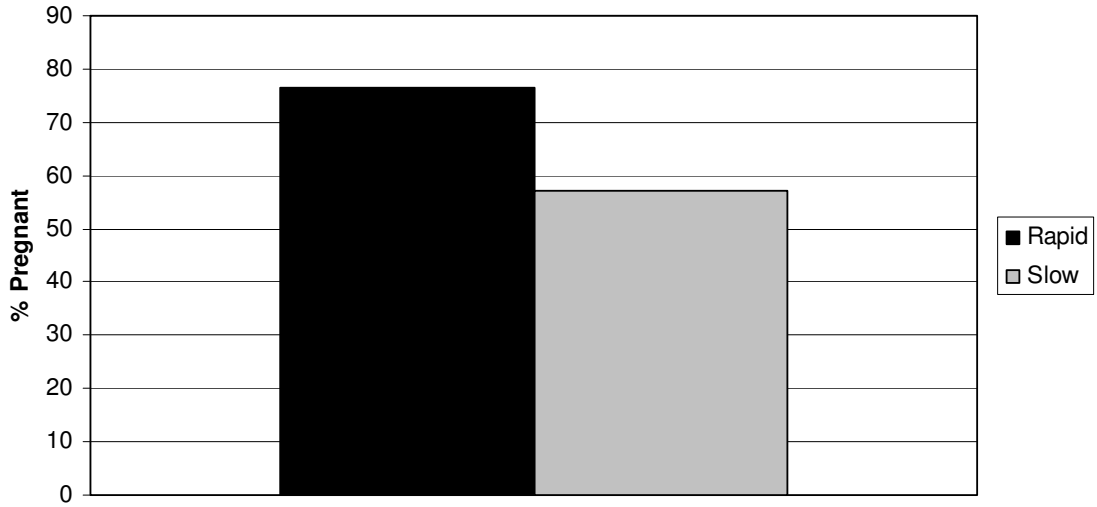

Figure 3.5. End of breeding season pregnancy rate for Brahman females returning to estrous cyclicity in ninety days or less (n = 34) and those returning to estrous cyclicity in ninety-one days or more (n = 7) (P = 0.2933).

Discussion

Similar to results found in Chapter II, Table 3.1 shows that efficient females were lower in both BW and BCS than their inefficient cohorts. Golden et al. (2008) found that inefficient feedlot steers fed either roughage or no-roughage diets had an increased number of eating sessions and increased feed consumption. If the same theory may be applied to females in a pasture setting, this may help explain why the efficient females had decreased BW and BCS compared to their inefficient counterparts. Bingham et al. (2009) supports these findings as inefficient Brangus heifers were found to consume 21.9% more feed and have an increased number of eating sessions daily than their efficient counterparts, while still achieving similar average daily gain and BW during the course of an RFI trial.

Inefficient females experienced both BCS gain and loss during the postpartum period. However; caution should be exercised when reviewing these results as all BCS changes recorded had less than 0.6 of one unit change. Changes in BW over time for females also varied between loss and gain. Efficient females experienced less dramatic change over time, suggesting that they are able to better adapt to the stress of calving and lactation than their inefficient cohorts. This supports the findings of Lents et al. (2008) who reported that crossbred *Bos taurus* females who were able to better maintain BW following parturition maximized reproductive potential.

Nonesterified fatty acid concentrations did not change significantly over time in postpartum females. This is not in agreement with McNamara (1991) who found that as a dairy female adjusts to the energy demands of lactation, less of her adipose tissue

energy reserves are required in order to sustain this process and thus NEFA concentrations begin to decline throughout lactation. However; findings of this study are in agreement with Ciccioli et al. (2003), who found that there was no effect of sampling time upon NEFA concentrations. Samples in this study were collected during the seven weeks prior to first estrus and thus may have been collected too late following parturition to be significant. Because the current study included only multiparous females, it is possible that the stress of lactation and meeting nutritional demands was less difficult than for primiparous females and thus no significant differences were detected. Although Flores et al. (2007) found negative correlations between NEFA concentrations and BCS change in Brahman-influenced cows; our data indicated only a tendency for a low correlation between NEFA concentrations and body condition score. This may be due in part to differences in sampling schedules.

