EFFECTS OF TIMING OF GAIN ON GROWTH, BODY COMPOSITION, REPRODUCTIVE PERFORMANCE, AND BLOOD METABOLITES IN BEEF REPLACEMENT HEIFERS

By

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To my loving family and friends for supporting and encouraging me through the achievement of my master's degree.

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EFFECTS OF TIMING OF GAIN ON GROWTH, BODY COMPOSITION, REPRODUCTIVE PERFORMANCE AND BLOOD METABOLITES IN BEEF REPLACEMENT HEIFERS

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The goal of any replacement heifer operation to optimize reproductive efficiency is to ensure that heifers attain adequate weight to initiate their first estrous cycle before the start of the breeding season while at the same time holding feed cost to a minimum. The experiment was conducted to evaluate the effect of delayed gain using round bale silage (RBS) and dried distillers grains (DDG) on the growth, body composition, reproductive performance, and blood metabolite responses in yearling beef heifers. The experiment utilized thirty Angus (276 $\pm$ 9.6 kg; age) and thirty Brangus (279 $\pm$ 9.6 kg) heifers. Heifers were stratified by BW, breed, and age and allocated to one of twelve pens (5 heifers per pen). On d 0 of the experiment, heifers were assigned to one of two treatments 1) RBS + DDG d 0 to 140 with heifers fed to gain 0.84 kg/d (CON); 2) RBS d 0 to 70 and fed to gain 0.24 kg/d and RBS + DDG d 70 to 140 and fed to gain 1.4 kg/d (LH). At the mid-point of the trial (d 70) CON heifers were 29.1 kg heavier (P = 0.04), had greater (P < 0.01) BCS, and a tendency (P = 0.08) for larger pelvic area (PA) compared to LH heifers. However at the end of the experiment (d 140), BW and PA were similar (P > 0.05) between treatments. Hip heights were greater (P < 0.01) for

Brangus compared to Angus throughout the entire experiment. On d 70, ribeye area (REA) and rib fat (RIBFT), were greater ($P < 0.01$) for CON compared to LH heifers. Additionally on d 140, REA, continued to be greater ($P < 0.01$) for CON compared to LH heifers. There were no breed effects ($P > 0.05$) on REA, rump fat (RMPFT), and RIBFT. However, intramuscular fat (IMF) was greater ($P < 0.01$) for Angus (4.07%) compared to Brangus (3.39%) on d 140 but there was no treatment effect ($P > 0.10$). Plasma urea nitrogen (PUN) concentrations were decreased ($P < 0.05$) for LH heifers, while NEFA concentrations were greater ($P < 0.05$) for LH heifers compared to CON animals from d 0 to 70 of the experiment. There was a tendency ($P = 0.08$) for a treatment x time effect on glucose concentrations. LH heifers had lower glucose concentrations from d 0 to 70 compared to CON heifers. A similar ($P > 0.05$) percentage of LH and CON as well as Brangus and Angus heifers attained puberty at the start of the breeding season. There were no treatment effects ($P > 0.05$) on, estrous response, conception rate, synchronized pregnancy rate, timed AI rate and overall pregnancy rate between treatments or breeds. Although body composition was altered due to delayed gain; the supplementation strategy did not alter percentage of pubertal heifers at breeding season as well as overall pregnancy rate. These results suggest that DDG supplementation for beef heifers only during the 70 d prior to breeding do not negatively affect reproductive performance of yearling heifers. This supplementation strategy may allow beef producers to develop heifers on low-cost nutritional programs without adversely affecting reproductive performance.

CHAPTER 1
INTRODUCTION

The cost of developing yearling replacement heifers is one of the major economic factors affecting the overall efficiency of a cow-calf operation (Bagley, 1993). The productivity of beef cattle herds improve when a greater percentage of heifers become pregnant early in their first breeding season (Lesmeister et al., 1973). Heifers should be bred approximately 2-3 wk before the cowherd so they have a longer postpartum rebreeding period (Funston and Deutscher, 2004). Therefore, one management tool a cow-calf operation can use to maximize production efficiency is to develop replacement heifers so a majority of heifers are cycling before the start of the breeding season. Heifers that are pubertal before the breeding season and bred on either their second or third estrus after puberty have greater pregnancy rates compared to heifers bred on their pubertal status (Byerley et al.,1987). Also, heifers that calve as two-year olds produce more calves during their lifetime compared to heifers that calve as three-year olds for the first time (Cundiff et al., 1974). In a review of worldwide literature, Morris et al. (1980) concluded that lifetime productivity could be increased by approximately 0.7 calves by breeding heifers for the first time as yearling as opposed to two-year olds. Nunez-Dominguez et al. (1991) observed a 6-8% decrease in costs per unit of output in heifers calving at two compared to three years of age, suggesting it is more beneficial to breed heifers at a year of age from an economic standpoint.

A number of factors can affect the ability of a heifer to reach puberty and calve at 2 years of age, such as the heifers age, BW, breed, management practices, forage quality and availability, and adaptation of heifers of different breed types to environmental conditions (Short et al., 1990). The importance that nutrition has on

attaining target BW to maximum reproductive performance of heifers is well documented (Short et al., 1990; Schillo et al., 1992; Patterson et al., 1992). Enhanced levels of nutrition hasten the onset of puberty (Warnick et al., 1956; Menge et al., 1960; Bellows et al., 1965; Ferrel, 1982)**;** whereas, low levels of nutrition during the prepubertal period delay the onset of puberty by inhibiting the development and maturation of the reproductive tract and endocrine system (Day et al., 1986b). Therefore, feeding heifers in order to attain a given target BW before the start of the breeding season is a practical management tool necessary to ensure that heifers attain puberty, and become pregnantduring the breeding season (Greer et al., 1983). Recommended guidelines for target BW in beef heifers is between 60-65% of mature BW, which is, dependent on heifer frame size (Patterson et al., 1992). As previously, indicated nutrition is one of the most important factors affecting cowherd profitability (Freetly et al., 2001). This is particularly true for replacement heifers development since feed cost is the primary economic cost associated with developing replacement heifers. Therefore, it is imperative that heifer development feeding programs allow heifers to achieve their target BW and conceive early in the breeding season at the lowest possible cost. One of the most common feeding programs utilized to decrease the total cost of developing a replacement heifer is an extended low gain period followed by a high gain period shortly before the start of the breeding season. The concept of the low- high gain program is that the high gain period will yield a high degree of compensatory gain. Clanton et al. (1983) and Lynch et al. (1997) suggested that compensatory gain can be used to reduce total feed inputs, thereby decreasing feeding

cost, and still be able to attain the minimum BW required for heifers to reach puberty before the start of the breeding season.

In summary, age at puberty is an important factor affecting production efficiency of the cowherd. Several factors can influence the age at which heifers attain puberty with the primary one being nutrition. Additionally, nutrition is one of the most expensive factors affecting profitability of the cowherd. Therefore, the use of feeding programs that allow yearling replacement heifers to achieve their target BW and conceive early in the breeding season at the lowest cost possible are desired.

Therefore, the objective of the research described in this thesis was to evaluate the timing of gain on the growth, body composition, and reproductive performance of *Bos taurus* and *Bos indicus* x *Bos taurus* yearling heifers developed on round bale silage and supplemented with dried distillers grain at strategic times during the developmental period of the heifers.

CHAPTER 2
REVIEW OF LITERATURE

Puberty

Puberty can be defined as the stage of development in which the first behavioral estrus is exhibited followed by ovulation and development of corpus luteum (Kinder et al., 1987). The goal of any replacement heifer operation is to breed heifers approximately 14 to 16 months of age so they can calve around 2 years of age, and to achieve this goal with maximum efficiency heifers should be pubertal before the start of the breeding season. Early attainment of puberty is extremely important to optimize production efficiency in beef cattle herds (Dow et al., 1982). Yearling heifers that are bred on the third estrus (78%) after puberty have greater pregnancy rates compared to heifers bred on the pubertal estrus cycle (57%; Byerley et al. 1987). Therefore, it is imperative that heifers achieve puberty around 12 months old to achieve optimal reproductive performance (Schillo et al., 1992). However, there are many factors that influence the timing of onset of puberty including heifer age, management, nutrition, body composition, breed, and season. All these factors can have either a positive and (or) negative effect on the onset of puberty and they will be discussed later in this review.

Endocrine Changes at Onset of Puberty

The attainment of puberty is due to changes that occur in the hypothalamic-pituitary-gonadal axis (Schillo et al., 1992). The changes leading to puberty occur in a gradual manner and originate before birth (Kinder et al., 1987). The main portions of the brain involved in the onset of puberty are the hypothalamus and pituitary. The hypothalamus is responsible for production and secretion of the decapeptide

gonadotrophin-releasing hormone (GnRH), which stimulates the release of gonadotrophic hormones from the pituitary gland. The pituitary gland is divided into the anterior and posterior pituitary gland, also known as adenohypophysis and neurohypophysis, respectively. The anterior pituitary is responsible for synthesis and secretion of the reproductive hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH). The release of FSH and LH is GnRH dependent. The frequency of the GnRH stimulus is the major factor in the regulation of synthesis and secretion of LH or FSH. Normally, less frequent pulses of GnRH lead to FSH secretion and more frequent pulses lead to LH secretion. GnRH is released into the hypothalamic-hypophyseal portal blood system, which connects the hypothalamus and anterior pituitary, and allows transport of GnRH to the anterior pituitary. GnRH stimulates the release of LH and FSH, which are released into the general circulation and travel to the ovary where they influence, recruitment, growth, maturation and ovulation of follicles (Ginther et al., 1989).

Kinder et al., (1987) suggested that the endocrine system of prepubertal heifers is functional before puberty. A pituitary response to exogenous GnRH in prepubertal heifers resulted in LH and FSH secretion (Schams et al., 1981); but, the magnitude of the secretions varied depending on the heifer's age. Kinder et al. (1987) reported that the frequency and amplitude of LH and FSH secretion was also dependent on animal age, but also the pubertal status of the heifer (Kinder et al., 1987). In other words, how close the heifers were to actually attaining puberty. Pulses of LH have been indentified in heifers as early as 1 month of age (Schams et al., 1981) but the pulses in prepubertal heifer are infrequent and occur once every 4 to 24 hours (Day et al., 1987). Day et al.

