RELATIONSHIPS BETWEEN RESIDUAL FEED INTAKE AND PERFORMANCE OF HEIFERS OF DIVERSE BREEDTYPES AND BRAHMAN COWS

A Thesis

by

ANDREA NICOLE LOYD

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 2009

Major Subject: Physiology of Reproduction

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ABSTRACT

Relationships Between Residual Feed Intake and Performance of Heifers of Diverse Breedtypes and Brahman Cows. (August 2009)

Andrea Nicole Loyd, B.S., University of Missouri – Columbia

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These studies were designed to evaluate the relationships between residual feed intake (RFI) and performance of growing heifers and Brahman cows. Residual feed intake was determined for 77 heifers of diverse breedtypes (Angus, Brahman, Hereford, Holstein, Jersey and F_1 crosses) during both the pre- and post-pubertal periods. Heifers were individually fed and allowed *ad libitum* access to feed for 84 ± 6 d during the pre-pubertal feeding trial and 90 ± 4 d during the post-pubertal feeding trial. Brahman-influenced heifers had lower RFI than heifers without Brahman influence during both the pre-pubertal ($P < 0.05$) and post-pubertal ($P < 0.0001$) periods. Residual feed intake determined during the pre-pubertal period was only a moderate predictor ($r = 0.48$; $P < 0.0001$) of RFI determined during the post-pubertal period.

Residual feed intake was determined for 38 Bonsmara heifers over a 70-d feeding period. Heifers were fed a high roughage diet at 2.65% of body weight (BW). Weekly blood serum samples were analyzed for progesterone concentration by radioimmunoassay (RIA) to determine puberty. There were no observed differences

between efficient and inefficient heifers for performance traits, age at puberty or conception, or cumulative achievement of puberty and conception.

The postpartum performance of Brahman primiparous (n=16) and multiparous (n=38) cows previously evaluated postweaning for RFI was investigated. Females were weighed and evaluated for body condition score (BCS) at 28-d intervals prior to the start of the 2008 calving season. Weekly weights and BCS were collected beginning 21 d after calving. Blood serum samples were also collected weekly for progesterone analysis by radioimmunoassay (RIA), non-esterified fatty acid (NEFA) analysis by enzymatic colorimetry, and insulin-like growth factor-I (IGF-I) analysis by RIA. Females were exposed to vasectomized marker bulls after calving to aid in estrus detection. Eight and ten d following observed estrus, females were evaluated using ultrasonography via rectal palpation to determine the presence of a corpus luteum (CL). Efficient cows exhibited estrus, developed functional corpora lutea, and exhibited estrus with CL formation earlier ($P < 0.05$) than inefficient cows. Furthermore, a greater percentage ($P < 0.05$) of efficient than inefficient cows were pregnant at the end of the breeding season.

DEDICATION

This thesis is dedicated to my dad, who taught me the value of hard work and perseverance, the importance of caring for the livestock whose care rests in our hands, and to appreciate the little things in life. He is my mentor, role model, and fishing buddy.

This thesis is also dedicated to my mom, who taught me how to love unconditionally, inspired me to be the best person I could be, and encouraged me to always follow my dreams. She is truly my inspiration and best friend.

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And last, but certainly not least, I want to express my immense gratitude for my family. I would not be where I am today without their unending love and constant support. To my parents, I would like to say "thank you" for being the best parents ever and for raising me to have strong values and a sincere love for agriculture. To my sister, thank you for always being there for me no matter what. I am so proud to call you my sister and friend.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Maximizing profit is the goal of most beef cattle operations. While there are many factors that influence the profit margin of a given production system, profitability can be simply defined as the difference between production outputs and inputs (Archer et al., 1999). Cattle selection has traditionally focused on output traits such as growth rate, reproductive performance, and carcass characteristics (Archer et al., 1998). Due to high feed, fuel and fertilizer prices in today's cattle industry, there has been renewed interest among cattle producers to utilize selection tools that are associated with input traits rather than the conventional output traits (Crews et al., 2005). Providing feed to cattle is an economically important input as feed expenses represent about 60-65% of the total cost of producing beef (Sainz and Paulino, 2004). Therefore, finding ways to decrease feed expenses are important to ensuring the continued profitability of beef cattle operations.

Identifying cattle that are more efficient at utilizing available nutrients may be one way to help reduce feed costs. While there are several methods that attempt to quantify feed efficiency in cattle, feed:gain ratio (F:G) has historically been the most commonly used measure of feed efficiency in both research and industry (Nkrumah et

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This thesis follows the style of Journal of Animal Science.

al., 2004). Although easy to calculate, there are fundamental problems associated with using F:G. As such, residual feed intake (RFI) was proposed as an alternative measure of feed efficiency (Koch et al., 1963). While many studies have evaluated RFI in young, growing animals (Herd and Bishop, 2000; Arthur et al., 2001a, b; Basarab et al., 2003; Nkrumah et al., 2004; Nkrumah et al., 2007), relatively few studies have investigated the relationships between RFI and the subsequent performance of cattle later in life (Arthur et al., 2005; Basarab et al., 2007). Furthermore, there is a lack of data investigating this relationship in *Bos indicus*-type cattle.

Nutrition has long been recognized as an important mediator of the events associated with reproduction (Guilbert, 1942; Asdell, 1949; Wiltbank et al., 1962; Randel, 1990; Short et al., 1990). Specifically, nutritional status can alter how quickly a heifer becomes pubertal (Wiltbank et al., 1969; Day et al., 1986) as well as the length of the postpartum interval in cows (Wiltbank et al., 1962; Dunn and Kaltenbach, 1980). Both of these anestrous periods can impact the profitability of cow-calf operations. Therefore, exploring the relationship between feed efficiency and these important reproductive traits is important to understanding how selection based on RFI might impact the overall performance of the cowherd.

Feed:Gain Ratio

Nkrumah et al. (2004) attributed the initial concept of F:G to Brody (1945). Feed:gain is a simple ratio of how much feed an animal consumes to how much weight the animal gains over a specified period. An animal with a low F:G uses less feed to

produce a unit of gain than an animal with a high F:G. Therefore, an animal with a lower F:G is termed more efficient than its contemporary with a higher F:G. As a result of its ease of calculation, F:G has been extensively used as a measure of feed efficiency in beef cattle. However, there is a significant problem associated with using F:G as a selection tool to improve feed utilization by cattle. Feed:gain has been negatively correlated with growth rate and BW in young, growing cattle (Mrode et al., 1990; Koots et al., 1994; Arthur et al., 2001b). Due to this correlation, selecting cattle based on F:G is similar to simply selecting for increased growth rate (Mrode et al., 1990) and tends to lead to the selection of cattle that are larger at maturity (Herd and Bishop, 2000). This increased mature size equates to increased feed requirements (Barlow, 1984), thus making it more expensive to maintain those cattle at maturity and thereby negating the objective of selecting for improved feed efficiency using F:G.

Residual Feed Intake

Koch et al. (1963) proposed RFI as an alternative to F:G to more appropriately evaluate feed efficiency in cattle. Residual feed intake is defined as the difference between an animal's actual feed intake and its expected feed intake (Arthur et al., 1996). Based on this definition, an animal with a negative RFI consumes less feed than expected and is thereby efficient. Conversely, an animal with a positive RFI consumes more feed than expected and is inefficient. Unlike F:G, RFI is by definition phenotypically independent of the animal's BW and growth rate (Kennedy et al., 1993; Herd and Bishop, 2000; Arthur and Herd, 2005). Therefore, selection for RFI should not

be accompanied by an increase in mature size or feed requirements (Arthur et al, 2001a; Arthur et al., 2004; Nkrumah et al., 2004). Several studies have confirmed the phenotypic independence of RFI from BW and growth rate with non-significant correlations among the traits (Arthur et al., 1996; Arthur et al., 2001a; Baker et al., 2006; Nkrumah et al., 2007).

Although RFI appears to have distinct advantages over F:G, its effective use as a selection tool for feed efficiency requires that it be a heritable trait in order to make genetic progress from one generation to the next. Several heritability estimates have been reported for RFI ranging from 0.14 (Fan et al., 1995) to 0.44 (Archer et al., 1997). These estimates of heritability suggest that genetic improvements in feed efficiency can be made by selecting for RFI. These estimates were substantiated in a study by Arthur et al. (2005) where divergent selection of RFI over 5 yr resulted in a 0.8 kg/d difference in mean RFI values between efficient (-0.3 kg/d) and inefficient (0.5 kg/d) Angus cattle. These data suggest that advancements can be made in feed efficiency by selecting cattle for reduced RFI.

Evaluating Cattle for RFI

Sex, age, and breedtype

Herd et al. (2004) reported that "efficiency is a function of the amount and type of feed eaten, the sex and breed of the animal, and the environmental conditions in which the animal is managed." Since there is no standard method for calculating RFI (Knott et al., 2008), care must be taken to address these issues when establishing a

protocol to evaluate cattle for RFI. As RFI is calculated as an index, selecting an appropriate group of cattle to be evaluated together for RFI is an important issue to consider. Sex differences in cattle performance have been established (Brinks et al., 1961; Bogart et al., 1963; Wilson et al., 1969). Therefore, cattle are typically sorted by sex class and managed differently or adjustments are made to account for these sex differences. When evaluating cattle for RFI, these differences should be considered so that cattle of the same sex are grouped together as cohorts.

The large variation that exists in maintenance requirements among cattle of different ages and breedtypes (NRC, 1996) often makes it challenging to make valid comparisons across different age ranges and breed compositions of cattle. One of the proposed advantages of using RFI as a measure of feed efficiency is that it attempts to account for this variation by including metabolic BW in the model for calculating RFI (Arthur et al., 2001b). This would theoretically allow for the comparison of RFI among cattle differing in age and breedtype. However, recent research questions the validity of this assumption. Breedtype differences in RFI have been reported by Schenkel et al. (2004) and Riley et al. (2007) suggesting that RFI cannot be compared across breedtypes. In addition, Crews et al. (2003) observed a moderate correlation ($r = 0.55$) in RFI evaluated on the same cohort of calves at 2 different ages. This was further substantiated when Johnston (2007) reported a similar correlation ($r = 0.59$) between postweaning RFI and feedlot RFI evaluated on the same calves. These results suggest that feed efficiency may be dependent on the stage of maturity of the animal (Arthur and

Herd, 2005). As such, it appears that RFI evaluation should be conducted on like-type animals to reduce errors in calculating RFI (Herd and Arthur, 2009).

Feed

The type and amount of feed provided to cattle may influence the outcome of an RFI evaluation. Fan et al. (1995) observed significant differences in RFI values calculated for Angus and Hereford bulls fed 2 different diets. Bulls fed a high concentrate diet had more positive RFI than bulls fed a high roughage diet (0.36 ± 0.12 vs. -1.67 ± 0.12 kg/d). Contrasting results were reported by Goonewardene et al. (2004) in crossbred steers. As the proportion of roughage increased, RFI became more positive. In addition, RFI became increasingly negative as the proportion of grain was increased. This suggests that animals may perform differently depending on the type of diet provided.

The amount of feed provided to cattle on RFI test may also impact the results. *Ad libitum* availability of feed allows cattle to express differences in appetite, whereas a limit-fed diet essentially eliminates the influence of appetite. Most RFI studies in cattle allow *ad libitum* access to the diet. While this may be appropriate for animals in a feedlot setting, this approach may not accurately reflect the true feed efficiency of heifers entering the cowherd as breeding females. While arguments have been made that RFI determined in a feedlot setting should be directly applicable to the cowherd (Arthur et al., 2001a; Arthur and Herd, 2005), few studies have actually investigated this issue. Herd et al. (1998) evaluated postweaning RFI in Angus heifers provided *ad libitum*

access to a high concentrate diet. The most efficient and least efficient heifers were re-evaluated for intake on pasture as 3-yr-old cows using intraruminal alkane capsules and fecal samples to determine dry matter intake (DMI). No differences were observed in the pasture DMI between the two groups. Similar results were reported by Meyer et al. (2008) where no significant differences were observed in pasture intake between previously determined efficient and inefficient cows. These results suggest that the amount of diet, as well as the type of diet, could potentially influence the outcome of an RFI evaluation.

Test duration

Determining the appropriate test duration is another factor that could alter the results of an RFI evaluation. Since there is such a large expense associated with feeding trials, reducing the test period as much as possible is ideal. Early estimates of the number of d required to accurately determine growth rate in cattle suggested a long test period of 112 d (Kemp, 1990; Brown et al., 1991). Archer et al. (1997) assessed the optimum length of the feeding period for RFI in Angus, Hereford and Shorthorn heifers and bulls by progressively increasing the test period from 7 to 119 d. They discovered there was little decrease in the variation of RFI following d 70. Archer and Bergh (2000) conducted a similar study to investigate the appropriate RFI test duration of cattle of differing biological types. Using young Angus, Hereford, Simmental, Afrikaner and Bonsmara bulls, they concluded that a 70 d feeding period was acceptable for these

breedtypes as well. From these studies, it appears that the feeding period can be reduced to as little as 70 d without sacrificing accuracy in determining RFI.

Estimated feed intake

Correctly estimating the expected feed intake of each animal is critical to the accuracy and usefulness of RFI. Two different methods exist for estimating feed intake in cattle. The method described by Koch et al. (1963) uses linear regression of actual feed intake on growth rate and mid-test BW to determine the expected feed intake of each animal. A modification of this model has been used in more recent studies where metabolic BW is used instead of actual BW (Arthur et al., 1996; Knott et al., 2008). This allows RFI to account for the wide variation in maintenance requirements that has been reported to exist between animals even at similar production levels (Montaño-Bermudez et al., 1990).

An alternative method of determining expected feed intake for RFI evaluation calculates expected feed intake from equations rather than from actual data. Under this concept, the net energy required for maintenance and growth is calculated from BW and growth rate using NRC estimates (Fann et al., 1995). Taking into consideration the nutrient content of the feed provided, the expected feed intake for each animal is then calculated. Studies that have employed this method have experienced problems with accurately estimating feed intake. In a study by Knott et al. (1998), this model overestimated feed consumption in 6-mo-old sheep and underestimated intake in 13-mo-old sheep. Furthermore, correlations between RFI and BW and growth rate have been

observed when using this system to estimate feed intake. Fann et al. (1995) reported correlations between RFI and average daily gain (ADG) of -0.50 in Hereford bulls and -0.58 in Angus bulls. Correlations were also observed between RFI and yearling weight of these Hereford (r = -0.44) and Angus (r = -0.53) bulls (Fan et al., 1995). Therefore, linear regression appears to be the more appropriate model for estimating feed intake for RFI calculation.

Indirect Measures of RFI

Insulin-like growth factor-I

Despite suggestions that the length of RFI trials can be reduced to as little as 70 d, feeding trials are still costly to conduct. Basarab et al. (2002) estimated that testing a single animal for RFI could cost as much as $188. This expense has been one of the main challenges to the widespread testing of cattle for RFI by producers. As a result, an indirect measure of RFI that is easy to test for and relatively inexpensive could help expedite the rate of genetic change in feed efficiency (Davis and Simmen, 1997). Circulating insulin-like growth factor-I (IGF-I) has been proposed as an indirect measure of feed efficiency in cattle. Insulin-like growth factor-I is a peptide hormone produced by the liver in response to episodic growth hormone release from the anterior pituitary. Upon release into the circulation, IGF-I travels to a variety of target tissues to cause glucose metabolism, protein synthesis and growth (Baxter, 1986).

Insulin-like growth factor-I has previously been correlated with growth traits in cattle (Bishop et al., 1989; Davis and Simmen, 1997), sheep (Roberts et al., 1990; Blair

et al., 2002) and pigs (Spicer et al., 1992; Bunter et al., 2002). Furthermore, circulating concentration of IGF-I is an easily quantifiable and heritable trait (Herd et al., 1995), thus justifying the concept of measuring circulating IGF-I as an indicator trait for RFI. Johnston et al. (2002) determined that circulating IGF-I concentration was positively correlated with RFI in *Bos taurus* cattle. In an economic evaluation conducted by Wood et al. (2002), the authors suggested that IGF-I concentration would be best used as a screening tool to determine which cattle should be further evaluated for RFI. However, no correlations were observed between RFI and IGF-I in Brangus heifers (Lancaster et al., 2007), suggesting that further research needs to be conducted to fully elucidate the relationship between RFI and IGF-I.

Genetic markers

Genetic indicators of RFI have also been identified in cattle. A whole-genome study of feedlot cattle of diverse breedtypes revealed 161 single nucleotide polymorphisms (SNP) that influence RFI (Barendse et al., 2007). Of these SNP, the 20 most significant accounted for 76% of the genetic variation in RFI. Pfizer Animal Genetics (2009) is currently marketing a genetic test consisting of 56 SNP for feed efficiency in cattle. Using 4 specific genetic markers in the bovine genome associated with RFI, the GeneSTAR® test estimates feed efficiency in sampled animals. Although Pfizer Animal Genetics (2009) reported a high genetic correlation between the genetic markers and RFI, the test still only accounts for 15% of the variation in feed consumption. Furthermore, a third party validation study conducted by the National

Beef Cattle Evaluation Consortium (2009) found that phenotypic RFI was correlated (r = 0.40; P = 0.02) in *Bos taurus* cattle but not correlated (P = 0.55) in *Bos indicus* cattle with results obtained with the Pfizer GeneSTAR® genetic evaluation. Therefore, caution should be exercised when interpreting the results of such genetic tests.

Estimated breeding values

Estimated breeding values (EBV) are estimations of an animal's genetic worth for a given trait and can be used to help guide producers when making breeding decisions. In Australia, BREEDPLAN is the most widely accepted measure of EBV in cattle (Sherman et al., 2009). Estimated breeding values exist for a variety of traits and include the major breeds that represent over 85% of the purebred bulls marketed in Australia (Exton et al., 1999). Although RFI is a fairly novel measure of feed efficiency, EBVs for RFI have been published since 2002 (Arthur and Herd, 2005). These EBVs were developed based on within and across herd comparisons of RFI measured in individual feeding trials (Sherman et al., 2009). While research is ongoing in order to expand BREEDPLAN, it appears that EBV may be a fairly reliable method to predict RFI. Richardson et al. (2004) reported a positive correlation (r = 0.35; P < 0.05) of RFI in Angus steers to their respective sire's RFI EBV. While it may be possible to incorporate EBVs into mating decisions, single trait selection for RFI is not recommended. Rather, Crews et al. (2005) suggested that RFI should be included as part of a multiple trait selection index.

Sources of Variation of RFI

Residual feed intake reflects differences in how animals use available nutrients for maintenance and production. Understanding the biological mechanisms that influence feed efficiency is important to understanding why feed consumption differs among cattle once maintenance and production requirements have been accounted for. Furthermore, identifying the underlying traits responsible for the phenotypic expression of feed efficiency may help identify indirect markers for feed efficiency. This could potentially eliminate the need for time-consuming and expensive feeding trials currently necessary to determine individual animal RFI. Historical data from other traits (growth performance, wool production, etc.) suggest that there is not a single mechanism that is responsible for controlling the phenotypic manifestation of feed efficiency (Oddy, 1999). Therefore, several biological mechanisms have been investigated for their potential roles in the expression of feed efficiency.

Composition of gain

Physiological maturity influences the proportion of bone, fat and muscle deposited (Robelin, 1986). As cattle mature, long bone growth and protein accretion slow while fat deposition increases. Therefore, faster maturing cattle deposit a greater proportion of fat than slower maturing cattle at a given chronological age. The energetic expense of depositing fat is higher than that of accruing protein, and a given amount of feed will support more lean tissue growth than adipose tissue (Trenkle and Willham, 1977). Gregory et al. (1962) noted that as cattle fatten, efficiency declines due to the

higher energetic cost of depositing adipose tissue. More recently, it has been suggested that differences in composition of gain are at least partly responsible for the variation observed in RFI in cattle. Herd and Bishop (2000) reported moderate, negative correlations between RFI and lean carcass content ($r = -0.22$; $P < 0.05$) and lean growth rate ($r = -0.33$; $P < 0.05$) suggesting that low RFI (efficient) cattle have a greater proportion of lean muscle as compared to their inefficient cohorts. Residual feed intake has also been positively correlated with final 12^{th} rib back fat thickness ($r = 0.20$; $P < 0.05$), gain in 12^{th} rib back fat thickness ($r = 0.30$; $P < 0.05$; Lancaster et al., 2009) and gain in empty body fat ($r = 0.22$; $P < 0.01$; Basarab et al., 2003). Furthermore, Richardson et al. (2001) observed more bone and protein and less fat content in low RFI steers, implying there may be small differences in the maturity patterns of low versus high RFI cattle. Despite these observations, body composition has been estimated to account for only 5 (Richardson and Herd, 2004) to 9 (Lancaster et al., 2009) percent of the total variation in RFI.

Feeding behavior

The feeding behavior of cattle fed under the same environmental conditions has been shown to be highly variable (Robinson et al., 1997; Gibb et al., 1998). Since the feeding behavior of an individual animal in a healthy state is generally consistent (Nkrumah et al., 2007), the between animal variation in feeding activity could be a potential source of the variation observed in RFI. Golden et al. (2008) reported that high RFI steers ate meals more frequently than low RFI steers (18.2 vs. 11.0 ± 0.75 eating

bouts per day; $P < 0.05$). In addition, feeding duration, head-down time and feeding frequency were greater for inefficient cattle (Nkrumah et al., 2007; Lancaster et al., 2009). Richardson et al. (2000) determined that RFI was positively correlated ($r = 0.32$) with daily pedometer count. Together, these data suggest that high RFI cattle spend more time walking to and from the feed bunk as well as more time eating. Since the time spent in eating activity (walking, chewing, ruminating, etc.), is related to the energetic cost of eating (Susenbeth et al., 1998), the increased physical activity of the high RFI steers could partly explain the reduced efficiency with which they utilize available energy.

Feed digestibility

Once feed has been consumed, the ability of the animal to digest and absorb the nutrients may also influence the efficiency with which the feed is utilized. Numerous studies have reported an increased daily feed intake by high RFI cattle as compared to low RFI cattle. As feed intake increases, ruminal passage rate accelerates (Grovum and Hecker, 1973), thereby decreasing the amount of time feed remains in the rumen for digestion. It has been theorized that the ability of high RFI cattle to digest feed is reduced as a result of their increased daily feed consumption and passage rate. Using acid insoluble ash as a digestibility marker, Krueger et al. (2008) concluded that low RFI heifers had higher ($P < 0.05$) dry matter (731 vs. 705 ± 12 g/kg dry matter) and crude protein (691 vs. 657 ± 13 g/kg dry matter) digestibility than high RFI heifers. Residual feed intake was negatively correlated ($r = -0.44$; $P < 0.05$) with metabolizable energy in

a study where high RFI steers recovered 10% less metabolizable energy than low RFI steers (Nkrumah et al., 2006). Richardson et al. (1996) also reported a trend ($P < 0.10$) for low RFI cattle to have increased nutrient digestibility than high RFI cattle. Although only a 1% difference in digestibility by RFI was reported in that study, the authors concluded that this difference could account for as much as 14% of the observed difference in feed efficiency.

Methane production

Of the total energy consumed by cattle, up to 6% may be lost to methane production in the rumen (Johnson and Johnson, 1995). Reducing this loss has been the target of research for many years in an effort to improve overall production. However, it has been difficult to reduce methane production without compromising the productivity of the animal (Hegarty et al., 2007). Methane production is largely influenced by feed intake and digestibility (Blaxter and Clapperton, 1965). Since low RFI cattle have reduced feed intake and improved apparent digestibility, researchers have theorized that these cattle might also have reduced methane production. Nkrumah et al. (2006) reported a positive correlation ($r = 0.44$; $P < 0.05$) between RFI and methane production. In addition, a 28% reduction in methane production was observed in the low RFI steers. Similarly, Hegarty et al. (2007) found that low RFI steers eructated 25% less methane than high RFI steers. This reduction in methane could explain part of the variation observed in feed efficiency. In addition, decreased methane production could have environmental benefits as well. Cattle have been targeted as contributors to global

warming because of their methane production (Johnson and Johnson, 1995). By selecting for low RFI cattle, methane production by cattle could be reduced by as much as 3% within 25 yr (Hegarty et al., 2007).