Efficient females obtained a much greater pregnancy rate during the breeding season than did their inefficient cohorts (64.71% vs. 20.83%, respectively; P = 0.0046). These findings closely mimic those of Loyd (2009) who noted similar results in end of season pregnancy rate in multiparous females. Although efficient females did have a lower BCS than their inefficient cohorts, the efficient females still were well above BCS 5, where optimum reproductive performance becomes negatively affected (Morrison et al., 1999; Lents et al., 2008). This is in agreement with Selk et al. (1988) who reported that an adequate BCS is necessary in order to achieve a high percentage pregnancy rate.

Conclusions

Although prepartum nutrition is more crucial than postpartum nutrition with regards to nutritional anestrus (Wiltbank et al., 1962; Dunn and Kaltenbach, 1980; Dziuk and Bellows, 1983; Randel, 1990), this does not mean that postpartum nutrition should be disregarded entirely. Similar to the conclusions of the prepartum period, a strong relationship exists between BW and BCS over time. Additionally, BCS was shown to be significantly different between efficient and inefficient cows, suggesting that efficient females are better able to maintain life processes at a lower BCS, perhaps due to decreased ingestion of feed as suggested by Golden et al. (2008) and Bingham et al. (2009). Changes in BW and BCS over time were not significant in efficient or inefficient cows. This is beneficial as Lents et al. (2008) reported that cows should be managed to calve in a moderate BCS and maintain BW following parturition in order to maximize fertility and achieve optimal pregnancy rates.

Efficient females achieved a significantly greater end of season pregnancy rate than their inefficient counterparts. Additionally, although not significant, females that returned to estrous cyclicity earlier following calving obtained greater end of season pregnancy rates as well. Although these current results did not prove to be statistically significant, these results combined with those in the literature infer that efficient females did not return to estrous cyclicity more quickly but were able to achieve greater pregnancy rates than their inefficient herd mates.

CHAPTER IV

CONCLUSIONS

Beef production compromises the largest segment of U.S. agriculture production and thus warrants significant attention. One of the most simplistic means for beef producers to maximize profit potential is to minimize input costs. Feedstuffs comprise the majority of any beef cattle production system and thus it would be beneficial to be able to identify animals which are more efficient in their utilization of nutrients. Residual feed intake has been utilized as a means by which individuals can be identified as consuming less feed than expected (efficient) or more feed than expected (inefficient). The next step taken by researchers has been to evaluate the relationships that exist between RFI status and other economic traits, including reproduction.

These studies suggest that BCS is influenced by RFI as efficient females have a lower BCS than their inefficient counterparts. However; the underlying endocrine and metabolic signals that allow for this lower BCS are not fully understood. Nonesterified fatty acids were not affected by or correlated with RFI status and thus alternative hormones and blood metabolites should be investigated. Selection for efficient females may improve end of season pregnancy rates in cows. A void exists concerning RFI research relative to reproductive performance and additional research is needed to investigate the effects that selection for RFI may have reproduction and other economically important traits affecting beef production.