(1984) reported that the decreased frequency of LH pulses prior to puberty is due to negative feedback of estradiol. It was also observed that LH pulse frequency increased with heifer age as negative feedback effects of estradiol deceased (Day et al., 1984). Seidel et al. (1971) also reported that exogenous administration of gonadotrophins leads to ovulation in calves up to two months of age supporting the hypothesis that the endocrine system of prepubertal heifers appears to be functional prior the onset of puberty. Although, the endocrine system is technically not functional at an early age since it is still not yet mature enough to stimulate a spontaneous ovulation on its own.

The main factor preventing the onset of puberty is the negative feedback effect of estradiol on the hypothalamus and pituitary (Dodson et al., 1988). Rodrigues et al. (2002) reported increased concentrations of LH in 10 month old in ovariectomized heifers compared to the decreased LH concentrations in ovariectomized heifers of similar age that received estradiol implant. In agreement, Day et al. (1984) reported that LH concentrations increased in ovariectomazied heifers, suggesting that estradiol produced by the ovary has a negative effect on LH secretion. Subsequent research showed that estradiol receptors in the anterior pituitary and hypothalamus decreased as the onset of puberty approaches (Day et al., 1987). A decrease in the number of estradiol receptors leads to a decrease in estradiol negative feedback because the number of estradiol binding sites is decreased. A decrease in the negative feedback caused by estradiol allows gonadotrophins to rise to concentrations sufficient to stimulate follicular growth and eventually the first ovulation (Dodson et al., 1988).

The onset of puberty is also accompanied by metabolites and metabolic hormone changes. During the prepubertal period, there are increases in the circulating

concentrations of GH, IGF-1, insulin, and leptin (Jones et al., 1991; Yelich et al., 1995; Monget and Martin, 1997; Garcia et al., 2002). These hormones have been shown to stimulate LH secretion (Schillo et al., 1992; Lin et al., 2000; Garcia et al., 2002). Hiney et al. (1996) administrated IGF-1 intraventricularly in rats and observed an increase in LH secretion as well as advancing the onset of puberty in the rats. Amstalden et al. (2002) infused leptin into the lateral ventricle of cows and observed an increase in GnRH and LH secretion. In addition, the nutritional status of prepubertal heifers has a major impact on onset of puberty as increased nutrient intake can stimulate the synthesis and secretion of LH, IGF-1, glucose and insulin; thereby, enhancing the onset of puberty. The relationships between nutrition, hormones and onset of puberty will be further discussed later this review.

The knowledge about the alterations in uterine and ovarian development before the onset of puberty and the role they have on onset of puberty is unclear. The ovaries are responsive to gonadotrophins and capable of ovulation shortly after birth (Howe et al., 1962; Seidel et al., 1971) suggesting that maturation of the ovaries is probably not a limiting factor in the onset of puberty. There were no major changes in ovary weight during the 130 d before puberty (Day et al., 1987). The number of small (<3mm), medium (3 to 6mm), or large (7 to 12mm) follicles also do not change as puberty approaches. However, the number of extra-large follicles (>12mm) was greater in heifers that were closest to puberty (Day et al., 1987). Furthermore, Evans et al. (1994) observed an increase in follicle diameter from 2 to 34 wk of age, with the greatest increase occurring between 2 and 8 wk of age. In contrast, Day et al., (1987) reported that uterine weight increases as puberty approaches probably due the increase in

estradiol concentrations produced by the ovary. The increased estradiol could be acting as a growth stimulator and increasing the development of the uterine tissue. It is likely that development of the reproductive tract is dependent on the activity of the hypothalamic-pituitary axis.

In summary, the attainment of puberty is a gradual process, and during this process there are many physiological and endocrine changes that occur, which eventually result in the maturation of the hypoyhalamic-pituitary axis. The primary endocrine factor associated with the onset of puberty is an increase in LH pulse frequency that allows for enhanced follicle development and culminating in ovulation. However, early in the heifers life the main factor preventing the onset of puberty is the negative feedback effect of estradiol on the hypothalamus and pituitary by suppressing LH secretion. The negative effect of estradiol is gradually decreased as the heifers become older. Furthermore, nutrition plays an important role in hastening the onset of puberty as increased nutrient intake can stimulate the synthesis and secretion of LH; thereby, enhancing the onset of puberty.

Factors Affecting Onset of Puberty

Age

Normally the age at which puberty is initiated is defined as the date of the first ovulatory estrus (Arije and Wiltbank,1971). Although several factors affect the onset of puberty, Nelsen et al. (1982) suggested that a minimum age is required for puberty. Moseley et al. (1982) divided crossbred *Bos taurus* beef heifers into heavy and light weight groups to study age at puberty, and observed that age at puberty was decreased by 9 d for heavy weight heifers. They suggested that onset of puberty could be limited by age in heavyweight heifers and by weight in lightweight heifers. Arije and Wiltbank,

(1971) reported that age at puberty is affected by average daily gain. Heifers that grew faster in the preweaning period reached puberty at an earlier age. Sire is also important factor that can influence the age at puberty and this effect is typically manifested in the scrotal circumference of the sire. The genetic correlation between a sires scrotal circumference and age at puberty in its offspring is -0.42 in Brahman (Vargas et al., 1998) and -0.39 in Hereford cattle (Toelle and Robison, 1985). This estimated genetic correlation indicates that sires of above average scrotal circumference should produce progeny that reach puberty at a younger age compared to those produced from sires of below average scrotal circumference. Short et al. (1990) concluded that age at puberty is primarily a function of genetics, level of nutrition, and (or) rate of gain from weaning to the start of the breeding period.

Body weight

Frisch and Revelle (1970) reported that a critical BW must be achieved in order for the attainment of puberty in women. An inverse relationship exists between age at puberty and BW at puberty in beef heifers (Short and Bellows, 1971; Yelich et al., 1995). Furthermore, Arije and Wiltbank (1971) reported that the correlation between age at puberty and BW at puberty was 0.64, suggesting that age at puberty increases when heifers have heavier mature BW. In a review of the literature conducted by Patterson et al. (1992), it was suggested that puberty can be expected to occur at a genetically predetermined target BW that is 55 to 65% of the mature BW of the heifer's damn, which may be dependent on the frame size of the dam (Patterson et al., 1992). Funston and Deutscher (2004) divided spring-born heifers into two groups to evaluate development of heifers fed to attain two different target BW, 55% or 60% at the start of the breeding season and its effect on reproductive performance. Heifers reached 53%

or 58% of their mature BW at the start of the breeding season and had similar pregnancy rates. The result suggested that when target BW fall within a range between 53 to 60% that the attainment of the target BW within that range will lead to similar reproductive performance. It should be noted that these results were obtained from research conducted in *Bos taurus* cattle and similar studies have not been thoroughly evaluated in cattle of *Bos indicus* × *Bos taurus* breeding. It is also important to state that BW alone is not responsible for onset of puberty. The actual physiological changes that occur during the achievement of a target BW, such as hormonal and metabolic changes, could have a significant effect on onset of puberty.

Body composition

Frisch et al. (1981) proposed that attainment of a critical lean to fat ratio is necessary for the onset of puberty and maintenance of regular ovulatory cycles in women. Wilen and Naftolin (1978) suggested an association between body protein content and onset of puberty in rodents. But, the correlation between body composition and onset of puberty in livestock are contradictory and not well understood. Richards et al. (1989) reported the importance that a certain percentage of body fat was necessary for maintenance of estrous cycles in non-lactating beef cattle. Furthermore, Hopper et al. (1993) suggested that the critical threshold levels of fat necessary for occurrence of puberty might be different between cattle breeds. Moore et al. (1985) investigated the relationship between body composition and the occurrence of estrus in ewe lambs divided into two nutritional groups, a low verses a high level of nutrition. Independent of the nutritional treatment, lambs that achieved puberty had greater internal fat compared to lambs that were not pubertal. However, some studies do not support the critical body composition hypothesis for the onset of puberty in livestock (Brooks et al., 1985). Hall et

al. (1994) reported that heifers did not attain puberty at similar body compositions, and there was no consistent change in body composition before puberty. Yelich et al. (1995) also observed different body composition for heifers fed at three different rates of gain and suggested that critical amount of body fat alone may not be responsible for initiation of puberty. Furthermore, lean and bone mass may be more closely related to onset of puberty than fat, since heifers attained puberty with the same bone and lean mass percentage irrespective of nutritional treatment or fat content (Yelich et al., 1995). Bronson and Manning (1991) suggested that available energy in the body modulates the activity of the GnRH pulse generator in regulating reproduction in prepubertal and adult females (Bronson and Manning, 1991). Therefore, total energy available rather than specific substrate, metabolites, hormones, and body composition, may mediate the GnRH pulse generator important for onset of puberty (Schillo et al., 1992).

Breed

The majority of cattle raised in Southeastern United States have some degree of *Bos indicus* breeding. Heifers that are *Bos indicus* or *Bos indicus x Bos taurus* breeds tend to mature slower and reach puberty at an older age compared to *Bos taurus* breed (Warnick et al., 1956; Plasse et al., 1968). However, age at puberty of *Bos indicus* cattle can be reduced when crossed with *Bos taurus*, which is a direct response of heterosis. The role of heterosis on onset of puberty will be discussed later on in this review. Stewart et al. (1980) reported that Brahman heifers were older at puberty compared to Angus, Hereford, Holstein and Jersey heifers. Reynolds et al. (1963) observed Angus, Brahman, Brangus, and Angus x Brahman heifers for onset of puberty over a four year period, and reported that Angus were the youngest heifers to reach puberty (433 d), followed by Angus x Brahman (460 d), Brangus (531 d), and Brahman (816 d). Mukasa-

Mugerwa (1989) reviewed data from around the world and reported that *Bos indicus* heifers reach puberty in a range from 15.6 to 40 months of age. In summary, *Bos indicus* heifers reach puberty at older ages compared to *Bos taurus* heifers, and the inclusion of *Bos taurus* breeding into a *Bos indicus* breeding program can result in heifers that reach puberty at younger ages.