Reproductive Performance

The traits associated with reproduction are reported to be at least 5 times more economically important than growth traits (Trenkle and Willham, 1977), thus indicating that reproductive success is crucial to the profitability of cow-calf operations. The role of the breeding female in a typical cow-calf operation is a very demanding one. Generally, a female is expected to become pregnant, carry the fetus to term, successfully raise the calf until weaning, and become pregnant again so that she calves within 1 yr of birthing the previous calf. Failure of the female to accomplish any one of these steps has a detrimental effect on the profitability of that female and the operation as a whole. One of the key factors influencing a female's reproductive success is her ability to become pregnant (Wiltbank et al., 1961). Since reproductive traits are lowly heritable (Davenport et al., 1965; Johnson and Notter, 1987), environmental factors have profound effects on reproductive success. Nutrition has been recognized for years as an important mediator of the events associated with reproduction (Guilbert, 1942; Asdell, 1949; Wiltbank et al., 1962; Randel et al., 1990; Short et al., 1990) and can be manipulated to influence reproductive performance. Residual feed intake reflects differences in how cattle use nutrients for life processes such as maintenance, growth, gestation and lactation (Kennedy et al., 1993). As a result, selection for RFI could alter the nutritional

control over reproduction and impact subsequent performance of the cowherd. Puberty and the postpartum period are especially sensitive to the nutritional status of the female and need to be monitored as selection for RFI is further investigated.

Puberty

Puberty can be defined in many ways. From a general view, puberty is the process by which an animal becomes capable of sexual reproduction (Robinson, 1977). More specific to cattle, puberty has been described as the first behavioral estrus accompanied by the development and maintenance of a corpus luteum (CL) on the ovary (Kinder et al., 1987). In the pubertal heifer, the pulsatile release of gondadotropin-releasing hormone (GnRH) from the hypothalamus into the portal blood stimulates the episodic release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) into systemic circulation. Follicle stimulating hormone promotes the recruitment and early growth of follicles on the ovary, while LH is responsible for the selection and maturation of a dominant follicle. The high circulating concentration of estradiol secreted by the dominant follicle causes estrus behavior as well as the LH surge responsible for ovulation and formation of luteal tissue.

Research indicates that inadequate stimulation of gonadotropes by GnRH limits the functionality of the hypothalamic-pituitary-gonadal axis in the prepubertal heifer. In 3, 6, and 9 mo old prepubertal heifers, treatment with exogenous GnRH caused an immediate release of LH and FSH within 20 min of infusion (Barnes et al., 1980). Gonadotropin release peaked within 20 min to 2 hr of treatment, and concentrations

were similar to those observed in untreated heifers in estrus (Dobson, 1978). These results suggest that the pituitary of prepubertal heifers is capable of responding to GnRH stimulation well before puberty occurs. Furthermore, treatment with FSH and LH caused successful ovulation in calves as young as 2 and 4 wk of age (Black et al., 1953; Seidel et al., 1971), thus indicating that the ovary is responsive to gonadotropin stimulation at a very young age.

With evidence supporting the functionality of the pituitary and gonads well before puberty, it appears that maturation of the hypothalamus controls when puberty occurs. A shift in hypothalamic neuron sensitivity to estradiol has been proposed as the mechanism responsible for initiating puberty. Ovariectomization (OVX) of prepubertal heifers caused mean LH concentrations and pulse frequency to increase significantly above that observed in intact heifers (Schillo et al., 1982; Day et al., 1984). When estradiol replacement was provided to OVX heifers, LH release was effectively blocked. This suggests that estradiol secreted from small follicles on the ovaries of prepubertal heifers inhibits the pulsatile release of GnRH that is observed in the pubertal heifer. Further experimentation by Schillo et al. (1982) revealed that the effectiveness of estradiol in blocking LH release was diminished as heifers approached puberty. It appears, therefore, that prepubertal females have heightened sensitivity to the negative feedback control of estradiol on GnRH release. Around the time of puberty, this sensitivity is altered, thus allowing the normal, pulsatile release of GnRH necessary to stimulate gonadotropin release and folliculogenesis.

The hypothalamic neurons associated with GnRH release are sensitive to a number of stimuli. The nutritional status of the maturing heifer is one such factor that has been shown to alter the timing of puberty in heifers. Dietary energy restriction in prepubertal heifers prevents LH release (Day et al., 1986; Kurz et al., 1990) and postpones puberty (Day et al., 1986). Alternatively, providing a high energy diet to heifers hastens the onset of puberty (Wiltbank et al., 1969). This is important as the age at which a heifer attains puberty is an important factor influencing her future reproductive success. Early attainment of puberty is particularly important in operations that have a specified breeding season and desire females to calve for the first time as 2-yr-olds. Heifers that calve at a younger age have higher lifetime productivity than those that calve at a later age (Lesmeister et al., 1973). Calves that are born later in the calving season are lighter at weaning (Evans et al., 1955) and reduce the overall productivity of the dam. In addition, cows that calve late one year tend to calve late or not at all the following year (Burris and Priode, 1958). Therefore, it is important that heifers are managed so that they breed as early as possible during the breeding season.

In order to accomplish this, it is recommended that heifers are managed to reach puberty at least 1 to 3 mo before the start of the breeding season (Short et al., 1990). The first estrus is often accompanied by a lack of luteal formation (Rutter and Randel, 1986). As a result, the fertility of the first pubertal estrus is much lower than the fertility of the third estrus following puberty (Byerley et al., 1987). Managing heifers to reach puberty prior to the breeding season helps ensure that heifers are estrous cycling and can become pregnant early in the breeding season. Management strategies involve targeted

feeding of heifers so that they are able to reach puberty prior to the start of the breeding season (Wiltbank et al., 1985). Feeding heifers to gain weight so that they achieve 65% of their mature weight prior to the breeding season is the recommended strategy for feeding heifers to reach puberty in a timely manner (Patterson et al., 1991). Since nutrition plays such a key role in timing puberty initiation, monitoring the effects of RFI selection on age at puberty is important.

Postpartum period

The postpartum interval is defined as the period of anestrous between parturition and the first estrus following calving (Short et al., 1990). The length of this period is an important determinant of the reproductive performance of cows, and thus the profitability of the enterprise. In general, most cows are expected to produce a calf each yr. If a cow spends approximately 285 d in gestation, she effectively has 80 d to become pregnant so that she will calve again within a year's time. A cow requiring longer than 365 d to produce one calf will calve later and later each year until she is no longer able to become pregnant during a defined breeding season (Burris and Priode, 1958). The primary reason cows are culled from the breeding herd is because they fail to become pregnant, thus decreasing herd productivity (Melton, 1995; Mathews and Short, 2001). One of the reasons cows fail to become pregnant is because they calve too late to initiate normal estrous cycles during the breeding season. Improved fertility has been associated with cows that initiate estrous cycles earlier in the breeding season (Thatcher and Wilcox, 1973; McNaughton et al., 2007), suggesting that the more estrous cycles a

female has, the more chances she will have to become pregnant. Therefore, minimizing the amount of time a cow spends in postpartum anestrous is important to maximizing reproductive performance.

Postpartum anestrous is similar in nature to the anestrous state of the pre-pubertal heifer (Wiltbank et al., 2002). During late gestation, high concentrations of estradiol exhibit a negative feedback on LH (Nilson et al., 1983), thus suppressing LH production by the anterior pituitary (Arije et al., 1974). Following parturition, the female regains the ability to produce and secrete LH at normal concentrations within 2 to 3 wk of calving (Williams and Ray, 1980). However, the normal, pulsatile release of GnRH is impaired, thus inhibiting the release of LH necessary for maturation and ovulation of a dominant follicle (Wettemann et al., 2003). It has been suggested that GnRH release is inhibited by estradiol negative feedback on the hypothalamus (Wiltbank et al., 2002). The sensitivity of the hypothalamic neurons to estradiol negative feedback is eventually diminished as the female approaches the time when normal estrous cycles are again observed.

As in the pre-pubertal heifer, the hypothalamic neurons controlling GnRH release in the postpartum cow are sensitive to a number of stimuli. One of the major factors regulating the postpartum anestrous period is the nutritional status of the female (Randel, 1990; Short et al., 1990). Evaluating the nutritional status of an individual can be monitored by evaluating BW and/or body energy reserves. Since BW is confounded by fetal growth and calf expulsion at parturition (Selk et al., 1988), BW may be an inappropriate measure to evaluate nutritional status in periparturient cows. However,

evaluating the energy reserves of these cows can be easily accomplished by evaluating body condition score (BCS). The traditional scoring system used in beef cattle production is based on a scale of 1 to 9, where 1 indicates an emaciated cow and 9 represents a very obese cow (Table 1.1; Wagner et al., 1988). Although BCS is a subjective measure, it is a satisfactory estimate of body energy reserves (Dziuk and Bellows, 1983) and has been well-documented for its association with reproductive performance (Wiltbank et al., 1962; Bishop et al., 1994; Spitzer et al., 1995).

The nutritional status of cows both before and after calving can influence postpartum reproductive performance. Multiple studies have reported that the BCS of cows at calving is a major factor influencing a variety of postpartum reproductive parameters in beef cows. Vizcarra et al. (1998) reported that a higher percentage of cows calving with a BCS of 6 had initiated luteal activity by the end of the breeding season as compared to cows that calved with a BCS of 5 or 4 (100%, 74% and 55%, respectively). Results from a study conducted by Richards et al. (1986) revealed that cows calving with a BCS <4 had a longer interval to estrus (61 vs. 49 d; $P < 0.01$) and extended interval from calving to pregnancy (90 vs. 84 d; $P < 0.05$) than cows with a BCS >4 at calving. Spitzer et al. (1995) reported that increased BCS of cows at parturition resulted in a greater percentage of those cows exhibiting estrus by d 40 and d 60 of the breeding season. By the end of the breeding season, 74, 90, and 98% of the

Table 1.1. Body condition scoring system[a]

Score	Description
1	SEVERELY EMACIATED. All ribs and bone structure easily visible and physically weak. Animal has difficulty standing or walking. No external fat present by sight or touch.
2	EMACIATED. Similar to 1 but not weakened.
3	VERY THIN. No palpable or visible fat on ribs or brisket. Individual muscles in the hind quarter are easily visible and spinus processes are very apparent.
4	THIN. Ribs and pin bones are easily visible and fat is not apparent by palpation on ribs or pin bones. Individual muscles in the hind quarter are apparent.
5	MODERATE. Ribs are less apparent than in 4 and have less than 0.5 cm of fat on them. Last two or three ribs can be felt easily. No fat in the brisket. At least 1 cm of fat can be palpated on pin bones. Individual muscles in hind quarter are not apparent.
6	GOOD. Smooth appearance throughout. Some fat deposition in brisket. Individual ribs are not visible. About 1 cm of fat on the pin bones and on the last two to three ribs.
7	VERY GOOD. Brisket is full. Tailhead and pin bones have protruding deposits of fat on them. Back appears square due to fat. Indentation over spinal cord due to fat on each side. Between 1 and 2 cm of fat on last two to three ribs.
8	OBESE. Back is very square. Brisket is distended with fat. Large protruding deposits of fat on tailhead and pin bones. Neck is thick. Between 3 and 4 cm of fat on last two to three ribs. Large indentation over spinal cord.
9	VERY OBESE. Description of 8 taken to greater extremes.

[a]Adapted from Wagner et al. (1988).

cows calving with a BCS of 4, 5, or 6 respectively, had expressed estrus. Furthermore, pregnancy rates after a 60 d breeding season were 56, 80 and 96% for cows with a BCS of 4, 5, or 6 at calving. Likewise, Lake et al. (2005) observed significantly higher ($P = 0.01$) pregnancy rates (89 vs. 64%) for cows calving in a BCS of 6 compared with cows calving with a BCS of 4.

It appears that the absolute BCS of cows at calving is a more significant factor influencing postpartum reproductive performance rather than the change in BCS leading up to calving. Ninety days prior to calving, Morrison et al. (1999) grouped heifers by BCS so that heifers with a BCS ≤ 4, BCS 5-6, and body condition score ≥ 7 were blocked separately. These heifers were then fed to achieve a BCS of 5 to 6 by calving so that cows gained, maintained or lost BCS prior to calving. The prepartum change in BCS did not affect ($P > 0.05$) the percentage of cows with luteal activity by the start of the breeding season, pregnancy rates at 20, 40 or 60 d of the breeding season, or the interval from calving to conception. In a separate study, Hess et al. (2005) re-evaluated data from a variety of reports to determine the effects of BCS at calving and prepartum change in BCS on postpartum interval. They reported that the length of the postpartum interval from calving to estrus was highly correlated ($r = 0.75$; $P < 0.001$) to BCS at calving but not correlated ($P > 0.10$) to change in BCS prior to calving. Therefore, it appears that the way in which cows achieve a certain BCS at calving does not have a significant impact on postpartum reproductive performance as long as cows have achieved adequate BCS by calving.

Postpartum nutritional management can also influence reproductive performance; however, this relationship is less clear than the prepartum nutritional status of beef cows. Stagg et al. (1995) fed multiparous cows either a high energy or low energy diet following calving. Cows fed the high energy diet had a shorter ($P < 0.05$) interval from calving to first ovulation (70 d) than cows fed the low energy diet (95 d). Rutter and Randel (1984) also reported a decrease in the interval from calving to estrus ($P < 0.01$) with increasing nutrient intake following calving. Furthermore, cows that maintained BCS had a much shorter interval to estrus than cows that lost BCS after calving (32 vs. 60 d; $P < 0.005$). Richards et al. (1986) fed postpartum multiparous cows to either maintain weight, gain weight, lose weight, or lose weight prior to a flush period. Results from this study indicate that the postpartum feeding regime did not influence the interval from calving to estrus or cumulative return to estrus. However, it appeared that postpartum nutrition did affect the reproductive performance of cows calving in poor body condition. Of the cows that calved with a BCS < 4, a greater percentage of cows fed to maintain or gain BW after calving exhibited estrus ($P < 0.01$) and became pregnant ($P < 0.05$) by 40 and 60 d after calving than cows that lost weight.

It appears that the ability of a cow to maintain BCS after calving may be an important determinant of postpartum reproductive performance (Rutter and Randel, 1984). This can be accomplished by providing increased nutrients to thin cows or by ensuring that cows have adequate energy reserves at calving. Although the reproductive performance of cows that are thin at calving can be improved with postpartum feeding, the performance of these cows may still not be as satisfactory as desired. Therefore,

Selk et al. (1988) suggested that the BCS of cows at calving is the largest determinant of whether or not a cow will become pregnant during the subsequent breeding season and recommended that cows should be managed so that they calve with at least a BCS of 5.

The rationale behind this concept likely stems from the biphasic nature of adipose tissue metabolism in the cow (McNamara and Hillers, 1986a). During early pregnancy, the cow stores adipose tissue in preparation for the extreme energy demands of fetal growth and lactation. As fetal growth increases exponentially during the latter part of gestation, hydrolysis of stored triglycerides is initiated to provide additional energy to meet the demands of the growing conceptus (Wiltbank et al., 1962; Lucy et al., 1991). The mobilization of adipose tissue continues through early lactation as energy demands remain high (McNamara and Hillers, 1986a). If cows are not in adequate body condition around the time of calving, this mobilization of adipose tissue can impede postpartum reproductive performance as previously discussed. Providing additional nutrients to these cows can help mitigate this negative effect; however, it is difficult and expensive to provide enough nutrients to overcome the negative energy balance of early lactation.

After peak lactation, cows eventually begin to replenish adipose reserves (McNamara and Hillers, 1986a). For cows in thin body condition at calving, it often takes too long to regain energy reserves to allow these cows to become pregnant during a defined breeding season. Therefore, it is imperative that cows are in adequate body condition at calving. A low correlation has been reported between RFI and lean carcass composition ($r = -0.22$; $P < 0.05$; Herd and Bishop, 2000) as well as RFI and back fat

thickness ($r = 0.20$; $P < 0.05$; Lancaster et al., 2009). This suggests that selection for RFI may slightly alter the body composition of cattle in favor of cattle with less body energy reserves. Therefore, it is important to monitor the impact of selection for RFI on BCS and subsequent reproductive performance of the cowherd.

Lipid metabolism

Lipid metabolism is an active cycle of free fatty acid (FFA) uptake into adipose tissue for storage as triglycerides and hydrolysis of stored triglycerides into non-esterified fatty acids (NEFA) and glycerol (McNamara, 1994). During late gestation and early lactation in the cow, lipid metabolism is altered to favor lipolysis over lipogenesis in order to provide an additional source of energy for the high energetic demands of fetal growth and lactation. This results in an increase in the breakdown of triglycerides stored in adipose tissue and an increase in circulating NEFA and glycerol. Both glycerol and NEFA concentrations in the blood can be used as indicators of adipose tissue metabolism. However, glycerol is only released from adipose tissue when a triglyceride is fully hydrolyzed. Non-esterified fatty acid concentration reflects total fatty acid hydrolysis from the adipose tissue and is a more appropriate measure than glycerol for monitoring total adipose tissue metabolism (McNamara and Hillers, 1986a). Furthermore, NEFA concentration has been correlated to fat loss in cattle (Trigg and Tops, 1981; Chilliard et al., 1984) and is a useful tool for determining negative energy balance.

Lipolysis and the subsequent release of NEFA into circulation are regulated by a number of systems. Growth hormone, catecholamines (epinephrine and norepinephrine), glucagon, and prolactin all stimulate lipolysis in the adipocyte while insulin promotes lipogenesis. In the lactating cow, growth hormone, norepinephrine and prolactin are the primary regulatory agents controlling lipid metabolism in the adipocyte (McNamara, 1994). These signals work in concert with one another to increase lipolysis during late gestation and early lactation to such an extent that adipocyte size is effectively reduced during early lactation (Chilliard et al., 1984). Once receptors on the adipocyte are bound by a lipolysis stimulator, adenyl cyclase is activated to increase cyclic AMP concentrations. This, in turn, activates protein kinase to phosphorylate and activate hormone sensitive lipase. Hormone sensitive lipase then acts to hydrolyze triglycerides and release NEFA from the adipocyte (McNamara, 1994).

Although it is well documented that lipolysis rates are increased in the lactating cow, information regarding the exact site(s) of lipolysis is less well documented. Arthur et al. (2005) noted a substantial decline in subcutaneous fat thickness over the ribs of cows from the beginning of the breeding season until weaning. Work done by McNamara and Hillers (1986b) also utilized subcutaneous fat as a source of adipocytes to study lipolysis and lipogenesis in lactating cows. While subcutaneous fat metabolism is different than other fat deposits (i.e. visceral, intermuscular, intramuscular, etc.) during growth, it has been suggested that subcutaneous fat metabolism is reflective of total body adipose metabolism due to the extreme requirement for a fast, massive movement of adipose tissue during this period (McNamara and Hillers, 1986a).

However, when lipolysis rates were analyzed in inner back fat, outer back fat, intermuscular fat from the high leg, omental fat and perirenal fat samples from Holstein steers, inner and outer back fat had the highest lipolytic rates suggesting different rates of metabolism in different areas of adipose tissue (McNamara and Hillers 1986b). It is important to note, though, that these samples were collected from a steer rather than a lactating cow and may not be reflective of adipose tissue metabolism during lactation.

CHAPTER II

EFFECT OF BREEDTYPE AND PHYSIOLOGICAL AGE ON RESIDUAL FEED INTAKE OF GROWING HEIFERS

Introduction

Providing feed to cattle represents a significant proportion of the costs associated with producing beef (Montaño-Bermudez et al., 1990). With current biofuel policies creating competition for feedstuffs traditionally used to feed livestock (Bottje and Carstens, 2009), high commodity prices are making it as challenging as ever for beef producers to operate at a profit. As a result, many beef producers are looking for ways to keep feed expenses to a minimum. Identifying and selecting cattle that are more efficient at utilizing feed resources has been receiving attention as a potential production practice to reduce feed expenses. Selection strategies have been shifting away from the traditionally used F:G ratio and are beginning to incorporate RFI as an indicator of feed efficiency in beef cattle.

Although the concept behind RFI was developed more than 45 yr ago (Koch et al., 1963), it has been only recently that most RFI research has been conducted. Arguably, a relatively small amount of this research has been directed towards investigating and developing appropriate guidelines to evaluate cattle for RFI. There are many variables that could potentially alter the outcome of an RFI evaluation. However, many of these can be controlled (ie: test duration, type and amount of diet provided, selection of cohorts of animals, etc.). Therefore, identifying procedures that yield

consistent, accurate results are necessary in order for RFI to be a useful production tool. As a result, the objective of this study was to investigate the potential consequences of cohort selection on the outcome of RFI trials. To accomplish this, a retrospective evaluation of RFI was conducted using heifers of diverse breedtypes during two different physiological periods (pre- and post-pubertal).

Materials and Methods

Animals and experimental design

The data used for this study were collected during 1973 and 1974 from the McGregor location of the Texas Agricultural Experiment Station. Heifers (n = 77) were obtained from a large crossbreeding program that utilized a five-breed diallel mating scheme. Breedtypes included straightbred Angus, Brahman, Hereford, Holstein and Jersey and F_1 Angus x Brahman, Angus x Hereford, Angus x Holstein, Angus x Jersey, Brahman x Hereford, Brahman x Holstein, Brahman x Jersey, Hereford x Holstein, Hereford x Jersey and Holstein x Jersey crosses (reciprocals pooled). Pre-pubertal heifers were individually penned at approximately 6 mo of age in 3 m by 10 m open, dirt-floored pens. Heifers were allowed *ad libitum* access to a balanced ration, and the ration was changed for each heifer after reaching puberty to reduce the energy density (Table 2.1). Feed intake and BW data were recorded monthly for 84 ± 6 d prior to puberty and for 90 ± 4 d after puberty for each heifer following the procedures described by Long et al. (1979). Puberty was defined as the first ovulatory estrus. Heifers were exposed to marker bulls during overnight exercise periods to aid in estrus detection.

Table 2.1. Pre- and post-pubertal diet compositions

Ingredient	Pre-pubertal	Post-pubertal
Sorghum, %	48.5	33.0
Cottonseed meal, %	20.0	10.0
Cottonseed hulls, %	25.0	50.0
Vegetable fat, %	4.0	4.0
Vitamin/mineral supplement, %	2.5	3.0

Heifers were also examined by rectal palpation every 3 wk and when marked by a bull to determine ovarian activity (Stewart et al., 1980).

RFI determination

In order to compare RFI during two distinct physiological periods, the pre-pubertal and post-pubertal periods were considered separately for RFI calculation. Initial BW and ADG were computed from linear regression of BW on day of test using the PROC REG function of SAS (2002). Mid-test BW was estimated using initial BW and ADG and adjusting for a 3% shrink. Considering all females as cohorts, RFI was determined for each heifer for each period as the residual from the linear regression of average daily feed intake (ADFI) on mid-test $BW^{0.75}$ and ADG using the GLM procedure of SAS (2002).

Statistical analysis

Heifers were assigned to breedtype groups for statistical analysis. The 0% *Bos indicus* group included heifers (n = 51) with no Brahman influence, whereas the ≥50% *Bos indicus* group included heifers (n = 26) that had at least 50% Brahman breeding. Using breedtype group as a class variable, data were analyzed by GLM to determine breedtype differences during the pre- and post-pubertal periods. The GLM procedure specific for repeated measures (SAS, 2002) was used to analyze differences in initial and final BW for the two periods. Pearson's correlations were determined using the CORR function of SAS to correlate pre- and post-pubertal RFI. Spearman's rank order

correlation was also used to evaluate the change in RFI rank during the pre- and post-pubertal periods (SPSS, 2005). Chi-square (SAS, 2002) was used to evaluate changes in RFI sign between the pre- and post-pubertal periods. In addition, daily net energy for maintenance (NE_m) requirements were estimated for each heifer using the equation $NE_m = 0.077Mcal / BW^{0.75}$ (NRC, 1996). Breed-specific adjustments were made to the NE_m requirements based on the adjustment factors shown in Table 2.2. For crossbred heifers, the average of the sire and dam adjustment factors was used. Residual feed intake was then co-varied with the NE_m requirements using the GLM procedure of SAS (2002) to determine if differences observed in RFI were attributable to breedtype differences in maintenance requirements.

Results

Heifers were 5.6 ± 0.6 mo of age at the start of the pre-pubertal feeding trial, 10.9 ± 2.0 mo of age at puberty, and 11.8 ± 2.0 mo of age at the start of the post-pubertal feeding trial (Table 2.3). Heifers gained 0.95 ± 0.17 kg/d while eating 6.0 ± 1.0 kg/d of feed during the pre-pubertal test period. Over the course of the post-pubertal feeding trial, heifers gained 0.67 ± 0.17 kg/d while consuming 9.0 ± 1.6 kg/d of feed. Mean RFI was 0.00 for both the pre- (SD = 0.57) and post-pubertal (SD = 1.11) periods.