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APPENDIX A

PROGESTERONE RADIOIMMUNOASSAY PROTOCOL FOR BOVINE SERUM

Reference:

Williams, G. L. 1989. Modulation of luteal activity in postpartum beef cows through changes in dietary lipid. J. Anim. Sci. 67:785

REAGENT PREPARATION

PBSG (0.1% Gelatin, pH 7.5)		1 Liter	2 Liters
1.	Monobasic Sodium Phosphate (Sigma, S-9638; FW 138.0)	0.070 g	0.140 g
2.	Dibasic Sodium Phosphate (Sigma, S-0876; FW 142.0)	1.350 g	2.700 g
3.	Sodium Chloride (Sigma, S-9888; FW 58.44)	8.812 g	17.624 g
4.	Sodium Azide (Sigma, S-2002; FW 65.01)	1.000 g	2.000 g
5.	Disodium EDTA: dihydrate (Sigma; ED2SS, FW 372.2)	0.372 g	0.744 g
6.	Gelatin (J.T. Baker, 2124-01)	1.000 g	2.000 g
7.	Double Distilled H_2O	1.00 liter	2.00 liter

Into dd H_2O, at about 90% of the final volume, weigh out and add all reagents except EDTA and gelatin. Mix and pH to 7.5 using 1.0 N HCl or NaOH. Bring to final volume in calibrated 2 L beaker or volumetric flask. Add EDTA and gelatin with continuous stirring over lowest heat until dissolved; this should take approx. 1 h. Transfer to storage bottle and store at 4°C. Replace at 30 to 40 d intervals. Sodium Azide is highly toxic – take appropriate precautions.

Charcoal Suspension for RIA. Prepare at least 1 d in advance of RIA and discard at 20 d intervals. Can be stored at 4 C in a sealed beaker and must be maintained at approx.

4°C during additions. Use an ice bath with continuous stirring if addition time exceeds 5 min.

Reagent/100mL PBSG	
Activated Charcoal (Sigma C-5260)	1.875 g
Dextran (Sigma D-4271)	0.188 g
Additional Volume (uL/tube)	750

Charcoal-Stripped Serum or Plasma Stock

1. Bleed, separate and collect 300+ mL sera or plasma from, preferably, an intact prepubertal female. Another reasonable source would be a mature female at 4 to 12 d postpartum. In cattle, "free-flow" bleeding with a large needle (14G), used-cleaned vacutainer tubes, and using intravenous pressure (i.e. no vacuum) will greatly reduce subsequent fibrin clots in sera stocks, both during and after processing.

2. Using a standard beaker that is ~200% of the pooled volume, pool the raw sera or plasma, and add a large magnetic stir bar. For each 100 mL sera or plasma, add: 9.375 g Sigma C-5260 activated charcoal and 0.938 g Sigma D-4751 dextran.

3. Cover and stir for 1.5 to 2 h at room temperature on stir plate.

4. While stirring by hand, pour suspension into 50 mL polycarbonate high-speed centrifuge tubes.

5. Centrifuge for 2.0+ h at 10,000 rpm x 4°C. Carefully remove tubes from rotor head and decant sera or plasma into a clean flask. Transfer only clear sera or plasma into this pool (e.g. leave the final 3 to 6 mL of charcoal-contaminated stock as waste).

6. Repeat centifugation (Step 5.) using fresh centrifuge tubes. Carefully decant and pool clear sera or plasma stock into fresh flask.

7. Filter stock using Sartorius vacuum-filtration setup and hand-cut filters (derived from Whatman nos. 43 or 41 ashless 15.0 cm filter papers). Ideally this step should be repeated until no charcoal residue if visible on filter after procedure (about 5X; use dissecting scope to examine filters). In practice, we generally repeat the procedure twice for a total of three filtrations.

8. Aliquot at 5 to 7 mL in peti-vials, cap, label and freeze at –20°C until use.

Trace Dilution Store at 4°C. Working dilution effective for at least 3 - 4 wks.

Stocks: #/RIA = n*

TRK.413 [1,2,6,7-^{3}H]-Progesterone, Amersham	(4 – 8; 3.5/RIA)
NET-370 [1,2,6,7-^{3}H] Testosterone, DuPont-NEN Research	(4 – 8; 3.5/RIA)
TRK.517 13,14-Dihydro-15-keto-^{3}H-$PGF_{2\alpha}$, Amersham	(40 -100; 33/RIA)
TRK.587 [2,4,6,7,16,17-^{3}H] Oestradiol, Amersham	(4 - 8; 3.5/RIA)

1. Using micropipet, or Hamilton syringe for PGF_m, introduce (n*) uL of ^{3}H-tracer stock into 25 mL PBSG; mix for 5 min on stir-plate and let stand for 10 min at 4°C.