Breeds with large mature size tend to be older and heavier at the onset of puberty compared to breeds with smaller mature size (Martin et al., 1992). Fajersson et al. (1991) observed a significant difference in average age at onset of puberty between Zebu (12.3 months) and Brown Swiss heifers (9.4 months). The difference between breeds for onset of puberty is mainly due genetic selection of the breed toward different production goals. For example, breeds that have been selected for milk production, such as Holstein and Simmental attain puberty at a younger age compared to breeds that are not selected for milk, such as Charolais and Chianina (Gregory et al., 1991; Martin et al., 1992).

Heterosis can also affect age at puberty. Based on Martin et al. (1992), heterosis is by definition the difference between the mean for reciprocal F1 crosses and the mean for parental pure breeds contributing to the cross. Nonadditive gene effects, such as dominance or epistasis, cause heterosis. Wiltbank et al. (1966) suggested that heterosis decreases age at puberty of beef cattle. One-half to three-fourth of the heterotic effect on age at puberty were still present after adjustment for average daily gain in a linear model. In general, crossbred cattle reach puberty at a younger age compared to purebred cattle (Wiltbank et al., 1966). Laster et al. (1976) studied the effects of heterosis on puberty in Hereford × Angus cows bred to Hereford, Angus,

Jersey, South Devon, Limousin, Charolais and Simmental sires, heterosis among breeds resulted in a decrease in age at puberty. Dow et al. (1982) reported that compared to purebred, 18% more of crossbred heifers were cycling at 11.5 months, 30% more at 15 months, and 12% more at 19.5 months. This agrees with Plasse et al. (1968), who observed that Brahman x British heifers achieved puberty at a younger age compared to Brahman heifers. Not only does cross breeding hasten the onset of puberty, there are carry-over effects on other reproductive treatits. Cundiff et al. (1974) reported that heterosis increased calf-crop weaned due to greater first conception rates and pregnancy rates in the crossbred cattle (Cundiff et al., 1974).

Season

Although cattle are not seasonal breeders like sheep and horses, certain aspects of cattle reproduction are affected by season (Schillo et al., 1983). Seasonal variation in sexual activity of *Bos indicus* cattle has been observed (Anderson, 1944). Reproductive activity in Brahman heifers, measured by presence of corpus luteum and uterine tone, increase during the spring, peaks during the summer, and decreases to a minimum during winter (Plasse et al., 1968). It was observed by Stahringer et al. (1990), that Brahman cows had higher incidence of anestrous in months with shortest photoperiods.

The results of the effect of season of birth on age at puberty are contradictory. Spring born heifers reached puberty earlier than fall and autumn born heifers (Menge et al.,1960; Roy et al., 1980). In contrast, Greer (1984) did not find any relationship between season of birth and age at puberty in beef heifers. Hansen (1985) suggested that the discrepancies among research results can be due to factors such as environment, nutrition and breed, since these factors can affect the age of sexual development. Comparisons are confounded, since heifer development occurs over

several seasons, and season of birth can be confounded with season of subsequent development. In an attempt to clarify the time where season influences sexual maturation, a study was done by Schillo et al. (1983) where 28 heifers born in either September or March were reared under natural conditions until 6 months of age. From 6 to 12 months of age, heifers were reared in environmental chambers programmed to stimulate seasonal changes in temperature and photoperiod characteristics of one of the two treatments: A) spring, summer and early autumn or B) autumn, winter and early spring. September born heifers reached puberty earlier than March born heifers, whereas heifers exposed to treatment A were younger than heifers from treatment B. Based on these results it is apparent that season influences onset of puberty during the earlier and later stages of sexual development.

It has also been noted that season has influences on LH secretion in cows and yearling heifers Harrison et al. (1982) reported that Brahman cows exhibited a greater incidence of LH surge and LH surges of greater magnitude during early spring than during winter. Similar observation was reported by Schillo et al. (1983), where heifers between 6 to 7 months of age born in September had greater mean LH concentrations compared to similar age heifers born in March. Hansen et al. (1982) reported that prepubertal ovariectomized heifers exposed to 18 hours light/d released more LH after estradiol injection compared to heifers exposed to natural winter photoperiods, suggesting that there is an effect of photoperiod on LH release. Ovariectomized cows exhibit seasonal fluctuations in LH concentrations both in the presence and in the absence of estradiol (Critser et al., 1983). In summary, it appears that season has some effect on LH secretion, the effects of d length on the mechanisms that control pulsatile

release of LH in the developing heifer is not well understood and remains to be elucidated. Additionally, it is unclear whether the effects of d length actually have a significant effect on animal productivity and overall reproductive performance.

Nutrition

It is well documented that growth rate influences the onset of puberty in beef heifers, and the onset of puberty is highly correlated with weight gain from birth to puberty (Warnick et al., 1956; Menge et al., 1960; Bellows et al., 1965; Ferrel, 1982). Therefore, nutrition of the growing heifer is an important factor that can influence the onset of puberty (Oyedipe et al., 1982). Bellows et al. (1965) fed crossbred heifers at three different levels of nutrition to attain ADG of 0.23, 0.5, and 0.67 kg/d, and observed that heifers fed to gain 0.67kg/d attained puberty 45 and 23 d earlier than heifers fed 0.23 and 0.5 kg/d, respectively. Ferrel (1982) studied the effect of postweaning nutritional levels characterized by low (0.4 kg/d), medium (0.6 kg/d) and high (0.8 kg/d) gain on the onset of puberty, and reported that heifers fed at low rate of gain had the onset of puberty delayed (387 d) compared to medium (365 d) and high (372 d) rate of gain. Normally, as nutrient intake is increased, resulting in greater ADG, age at puberty is decreased (Bellows et al., 1965). This is supported by Yelich et al. (1995), where heifers fed to gain 1.36 kg/d had greater BW and were younger at onset of puberty compared to heifers fed 0.68 kg/d or 0.23 kg/d for 16 wk followed by 1.36 kg/d. In summary, postweaning plane of nutrition is extremely important on onset of puberty. The age at the onset of puberty is inversely correlated with the level of nutrition a heifer is receiving where heifers are fed to gain at a constant rate of gain from weaning to the start of the breeding season. Additional heifer development strategies have been utilized where the timing of the rate of gain is modified. Heifers on these programs start

on low rates of gain and are shifted to increased rates of gain as the start of the breeding season nears. The purpose and effectiveness of these systems will be discussed later in this review.

Several studies have focused on endocrine mechanisms whereby nutrition influences timing of puberty onset in heifers (Schillo, et al., 1992; Day et al., 1986b; Kurtz et al., 1990). The nutritional status of developing heifers influences pulsatile release of LH. Heifers maintained on low-energy diets failed to exhibit an increase in LH pulse frequency at a time when heifers fed a diet adequate for growth exhibited increased LH pulse frequencies and attained puberty (Day et al., 1986b). Kurtz et al. (1990) reported similar results, indicating that restriction of dietary energy prevented the increase in LH pulse frequency in ovary-intact, ovariectomized and ovarectomized estradiol-treated heifers. Scott et al. (1990) concluded that regardless of the pituitary-ovarian axis status (intact, ovariectomized, or ovariectomized and estradiol –implanted), LH secretion responds rapidly to increased dietary energy. In an experiment conducted by Hall et al. (1994) heifers were fed 14.15 Mcal of ME/d attained puberty an average 53 d earlier than those fed 10.84 Mcal of ME/d. Heifers fed 14.15 Mcal of ME/d had greater LH pulse frequencies compared to heifers fed 10.84 Mcal of ME/d; however, plane of nutrition had no effect on LH pulse amplitude. Similar results were observed by Oyedipe et al. (1982) when heifers fed isocaloric diets with low (8.37% CP), medium (13.37% CP), or high (19.17% CP) level of protein were youngest and heaviest at puberty on the high protein diet. Furthermore, Scott et al. (1990) suggested that heifers developed in low plane of nutrition sustained estradiol inhibition of LH, preventing the increase of LH necessary for onset of puberty. The restriction of nutrient intake leads to

a decrease in LH release, which hastens the onset of puberty in heifers (Schillo et al., 1992). In agreement, Day et al. (1986b) observed that heifers maintained on a low-energy diet exhibited a decreased in LH pulse frequency compared to heifers fed a diet adequate. Therefore, reduced nutrient intake during the prepubertal period decreases LH pulse frequency, which can lead to a delayed onset of puberty in heifers (Day et al., 1986b; Hall et al., 1994).

The level of nutrition heifers receive can also impact insulin, glucose, growth hormone (GH), and insulin-like growth factor-1 (IGF-1) concentrations resulting in either a delayed or hastened onset of puberty depending on the level of nutition. Normally, an increased rate of gain leads to increases in IGF-1, glucose, and insulin concentrationbut decreased GH concentration. In addition, nutrient intake has been shown to be inversely related to GH concentrations in ewes (Foster et al., 1989; Kile et al., 1991) and heifers (Petitclerc et al., 1983) and possibly having a negative affect on the onset of puberty. According to these associations, it seems possible that GH is inhibitory, whereas IGF-I, insulin, and glucose are stimulatory to LH release (Schillo et al., 1992). Although, Harrison and Randel (1986) observed that insulin infusions did not influence LH secretion in heifers. In contrast, studies with nutritionally anestrous cows (Richards et al. 1989a,b) observed that decreased nutrient intake resulted in decreased glucose concentrations and the authors suggested that lack of glucose may not allow for adequate LH secretion. Therefore, it has been suggested that availability of metabolic fuels can influence hypothalamic-pituitary axis function (Hillman et al., 1991); however the mechanism of action is unclear. Additionally, mean IGF-1 concentrations have been shown to increase before puberty (Jones et al. 1991; Yelich et al., 1995). Granger et al.

(1989) indicated that decreased IGF-1 were positively associated with delayed puberty in heifers fed low-quality hay compared to heifers fed hay plus a grain supplement. However, the mechanism by which IGF-1 enhances the onset of puberty is unclear.