Table 2.2. Net energy for maintenance breed adjustment factors

Breed	Adjustment factor
Angus	1.0
Brahman	0.9
Hereford	1.0
Holstein	1.2
Jersey	1.2

Table 2.3. Pre- and post-pubertal summary statistics for growing heifers

	Pre-pubertal				Post-pubertal			
Trait[a]	Mean	SD	Min	Max	Mean	SD	Min	Max
n	77				77			
Initial age, mo	5.6	0.6	3.9	7.6	11.8	2.0	8.6	15.9
Age at puberty, mo					10.9	2.0	7.7	15.4
Test duration, d	84	6	78	119	90	4	84	94
Initial BW, kg	116.1	25.7	51.3	191.1	273.2	54.6	175.9	377.0
Final BW, kg	196.0	33.7	108.6	285.2	333.1	60.9	223.3	454.2
Mid-test metabolic BW, kg	43.0	6.1	26.1	59.3	70.8	10.1	52.3	88.8
ADG, kg/d	0.95	0.17	0.41	1.45	0.67	0.17	0.21	1.17
ADFI, kg/d	6.0	1.0	3.9	8.5	9.0	1.6	5.9	13.5
NE_m requirement, Mcal/d	3.50	0.51	2.41	4.79	5.77	0.84	4.17	7.83
Residual feed intake, kg/d	0.00	0.57	-1.47	1.32	0.00	1.11	-2.08	3.96

[a] NE_m = net energy for maintenance

Effects of breedtype

Average daily feed intake, ADG and NE_m did not differ ($P > 0.05$) by breedtype group during the pre-pubertal period (Table 2.4). Test duration tended to be different ($P < 0.10$) between the breedtype groups. Initial age, test duration, initial BW, final BW, mid-test metabolic BW, NE_m as a percentage of BW, RFI, and RFI covaried with NE_m all differed by breedtype group during the pre-pubertal period. Heifers without *Bos indicus* influence were older ($P < 0.05$) at the start of the pre-pubertal feeding period than heifers with at least 50% *Bos indicus* influence (5.7 ± 0.1 vs. 5.4 ± 0.1 mo). Heifers in the 0% *Bos indicus* group were lighter at the beginning of the feeding trial (109.9 ± 3.4 vs. 128.4 ± 4.8 kg; $P < 0.01$), lighter at the end of the feeding trial (187.2 ± 4.4 vs. 213.2 ± 6.2 kg; $P < 0.001$), and had lower metabolic mid-test BW (41.5 ± 0.8 vs. 46.1 ± 1.1 kg; $P < 0.01$) than heifers in the ≥50% *Bos indicus* group. 0% *Bos indicus* heifers had a higher NE_m requirement as a percentage of BW than *Bos indicus*-influenced heifers (2.46 ± 0.03 vs. 2.12 ± 0.04%; $P < 0.0001$). Pre-pubertal RFI (Figure 2.1) and RFI covaried with NE_m were higher ($P < 0.05$) for heifers without Brahman influence when compared to heifers with at least 50% Brahman breeding.

As in the pre-pubertal period, ADG, ADFI and NE_m requirements did not differ during the post-pubertal period between the breedtype groups (Table 2.5). There was a tendency ($P < 0.10$) for test duration to be shorter for the 0% *Bos indicus* group. Initial age, age at puberty, initial BW, final BW, mid-test metabolic BW, NE_m as a percentage of BW, RFI and RFI co-varied with NE_m were all different between the breedtype groups. Heifers without Brahman influence were younger ($P < 0.0001$) at puberty and at

Table 2.4. Age, performance traits and residual feed intake of 0% *Bos indicus* and ≥50% *Bos indicus* heifers during the pre-pubertal period

	Breedtype group		
Trait[a]	0% *Bos indicus*	≥50% *Bos indicus*	P-value
n	51	26	
Initial age, mo	5.7 ± 0.1[b]	5.4 ± 0.1[c]	0.0276
Test duration, d	83 ± 1	86 ± 1	0.0848
Initial BW, kg	109.9 ± 3.4[d]	128.4 ± 4.8[e]	0.0023
Final BW, kg	187.2 ± 4.4[f]	213.2 ± 6.2[g]	0.0010
Metabolic mid-test BW, kg	41.5 ± 0.8[d]	46.1 ± 1.1[e]	0.0012
ADG, kg/d	0.93 ± 0.02	0.99 ± 0.03	0.1707
ADFI, kg/d	5.9 ± 0.1	6.1 ± 0.2	0.2125
NE_m, Mcal/d	3.50 ± 0.07	3.51 ± 0.10	0.9816
NE_m as percentage of BW, %	2.46 ± 0.03	2.12 ± 0.04	<0.0001
RFI, kg/d	0.09 ± 0.08[b]	-0.18 ± 0.11[c]	0.0428
RFI covaried w/ NEm, kg/d	0.09 ± 0.76[b]	-0.18 ± 0.11[c]	0.0378

[a] NE_m = net energy for maintenance and RFI = residual feed intake.

[b,c] Least square means within a row differ ($P < 0.05$).

[d,e] Least square means within a row differ ($P < 0.01$).

[f,g] Least square means within a row differ ($P < 0.001$).

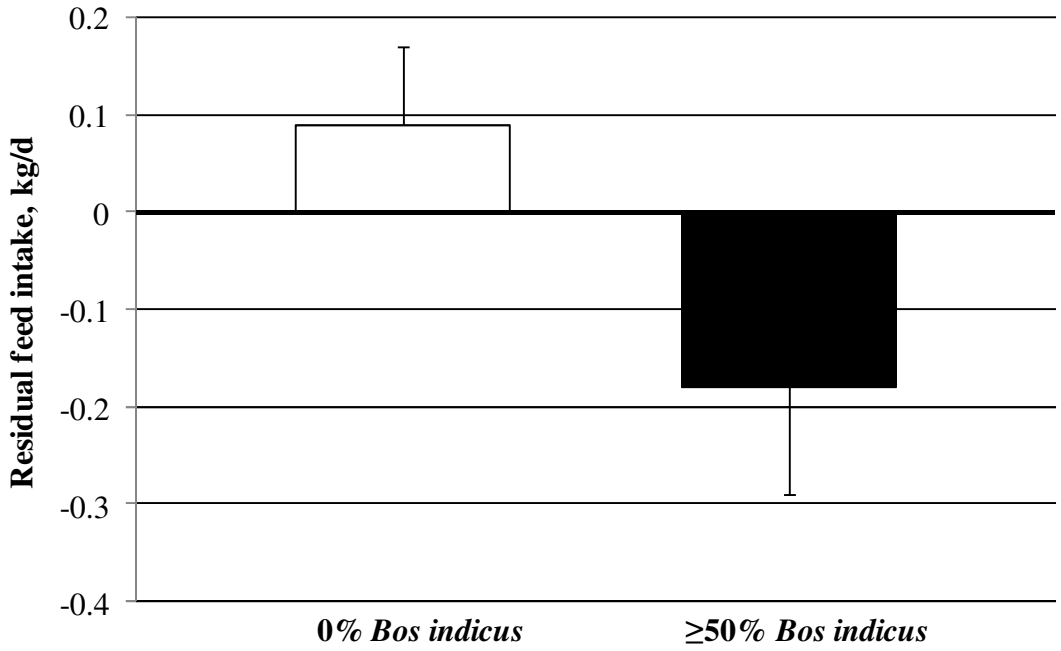

Figure 2.1. Pre-pubertal residual feed intake for 0% *Bos indicus* (n = 51) and ≥50% *Bos indicus* (n = 26) heifers.

Table 2.5. Age, performance traits and residual feed intake of 0% *Bos indicus* and ≥50% *Bos indicus* heifers during the post-pubertal period

	Breedtype group		
Trait[a]	0% *Bos indicus*	≥50% *Bos indicus*	P-value
n	51	26	
Initial age, mo	11.1 ± 0.3[b]	13.0 ± 0.4[c]	<0.0001
Age at puberty, mo	10.3 ± 0.3[b]	12.2 ± 0.4[c]	<0.0001
Test duration, d	89 ± 1.0	91 ± 1.0	0.0587
Initial BW, kg	252.9 ± 6.6[b]	313.1 ± 9.2[c]	<0.0001
Final BW, kg	312.3 ± 7.5[b]	373.8 ± 10.5[c]	<0.0001
Metabolic mid-test BW, kg	67.2 ± 1.2[b]	77.9 ± 1.7[c]	<0.0001
ADG, kg/d	0.67 ± 0.02	0.67 ± 0.03	0.9837
ADFI, kg/d	9.1 ± 0.2	8.8 ± 0.3	0.4344
NE_m, Mcal/d	5.68 ± 0.12	5.93 ± 0.16	0.2326
NE_m as percentage of BW, %	2.09 ± 0.02	1.78 ± 0.03	<0.0001
RFI, kg/d	0.36 ± 0.14[b]	-0.70 ± 0.20[c]	<0.0001
RFI covaried w/ NE_m, kg/d	0.39 ± 0.13[b]	-0.73 ± 0.19[c]	<0.0001

[a] NE_m = net energy for maintenance and RFI = residual feed intake.

[b,c] Least square means within a row differ ($P < 0.0001$).

the start of the post-pubertal feeding trial than Brahman-influenced heifers. 0% *Bos indicus* heifers had lighter initial BW (252.9 ± 6.6 vs. 313.1 ± 9.2 kg; $P < 0.0001$), lighter final BW (312.3 ± 7.5 vs. 373.8 ± 10.5 kg; $P < 0.0001$) and lighter mid-test metabolic BW (67.2 ± 1.2 vs. 77.9 ± 1.7 kg; $P < 0.0001$) than ≥50% *Bos indicus* heifers during the post-pubertal period. 0% *Bos indicus* heifers had higher NE_m requirements as a percentage of BW than *Bos indicus*-influenced heifers (2.09 ± 0.02 vs. 1.78 ± 0.03%; $P < 0.0001$). Heifers without *Bos indicus* influence had higher ($P < 0.0001$) RFI (0.36 ± 0.14 vs. -0.70 ± 0.20 kg/d; Figure 2.2) and RFI covaried with NE_m (0.39 ± 0.13 vs. -0.73 ± 0.19 kg/d) than heifers with at least 50% *Bos indicus* influence.

Effects of physiological age

Using Pearson's correlation, a moderate, positive correlation ($r = 0.48$: $P < 0.0001$) was detected between pre-pubertal and post-pubertal RFI (Appendix A). Spearman's rank order correlation revealed a similar correlation ($r = 0.46$: $P < 0.0001$) between RFI ranking of heifers during the pre- versus post-pubertal periods (Appendix B). Of the heifers evaluated, 32.5% had opposite RFI signs in the pre- and post-pubertal periods. Eleven heifers evaluated as efficient (negative RFI) during the pre-pubertal period were classified as inefficient (positive RFI) during the post-pubertal period. Fourteen of the inefficient heifers during the pre-pubertal period were efficient during the post-pubertal period. However, when analyzed by chi-square, there was no statistical difference ($P > 0.05$) observed in the percentage of heifers that had opposite RFI signs in

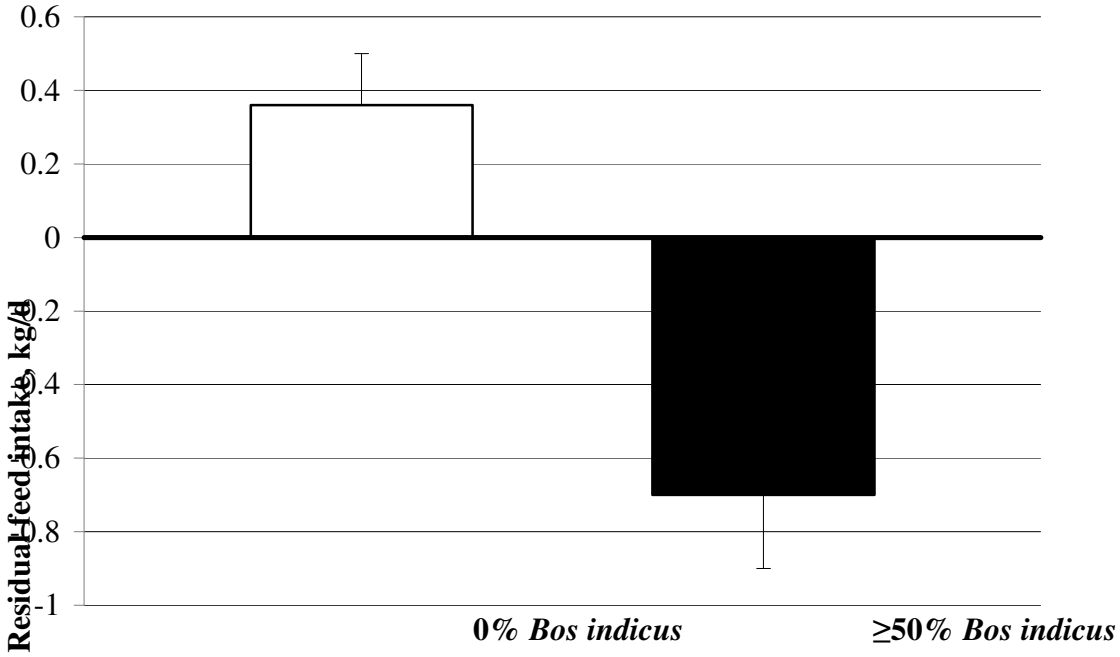

Figure 2.2. Post-pubertal residual feed intake for 0% *Bos indicus* (n = 51) and ≥50% *Bos indicus* (n = 26) heifers.

the pre- and post-pubertal periods and the percentage of heifers that had the same RFI sign during both periods.

Discussion

Effects of breedtype

The improved feed efficiency of ≥50% *Bos indicus* heifers over 0% *Bos indicus* heifers in both the pre- and post-pubertal periods agrees with other reports of breedtype differences in RFI. Riley et al. (2007) reported that Brahman heifers had more favorable RFI than Angus, Romosinuano and the F_1 crosses of these breeds. Breedtype differences were also observed when Charolais, Angus, Hereford, Limousin, Simmental and Blonde d'Aquitaine bulls were evaluated for RFI (Schenkel et al., 2004). Schenkel et al. (2004) reported breedtype differences for both ADG and ADFI that likely accounted for the breedtype variation in RFI. Other reports have observed strong correlations between ADFI and RFI (Archer et al., 1998; Arthur et al., 2001b; Nkrumah et al., 2007), suggesting that differences in feed intake account for part of the variation in RFI. However, no differences in ADG or ADFI were observed in this study between ≥50% *Bos indicus* heifers and 0% *Bos indicus* heifers, thus failing to account for the breedtype differences in RFI.

In an attempt to account for the breedtype variation in RFI, RFI was co-varied with NE_m requirements. Although Brahman-influenced cattle have 5-25% lower maintenance requirements than Angus-, Hereford-, Holstein- or Jersey-influenced cattle (NRC, 2000), RFI still differed by breedtype group in both the pre-pubertal and post-

pubertal periods when RFI was co-varied with NE_m requirements. This suggests that much of the variation in RFI across breedtypes could not be attributed to NE_m requirements. One possible explanation may lie in the difference in mid-test BW between the breedtype groups. ≥50% *Bos indicus* heifers weighed more than 0% *Bos indicus* heifers in both pre- and post-pubertal periods. As animals of different weights have different requirements for maintenance (Koch et al., 1963), the calculated NE_m requirements did not differ between the breedtype groups in either the pre- or post-pubertal periods despite the adjustments made for breed differences.

Since the difference observed in RFI between breedtype groups was not attributable to ADG, ADFI or calculated NE_m requirements, differences in RFI were possibly due to the differences in BW between the groups. ≥50% *Bos indicus* heifers had heavier initial BW, final BW and mid-test metabolic BW than 0% *Bos indicus* heifers in both the pre- and post-pubertal periods. Feed intake increases as cattle grow (Hicks et al., 1990), suggesting that heavier cattle have increased ADFI. Although heifers from Brahman breeding were heavier during both feeding periods, their ADFI did not differ from those heifers without Brahman influence. Therefore, it appears that ≥50% *Bos indicus* heifers may actually have been consuming less feed than expected for their given BW. Based on the definition for RFI, this would result in Brahman-influenced heifers having lower RFI values than heifers without Brahman influence, as was observed in this study.

This idea is further supported by the increased NE_m requirements of 0% *Bos indicus* heifers as compared to ≥50% *Bos indicus* heifers when NE_m was expressed as a

percentage of BW during both the pre- and post-pubertal periods. This suggests that the *Bos indicus*-influenced heifers required less energy to support maintenance requirements at a given BW than heifers without *Bos indicus* influence. Therefore, more nutrients could be diverted to other body processes, such as growth, in heifers with *Bos indicus* influence as compared to heifers without *Bos indicus* influence.

≥50% *Bos indicus* heifers reached puberty at significantly older ages than 0% *Bos indicus* heifers. This is consistent with results reported by Reynolds et al. (1963), Gregory et al. (1979), Morgan (1981) and Hearnshaw et al. (1994) where puberty occurred later in straightbred or crossbred Brahman heifers than in British breeds. *Bos indicus* cattle are generally larger at maturity and appear to reach puberty at a greater percentage of mature BW than *Bos taurus* cattle (NRC, 2000). As a result, puberty in Brahman and Brahman-influenced cattle is often delayed compared to *Bos taurus* cattle. Furthermore, high milk-producing Friesian breeds reach puberty earlier and at a lower percentage of BW than British breeds (NRC, 2000). This provides additional support for the difference in age at puberty observed between breedtype groups as a portion of the 0% *Bos indicus* heifers were Friesian-influenced.

Effects of physiological age

The moderate correlations observed between pre- and post-pubertal RFI as well as RFI rank suggest that RFI determined prior to puberty may only be a moderate predictor of RFI during the post-pubertal period. These results are consistent with other studies that evaluated RFI on the same cattle at different ages. The genetic correlation

between heifers evaluated for RFI post-weaning and again during their first lactation was 0.58 (Nieuwhof et al., 1992). Archer et al. (2002) reported a moderate phenotypic correlation of 0.40 between RFI measured in heifers during the post-weaning period and again after 2 parities. Arthur et al. (2001c) compared RFI measurements of weanling and yearling bulls and observed a phenotypic correlation of 0.43. A genetic correlation of 0.55 was reported between steers evaluated for RFI during the growing and finishing phases (Crews et al., 2003).

Physiological maturity has been implicated as a source of variation when determining feed efficiency (Mader et al., 2009). As cattle grow, their composition of gain shifts away from protein accretion toward fat deposition (Trenkle and Willham, 1977). Since the energetic expense associated with protein accrual is less than that for fat deposition (Ferrell and Jenkins, 1985), the efficiency with which cattle convert feed into BW gain is reduced as they mature. These changes in body composition associated with advancing physiological maturity could explain the differences observed in RFI evaluated in the same animals at different physiological ages.

Conclusion

One of the proposed benefits of using RFI as a measure of feed efficiency in beef cattle is that it accounts for between-animal variation in maintenance and growth (Arthur et al., 2001b). Under this principle, the comparison of RFI values from animals differing in age and breedtype should be valid. However, the results of this study suggest that RFI should not be used to compare cattle of differing breedtypes without further study and

adjustment due to the breedtype differences in RFI. Furthermore, the moderate correlation between pre- and post-pubertal RFI suggests that RFI determined during the post-weaning period may only be a moderate predictor of feed efficiency during the post-pubertal period. As a result, physiological maturity should also be considered when evaluating cattle for feed efficiency using RFI.

CHAPTER III

EFFECT OF SELECTION FOR RESIDUAL FEED INTAKE ON PUBERTAL CHARACTERISTICS AND CONCEPTION RATE OF BONSMARA HEIFERS

Introduction

Concerning the selection of beef cattle for feed efficiency, RFI has been proposed as an alternative measure to the conventionally used F:G ratio (Koch et al., 1963). Although calculation of F:G is simplistic when compared to RFI quantification, selection based on F:G tends to result in cattle with increased mature size being retained in the breeding herd (Herd and Bishop, 2000). At maturity, these cattle have increased feed requirements (Barlow, 1984), thus effectively negating the benefits of improved feed efficiency. Since RFI is by definition independent of BW and growth rate (Kennedy et al., 1993), there should be no increase in mature size associated with selection for RFI. Therefore, RFI appears to be an attractive tool for selecting feed efficient cattle.

However, there are a variety of other economically important traits that must be considered when evaluating the impact of selecting cattle based on RFI. Of particular importance are the traits associated with reproduction. The age at which a heifer reaches puberty is an important reproductive trait shown to influence her subsequent lifetime productivity (Lesmeister et al., 1973). Since nutrition is recognized as an important mediator of the events associated with puberty, careful evaluation of any consequences of selection for RFI on age at puberty is warranted. This study was conducted to

evaluate this relationship by comparing the differences in pubertal characteristics and first parity reproductive performance between high and low RFI Bonsmara heifers.

Materials and Methods

Animals and experimental design

Bonsmara heifers (n = 38) born in spring 2007 were trucked from the Texas *Agri*Life Research facility in Uvalde, TX to the Texas *Agri*Life Research facility in Overton, TX in December 2007. Upon arrival, heifers were weighed and group-fed a high roughage diet (Table 3.1; 12% CP and 55% TDN) for 1 wk. The amount of diet fed was incrementally increased over the first 3 d until heifers consumed approximately 2.65% of BW. Heifers were weighed again 7 d after arrival and were allocated to pens of 5 head based on BW. Each heifer was fitted with an electronic key worn around the neck to allow access to an individual feed bunk using the Calan gate system (American Calan, Northwood, NH). Heifers were trained to eat from the bunks over a 4 d acclimation period. On the first day, feed was placed in the feed bunks, but the doors were wired open so that heifers could freely eat from the bunks. The gates were closed on the next two days, but the latches were taped so that the gates would not lock. On the fourth day, gates were allowed to close and latch so that heifers could learn to identify their respective bunks and activate the gate with the electronic key. Three heifers did not learn to eat out of the Calan gates and were penned and fed individually. Data collection began 1 wk after heifers began eating from the Calan gates and continued for

Table 3.1. Diet composition

Ingredient	% of Diet
Cottonseed hulls, pelleted	30.00
Cottonseed hulls	25.00
Alfalfa, dehydrated	12.50
Soybean meal, 48%	10.47
Rice bran	10.00
Soybean hulls	7.45
Corn, crimped	2.00
Salt	0.85
Calcium carbonate	0.70
Magnesium	0.46
Potassium/magnesium/sulfate	0.32
855 Calf 2x	0.12
Dairy ADE	0.04
Vitamin A-30	0.04
Trace mineral premix	0.03
Zinpro 100	0.01
Selenium	0.01

70 d. On d 0, heifers were weighed and individual daily rations were calculated as 2.65% of BW. At this feeding level, heifers were expected to gain approximately 0.68 kg/d. Daily feed was weighed out for the week into plastic sacks that were stored in front of each heifer's bunk. Heifers were fed approximately half of the daily ration at 0800 h and the remaining half at 1600 h. Orts, if any, were collected weekly and used to adjust feed intake for the previous week. Heifers were weighed weekly and the amount of feed offered to each heifer was recalculated for the following week.

At the end of the feeding trial, heifers were evaluated for rib-eye area (REA), percent intramuscular fat (%IMF), and rump fat thickness by an Ultrasound Guidelines Council certified technician. The Aloka 500V real-time ultrasound machine (Corometrics Medical Systems, Wallingford, CT) was equipped with a 17.2 cm, 3.5 MHz linear transducer fitted with a Superflab (Designer Genes Technologies, Inc., Harrison, Arkansas) guide for image capture. Prior to ultrasound, animals were curried and 100% vegetable oil was applied as a coupling agent to ensure adequate transmission of ultrasound waves from the animal to the transducer. Images were stored on a personal computer and interpreted using Beef Image Analysis Pro Plus Software 2.0.3 (Designer Genes Technologies, Inc., Harrison, Arkansas).

After the 70-d feeding trial, heifers were hauled back to Uvalde where they were allowed to graze ryegrass until May 25. Fourteen head were then moved to dry land haygrazer pasture, whereas the other 24 head were allowed to graze irrigated haygrazer pasture. All heifers were supplemented with 20% CP cubes at approximately 2.3 kg/head/wk. Heifers were exposed to Bonsmara bulls for natural mating from April 15

to July 15. Approximately 45 d after the bulls were removed, heifers were examined by rectal palpation to determine pregnancy. Estimates for the number of days pregnant were made in order to determine approximate breeding dates for the heifers.