2. Prepare a triplicate set of scintillation vials containing the standard volume of cocktail (4-5 mL). Add a 100 uL aliquot of tracer solution base to each tube; mix by inversion, let stand 2 min and count for 1 min on LSC.

3. Calculate appropriate dilution. Currently 9500 - 10500 cpm/100 uL trace (i.e. mean cpm x original volume / 10500 = final volume)

4. Add appropriate volume of PBSG for working dilution of trace. Mix well and let stand overnight at 4°C before use.

Antibody Dilution: Prepare working dilutions daily from aliquoted storage dilutions. Store at 4°C.

Stocks: #337 anti-progesterone-11-BSA serum; Dr. G.D. Niswender, CSU, Ft Collins

#250 anti-testosterone-11-BSA serum; Dr. G.D. Niswender, CSU, Ft Collins (1:500/1:50,000 to 1:60,000 for extr.)

#133 (02/18/76) anti-$PGF_{2\alpha}$; Dr. Ray Haning, Women and Infants' Hospital of Rhode Island, Providence

#244 anti-estradiol-6-BSA; Dr. G.D. Niswender, CSU, Ft Collins (1:500/1:60,000)

1. Reconstitute lyophilized P_4 , T and E_2 anti-sera with 1.0 mL dd H_2O (1:1) and $PGF_{2\alpha}$ anti-sera with 10.0 mL dd H_2O (1:100). $PGF_{2\alpha}$ anti-sera should be aliquoted at 250 uL x 1:100. Always label and snap-freeze remainders in liquid N_2, parafilm vial caps and store frozen.

2. In order to minimize detrimental effects of repeated freeze-thaw cycles, use an aliquot of the full-strength antisera to prepare a second series of concentrated storage aliquots. Aliquot volumes should be appropriate for the simple preparation of adequate antisera to be used in a single RIA throughput.

   Recommended PBSG dilutions for concentrated anti-sera storage aliquots:
   1.0 mL x 1:46 for P_4
   250 uL x 1:500 for Testosterone and E_2
   250 uL x 1:100 for $PGF_{2\alpha}$ (as prepared in step 1).

3. Working dilutions are prepared independently for each RIA in PBSG to achieve 20 to 50% max binding (%Ref/TC). Pre-labeled urine specimen cups are generally ideal for this step.

   Recommended dilutions for anti-sera working stocks in 298-tube RIA:
   60.0 mL x 1:2760 for P_4 , (1 mL stock + 59 mL PBSG)
   30.0 mL x 1:60,000 for Testosterone and serum E_2(.250 mL stock + 29.75 mL PBSG)
   30.0 mL x 1:50,000 for extraction Testosterone (.30 mL stock + 29.70 mL PBSG)
   30.0 mL x 1:120,000 for (extracted serum and CL E_2) (.125 mL stock + 29.88 mL PBSG)
   30.0mL x 1:12,000 for $PGF2_{\alpha}$ (.250 mL stock + 29.75 mL PBSG)

RIA Standards: Prepare, aliquot at 1.0 mL and snap freeze in liquid nitrogen. Store at -20°C until required. Discard after 12 mo. Degradation may occur more rapidly in lowest concentrations of prepared standards. Minimal labeling requires analyte, concentration and date of preparation.

Progesterone Protocol:

Prepare or use P_4 Stock I @ 1.00 mg/mL EtOH. Construct by adding 0.025 g P_4 to 25 mL volumetric and Q.S. to 25.0 mL with EtOH. Mix and let sit overnight at 4°C before use or otherwise store at –20°C.