The hormonal and metabolic signals that communicate the level of energy to the hypothalamic-pituitary axis to hasten onset of puberty are still unclear; however it is known that leptin plays an important role on this mechanism. A linear increase in plasma leptin concentrations is observed in prepubertal heifers (Garcia et al., 2002); however, nutrient restriction can decrease leptin concentrations in cattle (Block et al., 2003; Brown et al., 2005), affecting onset of puberty in heifers. Chelikani et al. (2009), evaluated the effect of 0.50, 0.80, and 1.10 kg/d average daily gain on leptin secretion of prepubertal dairy heifers, and concluded that plasma leptin concentrations may not be a critical trigger for puberty in rapidly growing heifers, but a certain threshold of leptin concentrations appears important for puberty especially in heifers with normal or restricted growth rates.

In summary, nutrition can drastically affect onset of puberty. The nutritional status of developing heifers influences leptin secretion, which plays an important role signaling the level of body energy to hypothalamic-pituitary axis. Generally, heifers maintained in a low-plane of nutrition diet has delayed puberty due to decreased LH secretion, as well as decreased IGF-1, glucose and insulin concentrations. The ideal level of nutrition is essential to allow adequate LH pulses frequency for onset of puberty.

Management

Age at onset of puberty can be affected by management decisions (Greer et al., 1983), such as feeding programs, nutritional supplements, genetic selection, and progestogen treatments. The goal of any heifer development program should be to

obtain as many estrous cycling heifers as possible before the start of the breeding season. There are many products and management strategies that can be utilized on the heifer's development program to decrease age at puberty.

One of the most common strategies is sorting heifers into groups based on BW. This strategy allows nutritional requirements to be met more precisely, and it makes it easier for heifers to. attain the desired target BW before the start of the breeding season (Staigmiller and Moseley, 1981). Varner et al. (1977) reported that heifers sorted by BW attained puberty earlier and had greater pregnancy rates compared to unsorted heifers. Wiltbank et al. (1985) sorted heifers into two weight groups, and then divided each group into either a light or heavy target BW group. Both groups achieved their target BW before the breeding season. Puberty at the start of the breeding season and 20, 40, 60 and 93 d after the start of the breeding season did not differ between treatments. Therefore, separation of heifers based on BW may allow nutritional requirements to be met more precisely, and possibly decreased age at puberty and improved reproductive performance without overfeeding heavy weight heifers.

The use of ionophores, can also be used as a management tool to enhance growth and possibly the onset of puberty in heifers. Ionophores increase feed efficiency and rate of gain in cattle by altering the rumen microflora and decreasing the acetate:propionate ratio. Patterson et al. (1992) concluded that varying the quality or quantity of feedstuff may alter ruminal fermentation leading to an increase in propionate productions. Propionate can modify hormone secretion, such as increasing IGF-I, which subsequently influence the mechanism that regulate puberty Inclusion of ionophores into the diet of growing heifers has also been reported to decrease age at

puberty. Moseley et al. (1982) reported that supplementation of monensin (200 mg/head/d) with a high roughage diet decreased age at puberty for heifers in heavy weight class compared to lighter heifers. The authors suggested that decrease in age at puberty was not due to greater BW or ADG, but greater proprionate production, which altered the endocrine status and hastened the onset of puberty in heifers. Similarly, Purvis and Whittier (1996) observed a slight decrease in age at puberty in heifers fed monensin (200 mg/head/d; 425 d) compared to control heifers that did not receive monensin (433 d). Furthermore, they reported a tendency for greater first service conception rate for heifers fed monensin compared to control heifers. Lalman et al. (1993) observed that heifers fed monensin were on average 21 d younger at puberty compared to control heifers not fed monensin; however, no differences in overall breeding season pregnancy rates were observed between monensin (84%) and control treatment (82%).

Genetic selection of heifers can also be used as a management tool to decrease age at puberty. Even though the heritability estimates for age at puberty are low (0.20; Arije and Wiltbank, 1971), this trait should still respond to a certain extent to selection. Furthermore, selection of growth traits, such as height of heifers at puberty, is correlated with pubertal traits (Baker et al., 1988). Wolfe et al. (1990) indicated that heifers selected for growth traits were younger at puberty compared to heifers not selected for growth. It is important to understand that selection of growth traits means that heifers are being selected for faster growth. This difference from selection of animals for larger frame size and BW has a negative impact on onset of puberty, resulting in an increased age at puberty for heifers. Besides growth trait selection, one of the most practical

methods to decrease age at puberty in heifers is to select daughters of bulls with large SC (Smith et al., 1989). Moser et al. (1996) analyzed results from a selection study based on expected progeny difference (EPD) for SC in the Limousin breed, and reported that there was a 25 d advantage in age at puberty for heifer progeny of high-EPD SC sires compared to Low-EPD SC sires.

Progestogen used for estrous synchronization, such as melengestrol acetate (MGA), SynchroMate-B implant and controlled intravaginal releasing device (CIDR) can also be used to induce cyclicity in prepubertal heifers that are close to attaining puberty (Patterson et al.,1989). The progestogen treatments appear to stimulate LH secretion, which stimulates follicle development leading to ovulation after withdrawal of the progestogen treatment. Imwalle et al. (1998) tested the hypothesis that prepubertal heifers treated with MGA for 8 d would ovulate after MGA withdrawal due to rise of LH secretion. Prepubertal Angus heifers were randomly assigned to one of the two treatments, MGA treated heifers and untreated control heifers. After MGA withdrawal, all of the MGA-treated heifers had ovulated within 10 d, whereas only 44% of the control heifers had ovulated. The authors concluded that the MGA, stimulated LH pulse frequency during MGA treatment, and MGA withdrawal enhanced the onset of puberty by stimulating follicle development and eventually ovulation. Similar results were observed by Anderson et al. (1996) where 6 out of 7 prepubertal heifers treated with norgestomet implant ovulated within 10 d of implant withdrawal while none of the untreated control heifers ovulated during that period. Furthermore, Jaeger et al. (1992) evaluated puberty after MGA treatment in yearling beef heifers, and observed that 75% of heifers treated with MGA reached puberty before the start of the breeding season

compared to 45% of untreated heifers. Lucy et al. (2002) synchronized yearling beef heifers with a 7 d CIDR with PGF on day 6 of CIDR treatment and induced estrus in 48% of the heifers that were prepubertal at CIDR insertion.

Management strategies of replacement heifer development should focus on factors that enhance physiological processes that promote puberty. As we broaden our understanding of the basic principles that govern onset of puberty in the heifer and of the major factors that influence puberty, management practices will undoubtedly be modified to allow producers to enhance production efficiency and improve profitability of the cow-calf operation (Patterson et al., 1992).

Protein Supplementation

Beef cattle in Florida are mainly produced in forage-based systems. Low-quality forages are one of the most common available feed resources in Florida, as well as worldwide (Bandyk et al., 2001). To optimize the utilization of forage, especially low- to medium-quality forages, provision of additional nutrients is necessary (Köster et al., 1996). Protein is often one of the most limiting nutrients in diets of cattle raised in a forage-based system (Anderson et al., 1988a; Hughes and Hersom, 2009). Protein supplementation to cattle grazing low-quality forages has been shown to stimulate forage intake, digestion, and animal performance (DelCurto et al., 1990; Bandyk et al., 2001). Moore et al. (1991a) analyzed 637 samples of common Florida grasses and reported an average crude protein (CP) between 5 – 7 % on a dry matter basis. Based on NRC requirements, growing cattle require an average of 8.3 % of CP to sustain desirable growth rates (> 0.5 kg/d). Generally, animals raised on forages of less than 7% CP and offered a protein supplement have a significant improvement in performance, even though that can vary depending on environmental and management

effects (Mathis et al., 2000). The difference in improvement of animal performance from protein supplementation is also due the TDN to CP ratio of the diet (Moore at al., 1991a). When the TDN to CP ratios are low (< 8), there is an indication that there is adequate protein to match energy in the forage (e.g., when TDN=60%, and CP=12%, TDN:CP=5). However, when TDN to CP ratios are high (> 8) there is an indication that there is a deficiency of protein relative to energy (e.g., when TDN=54%, and CP=6%, TDN:CP=9) (Moore et al., 1991a). In addition, it is often difficult to determine the TDN to CP ratio of the diet on grazing cattle due the selective grazing nature of cattle (Kunkle et al., 1994). However, Moore et al. (1991a) suggested a TDN to CP ratio of 7 as the critical level for grazing cattle.

Dietary CP can be divided in two types. The first type is known as rumen degradable protein (RDP) or degradable intake protein (DIP), which is the protein that is degraded by the microbes in the rumen. This process will convert the RDP to either ammonia or amino acids that can be further utilized by rumen microbes to produce microbial protein (Butler, 1998). The remaining portion of CP is known as rumen undegradable protein (RUP) or undegradable intake protein (UIP), which is not degraded in the rumen and ultimately will be absorbed in the small intestine. Beef cattle need to meet RUP and RDP requirements so satisfactory animal performance and reproduction can be achieved. The microbial protein is responsible for only about 50% of the metabolizable protein required for beef cattle (NRC, 1996). Even though microbial protein has a high quality amino acid profile that complements the protein requirements for ruminants (Santos et al., 1998), alone it is not enough to promote desirable growth and reproductive performance of beef cattle produced in low-quality forage based

system. Furthermore, the synthesis of microbial protein is related to forage quality, thus microbial protein is likely to be lower on low forage quality (NRC, 1996).

Rumen Degradable Protein

Generally, RDP is the first limiting dietary component in the utilization of low-quality forages (Austin, 2009). Since protein supplementation can be quite costly, achieving the necessary amount of RDP to maximize animal performance is important (Köster et al., 1996). Hafley et al. (1993) conducted an experiment to evaluate supplementation of protein of various ruminal degradability on steers grazing warm-season grass and concluded that weight gains increased when RDP was offered. The improvement in cattle performance when supplemented RDP is probably due to the increase of forage digestibility, dry matter intake (DMI) and improvement of metabolizable energy use (Owens et al.,1991). Bandyk et al. (2001) observed a 62% increase in hay organic matter intake when steers were supplemented RDP, such as casein. Hannah et al. (1991) also reported benefits of RDP supplementation of steers consuming dormant bluestem-range forage; RDP supplementation promoted an increase in forage intake and flow of nutrients to the small intestine. In agreement, Guthrie and Wagner (1988) observed a quadratic increase of hay and total intake, as well as improved digestibility, when increasing amounts of soybean meal offered to heifers consuming prairie hay. Köster et al. (1996) also examined the effects of RDP on DMI and digestion of low-quality hay by beef cows. Cows were supplemented with sodium caseinate that provided 0, 180, 360, 540, and 720 g/d RDP. Forage DMI increased quadratically with the increasing supplemental RDP. The authors concluded that increasing RDP levels in the diet generally improves forage utilization.