RFI determination

RFI was calculated as described in Chapter II. Initial BW and ADG were computed from linear regression of BW on day of test. Mid-test BW was estimated using initial BW and ADG and adjusting for a 3% shrink. Considering all females as cohorts, RFI was determined for each heifer as the residual from the linear regression of ADFI on mid-test $BW^{0.75}$ and ADG.

Blood collection and assays

Approximately 15 mL (one 16 x 125 mm serum tube) of whole blood was collected weekly from heifers via coccygeal vessel puncture. Blood was refrigerated at 4°C and allowed to clot overnight. Blood samples were centrifuged at 1400 x g for 30 min. Serum was collected and stored in 2 aliquots at -20°C until analysis for progesterone and IGF-I. Weekly blood samples were analyzed for progesterone using a radioimmunoassay (Appendix C) adopted from Williams (1989). Intra- and interassay CV were 4.9 and 18.6%, respectively. A heifer was determined to have reached puberty when she had elevated blood progesterone concentrations above 1 ng/mL for 2 consecutive wk (Day et al., 1984). Day 0 and 70 blood samples were analyzed for IGF-I concentrations using a modification of the protocol (Appendix D) described by Bilby et

al. (1999) to determine the predictive ability of IGF-I for feed efficiency as determined by RFI. All IGF-I samples were analyzed in a single assay and the intraassay CV was 3.4%.

Statistical analysis

Heifers were grouped according to RFI sign, where a negative RFI was indicative of an efficient heifer and a positive RFI signified an inefficient heifer. Comparisons of IGF-I concentrations on d 0 and d 70 as well as BW on d 0 and d 70 were made between RFI groups using the GLM function of SAS (2002) specific for repeated measures. Using RFI group as a class variable, data were analyzed using the GLM function of SAS (2002) to determine differences by RFI. The CORR function (SAS, 2002) was also used to determine relationships among traits evaluated. Chi-square analysis (SAS, 2002) was used to determine differences in cumulative achievement of puberty and conception by RFI group.

Results

Heifers were 10.5 ± 0.7 mo of age at the start of the RFI trial, 12.3 ± 1.6 mo of age at puberty, and 15.5 ± 0.9 mo of age at conception (Table 3.2). Heifers averaged 278.8 ± 4.8 kg BW at the start of the RFI trial, 329.6 ± 5.7 kg BW at the end of the RFI trial, and had a metabolic mid-test BW of 71.1 ± 0.9 kg. Heifers consumed 8.0 ± 0.1 kg of feed per d and had an ADG of 0.7 ± 0.0 kg/d. Concentrations of IGF-I were 147.51 ± 4.30 ng/mL on d 0 and 189.85 ± 4.56 ng/mL on d 70. Use of ultrasound scanning at the

Table 3.2. Summary statistics for Bonsmara heifers

Trait	Mean	SD	Min	Max
n	38			
Test duration, d	70			
Initial age, mo	10.5	0.7	8.8	11.4
Age at puberty, mo	12.3	1.6	9.1	14.8
Age at conception, mo	15.5	0.9	13.8	17.9
Initial BW, kg	278.8	29.4	222.5	334.4
Final BW, kg	329.6	34.8	264.5	397.7
Mid-test metabolic BW, kg	71.1	5.6	60.4	81.8
ADG, kg/d	0.73	0.14	0.43	1.00
ADFI, kg/d	8.0	0.8	6.4	9.6
RFI, kg/d	0.00	0.07	-0.23	0.10
Day 0 IGF-I, ng/mL	147.51	26.53	83.89	197.16
Day 70 IGF-I, ng/mL	189.85	28.13	124.17	248.61
Rib-eye area, cm^2	63.3	8.8	48.5	86.6
Intramuscular fat, %	0.20	0.08	0.06	0.39
Rump fat, mm	7.71	2.20	4.05	12.29

end of the RFI trial revealed that heifers had 63.3 ± 1.4 cm^2 REA, 0.20 ± 0.01 %IMF, and 7.71 ± 0.36 mm of rump fat.

There were no significant differences observed ($P > 0.05$) between the efficient and inefficient heifers for initial age, age at puberty, age at conception, initial BW, final BW, mid-test metabolic BW, ADG, ADFI, d 0 IGF-I concentration, d 70 IGF-I concentration, REA, %IMF, and rump fat thickness (Table 3.3). However, RFI differed ($P < 0.0001$) between the two groups, with efficient heifers having lower RFI than the inefficient heifers. The percentage of heifers reaching puberty by 10, 11, 12, 13, 14, and 15 mo of age was not different ($P > 0.05$) between efficient and inefficient heifers (Figure 3.1). Furthermore, the percentage of heifers conceiving by 20, 40, 60, 80 and 95 d of the breeding season was not different between efficient and inefficient Bonsmara heifers (Figure 3.2).

Residual feed intake was not correlated ($P > 0.05$) with any of the traits evaluated (Table 3.4). Average daily gain was correlated with initial BW ($r = 0.45$; $P < 0.01$), final BW ($r = 0.66$; $P < 0.0001$), and mid-test metabolic BW ($r = 0.57$; $P < 0.001$). There was a strong correlation between initial BW and final BW ($r = 0.97$; $P < 0.0001$), initial BW and mid-test metabolic BW ($r = 0.99$; $P < 0.0001$), and final BW and mid-test metabolic BW ($r = 0.99$; $P < 0.0001$). Day 0 IGF-I was not correlated with any performance or carcass traits measured ($P > 0.05$). Day 70 IGF-I tended to be correlated with ADG ($r = 0.28$; $P < 0.10$), was highly correlated with d 0 IGF-I ($r = 0.75$; $P < 0.0001$), but was not correlated ($P > 0.05$) with any other parameters evaluated.

Table 3.3. Age, performance traits, residual feed intake, insulin-like growth factor-I and body composition traits of efficient and inefficient Bonsmara heifers

	RFI group		
Trait	Efficient	Inefficient	P-value
n	12	26	
Initial age, mo	10.5 ± 0.2	10.4 ± 0.1	0.6164
Age at puberty, mo	12.4 ± 0.5	12.2 ± 0.3	0.6932
Age at conception, mo	15.4 ± 0.3	15.6 ± 0.2	0.5400
Initial BW, kg	277.1 ± 8.6	279.5 ± 5.8	0.8195
Final BW, kg	330.2 ± 10.2	329.3 ± 6.9	0.9412
Mid-test metabolic BW, kg	71.1 ± 1.6	71.1 ± 1.1	0.9623
ADG, kg/d	0.76 ± 0.04	0.71 ± 0.03	0.3416
ADFI, kg/d	7.9 ± 0.2	8.0 ± 0.2	0.7172
Residual feed intake, kg/d	-0.06 ± 0.15[b]	0.03 ± 0.01[c]	<0.0001
IGF-I (d0), ng/mL	151.98 ± 8.84	142.88 ± 6.18	0.3948
IGF-I (d70), ng/mL	191.14 ± 8.35	182.33 ± 6.05	0.4000
Rib-eye area, cm^2	65.3 ± 2.6	62.4 ± 1.7	0.3479
Intramuscular fat, %	0.21 ± 0.02	0.20 ± 0.02	0.6817
Rump fat, mm	7.93 ± 0.64	7.60 ± 0.44	0.6769

[a] IGF-I (d0) = insulin-like growth factor-I concentration on day 0 and IGF-I (d70) = insulin-like growth factor-I concentration on day 70.

[b,c] Least square means within a row differ ($P < 0.0001$).

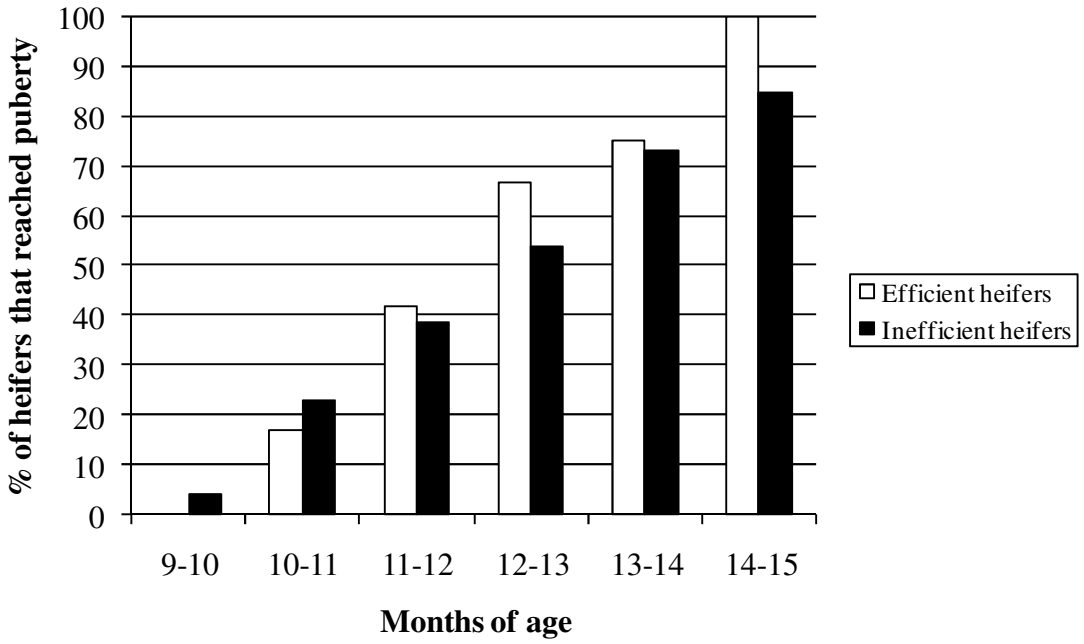

Figure 3.1. Cumulative achievement of puberty for efficient (n = 12) and inefficient (n = 26) Bonsmara heifers.

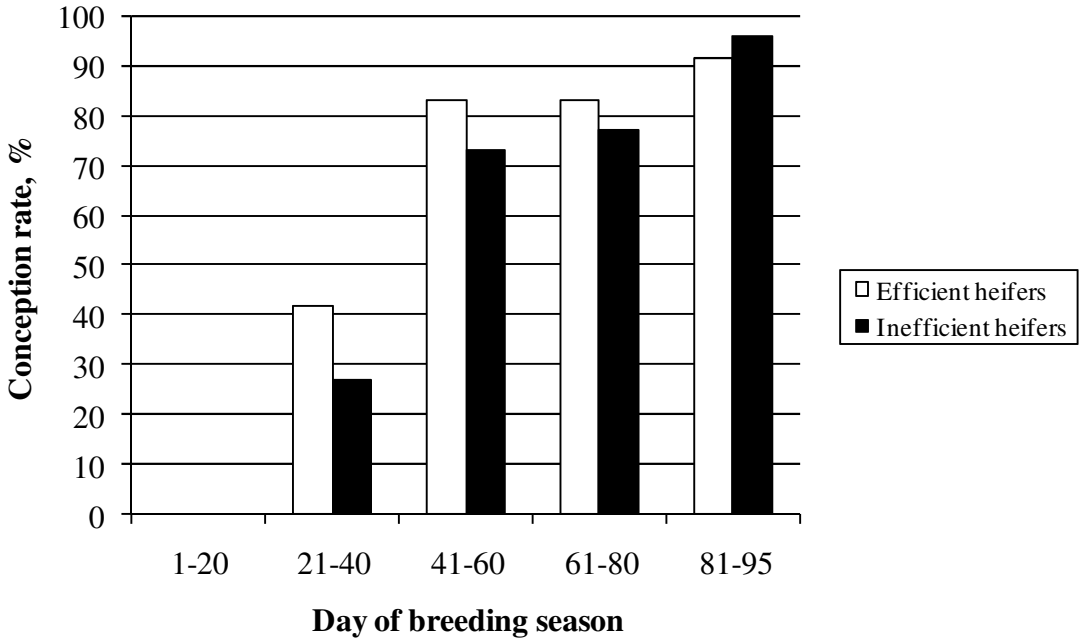

Figure 3.2. Cumulative conception rate for efficient (n = 12) and inefficient (n = 26) Bonsmara heifers.

Table 3.4. Partial correlation coefficients of performance traits, residual feed intake, insulin-like growth factor-I, and body composition traits in Bonsmara heifers

Trait	Initial BW	Final BW	$MBW^{0.75}$	ADG	ADFI	RFI	IGF-I (d0)	IGF-I (d70)	REA	%IMF	Rump fat
Initial BW	1.00	0.97[b]	0.99[b]	0.45[c]	-	0.00	0.15	0.09	0.62[b]	0.45[c]	0.36[d]
Final BW		1.00	0.99[b]	0.66[b]	-	0.00	0.17	0.16	0.60[b]	0.44[c]	0.38[d]
$MBW^{0.75}$			1.00	0.57[e]	-	0.00	0.16	0.13	0.61[b]	0.45[c]	0.38[d]
ADG				1.00	0.57[e]	0.00	0.14	0.28[f]	0.30[f]	0.22	0.28[f]
ADFI					1.00	0.08	0.17	0.15	0.60[b]	0.43[c]	0.37[d]
RFI						1.00	0.12	0.23	-0.13	-0.21	-0.05
IGF-I (d0)							1.00	0.75[b]	0.09	0.20	0.28[f]
IGF-I (d70)								1.00	0.16	-0.07	0.08
REA									1.00	0.41[c]	0.46[c]
%IMF										1.00	0.79[b]
Rump fat											1.00

[a] $MBW^{0.75}$ = mid-test metabolic BW, RFI = residual feed intake, IGF-I (d0) = insulin-like growth factor-I concentration on day 0, IGF-I (d70) = insulin-like growth factor-I concentration on day 70, REA = rib-eye area, and %IMF = percent intramuscular fat.
[b] Correlation is different than zero ($P < 0.0001$).
[c] Correlation is different than zero ($P < 0.01$).
[d] Correlation is different than zero ($P < 0.05$).
[e] Correlation is different than zero ($P < 0.001$).
[f] Correlations is different than zero ($P < 0.10$).

Strong correlations were observed between REA and initial BW ($r = 0.62$; $P < 0.0001$), final BW ($r = 0.60$; $P < 0.0001$), mid-test metabolic BW ($r = 0.61$; $P < 0.0001$), and ADFI ($r = 0.60$; $P < 0.0001$). Rib-eye area and ADG tended to be correlated ($r = 0.30$; $P < 0.10$). Likewise, %IMF was also correlated with initial BW ($r = 0.45$; $P < 0.01$), final BW ($r = 0.44$; $P < 0.01$), mid-test metabolic BW ($r = 0.45$; $P < 0.01$), and ADFI ($r = 0.43$; $P < 0.01$). In addition, a moderate correlation was observed between %IMF and REA ($r = 0.41$; $P < 0.01$). Rump fat thickness was correlated with initial BW ($r = 0.36$; $P < 0.05$), final BW ($r = 0.38$; $P < 0.05$), mid-test metabolic BW (0.38; $P < 0.05$), ADFI (0.37; $P < 0.05$), REA ($r = 0.46$; $P < 0.01$) and %IMF ($r = 0.79$; $P < 0.0001$). There was also a tendency ($P < 0.10$) for rump fat thickness to be correlated to ADG ($r = 0.28$) and d 0 IGF-I ($r = 0.28$).

Discussion

By definition, RFI is phenotypically independent of growth rate and body size (Herd and Arthur, 2009). As expected, no differences were observed between efficient and inefficient Bonsmara heifers for initial BW, final BW, mid-test metabolic BW and ADG. Furthermore, RFI was not correlated ($P > 0.05$) with any measure of BW or ADG. This finding agrees with multiple studies that failed to find any correlation between RFI and ADG or BW (Herd and Bishop, 2000; Arthur et al., 2001a; Arthur et al., 2001b; Baker et al., 2006). Conversely, Fan et al. (1995) reported moderate correlations in Hereford and Angus bulls between RFI and ADG ($r = -0.50$ and -0.58) and RFI and yearling BW ($r = -0.44$ and -0.53). It is important to note that expected feed

intake was estimated from equations in that study instead of from linear regression of actual data. Since these calculations are made based on animal BW and energy requirements for growth, correlations between RFI and BW or ADG would certainly not be unexpected.

Average daily feed intake was not different between RFI groups and was not correlated to RFI. Several studies have reported significant correlations between RFI and ADFI ranging from 0.49 (Arthur et al., 1996) to 0.70 (Herd and Bishop, 2000). Cattle in those studies were allowed *ad libitum* access to feed, whereas the Bonsmara heifers in this study were limit fed at 2.65% BW to gain approximately 0.68 kg/d. Since RFI is independent of BW and ADFI was a function of BW, it was expected that there would be no correlation between RFI and ADFI under this feeding strategy.

There have been conflicting reports attesting to the ability of circulating IGF-I concentration to predict RFI. Results from this study indicate there was no difference between RFI groups for serum IGF-I concentrations measured on d 0 or d 70 of the feeding trial. Likewise, RFI was not correlated with serum IGF-I concentration at either sample date. Caldwell (2009) reported no statistical differences or correlations between RFI and plasma IGF-I concentration in straightbred Angus, Brahman, Romosinuano, or the F_1 crosses of those breeds. Lancaster et al. (2007) also failed to find a significant correlation between RFI and circulating IGF-I concentration in Brangus heifers. Contrastingly, Johnston et al. (2002) and Moore et al. (2005) reported positive correlations between RFI and circulating IGF-I concentration in *Bos taurus* cattle,

suggesting that further research is warranted to determine the relationship among RFI, IGF-I and cattle breedtype.

Although this study failed to find a correlation between RFI and IGF-I, it does provide one of the first reports of circulating IGF-I concentrations in growing Bonsmara heifers in the United States. The IGF-I concentrations of the Bonsmara heifers from this study were similar to IGF-I concentrations reported in Brahman and tropically adapted Romosinuano heifers but higher than IGF-I concentrations in Angus heifers at weaning (Caldwell, 2009). Simpson et al. (1997) also reported elevated IGF-I concentrations in Brahman cows as compared to Angus cows. While the biology underlying this breedtype difference in circulating IGF-I concentration remains unclear, it has been suggested that tropically adapted cattle also have a greater activity of IGF binding protein 3 (IGFBP3) in circulation (Simpson et al., 1997). This would allow for an increased quantity of IGF-I to be present in the circulation and could at least partly explain the elevated IGF-I concentrations of tropically adapted cattle as compared to temperate cattle.

Ultrasound measurements were not different between efficient and inefficient Bonsmara heifers, and RFI was not correlated with any of the body composition traits. Similar results were reported by Baker et al. (2006) when they observed no correlations between RFI and carcass traits of purebred Angus steers. Residual feed intake was not correlated with loin muscle area, back fat thickness, or marbling score in a report by Mader et al. (2009). However, Basarab et al. (2003) reported weak, positive correlations between RFI and measures of fatness at harvest and weak negative correlations between

RFI and carcass percent lean. Herd and Bishop (2000) reported a weak, negative correlation (r = -0.22) between RFI and carcass lean content, while Lancaster et al. (2009) reported a weak, positive correlation (r = 0.20) between RFI and back fat thickness at harvest. These studies imply there may be a weak relationship between RFI and body composition. However, body composition traits in this study were moderately to strongly correlated with BW. Since RFI is independent of BW, it would be expected that RFI would not be correlated with measures of body composition.

Within a given breed and contemporary group, nutrition plays a significant role in determining when a heifer reaches puberty (Wiltbank et al., 1969; Day et al., 1986; Kurz et al., 1990). As a result, monitoring the effects of selecting cattle for RFI on the age at which replacement heifers reach puberty is important. The age at which Bonsmara heifers reached puberty in this study did not differ by RFI group. Furthermore, there was no difference in the percentage of inefficient and efficient heifers reaching puberty by 10, 11, 12, 13, 14, or 15 mo of age. This suggests that selection for RFI should not alter age at puberty in Bonsmara heifers. It is important to note; however, that the variation in RFI observed among the heifers was small. Heifer RFI values ranged from -0.23 to 0.10, which equates to a 0.33 kg/d difference in RFI between the most and least efficient heifers. Other studies that evaluated RFI where the animals were fed *ad libitum* observed significantly more variation in individual animal RFI. Residual feed intake of Angus steers ranged from -1.08 to 1.69 kg/d (Baker et al., 2006). Mader et al. (2009) reported a 3.14 kg/d difference in RFI of crossbred steers.

Residual feed intake values ranging of -2.46 to 2.58 kg/d resulted in a difference of 5.04 kg/d between the most efficient and least efficient Angus bulls (Lancaster et al., 2009).

One possible explanation for the small variation in RFI observed among the Bonsmara heifers could be provided by the way in which the heifers were fed. By providing feed at a set proportion of BW, heifers were not allowed to express appetite differences that may have otherwise resulted in a greater variation of RFI values. The relatively limited genetics of Bonsmara cattle in the United States could also explain, in part, the small variation observed in RFI in this study. With limited genetic diversity among Bonsmara cattle, small variation in expressed traits such as RFI would be expected.

Conclusion

Before RFI can be successfully implemented as a selection tool to improve feed efficiency in beef cattle, the relationship(s) of RFI to other economically important traits should be thoroughly investigated. The age at which a heifer reaches puberty is of great economic importance as it influences her lifetime productivity (Lesmeister et al., 1973). Since nutritional status is a large determinant of pubertal achievement, concern over how selection for RFI might influence age at puberty in heifers is warranted. Results from this study suggest that selection for RFI in Bonsmara cattle should not alter the age at which heifers reach puberty or pregnancy rates during a discrete breeding season.

CHAPTER IV

EFFECT OF SELECTION FOR RESIDUAL FEED INTAKE ON POSTPARTUM PERFORMANCE OF BRAHMAN COWS

Introduction

Recent studies have suggested that selection for lower RFI may result in small alterations in the body composition of beef cattle. Richardson et al. (2001), Basarab et al. (2003), and Lancaster et al. (2009) all observed reduced fatness in low RFI cattle when compared to high RFI cattle. Although these studies used finishing steers as their animal model, these changes in body composition may also carry over into the breeding herd if RFI is included as part of a selection system. Reducing fatness in the cowherd could potentially cause unfavorable changes in cow performance. Since puberty, the postpartum interval and calf performance are all affected either directly or indirectly by cow body energy reserves, altering the body composition of the cowherd could negatively impact these traits. Bennett and Williams (1994) provided support for this concept when they suggested that body composition changes could affect calf survival ability, puberty in heifers, and the postpartum interval in cows. Furthermore, they concluded that reduced fatness could potentially limit the reproductive performance and overall profitability of the cowherd. However, limited data are available regarding differences in reproductive performance of efficient versus inefficient cows, particularly of *Bos indicus* genetics. Therefore, this study was conducted to evaluate important

reproductive parameters in primiparous and multiparous Brahman cows to determine if selection for RFI might potentially alter the reproductive performance of the cowherd.

Materials and Methods

Animals and experimental design

Brahman primiparous (n = 16) and multiparous cows (n = 38) from the Texas *Agri*Life Research facility in Overton were utilized for this study. All females had been previously evaluated for RFI within their respective contemporary groups. Females were weighed and evaluated for BCS at 28-d intervals for 3 mo prior to the expected start of the 2008 calving season. Females were again weighed and evaluated for BCS 24 hr after calving. Beginning 21 d post-calving, females were weighed and evaluated for BCS weekly. Weight and BCS data were again collected on all females within 24 hr of calving. Calves born from these cows were weighed and tagged within 24 hr of birth. Twenty-one days following birth, the calves were weighed again. Calves were then weighed every 28 d until weaning to determine pre-weaning ADG and weaning weight (WW). Calf WW was adjusted for sex and parity of the cow according to BIF guidelines (BIF, 2002).

After calving, females were exposed to vasectomized bulls fitted with chin-ball markers and were visually observed at least once daily to detect estrus. A cow was determined to be in estrus when she allowed another cow or bull to mount her or when ink marks indicated she had been mounted. Eight and 10 d following observed estrus, cows were examined using real-time ultrasonography to determine the presence of a CL.

If a CL was present, weekly blood sampling, weighing and body condition scoring terminated. If no CL was detected, weekly blood sampling, BW and BCS data continued to be collected, as previously described, until a CL was detected. Beginning May 13 and continuing through June 30, females were artificially inseminated 12 hr following observed estrus. On July 1, cows were exposed to intact Brahman bulls fitted with chin-ball markers for natural mating until July 31. Approximately 45 d after the bulls were removed, examination by rectal palpation was utilized to determine pregnancy status and to estimate breeding dates for the cows.