Using above Stock I @ 1.00 mg/mL, prepare Stock II @ 1.00 ug/mL. Construct Stock II by adding 50 uL Stock I to 50 mL volumetric and Q.S. to 50 mL with EtOH. Mix and let sit overnight at 4°C before use or otherwise store at –20°C.

Using above Stock II @ 1.00 ug/mL, construct Std A @ 16.00 ng/mL by transfer of 400 uL of Stock II to a 25 mL volumetric flask, dry off EtOH under N_2 stream, and Q.S. to 25.0 mL with PBSG. Let Std A sit overnight at 4°C. Prepare 1:1 serial dilutions in

PBSG. These dilutions should be based on mass, rather than volume, to eliminate variability in volume associated with working with solutions at differing temperatures.

Range produced currently for P_4 :

A= 16.000 ng/mL
B= 8.000 ng/mL
C= 4.000 ng/mL
D= 2.000 ng/mL
E= 1.000 ng/mL
F= 0.500 ng/mL
G= 0.250 ng/mL
H= 0.125 ng/mL

General Protocol for Radioimmunoassay

Assay Preparation and Setup

Day before:
Array the appropriate number of samples (e.g. n=272) into 100-cell flats in consecutive sequence (priority; left to right, and front to back) with no empty cells for missing samples. This arrangement is critical and must be double checked, sample-for-sample, against records the day before assay. Store overnight at –20°C. Verify that adequate supplies are available for the RIA; these include stocks of appropriate tracer and antisera dilutions, pre-racked pipet tips, arrayed mini-scintillation vials (preferably loaded with cocktail) and 12 X 75 mm polypropylene culture tubes for standards, controls, and determinations. Label 12 X 75 mm culture tubes as follows:

TC	total counts
NSB	non-specific binding
	TB_o total (or max) binding, zero concentration reference for standard curve
$STD_{(x)}$	one/standard concentration (e.g. STD_{39}, STD_{78}, STD_{156}, etc.)
C(-), C(+)	one/negative or positive control

These tubes represent a single standard curve and should be racked independently . At least two (2) standard curves must be included with each RIA. When assay requires more than one centrifuge-spin (batch), a single standard curve should be included at the beginning of the first batch, at the end of the last batch and with each batch in between.

1 through (n) one reaction tube/sample determination (e.g. 1 through 272; racked separately at 80 tubes/rack)

Day 1:
As early as possible, remove prepared samples, standards and controls from freezer(s) and set out to thaw. Remove PBSG from refrigerator. Allow enough time for these materials to reach room temperature (otherwise volume "drift" will occur during pipetting operations). Pipette the following into each tube:

P_4 Series (uL*)

Tube	PBSG	CharPlasma	STD/CNTL/SMPL	3H	Ab	Char/Dext
TC	1200	--	--	100	--	--
NSB	300	100	--	100	--	750
TB_o	100	100	--	100	200	750
Standards	--	100	100 STANDARD	100	200	750
Controls	100	--	100 CONTROL	100	200	750
Samples	100	--	100 SAMPLE	100	200	750

* Modifications of these volumes may be necessary to bring reaction-tube mass of analyte within range of the standards; however, this is a good place to start.

Practical RIA Schedule

This general protocol is described to accommodate a "two-spin" RIA of approximately n=270 sample determinations per d, repeated daily until all sample determinations are acquired. Under these circumstances, it is possible for one person to complete the required work for this RIA within approximately 8 h. Therefore, from the standpoints of assistance and safety, it is important to get started early.

Each of the "spins" or batches, and their respective standards, are handled as a single unit, separated by exactly 60 min. throughout the protocols. (They are called "spins" because centrifuge capacity is the limiting factor within each batch.) Because of the tenuous nature of these RIA measurements, timing is absolutely critical for useable results. Many things can go wrong, for which we have marginal control – procedural timing is not one of them! Timing errors are, by far, the most common problem for graduate students working in this laboratory. Two to five minutes error during some steps is usually enough destroy the outcome of any of these RIAs. With this in mind, there are several multi-channel timers available in the laboratory; learn how to operate them and to depend on them rather than your wristwatch or wall clocks.