However, diets containing excess amounts of RDP are undesirable due to excess N, which has a negative impact on animal performance (Poos et al., 1979). Mathis et al. (2000) conducted a research study with canulated steers fed bermudagrass hay and supplemented with RDP (sodium caseinate) placed in the rumen with levels of 0.041, 0.082, and 0.124% of BW and observed an increase in ruminal ammonia N concentrations with increasing levels of RDP. The same result was observed by Krysl et al. (1989) with steers supplemented 0.5 kg of soybean meal daily, which had greater ruminal ammonia concentrations compared to non-supplemented steers. Excess of RDP supplementation appears to have a negative impact on reproductive performance of cattle through an associated decline in fertility. Elrod and Butler (1993) reported that heifers fed 50% excess of RDP requirements had greater plasma urea nitrogen (PUN) concentrations compared to control heifers fed to meet RDP requirements, 23.6 mg/dl and 14.8 mg/dl, respectively. Heifers fed excess RDP also had lower conception rates (61%), compared to heifers fed a total mixed ration to meet RDP requirements (82%). This result agrees with Kaim et al. (1983) where cows fed 20% CP diet that resulted in higher PUN concentrations (16.8 mg/dl) and decreased pregnancy rates compared to cows fed 15% CP. In contrast, Ferguson et al. (1991) suggested that PUN concentrations between 10 and 20 mg/dl did not influence pregnancy rates; however, PUN concentrations greater than 20 mg/dl were extremely detrimental to fertility. The reason for the decline in fertility is unclear but appears to be associated with changes in uterine pH (Butler, 1998). A decreased uterine pH might influence uterine secretory activity and alter embryo survival; however, how these alterations are related remains unclear (Elrod and Butler, 1993).

Rumen Undegradable Protein

There are many byproducts utilized as high RUP source in the ruminant diet, such as fish meal, meat and bone meal, feather meal, blood meal, corn gluten meal, dried distillers grains, and brewers dried grains (Santos et al., 1998). The supplementation of RUP can increase ADG and feed efficiency in growing cattle fed forage-based diets. Dhuyvetter et al. (1993) fed early and late calving beef cows supplements that were isonitrogenous containing either 25% or 50% RUP. Late-calving cows weight responses were unaffected by level of protein supplementation. However, early-calving cows fed 25% RUP lost 39 kg more weight compared to cows fed 50% RUP. The authors concluded that rumen function was optimized with the additional protein provided by the 50% RUP supplement. Hafley et al. (1993) supplemented yearling steers grazing native range with an energy control supplement, energy control supplement plus 100 g/d of RUP, energy control supplement plus 200 g/d of RUP, and a control group with no supplement. The ADG was greater for steers supplemented with 200 g/d of RUP (1.01 kg/d) compared to 100 g/d of RUP (0.93 kg/d), energy control (0.91 kg/d), and no supplement (0.86 kg/d) steers. Anderson et al. (1988a) also supplemented steers grazing smooth bromegrass with RUP. The treatments consisted of 0, 110, 230, and 340 g/head/d of RUP supplement. A linear increase in gain with increasing levels of RUP was observed with a trend for a quadratic response. Maximum gains were observed at the 230 g/head/d of RUP inclusion. Karges et al. (1992) also examined the effects of RUP supplementation in growing cattle grazing native summer pasture for 83 d. Treatments included negative control, energy control, three levels of RDP (0.15, 0.27, and 0.37 kg/d), and three levels of RUP (0.07, 0.14, and 0.21 kg/d). No response to the RDP supplementation was observed; however, supplementation of RUP caused a linear

increase in cattle gains. The authors suggested that the additional weight gains from supplemental RUP indicates that microbial protein synthesis may not be sufficient to satisfy the metabolizable protein requirement, thereby limiting gains by the cattle. Also, the variation of the amino acid profile on different RUP sources influences the response of animal performance. In agreement, Gutierrez-Ornelas and Klopfenstein (1991) reported that responses to supplemental RUP by steers grazing corn residues were not constant throughout the grazing season, suggesting that RUP supplementation plans should consider forage availability and diet composition.

In summary, RUP supplementation can increase performance in grazing cattle. Considerable variation in animal performance to RUP supplementation is evident. The variation in responses is mainly due to different basal diets, different levels of RUP included in the diet, and interactions of the aforementioned factors with the environment and management.

Even though RUP is related to positive effects on animal performance, excessive supplementation of RUP can have detrimental effects. High levels of RUP supplementation can cause changes in body metabolites, as well as ovarian and pituitary function, thereby, altering the reproductive and growth performance of cattle. Supplementation of high levels of RUP has been shown to increase concentrations of PUN primarily due to deamination of excess metabolizable protein being fed (Dhuyvetter et al.,1993). Lalman et al. (1993) reported high PUN concentrations for beef heifers fed an additional 250 g of RUP compared to heifers fed 94 g of RUP. Excess RUP in the diet increases PUN concentrations and this might lead to some reproductive problems in cattle. Accordingly to Butler (1998), as PUN concentrations increase they

decrease uterine pH. Furthermore, Kane et al. (2004) reported that ovarian and pituitary function in heifers may be influenced by RUP levels. Diets high in RUP (312 g/d) caused decreases in anterior pituitary LH and FSH concentrations compared to low (115 g/d) and medium (216 g/d) amounts of supplemental RUP.

Lalman et al. (1993) also reported low cholesterol concentrations for cattle feed blood meal and corn gluten meal, which are supplements with high RUP concentrations. The same result was observed by Lindberg (1984) in lactating cows fed a high protein diet. The underlying mechanism for the decreased cholesterol concentrations is unclear. Lalman et al (1993) suggested that the increased amino acid from the high RUP supplement might have an impact on cytochrome P-450 enzymes leading to an increase of steroid hormone synthesis; therefore, reducing the total cholesterol concentrations. Insulin concentrations are also altered in animals fed supplements high in RUP content. Normally, concentrations of insulin increase as RUP increases in the diet (Laven et al., 1999). Blauweikel and Kincaid (1986) reported greater serum insulin concentrations in cows fed a diet containing 19% CP compared to cows fed a diet with 14% CP. Lalman et al. (1993) also reported increased insulin concentrations for heifers fed a diet with a increased levels of RUP. Insulin seems to alter ovarian response by increasing ovulation rate in Brangus heifers administered pulsatile infusion of insulin and treated with FSH; although the exact role of insulin on ruminant reproduction is still unclear (Harrison and Randel, 1986).

Non-protein nitrogen supplementation

Dietary protein can be divided as natural protein and non-protein nitrogen (NPN). Natural proteins contain naturally occurring essential and non-essential amino acids. In contrast, NPN sources are not protein but can be converted into proteins by the rumen

microbes. NPN sources consist of urea, biuret, diammonium phosphate, and anhydrous ammonia, with urea being the most common source added to ruminant diets. Urea is not very palatable compared to other protein supplement sources. Schaadt et al. (1966) compared the palatability of soybean meal, urea, and diammonium phosphate. Soybean meal was more palatable than the other NPN supplements, but urea was more palatable than diammonium phosphate. Furthermore, the addition of urea in excess may lead to feed refusal (Farmer et al., 2004).

Non-protein nitrogen is rapidly dissolved and hydrolyzed to ammonia by microbes in the rumen and the ammonia is utilized by the microbes for synthesis of amino acids or absorbed across the rumen wall into the bloodstream. The amino acids synthesized by the microbes are converted to microbial protein, and the circulating ammonia is metabolized into urea in the liver. However, excess ammonia consumption and (or) rapid absorption can be toxic. The symptoms of ammonia toxicity are uneasiness, tremors, excessive salivation, rapid breathing, incoordination, bloat, and tetany. These symptoms usually occur in about the order listed. Tetany is the last symptom before death occurs.

A considerable amount of research has been conducted with NPN sources to find the effects of NPN supplementation on animal performance. Clanton (1978) compared the effects of urea and biuret to soybean meal and alfalfa supplementation on growing calves. Calves had improved weight gains for all protein supplement treatments. Across all experiments, gains were either less or not different as level of NPN increased. However, ADG were greater for soybean meal and alfalfa compared to NPN sources. Brown and Adjei, (2001) examined the effects of protein supplementation in molasses

based supplements on the performance of growing cattle fed limpograss. Cattle were supplemented with varying protein sources including molasses, molasses + urea, molasses + feather meal, and molasses + urea + feather meal. In years 1 and 2, ADG was not influenced by protein supplementation; however, in year 3, protein supplementation improved ADG. The response to RDP from urea was similar to that obtained by RUP from feather meal, suggesting that NPN could be a replacement for natural proteins sources. Stateler et al. (1995) also examined the effect of protein supplementation in molasses slurries on performance of growing cattle fed bermudagrass hay. Cattle supplemented with molasses + urea had the same performance as cattle fed only molasses. In contrast, supplementation with soybean meal + molasses and molasses + blood feather meal + feather meal had greater ADG compared to molasses + urea supplementation. The authors concluded that the use of NPN in growing cattle is not recommended. Even though, NPN sources in most cases do not provide the same animal performance as natural protein sources, it still provides an improvement in animal performance.

Furthermore, NPN is mainly recommend for use in high concentrate diets instead of high-roughage diets for cattle due to the decreased TDN content of high roughage diet. Diets high in digestible energy such as high grain diets result in good urea utilization. In conclusion, NPN can be added to the diet as alternative protein source to decrease production cost due to the lower cost of NPN products. However, NPN should be offered continuously to animals and within adequate levels to prevent ammonia toxicity.