Blood collection and assays

Serum samples were collected weekly beginning 21 d after calving until a functional CL was detected via ultrasonography. Blood samples were also collected when ultrasonography was performed to ensure the observed CL was functional. Approximately 15 mL (one 16 x 125 mm serum tube) of whole blood was collected at each sampling via coccygeal vessel puncture. Blood was refrigerated at 4^{o}C and allowed to clot overnight. Blood samples were centrifuged at 1400 x g for 30 min. Serum was collected and stored in 2 aliquots at -20^{o}C until analysis for progesterone, NEFA and IGF-I. All blood samples were analyzed for progesterone using a radioimmunoassay (Appendix C) adapted from Williams et al. (1989). Intra- and interassay CV were 9.1 and 8.9%, respectively. Formation of a functional CL was determined when a cow had elevated blood progesterone concentrations above 1 ng/mL for 2 consecutive wk (Day et al., 1984). To illustrate the energy balance of cows, weekly blood samples were

analyzed for NEFA concentration by enzymatic colorimetric analysis (Appendix E) using a commercially available kit (NEFA-C, Wako Chemicals USA, Inc., Richmond, VA). Intra- and interassay CV were 9.9 and 11.3%, respectively. Day 21 serum samples were analyzed for IGF-I using a modification of the protocol (Appendix D) described by Bilby et al. (1999) to determine if there were differences in IGF-I production early in the postpartum period. All IGF-I samples were analyzed in a single assay and the intraassay CV was 3.4%.

Reproductive parameters

Multiple parameters were used to evaluate the post-calving performance, including:

1) The number of d from calving to first observed estrus
2) The number of d from calving to CL formation
3) The number of d from calving to observed estrus with subsequent CL formation
4) First service conception rate
5) Julian date of conception
6) Overall pregnancy rate
7) Calf pre-weaning growth performance (actual and adjusted)
8) Calf weaning weight (actual and adjusted)
9) Proportion of calf weaned (actual and adjusted; expressed as a percentage of cow BW)

Statistical analysis

Since numerical RFI values cannot be compared across contemporary groups, females were assigned to an RFI grouping for statistical analysis. A negative RFI indicated an efficient female, whereas inefficient females had a positive RFI. Cows were also stratified by parity where primiparous cows were grouped together and multiparous cows were grouped together. Using the GLM specific for repeated measures function of SAS (2002), BW, BCS, NEFA and calf weight data were analyzed with RFI group and parity as class variables. There were no interactions between RFI group and parity, so the interaction was excluded from the model. Reproductive performance, IGF-I concentration as well as changes in BW, BCS, and NEFA were analyzed by RFI group and parity using the GLM procedure of SAS (2002). Since there were no interactions between RFI group and parity, the interaction was not included in the model. Considering multiparous and primiparous cows separately, differences in cumulative return to estrus, CL formation, estrus with CL formation, and conception were analyzed by RFI group using the chi-square function of SAS (2002). Within parity group, chi-square was also used to evaluate differences in first service conception rate and pregnancy rate between the efficient and inefficient females.

Results

Cow mean BW ranged from 501.1 ± 82.5 to 534.4 ± 68.8 kg and mean BCS ranged from 5.9 ± 0.7 to 6.2 ± 0.8 (Table 4.1). Mean Julian date of calving was 102 ± 21.1 d, or April 29. Julian date of conception averaged 169 ± 20.0 d, or June 17. On

Table 4.1. Summary statistics for primiparous and multiparous Brahman cows

Trait[a]	Mean	SD	Min	Max
n	54			
Pre-calving BW1, kg	506.3	90.5	350.6	681.3
Pre-calving BW2, kg	506.0	84.2	361.1	675.0
Pre-calving BW3, kg	509.1	89.4	357.4	692.6
24 hour post-calving BW, kg	501.1	82.5	318.0	664.5
21 day post-calving BW, kg	507.0	82.8	338.4	689.9
BW at estrus with CL formation, kg	534.4	68.8	381.9	689.9
Pre-calving BCS1	6.0	0.7	5.0	7.5
Pre-calving BCS2	6.0	0.8	4.5	8.0
Pre-calving BCS3	6.2	0.8	5.0	8.0
24 hour post-calving BCS	5.9	0.7	4.5	7.5
21 day post-calving BCS	5.9	0.8	4.5	7.5
BCS at estrus with CL formation	6.0	0.7	4.5	7.0
Julian date of calving, d	102	21.1	54	137
Julian date of conception, d	169	20.0	134	208
Days to first estrus	59	25.7	22	125
Days to CL formation	55	25.1	22	115
Days to estrus with CL formation	56	25.0	22	115
Day 21 IGF-I, ng/mL	82.86	28.30	37.83	178.68
Day 21 NEFA, mEq/L	0.46	0.26	0.07	1.07
Peak NEFA, mEq/L	0.63	0.27	0.17	1.30
NEFA at estrus with corpus luteum, mEq/L	0.32	0.18	0.07	0.86
Calf birth weight, kg	34.5	5.4	23.6	49.9
Calf weaning weight, kg	181.4	28.9	122.9	259.5
Adjusted calf weaning weight, kg	201.6	17.4	160.1	249.2
Calf ADG, kg/d	0.91	0.13	0.62	1.28
Adjusted calf ADG, kg/d	1.05	0.14	0.78	1.40
Proportion calf weaned, %	36.8	5.3	27.5	53.0
Adjusted proportion calf weaned, %	41.3	6.3	32.2	58.9

[a] CL = corpus luteum, BCS = body condition score, IGF-I = insulin-like growth factor-I, and NEFA = non-esterified fatty acid.

average, cows returned to estrus within 59 ± 25.7 d of calving, developed corpora lutea within 55 ± 25.1 d of calving, and exhibited estrus with subsequent functional CL formation within 56 ± 25.0 d of calving. Mean IGF-I concentration was 82.86 ± 28.30 ng/mL on d 21 following parturition. Mean NEFA concentrations were 0.46 ± 0.26 mEq/L 21 d after calving, 0.63 ± 0.27 mEq/L at peak concentration, and 0.32 ± 0.18 mEq/L just prior to estrus with subsequent luteal formation. Calves born from these cows averaged 34.5 ± 5.4 kg at birth and 181.4 ± 28.9 kg at weaning, resulting in a pre-weaning ADG of 0.91 ± 0.13 kg/d. Cows were able to wean 36.8 ± 5.3% of their own BW. When WW was adjusted for sex of calf and age of dam, calves were 201.6 ± 17.4 kg at weaning, had an ADG of 1.05 ± 0.14 kg/d, and accounted for 41.3 ± 6.3% of cow BW.

Effects of time

Using repeated measures analysis for all cows, mean BW changed over time ($P < 0.01$). Likewise, mean BCS differed over time ($P < 0.0001$). Changes in mean NEFA concentration were also observed over time ($P < 0.01$).

Effects of parity

Multiparous cows were significantly heavier ($P < 0.0001$) than primiparous cows at each weighing (Table 4.2). There was no difference ($P > 0.05$) in BW change during the pre-calving period. However, primiparous cows gained more weight than multiparous cows (32.6 ± 7.4 vs. 8.0 ± 3.2 kg; $P < 0.05$) following calving. This also

Table 4.2. Body weight and body condition score of primiparous and multiparous Brahman cows

	Parity		
Trait[a]	Primiparous	Multiparous	P-value
n	16	38	
Pre-calving BW1, kg	405.1 ± 24.1[b]	554.3 ± 10.5[c]	<0.0001
Pre-calving BW2, kg	419.6 ± 24.7[b]	547.1 ± 10.8[c]	<0.0001
Pre-calving BW3, kg	409.9 ± 24.1[b]	555.6 ± 10.5[c]	<0.0001
24 hour post-calving BW, kg	409.4 ± 22.2[b]	543.2 ± 9.7[c]	<0.0001
21 day post-calving BW, kg	421.0 ± 22.5[b]	548.3 ± 9.8[c]	<0.0001
BW at estrus with CL formation, kg	442.0 ± 21.9[b]	551.2 ± 9.5[c]	<0.0001
Pre-calving change in BW, kg	4.2 ± 3.7	1.0 ± 2.4	0.4786
Post-calving change in BW, kg	32.6 ± 7.4[d]	8.0 ± 3.2[e]	0.0037
Pre-calving BCS1	6.1 ± 0.3	6.0 ± 0.1	0.8200
Pre-calving BCS2	6.1 ± 0.3	6.0 ± 0.1	0.7328
Pre-calving BCS3	6.5 ± 0.3	6.2 ± 0.1	0.2909
24 hour post-calving BCS	5.5 ± 0.3[f]	6.0 ± 0.1[g]	0.0693
21 day post-calving BCS	5.6 ± 0.3[h]	6.2 ± 0.1[i]	0.0477
BCS at estrus with CL formation	5.4 ± 0.2[h]	6.1 ± 0.1[i]	0.0123
Pre-calving change in BCS	0.4 ± 0.1[f]	0.1 ± 0.1[g]	0.0946
Post-calving change in BCS	-0.1 ± 0.2	-0.0 ± 0.1	0.6240

[a] BCS = body condition score
[b,c] Least square means within a row differ ($P < 0.0001$).
[d,e] Least square means within a row differ ($P < 0.01$).
[f,g] Least square means within a row differ ($P < 0.10$).
[h,i] Least square means within a row differ ($P < 0.05$).

was shown by a weight x parity interaction ($P < 0.01$) suggesting that primiparous cows gained weight over time while multiparous cows remained at a constant body weight (Figure 4.1). Pre-calving BCS was similar ($P > 0.05$) for primiparous and multiparous cows. However, primiparous cows tended ($P < 0.10$) to gain more body condition than multiparous cows (0.4 ± 0.1 vs. 0.1 ± 0.1) prior to calving. Multiparous cows tended ($P < 0.10$) to be in better body condition within 24 hr of calving when compared to primiparous cows. Multiparous cows also had higher ($P < 0.05$) BCS 21 d following calving and at estrus with subsequent CL formation. These differences in BCS were manifested in a BCS x parity interaction ($P < 0.0001$; Figure 4.2).

Reproductive performance differences by parity are shown in Table 4.3. Julian date of calving was not different ($P > 0.05$) between primiparous and multiparous cows. However, Julian date of conception was significantly later ($P < 0.001$) for primiparous cows (198 ± 9 d) than for multiparous cows (164 ± 3). Primiparous cows had an extended interval ($P < 0.0001$) from calving to first estrus (91 ± 5 vs. 48 ± 3 d), CL formation (94 ± 6 vs. 46 ± 3 d), and estrus with subsequent CL formation (94 ± 6 vs. 48 ± 3 d) as compared to multiparous cows. None of the primiparous cows conceived at first service, but 50% of the multiparous cows became pregnant at first breeding ($P < 0.001$). Furthermore, only 25% of the primiparous cows were pregnant at the end of the breeding season compared to 79% of the multiparous cows ($P < 0.001$).

Insulin-like growth factor-I concentrations tended ($P < 0.10$) to be higher for primiparous cows than multiparous cows (92.53 ± 7.01 vs. 78.09 ± 4.52 ng/mL; Table 4.4). Post-calving d 21 NEFA concentrations did not differ ($P > 0.05$) by parity. Peak

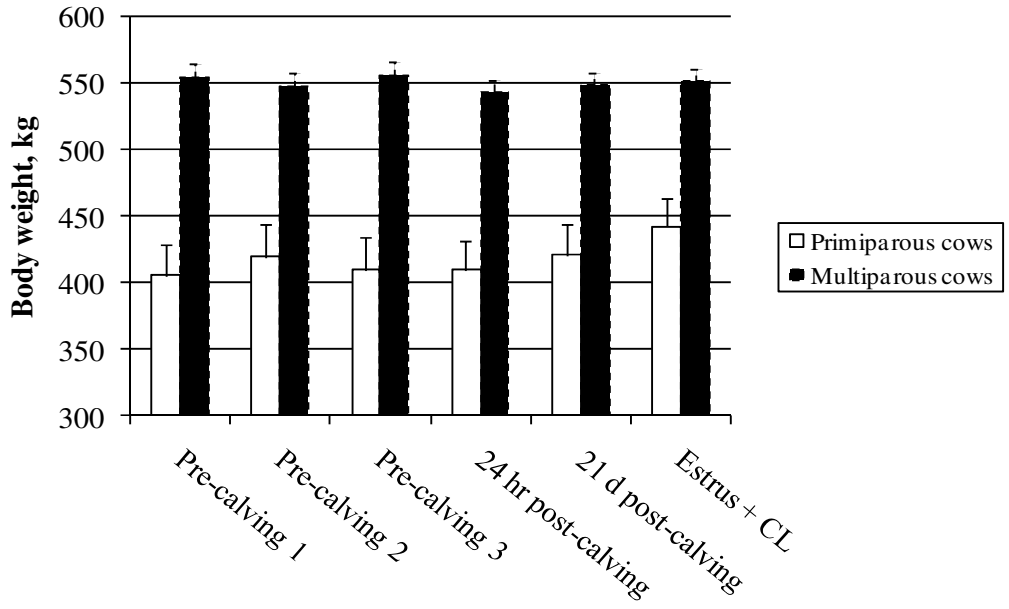

Figure 4.1. Pre- and post-calving mean body weights for primiparous (n = 16) and multiparous (n = 38) Brahman cows. Parity effect $P < 0.0001$, time effect $P < 0.01$, and time x parity effect $P < 0.01$, pooled SEM = 16.7.

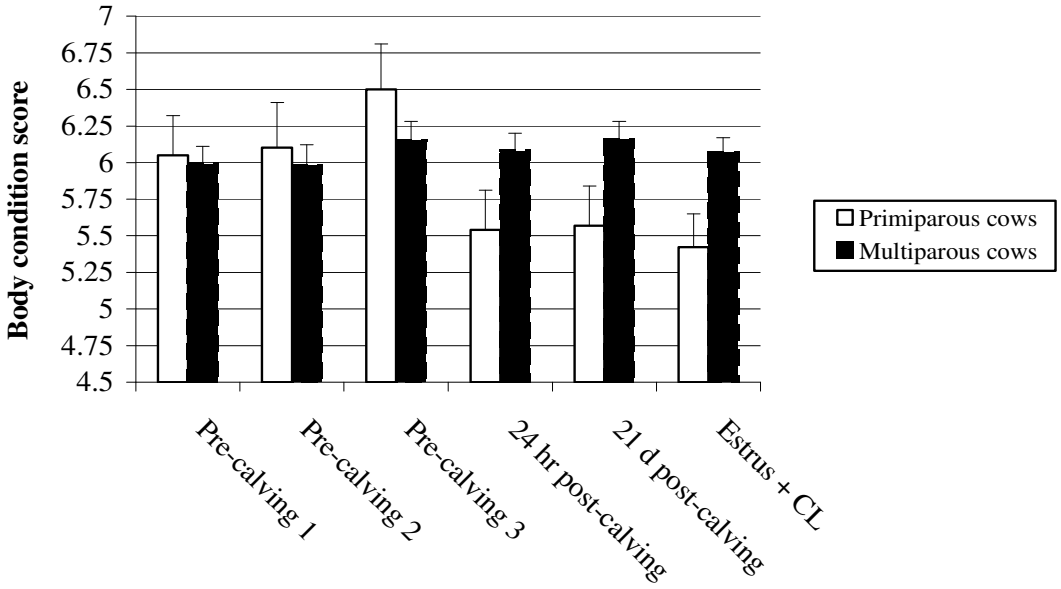

Figure 4.2. Pre- and post-calving mean body condition scores for primiparous (n = 16) and multiparous (n = 38) Brahman cows. Parity effect P = 0.43, time effect P < 0.0001, and time x parity effect P < 0.0001, pooled SEM = 0.2.

Table 4.3. Reproductive performance of primiparous and multiparous Brahman cows

	Parity		
Trait[a]	Primiparous	Multiparous	P-value
n	16	38	
Julian date of calving, d	106 ± 5	102 ± 3	0.5788
Julian date of conception, d	198 ± 9[b]	164 ± 3[c]	0.0008
Days to first estrus	91 ± 5[d]	48 ± 3[e]	<0.0001
Days to CL formation	94 ± 6[d]	46 ± 3[e]	<0.0001
Days to estrus with CL formation	94 ± 6[d]	48 ± 3[e]	<0.0001
First service conception rate, %	0[b]	50[c]	0.0004
End of breeding season pregnancy rate, %	25[b]	79[c]	0.0002

[a] CL = corpus luteum.
[b,c] Least square means within a row differ (P < 0.001).
[d,e] Least square means within a row differ (P < 0.0001).

Table 4.4. Insulin-like growth factor-I and non-esterified fatty acid concentrations of primiparous and multiparous Brahman cows

	Parity		
Trait[a]	Primiparous	Multiparous	P-value
n	16	38	
Day 21 IGF-I, ng/mL	92.53 ± 7.01[b]	78.09 ± 4.52[c]	0.0877
n	7	36	
Day 21 NEFA, mEq/L	0.53 ± 0.11	0.43 ± 0.04	0.3982
Peak NEFA, mEq/L	0.85 ± 0.12[d]	0.58 ± 0.04[e]	0.0386
NEFA at estrus with CL formation, mEq/L	0.18 ± 0.08 [b]	0.34 ± 0.03 [c]	0.0654
Change in NEFA from day 21 to estrus with CL formation, mEq/L	-0.29 ± 0.10[b]	-0.09 ± 0.04[c]	0.0827
Change in NEFA from peak to estrus with CL formation, mEq/L	-0.64 ± 0.10[f]	-0.24 ± 0.04[g]	0.0007

[a] IGF-I = insulin-like growth factor-I, NEFA = non-esterified fatty acid, and CL = corpus luteum
[b,c] Least square means within a row differ ($P < 0.10$).
[d,e] Least square means within a row differ ($P < 0.05$).
[f,g] Least square means within a row differ ($P < 0.001$).

NEFA concentrations were higher ($P < 0.05$) for primiparous cows (0.85 ± 0.12 mEq/L) than for multiparous cows (0.58 ± 0.04 mEq/L). Concentrations of NEFA just prior to estrus with subsequent CL formation tended ($P < 0.10$) to be lower for primiparous cows when compared to multiparous cows (0.18 ± 0.08 vs. 0.34 ± 0.03 mEq/L). The change in NEFA concentrations from d 21 post-calving to estrus with CL formation tended ($P < 0.10$) to be greater in magnitude for primiparous cows than for multiparous cows (-0.29 ± 0.10 vs. -0.09 ± 0.04 mEq/L). Furthermore, primiparous heifers had a greater change in NEFA concentrations from peak to estrus with CL formation than multiparous cows (-0.64 ± 0.10 vs. -0.24 ± 0.04 mEq/L; $P < 0.001$). A NEFA x parity interaction ($P < 0.01$) was observed as a result of these differences (Figure 4.3).

Primiparous cows had smaller calves at birth (30.5 ± 1.2 vs. 35.9 ± 0.8 kg; $P < 0.001$) and at weaning (153.9 ± 5.9 vs. 192.1 ± 3.8 kg; $P < 0.0001$) than did multiparous cows (Table 4.5). Calves born from primiparous cows grew slower ($P < 0.0001$) during the pre-weaning period than did calves born from multiparous cows (0.79 ± 0.03 vs. 0.96 ± 0.02 kg/d). However, there was no difference ($P > 0.05$) in the proportion of calf weaned as a function of cow BW. Despite WW adjustments made for sex of calf and age of dam, primiparous cows still weaned lighter calves than multiparous cows (191 ± 4.1 vs. 205.6 ± 2.6 kg; $P < 0.001$). Calf ADG calculated using adjusted calf WW was not different ($P > 0.05$) between primiparous and multiparous cows. When the proportion of calf weaned was calculated using adjusted calf WW, primiparous cows weaned more ($P < 0.0001$) kg of calf as a proportion of their own BW than did multiparous cows (47.2 ± 1.2 vs. 38.7 ± 0.8%).

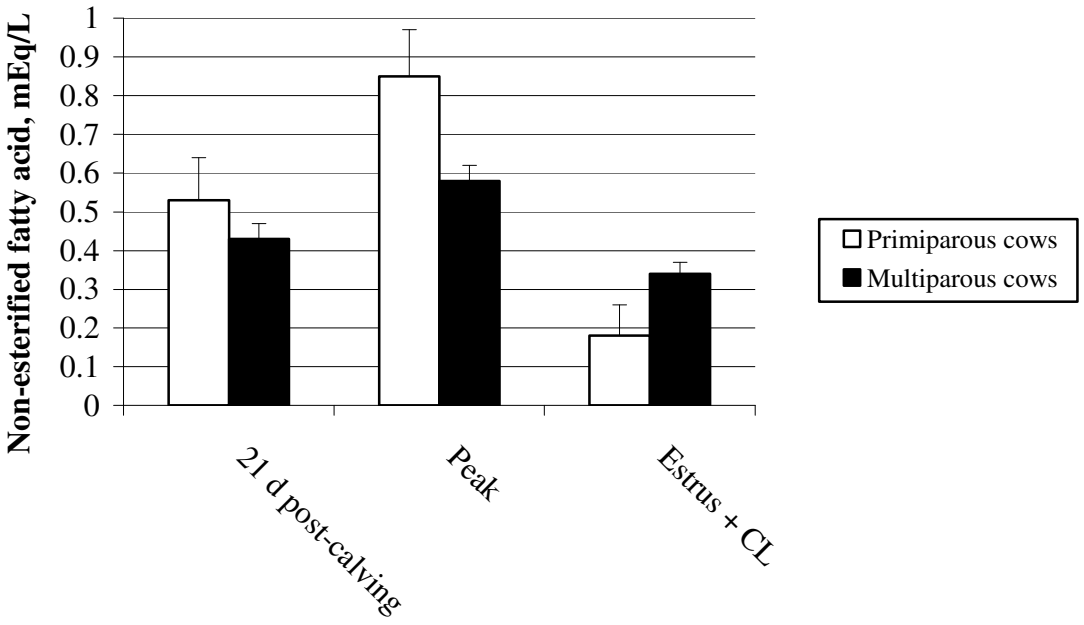

Figure 4.3. Post-calving mean serum non-esterified fatty acid concentrations for primiparous (n = 7) and multiparous (n = 36) Brahman cows. Parity effect $P = 0.43$, time effect $P < 0.0001$, and time x parity effect $P < 0.01$, pooled SEM = 0.07.

Table 4.5. Calf performance of primiparous and multiparous Brahman cows

	Parity		
Trait	Primiparous	Multiparous	P-value
n	16	38	
Calf birth weight, kg	30.5 ± 1.2[a]	35.9 ± 0.8[b]	0.0003
Calf weaning weight, kg	153.9 ± 5.9[c]	192.1 ± 3.8[d]	<0.0001
Adjusted calf weaning weight, kg	191.0 ± 4.1[e]	205.6 ± 2.6[f]	0.0040
Calf ADG, kg/d	0.8 ± 0.0[c]	1.0 ± 0.0[d]	<0.0001
Adjusted calf ADG, kg/d	1.0 ± 0.0	1.1 ± 0.0	0.7634
Proportion calf weaned, %	38.1 ± 1.3	36.0 ± 0.9	0.1745
Adjusted proportion calf weaned, %	47.2 ± 1.2[c]	38.7 ± 0.8[d]	<0.0001

[a,b] Least square means within a row differ ($P < 0.001$).
[c,d] Least square means within a row differ ($P < 0.0001$).
[e,f] Least square means within a row differ ($P < 0.01$).

Effects of RFI group

There were no differences (P > 0.05) in pre- or post-calving BW between efficient and inefficient cows (Table 4.6). However, inefficient cows gained more weight (P < 0.05) than efficient cows from calving to estrus with CL formation (26.4 ± 4.6 vs. 14.2 ± 5.4 kg). There was no BW x RFI group interaction (P > 0.05; Figure 4.4). Inefficient cows tended (P < 0.10) to be in better body condition than efficient cows at the first pre-calving BCS evaluation (6.2 ± 0.2 vs. 5.8 ± 0.2). Inefficient cows had higher BCS (P < 0.05) at the second pre-calving measurement than efficient cows (6.3 ± 0.2 vs. 5.8 ± 0.2). However, inefficient and efficient cows had similar (P > 0.05) BCS at the last pre-calving measurement, 24 hr after calving, and 21 d after calving. Inefficient cows were again in better body condition just prior to estrus with CL formation when compared to efficient cows (5.9 ± 0.1 vs. 5.6 ± 0.2; P < 0.05). There were no differences (P > 0.05) in BCS change during either the pre- or postpartum period between efficient and inefficient cows. Furthermore, there was no interaction between BCS and RFI group (P > 0.05; Figure 4.5).