Get the samples, standards and control stocks to room temperature and begin pipetting by 0900. Turn down centrifuge bowl temperature to 4 to 6 C. During the thaw, load mini-scintillation vials with Ecolite(+) if this was not done the previous day.

Begin with the careful setup of all standard curves needed for the RIA using the table above. Components should be pipetted in this order: Standards, PBSG, Charcoal-Stripped Plasma or Sera. Re-freeze the standard and plasma/sera stocks before continuing; hold the PBSG at room temperature on the bench.

Pipette the samples. The cell sequence of the storage flats should be used as the reference for the reaction tube sequence (e.g. sample of cell #4 pipetted into reaction tube #4). Rack individual "spins" as you work and group each with their respective standard curves so that they may be handled independently during the remainder of the protocol. For example, you may have two batches of n=135 samples plus n=13 standards that will require centrifugation. (Centrifuge capacity equals 148 tubes; TC tubes are not centrifuged.) With practice, this should require 1.5 to 2.0 h to complete.

Using the Eppendorf repeating pipette and the appropriate Combi-tip, add the appropriate volume of PBSG to the sample tubes (listed in table), shake each rack to mix. Set aside at room temperature.

Referring to the table above, begin the reaction of Spin 1 at exactly 1030 h, regardless of whether the sample pipetting operation is complete. Using the Eppendorf repeating pipette and the appropriate Combi-tips:

> Pipette the appropriate volume of 3H-Tracer into all tubes,
>
> Pipette the appropriate volume of antisera into all tubes except TC and NSB tubes.
>
> Shake racks vigorously or vortex. Place racks in plastic bags or parafilm the tubes.

Incubate all tubes within each batch for exactly 90 min at room temperature.

Transfer all tubes within each batch to refrigerator and incubate at 4°C for exactly 75 min.

Remove Dextran/Charcoal suspension from refrigerator and place on a stir plate, at setting 5, for approximately 1 min before use. Referring to the table above, and using the Eppendorf repeating pipette and the appropriate Combi-tip, add Dextran/Charcoal suspension to all tubes, except TC. Precise timing on this step is absolutely essential. Start timer for 30 min countdown, then shake racks vigorously and return to the refrigerator for incubation at 4°C.

At 30 min, remove batch from refrigerator and load all tubes, except TC, into centrifuge carriers (starting with standard curve) and centrifuge at 4000 rpm X 20 min X 4°C.

Re-rack tubes (behind TC; in the same sequence as Step 8) and carry to the isotope lab (#138) for decanting. The reaction tubes must be handled carefully from this point. Protect them from mechanical or thermal shock that might otherwise disturb the charcoal pellet. If this happens to a sample tube, take note, it must now be considered rerun. If this happens to a Standard tube, see step 1.

Starting with the standard curve, rack the tubes (in sets of 10) into the decanting bar and carefully decant supernatant into the 7 mL scintillation vials. Allow 10 seconds for complete pour-off and touch the rims of the reaction tubes to the surface of the cocktail to remove the last droplets. This step should be done precisely the same way for each bar of standards or samples across both batches.

Place the flat of scintillation vials on a tray and carry them to main lab (139) for capping, labeling and mixing. Cap the entire set. Label the cap of each standard vial with its ID or concentration. Label the cap of every fifth sample vial with its sequence number within the RIA (e.g. flat one = standard curve #1 plus samples 1 through 135; flat two = standard curve #2 plus samples 136 through 270). Place entire flat between two trays and mix thoroughly by 15 to 20 inversions. Leave the covered trays overnight in lab #138.

Day 2:
Re-mix the flats by inversion and count for 1.0 min each on TR2100 beta counter. Be sure to use the appropriate protocol-definition clip on the first cassette.