Energy Supplementation

Energy requirements for growing beef cattle can vary due to different seasons of the year (Senft et al., 1987), production level (Caton and Dhuyvetter, 1997), gender, age, temperature, physiological state, and previous nutrition (NRC, 1996). Lauren et al. (1991) also observed differences in energy requirements between breeds where Simmental had greater requirements compared to Angus cattle. Based on NRC (1996) *Bos indicus* beef breeds require about 10 percent less energy compared to *Bos taurus* beef breeds for maintenance, with crossbreds in between. There is also a difference in energy requirement between grazing and confined ruminants. Data from Osuji (1974) indicate that energy expenditure by grazing sheep can be 30% greater compared to confined sheep. Energy expenditure related to grazing is associated with forage availability, as forage availability declines grazing time increases and energy requirements increase (Caton and Dhuyvetter, 1997). It has also been shown by Krysl and Hess (1993) that increasing levels of energy supplementation decrease grazing time; therefore, if grazing time is decreased, energy demand should also decrease.

Energy supplementation for grazing ruminants is necessary in situations where energy availability is too low to meet desired production levels, which is normally due to large fluctuations in the amount of available forage. (Horn et al., 1995; Caton and Dhuyvetter, 1997). The main source of energy in supplements for ruminants is carbohydrates. Carbohydrates can be classified as structural carbohydrates (SC) and non-structural carbohydrates (NSC). The SC are the fibrous portions of plants located within the plant cell wall. The main function of SC are to provide structure to the plant; examples of structural carbohydrates are hemicellulose, cellulose, lignin, pectins, and beta-glucans. These components are known for slow digestion and they are accounted

for as neutral (NDF) and acid (ADF) detergent fiber in an analysis. The NSC are located in the leaves and seeds of plants. Examples of NSC are starches, simple sugars, organic acids, and fructans, which are rapidly degraded in the rumen.

High amounts of NSC supplementation may cause detrimental effects on animal performance; however, animal responses to high amounts of NSC energy supplementation is not consistent due to variation in the amount and source of supplement (Caton and Dhuyvetter, 1997). For example, the starch in processed wheat, oats, and barley is generally more degradable compared to the starch in processed corn. The digestibility of starch from sorghum is the lowest of the commonly used grains (Nocek, et al., 1991). Supplements containing high amounts of NSC, such as grains, when supplemented in excess may increase the amount of volatile fatty acids (VFA) produced during fermentation by the rumen microbes. Furthermore, high concentrations of VFA can negatively affect the buffering and absorption capacity of the rumen leading to a decrease in ruminal pH, which can be detrimental to fibrolytic bacteria resulting in decreased forage digestibility (Aldrich et al., 1993; Knowlton et al., 1998). Ulmer et al. (1990) observed a linear decrease in 16- and 48-h in situ NDF digestion from increasing barley supplementation in beef steers fed medium quality hay. The same result was observed by DelCurto et al. (1990) where NDF digestibility tended to be depressed with increased amounts of energy supplementation in the form of sorghum grain. However, Pordomingo et al. (1991) observed no effect on rumen pH of beef steers grazing summer blue grama rangeland and supplemented corn at 0.2, 0.4, and 0.6% of their BW, probably because the amount of NSC was not enough to have an effect on the rumen environment. Mertens (1977) suggested that forage digestibility decreases when

rumen pH falls below 6.7. Furthermore, Russell et al. (1979) observed that populations of cellulolytic bacteria decreased when pH ranged from 5.8 to 6.2, whereas soluble carbohydrates fermenting bacteria did not have a considerable decrease until ruminal pH ranged from 4.6 to 4.9. Decrease in forage intake is also observed when cattle on a forage based diet are fed supplements composed of high amounts of NSC. Bodine and Purvis (2003) observed reduced feed intake in steers grazing native tallgrass prairie and supplemented dry-rolled corn compared to steers not supplemented with corn.

Usually, supplementing grazing cattle with low to moderateamounts of NSC does not greatly affect the rumen pH (Caton and Dhuyvetter, 1997). In addition, the adverse effects of a diet containing high amounts of NSC on grazing cattle performance can be prevented by using high-fiber byproduct feeds in the diet, such as soybean hulls and corn gluten feed, which can be an alternative to formulating energy supplements with fairly high energy densities (Horn et al., 1995). Anderson et al. (1988b) evaluated the use of soybean hulls as an alternative to corn for energy supplementation of grazing cattle and reported that heifers fed soybean hulls had the same ADG to heifers fed corn. Horn et al. (1995) also observed no differences in daily gain and feed conversion between steers supplemented ground corn or soybean hulls.

Energy requirements can also be achieved with high-fat supplement, even though grazing ruminants do not consume significant quantities of fat under natural conditions. The addition of fat in the diet has been a common practice to meet the desired energy requirement of the diet. Fats are classified as lipids, which are biological compounds that can be dissolved in organic solvents. Lipids include cholesterol, phospholipids, and triglycerides. Fat used in ruminant diets is most commonly in the form of triglycerides.

After ingestion, bacterial enzymes in the rumen break down the triglycerides to free fatty acids and glycerol. The glycerol is fermented to VFA such as propionic acid. Fatty acids can be classified as unsaturated and saturated fatty acids. Saturated fatty acids contain no double bonds and are solid at room temperature; whereas, unsaturated fatty acids have at least one double bond, vary in melting point, and tend to be liquid at room temperature. Unsaturated fatty acids are rapidly modified to saturated fatty acids in the rumen, a process called biohydrogenation. According to Chilliard (1993), 70 to 90% of polyunsaturated fatty acids are transformed to saturated fatty acids in the rumen.

Many different types of fat sources can be offered as energy supplements for cattle, such as tallow, vegetable fat, and whole oilseeds (cottonseed, soybeans, canola seeds). The fatty acid profile of these fat sources varies widely. Most of the naturally occurring plant oils contain a large profile of unsaturated fatty acids. For example, whole soybeans and whole cottonseed have a fatty acid profile of 85% and 71% of unsaturated fatty acids and 15% and 29% of saturated fatty acids, respectively.

The effects of fat supplementation in ruminants has been investigated intensively in the past decade (Hess et al., 2008). Banta et al. (2008) reported that there was no effect on OM digestibility of cows fed bermudagrass hay and supplemented whole soybean. However, fat supplementation often leads to a decrease in fiber digestion, especially when added to more than 5% of total dry matter intake (Kowalczyk et al., 1977; Doreau and Chilliard, 1997; Williams et al., 2000). This is due the alteration of some rumen microorganisms, particularly cellulolytic bacteria (Kowalczyk et al., 1977). Another effect of fat supplementation is the decrease of the protozoa population in the rumen, which contribute to cellulolysis (Doreau and Chilliard, 1997). It has also been

observed that modifications of fiber digestion are influenced by fatty acid composition, where short-chain fatty acids lead to greater depression in fiber digestion compared to long-chain fatty acids. Furthermore, unsaturated fatty acids cause a greater reduction in fiber digestion compared to saturated fatty acids (Macleod and Buchanan-Smith, 1972; Clapperton and Steele, 1983).

The effects of fat supplementation on reproduction are unclear due many uncontrolled management factors (Staples et al., 1998). Bilby et al. (2005) stated that supplementation of diets with several fat sources varying in fatty acid profile improved overall pregnancy rates of dairy cattle due to beneficial effects on the follicle, oocyte, embryo, and uterus. Thomas et al. (1997) observed that polyunsaturated fatty acids enhanced follicular growth more than saturated fatty acids or highly unsaturated fatty acids; whereas Beam and Butler (1997) reported that supplemental fat in the diet increased dominant follicle size in dairy cattle. Similar results were observed by De Fries et al. (1998), where cows fed rice-bran supplement, which is rich in oleic and linoleic acid, enhanced follicular development due the stimulation of larger follicles into ovulatory-sized follicles. Mattos et al. (2000) also reported that the benefits of fat supplementation in reproduction were related with increased steroid and eicosanoid secretion. Eicosanoids are biologically active, oxygenated metabolites from fatty acids. They act as signaling molecules with either autocrine or paracrine functions and serve as important mediators in reproduction, immune function, and ion transport. The increase in steroid and eicosanoid secretion can alter uterine and ovarian function; however, this effect is somewhat independent of energy. Other reported effects of fat supplementation are increases in conception rates, pregnancy rates, and cows

exhibiting stronger signs of estrus, and decreased secretion of $PGF_{2\alpha}$. (Scott et al., 1995; Staples et al., 1998). The decrease in uterine $PGF_{2\alpha}$ secretion due fat supplementation is mainly due the inhibition of enzymes involved on $PGF_{2\alpha}$ synthesis. As a result, secretion of $PGF_{2\alpha}$ can be suppressed, thus potentially preventing early embryonic death and decreasing the sensitivity of the corpus luteum to $PGF_{2\alpha}$ (Staples et al., 1998).

Dried Distillers Grain with Solubles as Supplement

Corn had been used as a feedstuff for cattle for a long time. However, during the past years a greater amount of corn production has been used mainly for ethanol production in the United States. A byproduct in the corn dry milling process associated with ethanol production is dried distillers grain with solubles (DDG), which can be used as a corn substitute in beef cattle supplementation (Leupp et al., 2009). Dried distillers grains could be an effective supplement for forage-based beef productions systems because it provides high energy and protein concentrations as well as high phosphorus content. This is important since it would reduce the need of phosphorus supplementation for many forage types (MacDonald and Klopfenstein, 2004).

Dried distillers grain is produced from the corn's starch fermentation during ethanol production. About two thirds of the corn is starch. Starch is fermented during the dry milling process and the remaining nutrients are increased 3-fold compared to corn on a percentage basis. Protein increases from about 10 to 30%, fat from 4 to 12%, NDF from 12 to 36%, and P from 0.3 to 0.9% of DM (Klopfenstein et al., 2008). Because of the increased protein concentration of DDG compared to corn, DDG is primarily used as a protein source supplement (Klopfenstein et al., 2008). Dried distillers grain contains

more than 50% of RUP with values ranging from 47% to 69% (Schingoethe, 2006). The main protein in DDG is zein, which is highly undegradable (McDonald, 1954). Most of the degradable protein in corn is degraded during the ethanol fermentation process, which results in increased concentrations of RUP in DDG (Schingoethe, 2006).