Julian date of calving was not different (P > 0.05) between efficient and inefficient cows (Table 4.7). However, inefficient cows conceived sooner (175 ± 5 vs. 188 ± 6 Julian d; P < 0.05) than efficient cows. Efficient cows had shorter intervals from calving to first estrus (63 ± 4 vs. 76 ± 4 d; P < 0.05), functional CL formation (63 ± 5 vs. 77 ± 4 d; P < 0.05), and estrus with subsequent luteal formation (64 ± 5 vs. 77 ± 4 d; P < 0.05) than inefficient cows. Although more efficient cows were pregnant at the end of

Table 4.6. Body weight and body condition score of efficient and inefficient Brahman cows

	RFI group		
Trait[a]	Efficient	Inefficient	P-value
n	23	31	
Pre-calving BW1, kg	468.8 ± 17.7	490.7 ± 14.9	0.2671
Pre-calving BW2, kg	472.1 ± 18.2	494.6 ± 15.3	0.2687
Pre-calving BW3, kg	472.0 ± 17.7	493.4 ± 14.9	0.2796
24 hour post-calving BW, kg	473.8 ± 16.3	478.8 ± 13.8	0.7815
21 day post-calving BW, kg	476.4 ± 16.6	492.9 ± 14.0	0.3703
BW at estrus with CL formation, kg	488.0 ± 16.1	505.1 ± 13.6	0.3385
Pre-calving change in BW, kg	3.3 ± 3.3	1.8 ± 2.8	0.7277
Post-calving change in BW, kg	14.2 ± 5.4[b]	26.4 ± 4.6[c]	0.0480
Pre-calving BCS1	5.8 ± 0.2[d]	6.2 ± 0.2[e]	0.0615
Pre-calving BCS2	5.8 ± 0.2[b]	6.3 ± 0.2[c]	0.0455
Pre-calving BCS3	6.2 ± 0.2	6.5 ± 0.2	0.2097
24 hour post-calving BCS	5.7 ± 0.2	5.9 ± 0.2	0.4896
21 day post-calving BCS	5.7 ± 0.2	6.0 ± 0.2	0.1122
BCS at estrus with CL formation	5.6 ± 0.2	5.9 ± 0.1	0.0487
Pre-calving change in BCS	0.3 ± 0.1	0.1 ± 0.1	0.2161
Post-calving change in BCS	-0.2 ± 0.2	0.1 ± 0.1	0.1928

[a] CL = corpus luteum and BCS = body condition score.
[b,c] Least square means within a row differ ($P < 0.05$).
[d,e] Least square means within a row differ ($P < 0.10$).

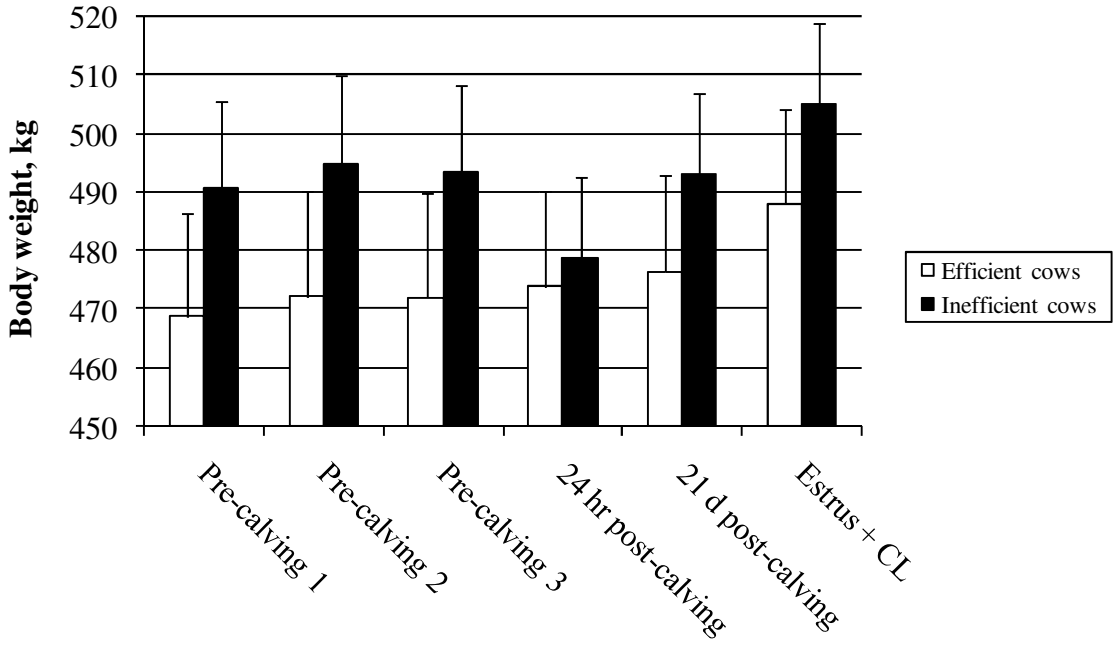

Figure 4.4. Pre- and post-calving mean body weights for efficient (n = 23) and inefficient (n = 31) Brahman cows. RFI effect P = 0.35, time effect P < 0.01, and time x RFI effect P = 0.15, pooled SEM = 15.8.

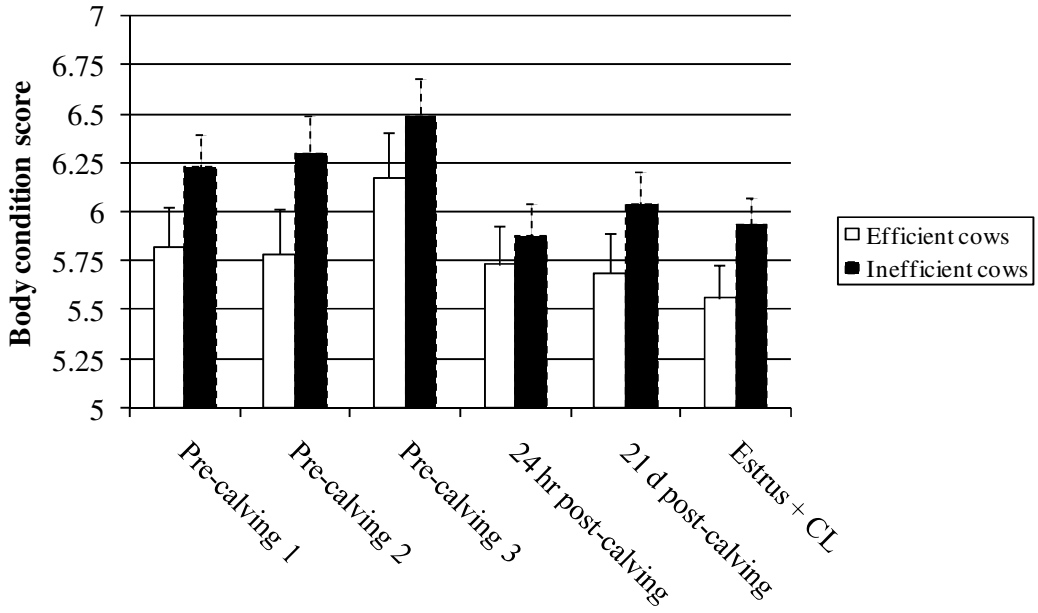

Figure 4.5. Pre- and post-calving mean body condition scores for efficient (n = 23) and inefficient (n =31) Brahman cows. RFI effect $P < 0.10$, time effect $P < 0.0001$, and time x RFI effect $P = 0.40$, pooled SEM = 0.2.

Table 4.7. Reproductive performance of efficient and inefficient Brahman cows

	RFI group		
Trait[a]	Efficient	Inefficient	P-value
n	23	31	
Julian date of calving, d	108 ± 5	100 ± 4	0.1461
Julian date of conception, d	187 ± 6[b]	175 ± 5[c]	0.0451
Days to first estrus	63 ± 4[b]	76 ± 4[c]	0.0122
Days to CL formation	63 ± 5[b]	77 ± 4[c]	0.0103
Days to estrus with CL formation	64 ± 5[b]	77 ± 4[c]	0.0181
First service conception rate, %	39	32	0.6010
End of breeding season pregnancy rate, %	78	52	0.0449

[a] CL = corpus luteum.
[b,c] Least square means within a row differ ($P < 0.05$).

the breeding season than inefficient cows (78 vs. 52%: $P < 0.05$), there were no significant differences observed for first service conception rate by RFI group.

Since reproductive parameters also differed by parity, primiparous and multiparous cows were separated for chi-square analysis of cumulative return to estrus, CL formation, estrus with CL formation, and conception, in addition to first service conception rate and end of breeding season pregnancy rate. Cumulative return to estrus for multiparous cows results are presented in Figure 4.6. Similar proportions ($P > 0.05$) of efficient and inefficient multiparous cows had expressed first estrus between d 21 and 30 following calving. Between d 31 and 40, 65% of efficient multiparous cows had expressed estrus as compared to 29% of inefficient multiparous cows ($P < 0.05$). There was a tendency ($P < 0.10$) for more efficient multiparous cows to have expressed first estrus by d 41 to 50 (71 vs. 43%). Ninety-four percent of efficient multiparous cows compared to 62% of inefficient multiparous cows had shown estrus by d 51-60 ($P < 0.05$). Another tendency was observed between d 61 and 70 for more efficient multiparous cows to have expressed estrus than inefficient multiparous cows (100 vs. 81%; $P < 0.10$). The proportion of multiparous cows showing estrus by d 71 to 80 was not statistically different by RFI group, although 2 multiparous inefficient cows never expressed estrus within 80 d of calving.

There was no observed difference between RFI groups for the percentage of multiparous cows forming corpora lutea between d 21 and 30 after calving (Figure 4.7). More ($P < 0.05$) efficient than inefficient multiparous cows developed functional corpora lutea by d 31 to 40 (71 vs. 33%), d 41 to 50 (84 vs. 48%), and d 51 to 60 (100 vs. 71%).

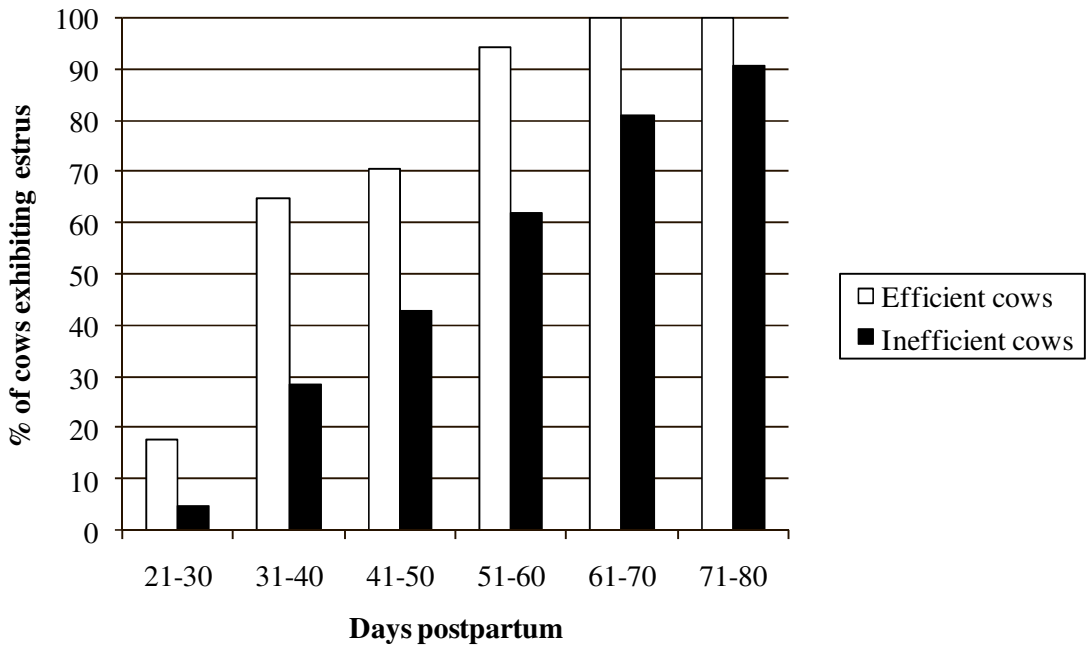

Figure 4.6. Cumulative return to estrus for efficient (n = 17) and inefficient (n = 21) multiparous Brahman cows.

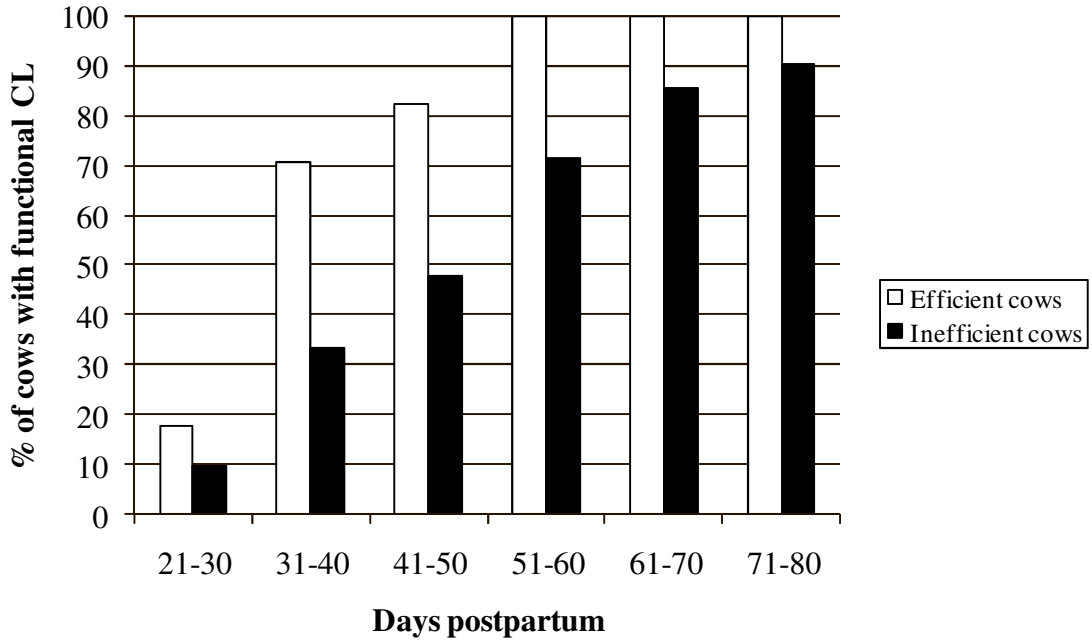

Figure 4.7. Cumulative achievement of corpus luteum formation for efficient (n = 17) and inefficient (n = 21) multiparous Brahman cows.

There were no statistical differences ($P > 0.05$) between RFI groups for the proportion of multiparous cows that had developed a functional CL by d 61 to 70 or d 71 to 80. Although not statistically different, it is important to note that 2 of the inefficient multiparous cows did not develop corpora lutea within 80 d after calving.

No difference was detected ($P < 0.05$) between the proportion of efficient and inefficient multiparous cows that had expressed estrus and subsequently developed a functional CL by d 21 to 30 after calving (Figure 4.8). By d 31 to 40, more efficient multiparous cows ($P < 0.05$) had exhibited estrus with CL formation than inefficient multiparous cows (65 vs. 29%). There was a tendency ($P < 0.10$) for a greater proportion of efficient multiparous cows to have shown estrus and developed a CL by d 41 to 50 (71 vs. 43%). More ($P < 0.05$) efficient than inefficient multiparous cows were detected in estrus with subsequent luteal formation by d 51 to 60 (94 vs. 62%) and by d 61 to 70 (100 vs. 71%). Although 4 inefficient multiparous cows never expressed estrus with subsequent CL formation, statistically similar proportions ($P > 0.05$) of efficient and inefficient multiparous cows had achieved estrus with CL formation by d 71 to 80.

As depicted in Figure 4.9, there were no differences observed in first service conception rate between efficient and inefficient multiparous cows. Furthermore, there were no differences ($P > 0.05$) between RFI groups in pregnancy rate by d 1-20, d 21-40, or d 41-60 of the breeding season (Figure 4.10). However, a greater proportion of efficient multiparous cows had conceived by d 61 to 80 (100 vs. 62%; $P < 0.01$) resulting in a significantly higher proportion ($P < 0.01$) of efficient than inefficient multiparous cows (100 vs. 62%) confirmed pregnant (Figure 4.11).

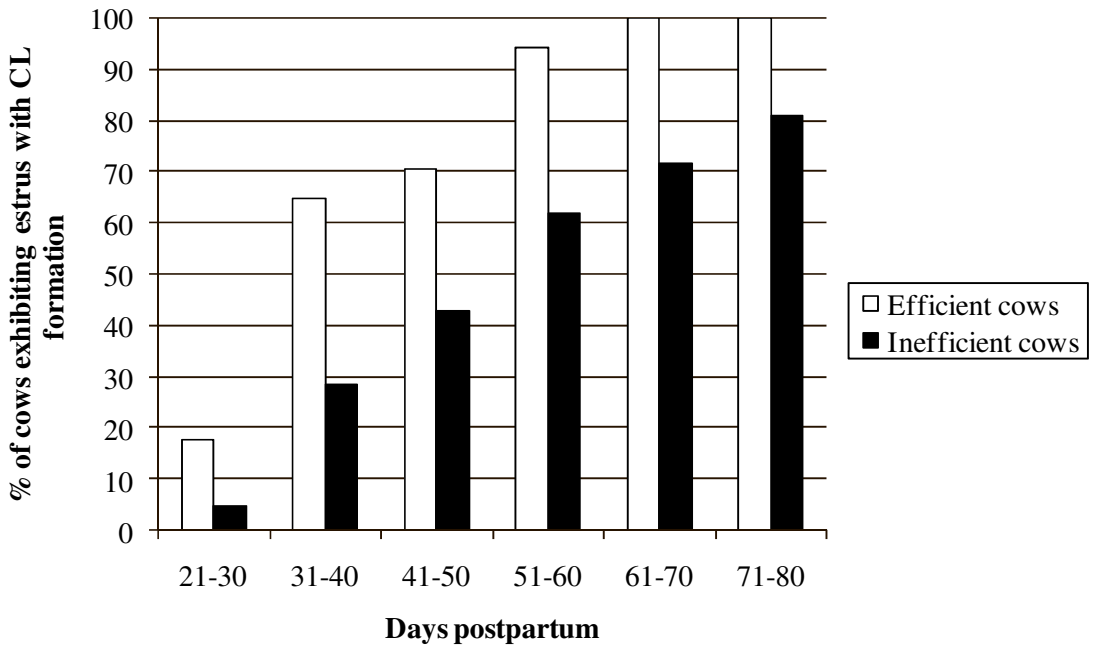

Figure 4.8. Cumulative return to estrus with subsequent corpus luteum formation for efficient (n = 17) and inefficient (n = 21) multiparous Brahman cows.

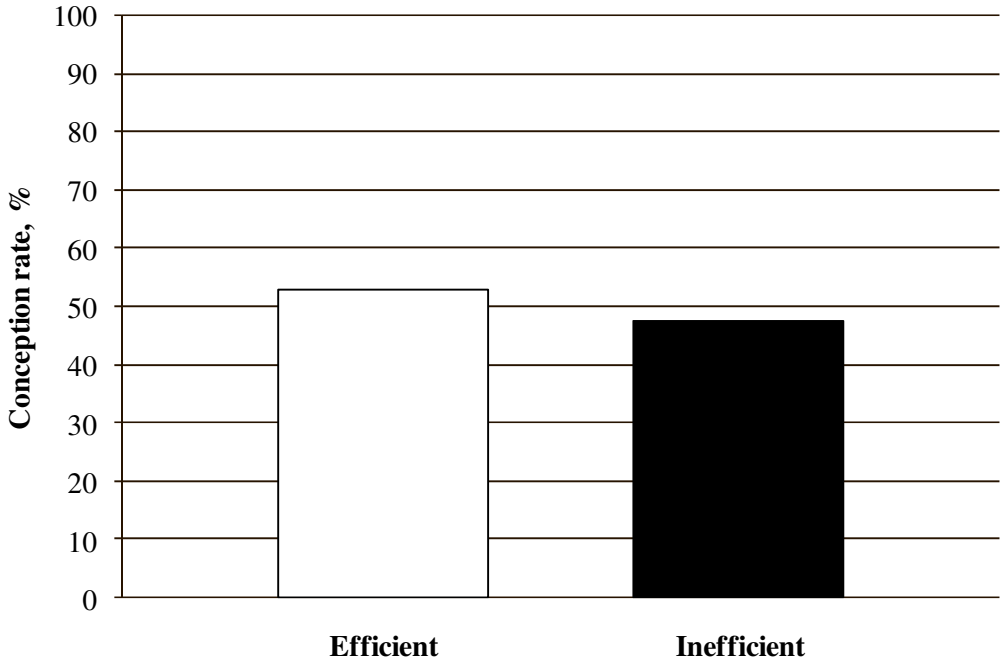

Figure 4.9. First service conception rate for efficient (n = 17) and inefficient (n = 21) multiparous Brahman cows.

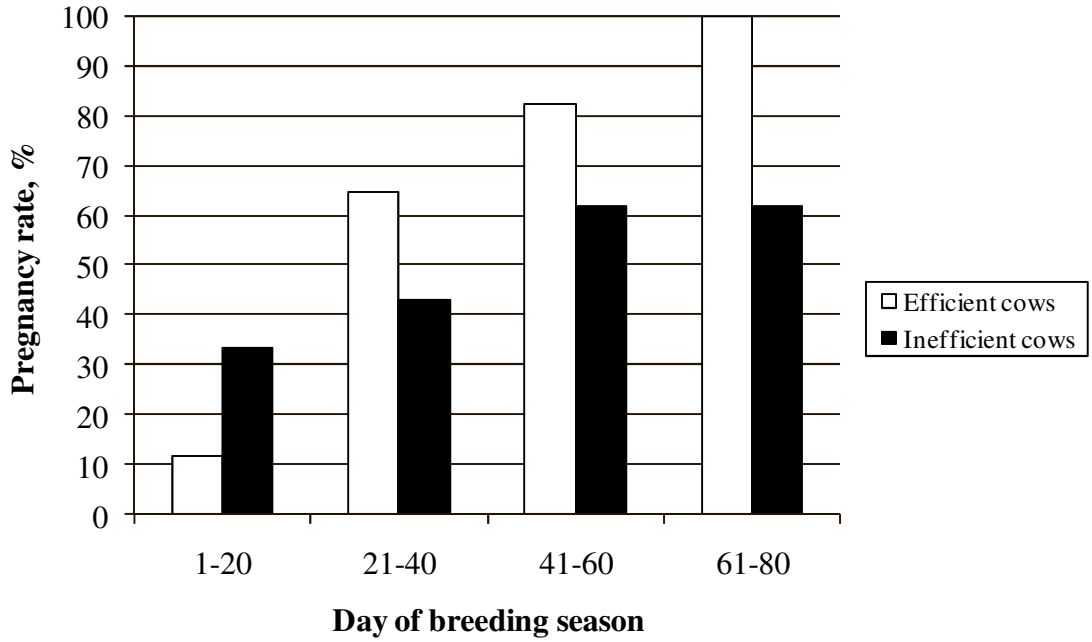

Figure 4.10. Cumulative pregnancy rate for efficient (n = 17) and inefficient (n = 21) multiparous Brahman cows.