Transfer the quantification data from the TR2100 to a desktop PC and match the sequence of the RIA to the sequence of the sample array.

Transfer the counted vials to radioactive waste storage. Vials and solids (reaction tubes, paper wastes, etc.) must be boxed separately.

	SPIN 1	Spin 2	Spin 3
Begin Reaction Add 3H Tracer Add Antisera Incubate @ R.T.	10:30	11:30	12:30
Transfer to Refrigerator Incubate @ 4 C	12:00	1:00	2:00
Add Charcoal/Dextran Suspension Incubate @ 4 C Start multi-channel timer	1:15	2:15	3:15
Centrifuge 4000 rpm X 20 min X 4 C	approx. 1:55	approx. 2:55	Approx. 3:55
Decant	approx. 2:25	approx. 3:25	approx. 4:25

APPENDIX B

NON-ESTERIFIED FATTY ACID (NEFA) PROTOCOL FOR BOVINE SERUM (FOR USE WITH WAKO HR SERIES NEFA-HR(2) STANDARDS AND REAGENTS)

Reagent Preparation:

1) Standard Dilution

Stock solution = 1 mEq/L (Wako 276-76491)

1:1 serial dilution with double-distilled water (ddH2O) to 0.0625 mEq/L:

NEFA STD	mEq/L
STD A	1.0
STD B	0.5
STD C	0.25
STD D	0.125
STD E	0.0625

2) Color Reagent Reconstitution

Open dry color reagents VERY slowly to prevent release of powder.

Using connector provided, attach the dry Color Reagent A (Wako 999-34691) container to the Solvent A (Wako 995-34791) container and invert several times until the reagent is completely dissolved. Use solvent to wash powder off cap and into solution.

Using connector provided, attach the dry Color Reagent B (Wako 991-34891) container to the Solvent B (993-35191) container and invert several times until the reagent is completely dissolved. Use solvent to wash powder off cap and into solution.
(**Solvent B can be hard to get into solution. Make sure it is completely dissolved.)

NOTE: Mix only enough color reagent as needed as reconstituted reagents are only stable for 10 days.

NEFA Assay Protocol:

1) Turn on plate reader and open NEFA protocol (File → Open → File type:Endpoint protocol (*.epr) → NEFA → Open)

2) Make sure correct parameters are set:
   Reading type: Endpoint
   Dual Measurement Wavelength at 540nm and 655nm.
   Incubator set for 37° C
   Wait time = 300 sec (5 minutes)
   Template is correct (see attached diagram for correct setup)
   Sample dilutions are correct
   Reports: raw data, absorbance data, standard curve, unknown concentrations

3) Turn on incubator (37° C).

4) Run all standards, pools and samples in duplicate.

5) Pipette 5µL ddH2O (blank), standards, pools, and samples into 96-well plate (diagram below for sample layout of 96-well plate).

6) Add 200µL Color Reagent A using multi-pipette.

7) Place on plate shaker for 30 seconds to mix. Return Color Reagent A to refrigerator while mixing.

8) Place plate in plate reader and press RUN. This will incubate plate at 37° C for 5 minutes before measuring absorbance at 540 nm (Sub:655nm). Save the raw and absorbance data from this reading for future corrections if needed.

9) Add 100µL Color Reagent B using multi-pipette.

10) Place on plate shaker for 30 seconds to mix. Return Color Reagent B to refrigerator while mixing.

11) Place plate in plate reader and press RUN. This will incubate plate at 37° C for 5 minutes before measuring absorbance at 540 nm (Sub:655nm).

12) Plot and print standard curve from second absorbance.

13) Calculate concentration of the unknowns from standard curve.

14) Calculate coefficient of variances (CV = standard deviation / mean *100).

15) Reanalyze samples with CV > 20% and those samples that have concentrations outside of the standard range (0.0625 to 1.0 mEq/l).