Additionally, the elevated level of RUP is related to the drying process of the byproduct after extraction of ethanol and the high temperatures during the drying process may cause denaturation of proteins. Firkins et al. (1984) reported 47% RUP for wet distillers grain (WDG) and 54% RUP for DDG, indicating that almost half of the protein is probably denatured during the drying process. Furthermore, research was conducted by Ham et al. (1994) to determine the effect of three levels of heat damage on DDG protein and energy utilization in steers utilizing diets with low, medium and high protein heat damage measured by acid detergent insoluble nitrogen (ADIN). They conclude that levels of ADIN did not affect daily gain, dry matter intake, or feed efficiency of steers.

Martin et al., (2007) conducted an experiment to determine if high levels of RUP from a DDG supplement would affect growth and reproduction of yearling heifers compared to hefiers fed corn gluten feed plus whole corn germ, which is slightly lower in RUP compared to DDG. Heifer growth was not affected by nutritional treatment; however, heifers fed DDG had greater AI pregnancy rates compared to corn gluten plus whole corn germ fed heifers. Engel et al. (2008) evaluated the reproductive performance of heifers fed hay and supplemented with DDG or soybean hulls, and observed greater pregnancy rates for DDG heifers (94%) compared to soybean hulls (84%), suggesting that the better reproductive performance was due to the high fat and

RUP content from DDG. Furthermore, Harris et al., (2008) compared whole soybeans (WSB) to DDG and concluded that heifers fed DDG had greater ADG compared to WSB fed heifers during the trial period. In summary, the results aforementioned suggest that supplementing growing heifers with DDG might have a positive impact on reproductive and growth performance.

As with most of corn products, lysine concentrations are low and the first limiting amino acid in DDG is lysine (Spiehs et al., 2002; Schingoethe, 2006;). The mean amino acid composition of DDG is 0.85% Lysine, 0.55% Methionine, 1.20% Arginine, 0.76% Histidine, 1.47% Phenylalanine, 0.25% Trypyophan and 1.13% Threonine. Lysine and methionine are the two most variable amino acids in DDG, ranging from 0.72% to 1.02% and 0.49% to 0.69%, respectively (Spiehs et al., 2002). In agreement, Cromwell et al. (1993) reported that lysine values were extremely variable in DDG, with more than twice the difference among DDG sources, and lysine decreased as crude protein concentration decreased with a correlation coefficient of 0.80.

Dried distillers grain is a unique supplement because it can be used not only as a protein source it can be used as an energy supplement since it is high in NDF and fat content. In addition to protein, NDF is also concentrated in DDG, but with low amounts of lignin (Klopfenstein et al., 2008). Spiehs et al. (2002) reported NDF values of 36.8% to 44.5% and ADF values of 13.8% to 18.5% in DDG. Thus, DDG is a source of readily fermentable fiber for beef cattle diets and can supply energy for growing cattle without decreasing the rumen pH, which is what occurs when corn is fed because of its increased starch content. However, because of the small particle size of DDG, itmay lack sufficient effective fiber (Schingoethe, 2006). Moreover, when DDG is fed as an

energy source (> 15% of the diet dry matter intake), protein is commonly supplied in excess of requirements (Martin et al., 2007).

In addition to fiber, fat is also energy source found DDG. Spiehs et al. (2002) reported mean crude fat content of DDG of 10.9 % and Cromwell et al. (1993) reported values of 9.3% and 9.7%. The fat content profile of DDG is mostly unsaturated fatty acids. Vander Pol et al. (2009) conducted a feedlot study and observed increased unsaturated fatty acid concentrations (30.9% of total fat) in duodenal content of steers fed WDG compared to animals fed corn oil (10.8% of total fat). This result suggests that some fat from WDG is protected from biohydrogenation in the rumen; therefore, a similar effect may be occur with DDG.

The utilization of DDG in the beef cattle diets and its positive effects on growth and reproductive performance has been documented by several researchers (Martin et al., 2007; Harris et al., 2008; Engel et al., 2008). However, it is important to utilize the right amount of DDG in the diet so performance can be maximized. Buckner et al. (2007) conducted a feedlot study comparing 10%, 20%, 30%, and 40% levels of DDG as a percentage of the total diet compared to a control corn diet. There was a trend for a quadratic response for feed efficiency, and the optimal inclusion rate was 20% of the diet on a dry matter basis. Leupp et al. (2009) also analyzed different levels of DDG in the diet (0%, 15%, 30%, 45% and 60% of dry matter) and concluded that 45% of DDG in the diet dry matter maximized digestion and fermentation of growing steers. Nichols et al. (2009) reported similar results when wet distillers grain (WDG) was added to finishing steer diets. They observed a linear decrease in DMI as WDG were added to the diet and concluded that 40% of WDG would maximize animal performance. In

summary, most researches (Bucker et al., 2007; Leupp et al., 2009; Nichols et al., 2009) reported 40% to 50% of diet dry matter being the ideal level of DDG inclusion in the beef cattle diet for optimal performance. However, all of the aforementioned studies were conducted with steers or finishing beef cattle; therefore the recommendations above are not for cattle raised on a high-roughage diet. Morris et al. (2005) conducted a study to evaluate the effect of increasing levels of DDG on forage intake and performance of heifers consuming either a low or high quality forage diet. Heifers were fed smooth brome grass hay, a low-quality forage, or alfalfa hay and sorghum silage mix, a high-quality forage and supplemented with 0, 0.75, 1.35, 2.05 or 2.75 kg/head/d of DDG. It was observed that forage intake decreased as DDG concentration increased; furthermore, ADG increase linearly with DDG supplementation. Loy et al. (2007) also observed a decrease in forage DMI when heifers were supplemented DDG at 0.4% of their BW. In summary, DDG appears to be a viable supplement in cattle on forage-based diets, resulting in increased animal performance but decreased forage intakes. Furthermore, it is difficult to predict how much DDG should be supplemented in beef cattle raised on high-roughage diets, since the nutritional value and availability of the forage varies drastically throughout the year.

As previously indicated, excess DDG in cattle diets may cause decreased cattle performance due to the high fat content and quite possibly the high sulfur content of DDG. Sulfur is highly concentrated in DDG primarily due the use of sulfuric acid by ethanol plants to control pH during fermentation, and secondly due the concentration of nutrients as a result of starch fermentation during the ethanol production. High concentrations of sulfur in the diet for a prolonged period of time may increase sulfide

gas formation in the rumen, which can be toxic to the cattle and in severe cases cause polioencephalomalacia (Kelzer and Maddock, 2009). Polioencephalomalacia is a neurologic disease that manifests itself in blindness, recumbency, tonic-clonic seizures, and coma in cattle. However, sulfur toxicity issues are not common on forage-based diets since these diets normally contain less than 0.5% of sulfur (Klopfenstein, et al. 2008). Another problem with high levels of DDG in the diet is the decrease of DMI due the high fat content; but this mechanism is still unclear, and does not affect forage-based diet cattle too often since the levels of roughages are high in the diet (Banta et al., 2008).

Supplementation Strategies

The goal of a supplementation program for replacement beef heifer development programs should be to use the most efficient supplementation strategy to minimize input cost (DelCurto, et al. 2000) and maximize the number of heifers that attain puberty before the start of the breeding season. Lesmesiter et al. (1973) indicated that yearling beef heifers that conceive early in their first breeding season will calve early, and have a greater lifetime productivity compared to heifers that calve at older ages. In order to ensure that heifers conceive and calve early, it is critical that an adequate BW is achieved so heifers exhibit their first estrous cycle before the start of the breeding season (Lynch, et al. 1997).

Most supplementation programs are based on the target BW principal, where heifers must be 60 to 65% of their mature BW in order to attain puberty before the breeding season (Lynch et al., 1997). Greer et al. (1983) suggested that a target BW is a useful monitor for predicting when heifers will attain puberty, and feeding to a target BW is a management tool to ensure high fertility in the herd. Target BW can vary from

53% to 66% depending on the breed type and frame size of the heifer (Patterson et al., 1992; Funston and Deutscher, 2004). The target BW principal allows producers to determine the nutrient intake and rate of gain necessary to achieve the desired BW so heifers are pubertal before the breeding season.

Forages are the base of almost every beef heifer development program. To minimize supplementation, use of forage supplies is highly encouraged. Pasture is the most common forage source for beef cattle raised on forage based systems, followed by hay and silage. Warm season perennial grasses are the foundation of pastures in Florida. Bahiagrass (*Paspalum notatum*) is predominantly used in Florida, since it is the primary pasture available (Chambliss et al., 2001). Other grasses available in Florida include Bermudagrass (*Cynodon dactylon*), Stargrass (*Cynodon* spp.) and Limpograss (*Hermathria altissima*) (Arthington and Brown, 2005). Moore et al. (1991a) analyzed 637 forage samples from Florida, including Bermudagrass, Bahiagrass, Limpograss, and Stargrass, and reported that most forages contained 5-7% CP (DM basis) and 48-51% TDN (DM basis). Based on NRC (1996) growing heifers have a requirement of 8% CP and 55% TDN. Therefore, to meet the nutritional requirements of growing heifers, additional supplementation is necessary.

There are many supplementation strategies that can be used in beef heifer development programs. Clanton et al. (1983) suggested that there are several replacement beef heifer development methods that can be used as long as the heifers reach the necessary BW by the beginning of the breeding season. The most common supplementation strategy for replacement beef heifers is the constant rate gain from weaning to breeding, which allows adequate body growth and moderate body condition.

The most common target rate of gain rate in a constant rate program is approximately 0.68 to 0.90 kg/d. The rate of gain needed to reach the specified TW can depend on numerous factors including breed, frame size, and environmental conditions.