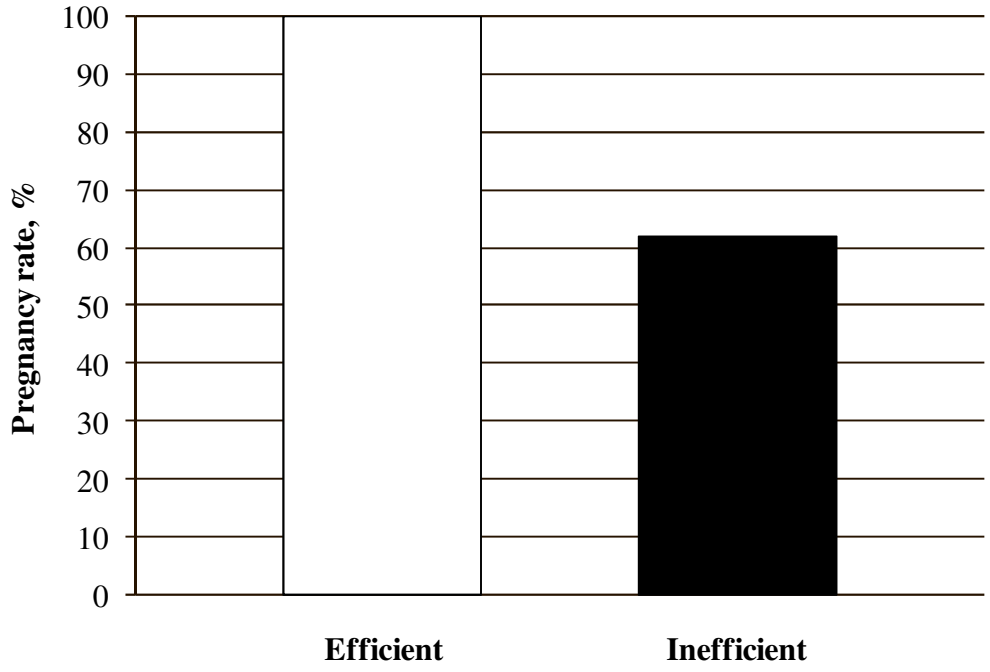

Figure 4.11. End of breeding season pregnancy rate for efficient (n = 17) and inefficient (n = 21) multiparous Brahman cows.

None of the primiparous cows had expressed estrus by d 60 following calving (Figure 4.12). There was a tendency ($P < 0.10$) for a higher proportion of efficient primiparous cows to have exhibited estrus by d 61 to 70 (33 vs. 0%) and by d 71 to 80 (50 vs. 10%) in comparison with inefficient primiparous cows. Three of the efficient and 9 of the inefficient primiparous cows had not expressed estrus by 80 d following calving. None of the primiparous cows had developed a CL by d 70 after calving (Figure 4.13). By d 71 to 80, 17% of the efficient primiparous cows and 10% of the inefficient primiparous cows had developed corpora lutea; however, these results were not statistically different ($P > 0.05$). Within 80 d of calving, 5 efficient and 9 inefficient primiparous cows had not developed corpora lutea. Identical results for cumulative return to estrus with subsequent CL formation are presented in Figure 4.14. None of the primiparous cows, regardless of RFI group, conceived to the first mating. Furthermore, there were no observed differences between efficient and inefficient primiparous cows for cumulative pregnancy rate as shown in Figure 4.15. As a result, pregnancy rate at the end of the breeding season did not differ by RFI group in primiparous cows (Figure 4.16).

For both primiparous and multiparous cows, no significant differences were observed for IGF-I or NEFA concentrations between efficient and inefficient cows (Table 4.8). In addition, there was no observed interaction between NEFA concentration and RFI group (Figure 4.17). Calf performance traits are presented in Table 4.9. Inefficient cows gave birth to heavier calves (34.59 ± 1.04 vs. 31.83 ± 0.88 kg; $P < 0.05$)

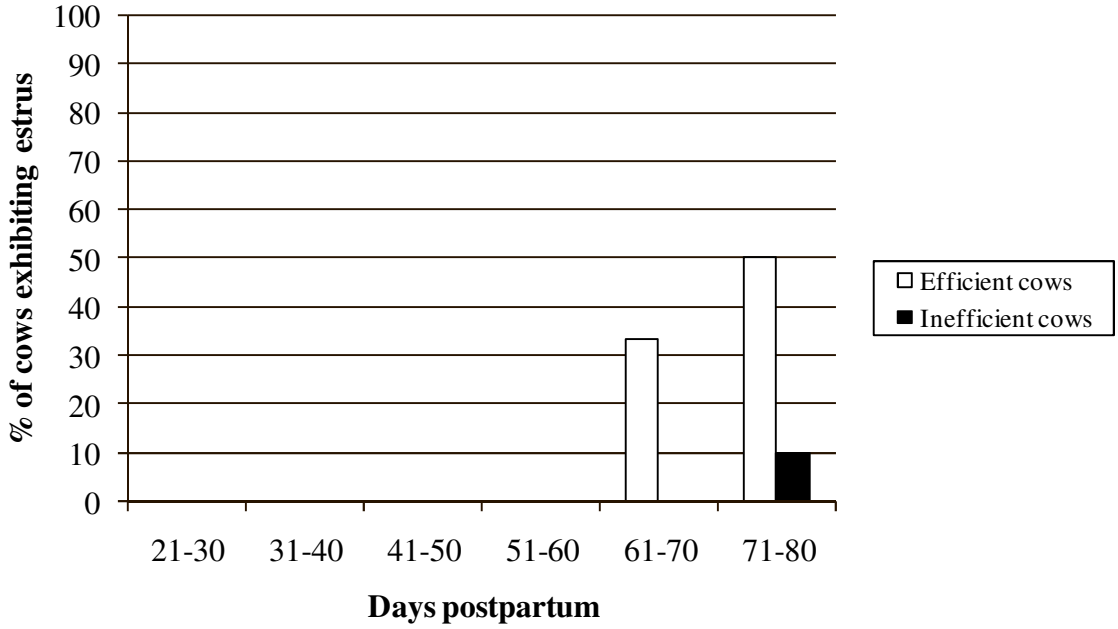

Figure 4.12. Cumulative return to estrus for efficient (n = 6) and inefficient (n = 10) primiparous Brahman cows.

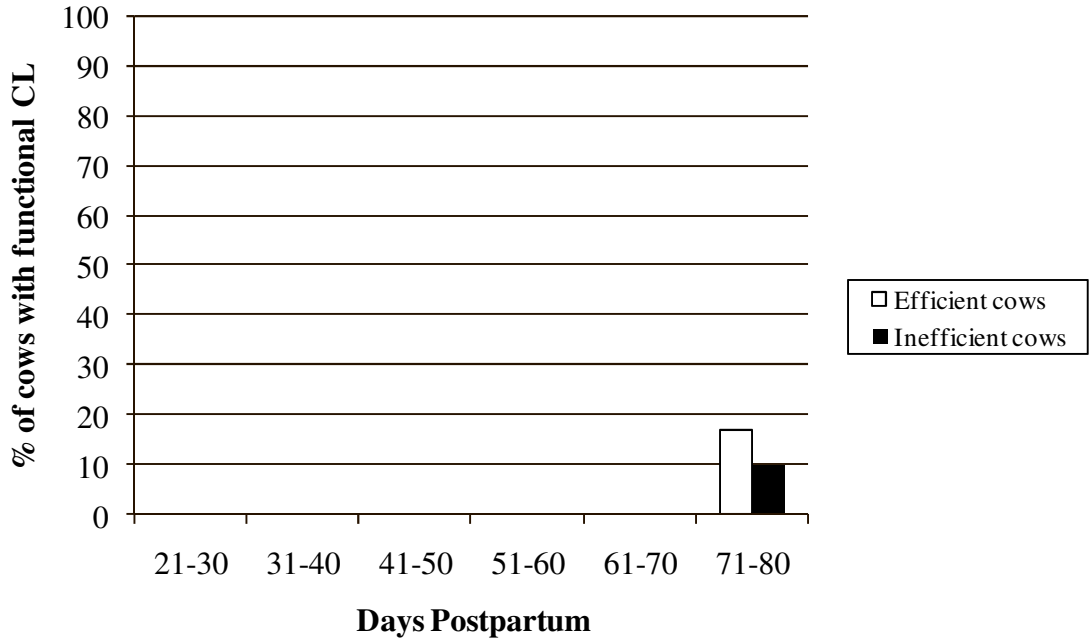

Figure 4.13. Cumulative achievement of corpus luteum formation for efficient (n = 6) and inefficient (n = 10) primiparous Brahman cows.

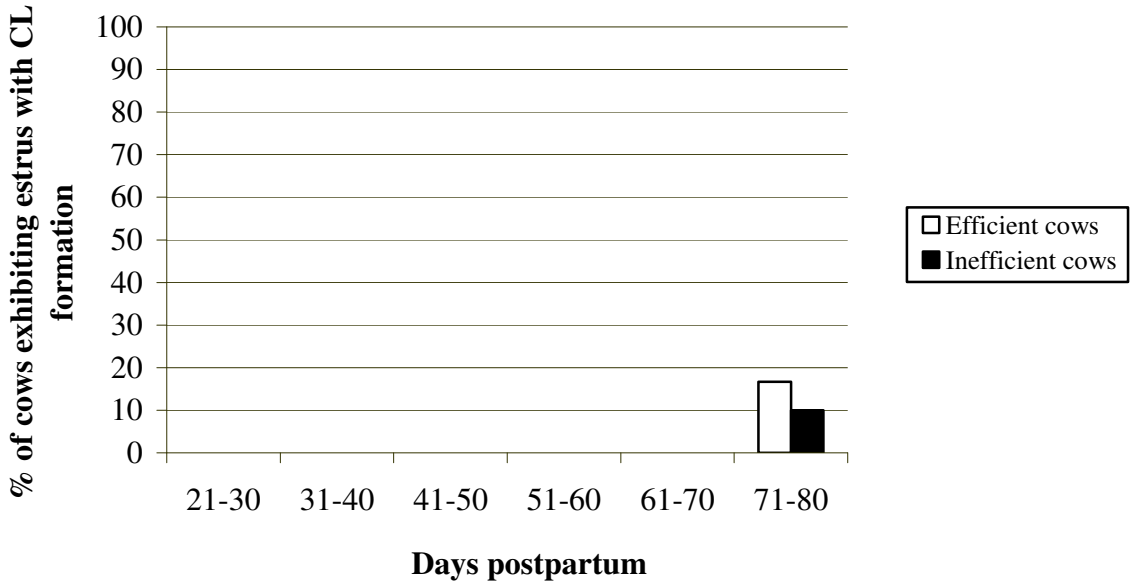

Figure 4.14. Cumulative return to estrus with subsequent corpus luteum formation for efficient (n = 6) and inefficient (n = 10) primiparous Brahman cows.

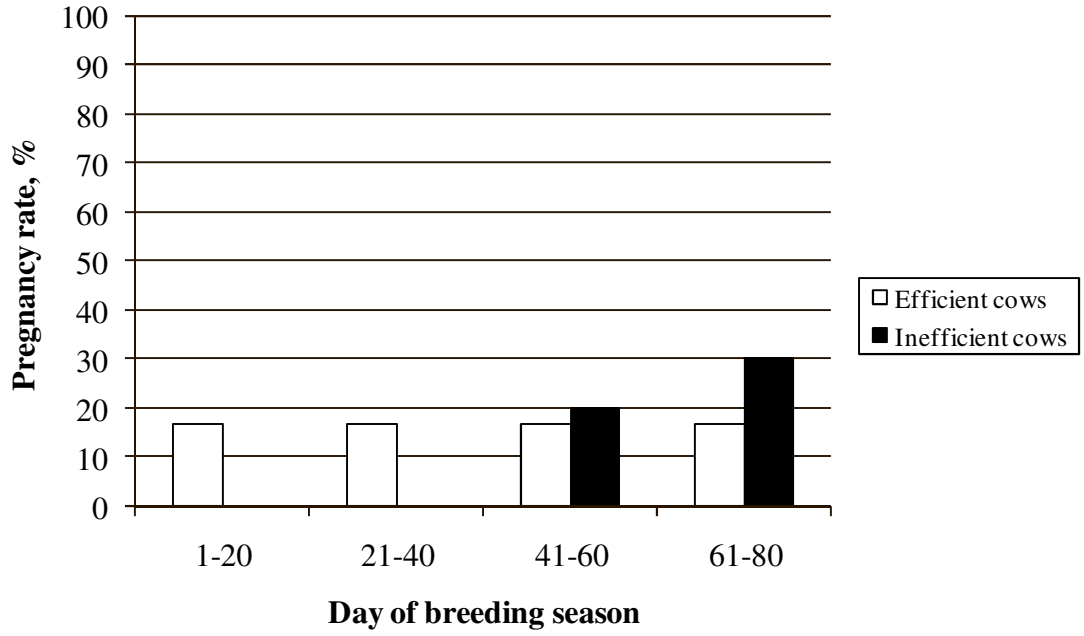

Figure 4.15. Cumulative pregnancy rate for efficient (n = 6) and inefficient (n = 10) primiparous Brahman cows.

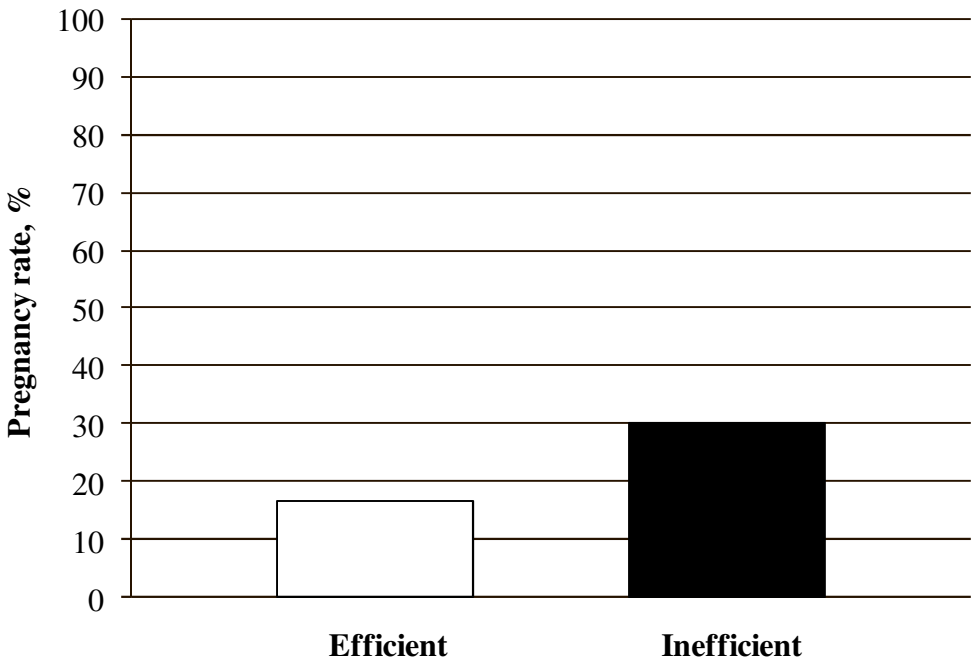

Figure 4.16. End of breeding season pregnancy rate for efficient (n = 6) and inefficient (n = 10) primiparous Brahman cows.

Table 4.8. Insulin-like growth factor-I and non-esterified fatty acid concentrations of efficient and inefficient Brahman cows

	RFI group		
Trait[a]	Efficient	Inefficient	P-value
n	23	31	
Day 21 IGF-I, ng/mL	82.00 ± 6.12	88.63 ± 5.20	0.3908
n	19	24	
Day 21 NEFA, mEq/L	0.52 ± 0.07	0.44 ± 0.07	0.3284
Peak NEFA, mEq/L	0.69 ± 0.08	0.73 ± 0.07	0.6193
NEFA at estrus with CL formation, mEq/L	0.27 ± 0.05	0.24 ± 0.05	0.5998
Change in NEFA from day 21 to estrus with CL formation, mEq/L	-0.22 ± 0.07	-0.16 ± 0.06	0.5336
Change in NEFA from peak to estrus with CL formation, mEq/L	-0.41 ± 0.08	-0.47 ± 0.06	0.4857

[a] IGF-I = insulin-like growth factor-I, NEFA = non-esterified fatty acid, and CL = corpus luteum

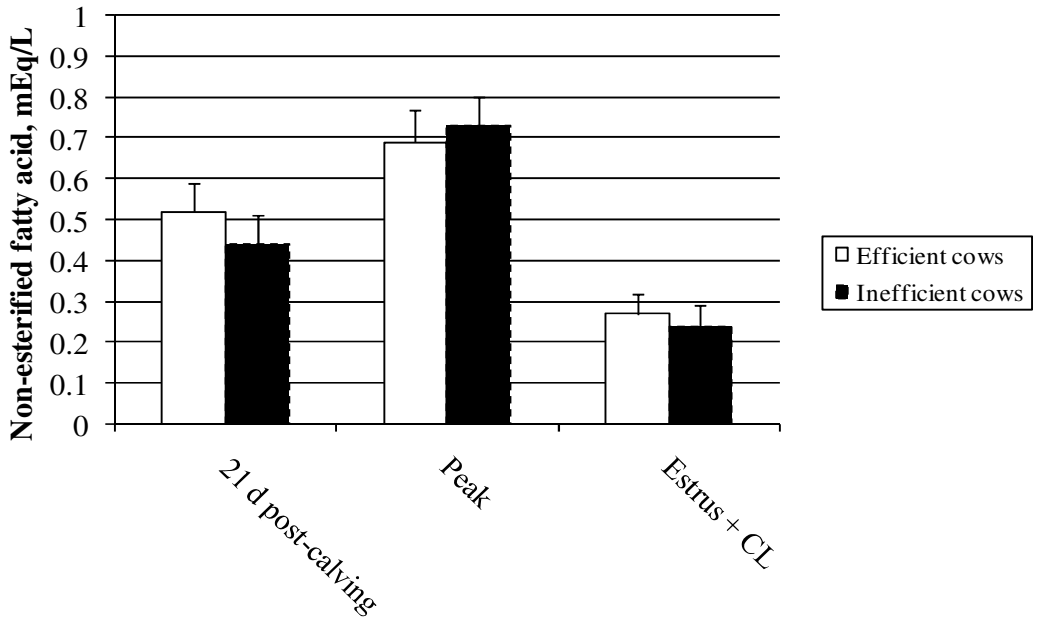

Figure 4.17. Post-calving mean serum non-esterified fatty acid concentrations for efficient (n = 19) and inefficient (n = 24) Brahman cows. RFI effect P = 0.70, time effect P < 0.0001, and time x RFI effect P = 0.35, pooled SEM = 0.07.

Table 4.9. Calf performance of efficient and inefficient Brahman cows

	RFI group		
Trait	Efficient	Inefficient	P-value
n	23	31	
Calf birth weight, kg	31.8 ± 1.0[a]	34.6 ± 0.9[b]	0.0386
Calf weaning weight, kg	168.4 ± 5.1	177.5 ± 4.3	0.1616
Adjusted calf weaning weight, kg	195.8 ± 3.6	200.7 ± 3.0	0.2729
Calf ADG, kg/d	0.9 ± 0.0	0.9 ± 0.0	0.2182
Adjusted calf ADG, kg/d	1.0 ± 0.0	1.1 ± 0.0	0.7076
Proportion calf weaned, %	36.1 ± 1.2	37.9 ± 1.0	0.2292
Adjusted proportion calf weaned, %	42.6 ± 1.1	43.3 ± 0.9	0.6298

[a,b] Least square means within a row differ ($P < 0.05$).

than efficient cows. However, no further differences in calf performance were observed between efficient and inefficient cows.

Discussion

Effects of parity

At each measurement of BW pre- and post-calving, multiparous cows weighed more than primiparous cows. This was expected as primiparous cows typically have not reached mature size and continue to grow through their first lactation. Evidence for the continued growth of primiparous cows in this study was provided by the difference in BW gain observed between parity groups. Primiparous cows gained significantly more weight after calving as compared to multiparous cows. However, multiparous cows were in better body condition following calving, as primiparous cows lost considerable condition after calving. This is likely the result of the increased energy demands to support both lactation and growth in the primiparous cows (Spitzer et al., 1995). It appears that the primiparous cows were mobilizing adipose tissue reserves in order to meet these energy demands.

Although Julian date of calving did not differ by parity, Julian date of conception was significantly later for primiparous cows than multiparous cows. This is consistent with the extended postpartum interval often experienced by primiparous cows. Wiltbank (1970) reported that first-calf heifers have a 25 to 40 d longer interval from calving to first estrus than older cows. Primiparous cows in this study exhibited first estrus, developed corpora lutea, and expressed estrus with subsequent CL formation more than

40 d later than multiparous cows. Furthermore, none of the primiparous cows conceived to the first service. This, combined with the extended postpartum interval, likely contributed to the low pregnancy rate observed at the end of the breeding season for primiparous cows as compared to multiparous cows. Because of the restricted breeding season, primiparous cows simply did not have enough time to initiate estrous cycles and become pregnant after calving.

Primiparous cows tended to have higher circulating concentrations of IGF-I than multiparous cows 21 d after calving. This conflicts with the suggestion that circulating IGF-I concentration increases with age (Plouzek and Trenkle, 1991). However, since primiparous cows were still growing after calving, it is possible that growth hormone stimulation of IGF-I production was elevated as compared to multiparous cows. Circulating NEFA concentrations did not differ by parity 21 d after calving, although primiparous cows had higher peak circulating concentrations of NEFA than multiparous cows after calving. The discrepancy in NEFA concentrations between parity groups on d 21 and at peak NEFA concentration may be a reflection of the effects of lactation. Peak lactation in Brahman cattle appears to occur 30 to 50 d after calving (Neidhardt et al., 1979). During this time, the energy demands experienced by primiparous cows are high. As a result, primiparous cows often enter into a state of negative energy balance because the energy available in the diet is insufficient to support the energetic demands of lactation and growth (Wiltbank et al., 2002). When this occurs, body lipid stores are hydrolyzed into NEFA and glycerol to provide additional energy to the female (Wettemann et al., 2003). As primiparous cows often experience greater negative

energy balance than multiparous cows, it would be expected that peak circulating NEFA concentrations of primiparous cows would exceed that of multiparous cows around the time of peak lactation.

Calf birth weight was significantly different between parity groups. This finding agrees with Burris and Blunn (1952) and Eriksson et al. (2004) who reported that calves born from primiparous cows were lighter at birth than calves born from multiparous cows. Calf pre-weaning growth performance also differed between parity groups. Calves born from primiparous cows had lower ADG than calves born from multiparous cows. Because of the lighter birth weight and reduced growth rate, calves born from primiparous cows were also lighter at weaning. However, when calf WW was expressed as a percentage of cow BW at weaning, there was no difference between parity groups. This is likely the result of the reduced BW of primiparous cows as compared to multiparous cows. When adjustments for sex of calf and age of dam were made for calf WW, primiparous cows still weaned lighter calves than multiparous cows. The actual WW of calves born from primiparous cows may have been lower than what was accounted for when making the age of dam adjustment to WW. As a result, the adjustment was unable to completely overcome the bias of age of dam on WW. However, when expressed as a proportion of cow BW, primiparous cows weaned a higher percentage of their BW than did multiparous cows. This is likely due to the inflation of the adjusted WW over actual WW for calves born from primiparous cows as a result of the age of dam adjustment.

Effects of RFI group

Body weight was not different between efficient and inefficient cows at any time pre- or post-calving. This finding is in agreement with a report made by Arthur et al. (2005) who observed no statistical difference in BW between high and low RFI Angus cows at any stage of the production cycle over 3.5 yr. However, inefficient cows gained more weight than efficient cows from calving to estrus with CL formation. Inefficient cows were also in better body condition when they first expressed estrus with subsequent CL formation, which may have resulted in the increased weight gain of inefficient cows post-calving.

Although Julian date of calving did not differ by RFI group, inefficient cows conceived an average of 13 d sooner than efficient cows. This was the only advantage inefficient cows displayed over efficient cows with regard to reproductive performance. Efficient cows exhibited first estrus, developed functional corpora lutea, and expressed estrus with functional CL development 13 to 14 d earlier than inefficient cows. Oftentimes, such differences can be attributed to differences in BCS at calving (Morrison et al., 1999; Hess et al., 2005). However, efficient and inefficient cows were in similar body condition at calving. Furthermore, pregnancy rate at the end of the breeding season was 26% higher for efficient cows than inefficient cows. As first service conception rate was not different by RFI group, it appears that the ability of efficient cows to initiate estrous cycles sooner than inefficient cows afforded them more opportunities to become pregnant during the defined breeding season. This agrees with

a report made by Thatcher and Wilcox (1973) where cows that exhibited more estrous cycles had improved fertility.