NOTE: For samples > 1.0, dilute 1:2 with ddH2O. For samples < 0.0625, further dilute standards.

Other Notes

**Handle plates on sides, NOT on the top or bottom.
**Label a plate diagram with sample #s prior to pipetting to double check wells as you pipette.
**Using a colored sheet of paper under plate will help you see which wells have been pipetted.
**Save ALL data for future reference.

Sample NEFA Plate Setup

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	STD 0.0625	STD 0.0625	STD 0.125	STD 0.125	STD 0.25	STD 0.25	STD 0.5	STD 0.5	STD 1.0	STD 1.0
B	Welsh Pool	Welsh Pool	Smpl 1	Smpl 1	Smpl 2	Smpl 2	Smpl 3	Smpl 3	Smpl 4	Smpl 4	Smpl 5	Smpl 5
C	Smpl 6	Smpl 6	Smpl 7	Smpl 7	Smpl 8	Smpl 8	Smpl 9	Smpl 9	Smpl 10	Smpl 10	Smpl 11	Smpl 11
D	Smpl 12	Smpl 12	Smpl 13	Smpl 13	Smpl 14	Smpl 14	Smpl 15	Smpl 15	Smpl 16	Smpl 16	Smpl 17	Smpl 17
E	Smpl 18	Smpl 18	Smpl 19	Smpl 19	Smpl 20	Smpl 20	Smpl 21	Smpl 21	Smpl 22	Smpl 22	Smpl 23	Smpl 23
F	Smpl 24	Smpl 24	Smpl 25	Smpl 25	Smpl 26	Smpl 26	Smpl 27	Smpl 27	Smpl 28	Smpl 28	Smpl 29	Smpl 29
G	Smpl 30	Smpl 30	Smpl 31	Smpl 31	Smpl 32	Smpl 32	Smpl 33	Smpl 33	Smpl 34	Smpl 34	Smpl 35	Smple 35
H	Smpl 36	Smpl 36	Smpl 37	Smpl 37	Smpl 38	Smpl 38	Smpl 39	Smpl 39	Smpl 40	Smpl 40	Smpl 41	Smpl 41

APPENDIX C

BODY CONDITION SCORE TABLE

1	Severely emaciated, muscle atrophy, no detectable fat. Tail head and ribs very evident. Animal is physically weak.
2	Very poor condition with muscle atrophy and no detectable fat. Tail head and ribs evident.
3	Thin condition with slight muscle atrophy and very little detectable fat. All ribs evident
4	Intermediate condition. Outline of spine still slightly visible as well as 3-5 ribs. Some fat detectable over rib and hip area.
5	Moderate condition and appearance. Spine is no longer visible, hips have some fat coverage but slightly visible, and outline of 1-2 ribs still visible.
6	High-moderate condition with ribs and spine no longer visible. Pressure must be applied to feel bone structures such as: spine, ribs, hips and tail head. Some fat deposits apparent in brisket and flank region.
7	Healthy, fleshy in appearance. Hips still slightly visible. Fat deposits in udder and tail head regions as well as brisket and flank regions.
8	Fat, fleshy and overly conditioned. Bone structures no longer visible on body. Large fat deposits visible near brisket, ribs, flank, udder and tail head.
9	Extremely obese. Mobility impaired. Bone structure definitely not visible. Extreme fat deposits located across body.

APPENDIX D

RAINFALL TABLE

Texas *Agri*Life Research
Overton, Texas

	2008	2009	Monthly Average since 1968
January	2.4	1.41	3.62
February	3.58	2.24	4.07
March	7.86	6.28	4.15
April	2.99	3.44	3.7
May	8.25	2.67	4.51
June	3.58	1.49	4.34
July	0	4.25	2.82
August	7	1.82	2.22
September	3.92	6	3.55
October	4.63	12.81	4.41
November	3.2	1.71	4.09
December	1.57	4.82	4.17
Total	48.98	48.94	45.6

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