The exact reasons for the early resumption of estrous cycles by the efficient cows as compared to the efficient cows are not known. Body condition score has been shown to be an important determinant of the length of the postpartum interval in cows (Rutter and Randel, 1984; Richards et al., 1986; Morrison et al., 1999; Hess et al., 2005). Selk et al. (1988) suggested that cows should be managed to calve in a minimum BCS of 5 in order to minimize the impacts of postpartum negative energy balance on the length of the postpartum interval. There was no difference in the mean BCS of efficient and inefficient cows at the time of calving or NEFA concentrations after calving. Therefore, it would appear that BCS and energy balance are not the primary contributors to the difference observed in the length of the postpartum interval between the efficient and inefficient cows. However, the minimum BCS required for resumption of estrous cycles during the postpartum period may be lower for the efficient cows than for the inefficient cows. If this is in fact the case, efficient cows in similar body condition as inefficient cows would be expected to resume estrous cycles earlier in the postpartum period than inefficient cows. However, this possible difference in maintenance requirements between efficient and inefficient cattle has not been investigated due to its expense and difficulty to measure.

The ability of efficient cows to initiate estrous cycles earlier than inefficient cows was further substantiated when primiparous and multiparous cows were analyzed separately for cumulative reproductive performance differences by RFI group. A greater

percentage of efficient multiparous cows had exhibited estrus, developed a functional CL, and exhibited estrus with subsequent CL formation earlier in the postpartum period than inefficient multiparous cows. Furthermore, 100% of the efficient cows compared to only 81% of the inefficient cows had expressed estrus with CL formation by 80 d after calving. The ability of efficient multiparous cows to initiate estrous cycles earlier than inefficient multiparous cows resulted in a 100% pregnancy rate for efficient multiparous cows as compared to a 62% pregnancy rate for inefficient multiparous cows 80 d after calving. This suggests that efficient multiparous cows are more likely to maintain a 365-d calving interval than inefficient multiparous cows.

No statistical differences were observed between efficient and inefficient primiparous cows for cumulative return to estrus, CL formation, estrus with CL formation, or conception. Furthermore, only 30% of the efficient primiparous cows and 17% of the inefficient primiparous cows were confirmed pregnant at the end of the breeding season. This is likely a reflection of the failure of the majority of primiparous cows to initiate estrous cycles prior to the end of the breeding season. It is important to note that the primiparous cows in this study calved for the first time as 2-yr-olds. Most Brahman cows do not calve for the first time until 3 yr of age. Therefore, it appears that regardless of RFI group, the primiparous cows in this study were simply not physiologically ready to conceive and support pregnancy during the restricted breeding season following their first parity.

For both primiparous and multiparous cows, there were no statistical differences detected for circulating IGF-I or NEFA concentrations between efficient and inefficient

cows. While there is a lack of data investigating circulating hormone and metabolite concentrations in high versus low RFI cows, several studies have evaluated growing animals for differences in circulating IGF-I concentration by RFI group. Results from *Bos taurus* cattle suggest that circulating IGF-I concentrations differ between high and low RFI cattle (Johnston et al., 2002). While this finding conflicts with the current study, our results are in agreement with studies that evaluated circulating IGF-I concentrations in *Bos indicus*-influenced cattle. Lancaster et al. (2007) reported that circulating IGF-I concentrations were not correlated with RFI in Brangus heifers. Furthermore, Caldwell (2009) reported circulating IGF-I concentrations in Brahman heifers were not different among RFI groups. No statistical difference in RFI was observed between low and high IGF-I bull and heifer calves born from Angus cattle divergently selected for IGF-I concentration for 13 yr (Lancaster et al., 2008).

Inefficient cows gave birth to 3 kg heavier calves than efficient cows. These results conflict with results published by Basarab et al. (2007) who observed no statistical differences in calf birth weight among *Bos taurus* dams that produced high, medium, or low RFI calves. No further differences in calf performance were detected between efficient and efficient cows, suggesting that selection for RFI in Brahman cattle should not impact calf pre-weaning performance.

Conclusion

Nutrition is an important mediator of the events associated with reproduction (Guilbert, 1942; Asdell, 1949; Wiltbank et al., 1962; Randel et al., 1990; Short et al.,

1990). Specific to the postpartum period, nutrition exerts its influence over reproductive performance primarily through cow body condition (Wiltbank et al., 1962; Selk et al., 1988; Spitzer et al., 1995). Recent trends in the beef cattle industry have shifted the focus of selection strategies towards the inclusion of input traits such as RFI. Weak correlations between RFI and cattle fatness (Richardson et al., 2001; Basarab et al., 2003; Lancaster et al., 2009) have sparked concern for the potential impact selecting cattle for improved RFI might have on subsequent reproductive performance by altering body condition. However, the results from this study suggest that selection of Brahman cattle for RFI should not impede reproduction in multiparous cows. Instead, it appears that selection of low RFI cattle actually shortens the interval from calving to first estrus, CL formation, and estrus with CL formation and also improves pregnancy rates in multiparous Brahman cows without altering calf performance. Reproductive performance differences between efficient and inefficient primiparous cows were not detected because the young age at which the primiparous cows calved was apparently limiting postpartum reproductive performance. Therefore, results from this study suggest that selection of efficient cattle using RFI as a selection tool may result in a shorter postpartum interval and improved pregnancy rates in multiparous Brahman cows.

CHAPTER V

CONCLUSIONS

Recent increases in the input costs associated with beef production have led cattle producers and researchers to seek methods of reducing input costs. Since providing feed to cattle represents the majority of the cost of beef production, finding ways to decrease feed costs is economically important. Identifying and selecting cattle that are more efficient at utilizing available nutrients is one method that may help reduce feed expenses. Although F:G has been the traditional model used to evaluate cattle for feed efficiency, its correlations with BW and growth rate result in cattle that are larger at maturity being retained in the breeding herd. Residual feed intake is an alternative measure of feed efficiency that is not phenotypically correlated with either BW or growth rate and may be a more appropriate measure of feed efficiency than F:G. However, there is a void of research investigating relationships among RFI, other traits of economic importance, and the performance of cattle later in life.

Results from these studies suggest that caution must be exercised when interpreting the results of an RFI evaluation. Cattle of different breedtypes should not be evaluated together for RFI. In addition, RFI may be influenced by the physiological age of the animals being evaluated suggesting that cattle of similar physiological age should be evaluated together for RFI. Furthermore, RFI evaluated during one physiological period may not necessarily be a true reflection of RFI of an animal during a different

physiological period. Further research to determine whether or not RFI of a given animal remains constant over time is warranted.

These studies also suggest that selection for lower RFI (improved feed efficiency) in Bonsmara cattle should not alter the age at which heifers reach puberty. Selection for lower RFI in Brahman cattle may shorten the postpartum interval and improve pregnancy rates in multiparous cows. Since BW, BCS, NEFA or IGF-I concentrations, and calf performance were not different between efficient and inefficient Brahman cows, it is possible that there is a difference in maintenance requirements between the high and low RFI cows. However, further research is needed to determine if this difference actually exists.

Although these studies provide important information regarding the potential use of RFI as a selection tool, there is still a lack of research investigating the relationships among RFI and other economically important traits. Determining how selection for RFI might impact other aspects of beef cattle production is necessary so that other traits are not hindered by the selection of cattle for lower RFI.

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APPENDIX A

PRE- AND POST-PUBERTAL RESIDUAL FEED INTAKE OF HEIFERS

Animal ID	Pre-pubertal RFI	Post-pubertal RFI
32	+0.1069	0.5335
33	+0.7979	0.1775
36	+1.2134	3.9569
46	+0.3788	0.3054
52	+0.1393	-0.4142
54	+0.2162	+1.6644
63	-0.5605	-1.1591
64	-0.1959	-0.0488
116	-0.6590	-0.0440
177	-0.4850	-0.7933
243	-0.8519	-1.7320
383	+0.0517	+0.6565
385	-1.4684	-0.8233
386	+0.4392	-0.1739
391	+0.9570	-0.0455
415	-0.8893	-0.8991
419	-1.0107	-1.6597
421	-0.9387	+0.0390
458	-0.1158	-0.9606
478	-0.4796	+0.5441
515	+0.4280	+0.2475
523	-0.0997	-0.7616
565	-0.2893	-0.7496
572	-0.0725	-1.9500
576	-0.5006	-0.5766
579	-0.9321	-1.2636
709	-0.5954	-0.7776
848	-0.3550	-0.8104
851	-0.7546	-2.0758
853	-0.5423	-0.8876
855	-0.0986	-1.4675
856	-0.0043	-0.3271
859	-0.2142	+0.0293
936	+0.3346	+1.5429
958	+0.3195	-0.1038
1032	+0.9379	+2.1598
1184	+0.0402	+0.2296
1202	+0.1234	+0.5572
1210	-0.1305	+0.1542

Animal ID	Pre-pubertal RFI	Post-pubertal RFI
1211	+0.5596	+2.1722
1212	-0.7533	-0.4680
1228	+0.7432	+1.7923
1233	+0.1770	-0.6573
1237	+0.2327	-0.4114
1238	-0.3205	-1.5381
1239	-0.1379	-0.3022
1240	+0.4403	+0.0990
1250	-0.0052	+0.5294
1374	+0.2536	+2.5098
1394	+0.6469	+0.0058
1492	-0.3733	+0.2050
1605	-0.7138	+1.0973
1609	+0.5117	+2.6940
1619	-0.3089	+1.0461
1620	-0.0908	+0.7223
1625	+0.2317	+1.0238
1655	+0.5130	-0.3505
1668	+0.5682	+0.5420
1765	+1.0038	-0.1244
1857	+0.4183	+1.2677
1985	+0.5508	+0.1288
1991	-0.1894	-0.7431
2004	-0.1835	-0.7722
2036	+0.1729	-0.7253
2403	-0.3462	-0.7250
2410	-0.1857	-0.7890
2412	-0.9224	-0.7752
2416	+0.1045	-0.8464
2428	+0.0354	-0.2363
2429	+0.1141	+1.1420
2430	+0.4606	+0.8847
3183	+0.3301	+0.3786
3270	-0.3896	+0.4891
3280	+0.7514	-1.1208
3293	+0.8576	-0.8734
3301	+1.3162	-0.4197
3396	-0.3134	+0.8553

APPENDIX B

HEIFER RANK BY PRE- AND POST-PUBERTAL RESIDUAL FEED INTAKE

Rank (low to high RFI)	Animal ID Pre-pubertal	Animal ID Post-pubertal
1	385	851
2	419	572
3	421	243
4	579	419
5	2412	1238
6	415	855
7	243	579
8	851	63
9	1212	3280
10	1605	458
11	116	415
12	709	853
13	63	3293
14	853	2416
15	576	385
16	177	848
17	478	177
18	3270	2410
19	1492	709
20	848	2412
21	2403	2004
22	1238	523
23	3396	565
24	1619	1991
25	565	2036
26	859	2403
27	64	1233
28	1991	576
29	2410	1212
30	2004	3301
31	1239	52
32	1210	1237
33	458	1655
34	523	856
35	855	1239
36	1620	2428
37	572	386

Rank (low to high RFI)	Animal ID Pre-pubertal	Animal ID Post-pubertal
38	1250	1765
39	856	958
40	2428	64
41	1184	391
42	383	116
43	2416	1394
44	32	859
45	2429	421
46	1202	1240
47	52	1985
48	2036	1210
49	1233	33
50	54	1492
51	1625	1184
52	1237	515
53	1374	46
54	958	3183
55	3183	3270
56	936	1250
57	46	32
58	1857	1668
59	515	478
60	386	1202
61	1240	383
62	2430	1620
63	1609	3396
64	1655	2430
65	1985	1625
66	1211	1619
67	1668	1605
68	1394	2429
69	1228	1857
70	3280	936
71	33	54
72	3293	1228
73	1032	1032
74	391	1211
75	1765	1374
76	36	1609
77	3301	36

APPENDIX C

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

PROTOCOL FOR BOVINE SERUM

Reagent Preparation:

1) IGF-I Assay Buffer

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

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

2) 1M Glycine

75.07 g Glycine (Sigma G-8898)

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

3) 0.5N NaOH

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

4) 12.5% Polyethylene Glycol (PEG)

250 g Carbowax PEG 8000 (Sigma P-2139)

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

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

(Source: A. F. Parlow, National Hormone and Peptide Program, Harbor-UCLA Medical Center, 1000 West Carson St., Torrance, CA 90509)

Antibody comes lyophilized at a 1:10 dilution in PBS. Use antibody at a final dilution of 1:120,000 in IGF-I Assay Buffer (7.5 µL stock into 90 mL IGF-I Assay Buffer). Prepare fresh daily at least one hour before use and store at 4ºC.

6) Secondary Antibody (GARGG)

(Source: Calbiochem, San Diego, CA; Goat Anti-Rabbit γ-Globulin cat#. 539845)

Add 667 µL of stock GARGG to 39.33 mL IGF-I Assay Buffer (1:60 dilution). Prepare fresh daily and store at 4ºC.

7) Normal Rabbit Serum (NRS) (IgG Corporation, Cat# IgG-NRS)

Prepare at 1:100 dilution in IGF-I Assay Buffer (500 µL stock into 49.5 mL buffer).

8) [^{125}I] Tracer

(Source: MP Biomedicals Inc., Cat# 68128)

Calculation of required activity:
1µCi isotope = 22.20 x 10^6 dpm.
2.22 x 10^6dpm = 1.665 x 10^6cpm (at 75% counting efficiency estimate [^{125}I]).

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

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

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

9) IGF-I Standards

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

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

Reconstitute lyophilized standard stock with 1.00 mL ddH_2O (IGF-1 STD Stock I). Note: this resulted in a previous shipment of this specific standard (sample #1168) having a concentration of 134 µg/mL (vial's specific mass listed on some by FJP).

Construct 1 µg/mL IGF-1 STD Stock II. Transfer 74.63 µL (i.e. 10 µg) to a 10 mL volumetric containing approximately 8.0 mL IGF-1 RIA buffer. Bring to volume and allow for equilibration. Aliquot and freeze if not used immediately.

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

IGF-I Standards chart:

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

10) IGF-1 Composite Pools for RIA:

For verification of inter-and intra-RIA performance over the expected biological IGF-1 concentration range, construct a "normal" pool and a "high" pool from a composite sub-set of acidified serum samples.

Pool (n = 30) 200 µL aliquots from a random set of acidified serum samples. Pipette 3.00 mL of this to a 13 x 100 mm PP culture tube (to be used for the "high" pool preparation). Aliquot remainder of "normal" pool at 500 µL, freeze and store until use.

For the "high" pool, prepare a 100 ng/mL IGF-1 stock through a 1:10 dilution of 1 µg /mL IGF-1 Standard Stock II. Pipette 20 µL of the 100 ng/mL stock into 2980 µL of the "normal" pool aliquot to produce the "high" pool stock (e.g.

back-pipette 20 µL from the 3.0 mL and replace). This results in the addition of 0.667 ng/mL at RIA or, after accounting for the 1:200 final sample dilution following RIA, a delta at RIA of 133.3 ng/mL (e.g. "normal" IGF-I concentration plus 133 ng/mL at the serum level). Vortex and aliquot "high" pool at 500 µL, freeze and store until use. Laboratory wide "Welsh" pool should be acid extracted along with unknown samples.

IGF-I Assay Protocol:

A) Acidification of Samples

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

B) Assay Procedure

1) Each assay should include at least triplicate tubes of total (T), non-specific binding (NSB), zero tubes (B_0), standards, and pools. Single acidified unknown samples should be in duplicate for the RIA.
2) Pipette 400 µL of IGF-I Assay Buffer into NSB tubes.
3) Pipette 300 µL of IGF-I Assay Buffer into B_0tubes.
4) Pipette 200 µL of IGF-I Assay Buffer into standard tubes.
5) Pipette 200 µL of IGF-I Assay Buffer into all sample tubes and pools.
6) Add 100 µL of each standard to each designated standard tube.
7) Add 100 µL of each acidified serum sample into each designated tube pair.
8) Add 100 µL of acidified pools into control pool tubes.
9) Pipette 100 µL of primary antibody to all tubes except NSB and T.
10) Carefully shake tubes to mix and cover with foil.
11) Incubate for 24 hours at 4ºC.
12) Pipette 100 µL of [^{125}I]-IGF-I Tracer to all tubes. Cover tubes with foil and shake the tubes carefully to mix.
13) Incubate for 16 hours at 4ºC.
14) Pipette 50 µL of NRS to all tubes except totals.
15) Pipette 50 µL of GARGG to all tubes except totals.
16) Pipette 300 µL of PEG to all tubes except totals.
17) Carefully shake tubes to mix and cover with foil.
18) Incubate tubes at room temperature for 30 minutes. (NO LONGER)

19) Centrifuge tubes at 3000 rcf for 25 minutes at 4ºC. (3220 rpm on Sorvall RC3C)
20) Decant tubes (except totals) immediately into radioactive waste container.
21) Allow tubes to remain upside down on absorbent towels for 5 minutes.
22) Remove all visible droplets by tapping tube bottoms.
23) Count tubes on Beckman gamma counter for 1 minute per sample.
24) Use AssayZap to calculate concentrations of unknowns in comparison to a known standard curve. (Final ng/mL concentrations are determined by multiplying mean unknown by 1000.)

APPENDIX D

PROGESTERONE RADIOIMMUNOASSAY PROTOCOL FOR BOVINE SERUM

Reagent Preparation:

1) Phosphate Buffered Saline (PBSG)

0.070 g Monobasic sodium phosphate (Sigma S-9638)
1.350 g Dibasic sodium phosphate (Sigma S-0876)
8.812 g Sodium chloride (Sigma S-9888)
1.000 g Sodium azide (Sigma S-2002)
0.372 g Disodium EDTA; dihydrate (Sigma, ED2SS)
1.000 g Gelatin (J.T. Baker, 2124-01)
1.00 L double-distilled water (ddH_2O)

Into ddH_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 approximately one hour. Transfer to storage bottle and store at 4°C. Replace at 30 to 40 d intervals. **(Caution: Sodium azide is highly toxic.)**

2) Charcoal suspension

0.188 g Dextran (Sigma D-4271)
1.875 g Activated charcoal (Sigma C-5260)
100 mL PBSG

Add Dextran to PBSG and mix until in solution; add charcoal and stir. Prepare at least one day in advance of RIA and discard at 20 d intervals. Can be stored at 4°C in a sealed beaker. Suspension must be maintained at approximately 4°C during additions. Use an ice bath with continuous stirring if addition time exceeds 5 min.

3) Charcoal-Stripped Serum or Plasma Stock

Bleed, separate and collect 300+ mL sera or plasma from (preferably) an intact prepubertal female. Another reasonable source would be a mature female at 4-12 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.

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.

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

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

Centrifuge for 2.0+ hours at 10,000 rpm x 4^{o}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).

Repeat centrifugation using fresh centrifuge tubes. Carefully decant and pool clear sera or plasma stock into fresh flask.

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 is 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 3 filtrations.

Aliquot at 5 to 7 mL in peti-vials. Cap, label and freeze at -20^{o}C until use.

4) Trace Dilution

Stock: TRK.413 [1, 2, 6, 7-^{3}H]-Progesterone, Amersham

Using micropipette, introduce 4 to 8 µL of ^{3}H-tracer stock into 25 mL PBSG; mix for 5 min on stir plate and let stand for 10 min at 4^{o}C.

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

Calculate appropriate dilution. Currently 9,500 – 10,500 cpm//100 µL trace (i.e. mean cpm x original volume / 10,500 = final volume)

Add appropriate amount of PBSG for working dilution of trace. Mix well and let stand overnight at 4^{o}C before use.

5) Antibody Dilution

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

Reconstitute lyophilized anti-sera with 1.0 mL ddH_2O (1:1).

In order to minimize detrimental effects of repeated freeze-thaw cycles, use an aliquot of the full-strength anti-sera to prepare a second series of concentrated storage aliquots. Aliquot volumes should be appropriate for the simple preparation of adequate anti-sera to be used in a single RIA throughput. The recommended PBSG dilution for concentrated anti-sera storage aliquot is 1.0 mL x 1:46.

Working dilutions should be prepared daily from aliquoted storage dilutions and stored at 4°C. Working dilutions are prepared in PBSG to achieve 20 to 50% maximum binding (%Ref/TC). Pre-labeled urine specimen cups are generally ideal for this step. The recommended dilution for anti-sera working stock in 298-tube RIA is 60.0 mL x 1:2760 (1 mL stock + 59 mL PBSG).

6) RIA Standards

Prepare or use Stock I @ 1.00 mg/mL EtOH. Construct by adding 0.025 g progesterone 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 µg/mL. Construct Stock II by adding 50 µL 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 µg/mL, construct Std A @ 16.00 ng/mL by transfer of 400 µL 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. Continue serial dilutions through STD H:

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

Progesterone Assay Protocol:

A) Day before assay

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 the assay. Store overnight at -20°C. Verify that adequate supplies are available for the RIA. These include stocks of appropriate tracer and anti-sera dilutions, pre-racked pipette tips, arrayed mini-scintillation vials (preferably loaded with cocktail) and 12 x 75 mm polypropylene culture tubes for standards, controls, and determinations. Label culture tubes as follows:

TC	total counts
NSB	non-specific binding
TB_0	total (or maximum) binding; zero concentration reference for standard curve
$STD_{(x)}$	one/standard concentration (e.g. $STD_{0.125}$, $STD_{0.250}$, $STD_{0.500}$, etc.
C(-), C(+)	one/negative or positive control

(These tubes represent a single standard curve and should be racked independently. At least 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)

B) 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:

Tube	PBSG	CharPlasma	STD/CNTL/SMPL	^3H	Ab	Char/Dext
TC	1200	--	--	100	--	--
NSB	300	100	--	100	--	750
TB_0	100	100	--	100	200	750
Standards	--	100	100 STD	100	200	750
Controls	100	--	100 CNTL	100	200	750
Samples	100	--	100 SMPL	100	200	750

NOTE: All volumes are reported in µL. 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 day. This can be 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 hours. 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 minutes throughout the protocol. (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! Two to five minutes error during some steps is usually enough to destroy the outcome of this RIA.

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

2) Begin with the careful setup of all standard curves needed. 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.

3) 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 2 batches of n = 135 samples plus n = 13 standards that will require centrifugation. (Centrifuge capacity holds 148 tubes; TC standards are not centrifuged.) This should take 1.5 to 2.0 hours to complete.

4) Add 100 µL of PBSG to the sample tubes. Shake each rack to mix. Set aside at room temperature.

5) Referring to the series table above, begin the reaction of Spin 1 at exactly 1030 h regardless of whether the sample pipetting operation is complete. Pipette the appropriate volume of ^{3}H-tracer into **all** tubes. Then, pipette the appropriate volume of anti-sera into all tubes **except TC and NSB**. Shake racks vigorously or vortex. Place racks in plastic bags or parafilm the tubes.

6) Incubate all tubes within each batch for exactly 90 minutes at room temperature.

7) Transfer all tubes within each batch to refrigerator and incubate at 4^{o}C for exactly 75 minutes.

8) Remove charcoal/dextran suspension from refrigerator and place on a stir plate for approximately 1 minute before use. Referring to the series table above, add 750 µL charcoal/dextran solution to all tubes **except TC**. Precise timing on this step is absolutely essential. Start timer for 30 minute countdown, then shake racks vigorously and return to the refrigerator for incubation at 4^{o}C.

9) At 30 minutes, remove batch from the refrigerator and load all tubes, except TC, into centrifuge carriers (starting with standard curve), and centrifuge at 4,000 rpm for 20 minutes at 4^{o}C.

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

11) 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 all batches.

12) Place the flat of scintillation vials on a tray and carry them to the main lab 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 1 = standard curve #1 plus samples 1 through 135; flat 2 = standard curve #2 plus samples 136 through 270). Place entire flat between 2 trays and mix thoroughly by 15 to 20 inversions. Leave the covered trays overnight.

C) Day 2

13) Re-mix the flats by inversion and count for 1 minute each on TR2100 beta counter. Be sure to use the appropriate protocol-definition clip on the first cassette.

14) 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.

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

	Spin 1	**Spin 2**
Begin reaction Add ^{3}H tracer Add anti-sera Incubate at room temp	10:30	11:30
Transfer to refrigerator Incubate at 4°C	12:00	1:00
Add charcoal/dextran suspension Incubate at 4°C Start timer	1:15	2:15
Centrifuge 4,000 rpm x 20 min x 4°C	approximately 1:55	approximately 2:55
Decant	approximately 2:25	approximately 3:25

APPENDIX E

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 (ddH_2O) 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 ^{o}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^{o}C).

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

5) Pipette 5µL ddH_2O (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^{o}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^{o}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 ddH_2O. 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	Smpl 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

VITA

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