The stair-step strategy is also utilized in replacement beef heifer development programs. The aforementioned strategy consists of a period of development where heifers are fed to gain at a slower rate, followed by a faster gain rate. The slower rates are approximately 0.5 kg/d and the faster rates are > 1 kg/d. The stair-step method of gain strategy relies on the potential for compensatory gain, which Park et al. (1987) described as a phenomenon that occurs when animals fed on a low nutritional level diet are followed by a phase where they are fed a higher nutritional level diet. During the compensatory gain period animals have a greater rate of gain than normal due to increased feed efficiency compared to similar, but continuously fed animals. Freetly et al. (2001) described that the overall feed efficiency due to compensatory gain may vary depending on the duration and severity of feed restriction as well as duration and level of feed intake during the realimentation phase. Furthermore, the quantity of feed necessary after the restriction phase to increase BW also depends on the magnitude and length of feed restriction and realimentation period (Freetly et al., 2001). Hersom et al. (2004a) studied the effect of different rates of gain during winter grazing on subsequent growth during feedlot finishing phase. Steers were allotted to one of the treatments before the finishing period 1) high rate of BW gain grazing winter wheat (HGW), 2) low rate of BW gain grazing winter wheat (LGW), or 3) grazing dormant tallgrass native range (NR) supplemented with 0.91 kg/d of cottonseed meal. During the finishing period, NR and LGW steers had greater DMI compared to HGW, suggesting

that NR and LGW steers were able to compensate during feedlot finishing phase. Furthermore, Clanton et al. (1983) studied the effect of the time of gain after weaning on the development of replacement beef heifers. Their objective was to determine when was the best time for the replacement heifers to gain the weight necessary to achieve puberty before the breeding season. Heifers were assigned to one of the three treatments from 45 d after weaning until the breeding season: (1) low-high treatment, where heifers were fed for no gain during period 1 followed by a second period where they were fed to gain 0.91 kg/head/d; (2) control treatment, where heifers were fed to gain 0.45 kg/head/d during the entire trial period; (3) high-low treatment, where heifers were fed to gain 0.91 kg/head/d during period 1 followed by a period of no gain. The BW of the heifers were different among treatments at the end of period 1; however, BW did not differ at the end of the trial. There were no differences for any of the reproductive data evaluated including age at puberty (403; 394; 392 d) and pregnancy rates (75; 82; 73%) for treatments 1, 2, and 3, respectively.. Lynch et al. (1997) studied the stair-step program by feeding a group of Angus x Hereford heifers to gain 0.45 kg/d for 159 d (Control) compared to a second group fed to gain 0.11 kg/d from d 0 to 112 followed by feeding to gain 0.91 kg/d from d 112 to 159 (late-gain). Heifers were expected to achieve 65% of target BW before the breeding season. The objective was to determine if the majority of developmental weight gain could be delayed, and if heifers could be developed with less total feed inputs. Heifers had similar skeletal growth and overall pregnancy rates between treatments. The authors concluded that delaying the majority of weight gain until late in heifer development may decrease costs without detrimental effects on reproductive performance. Grings et al. (1999) reported no effect of stair-step

supplementation on beef heifer pregnancy rates when heifers were fed a diet intended to supply energy to produce weight gains at 120% of the rate of the control group for 55 d followed by 70% rate of controls for 84 d and 120% rate of controls for the last 30 d before breeding season. They concluded that alteration of rate of gain from weaning to breeding season has no effect on the reproductive performance of beef heifers; therefore, there is flexibility in supplementation strategies leading to alterations and perhaps a decrease in diet cost. In conclusion, the stair-step system can be used in the replacement heifer development programs without altering heifer growth and pregnancy rates, as long as the target BW of 55%-65% is achieved before the breeding season (Freetly et al., 2001).

Feed Restriction and Compensatory Gain

Compensatory gain can be defined as the physiological process whereby an animal accelerates its growth after a period of restricted development, usually due to reduced feed intake, in order to reach the weight of animals whose growth was never affected (Hornick et al. 2000). Compensatory growth is expressed for a variable period of time as a higher rate of gain compared to continuously fed animals (Park et al. 1987). For example, Yambayamba et al. (1996) exposed heifers to a 95 d feed restriction period followed by realimentation period and observed that heifers gained up to 2 kg/d. Ryan et al. (1993a,b) suggested that compensatory growth is due to increased protein deposition, reduced maintenance requirements, and greater feed intake. The reduction in maintenance requirements and greater feed intake lead to conversion of feed more efficiently (Ryan et al., 1993a). All the physiological reasons behind the compensatory growth are complex, and it has not been satisfactory explained yet (Fox et al.1972; Hayden et al. 1993; Klopfenstein et al. 1999). However, it is known that the magnitude

of compensatory growth depends on the length and severity of the restriction period as well as the combination of dietary components utilized during the realimentation period (Fox et al., 1972; Park et al., 1987, Freetly et al. 2001).

Body Composition

Both feed restriction and compensatory growth can alter the metabolic and catabolic actions of numerous tissues of an animal, which can significantly alter the animals body composition. However, there is conflicting evidence on the changes of body composition. During normal growth, muscle has the highest growth rate followed by adipose tissue. When growth rate is reduced due to feed restriction, fat deposition is more affected than protein deposition; thus, the body becomes leaner (Hornick et al., 2000). Other tissues can also be affected by feed restriction depending on their metabolic activity. Metabolically active tissues such as the digestive tract and liver are likely to be reduced in size resulting in decreased maintenance requirements (Ryan et al., 1993b; Hornick et al., 2000).

The variation in changes in body composition due to feed restriction and compensatory growth depends on many factors. Ryan et al. (1993b) reported that cattle lost weight during feed restriction from their lung, liver, and digestive tract, and gained weight in their head and feet. There was no difference in body composition (protein, water, fat, and ash) at the end of the experiment between control and restricted cattle. However, during the restriction phase, the ratio of fat to protein mobilized was 1.7:1 indicating a high level of protein mobilization and suggesting that during the refeeding period there was an increase in protein deposition as the body returned to its original body composition. Murphy and Loerch (1994) also observed reductions in carcass fat content accompanied by increases in carcass water and protein for feed

restricted animals. Hornick et al. (1998a) conducted an experiment with Belgian Blue bulls to study the effects of three durations (115, 239, and 411 d) of growth restriction on carcass characteristics. They concluded that bulls that were exposed to a feed restriction diet for 411 d yielded more lean meat and less intramuscular fat compared to the other treatments. In addition, Coleman and Evans (1986) conducted an experiment where Angus and Charolais steers were randomly assigned to either a non-restricted control diet or restricted diet during the growing phase of the trial followed by finishing phase on a conventional feedlot diet. Carcasses from steers fed the control growing diet were heavier at the end of the experiment compared to steers fed the restricted diet. In comparison, Hayden et al. (1993) showed that there was no difference in carcass weight between steers fed a restricted (RES) diet with 2.13 Mcal ME/kg for 92 d and non-restricted (NR) fed steers, even though there was a slight reduction in carcass weight of non-restricted steers (NR 362.9 kg vs. RES 372.5 kg). Furthermore, the study showed that treatments did not differ for longissimus muscle area (RES 34.0 cm^2 vs. NR 37.0 cm^2) and subcutaneous fat (RES 0.93 cm vs. NR 0.86 cm); however, there was a trend for a slight reduction in longissimus muscle area and an increase in subcutaneous fat for restricted fed steers. In agreement, Henricks et al. (1994) also observed smaller longissimus muscle in bull calves that were exposed to a 84 d restricted diet followed by a 91 d realimentation period compared to control calves.

In conclusion, changes in body composition during feed restriction and throughout realimentation periods are hard to predict and depend on numerous factors including physiological maturity of the animal, animal age, breed, stage of the growth in which realimentation occurs, and endpoint determination. Also, body composition can vary

widely depending on the duration and magnitude of both the nutrient restriction and realimentation periods.

Endocrine Changes

During feed restriction, basal metabolism is reduced and metabolic and endocrine alterations occur (Ryan et al., 1993b). These reactions result in decreased circulating concentrations of anabolic hormones and increased concentrations of circulating catabolic hormones (Hornick et al., 2000). Several studies (Fox et al., 1974; Blum et al., 1985; Hayden et al., 1993; Yambayamba et al., 1996) have been conducted to better understand the effect of feed restriction and compensatory gain on hormones and metabolites and the interrelationships between the two.

Hayden et al. (1993) observed a decrease in concentrations of triiodothyronine (T3; 29%), reverse triiodothyronine (rT3; 35%) and thyroxine (T4; 36%) in energy-restricted steers during the restriction phase and concluded that the shared decrease in T3, T4 and rT3 concentrations suggests that T4 concentrations may be metabolized to T3 and rT3 at a comparable rate during feed restriction. In agreement, Hersom et al. (2004b) observed decreased concentrations of T3 and T4 in response to decreased nutrient intake. Blum et al. (1985) also observed decreased concentrations of T4 and T3 in feed restricted animals compared to control animals during the restriction period and for the first days of realimentation, no treatment differences were observed in rT3 concentrations throughout the experiment. The low concentrations of T3 and T4 during the first day of realimentation are probably due to the low energy requirements during the initial phase of compensatory growth (Fox et al. 1974). Pethes et al. (1985) observed decreased T3 concentrations in cows fed 20% less energy per day than

control cows, suggesting reduced maintenance requirements with lower energy intakes for the cattle consuming less energy. Therefore, the decreased T3 and T4 concentrations observed during restriction periods indicate a lower metabolic rate during maintenance period (Murphy and Loerch, 1994), due to the role of thyroid hormones on stimulating mitochondrial oxygen consumption and production of ATP (Ismail-Beigi and Edelman, 1970). Consequently, a lower metabolic rate would result in less energy being required during the restriction period (Ryan et al., 1993b). The decreased T3 and T4 concentrations observed during restriction periods could be associated with reduced thyroid-stimulating hormone secretion and increased degradation rates of T3 and T4 (Wrutniak and Cabello, 1987; Hornick et al., 1998b).

Nutritional status also affects GH concentrations. Several reports have demonstrated an elevation of circulating GH concentrations in cattle during feed restriction; although, the reasons for the elevation are unclear (Blum et al., 1985; McCann and Hansel, 1986; Ellenberger et al., 1989; Lobley, 1992; Hayden et al., 1993). Circulating concentrations of GH is the balance between pituitary secretion and clearance of GH from the circulation. Trenkle (1976) suggested that elevated concentrations of GH during feed restriction may be due to an increased half-life of circulating GH, which is probably due the fact that liver and kidney are less active in metabolizing of GH in feed restricted animals. The longer half-life of GH due to decreased clearance rates could also be associated with the decreased synthesis of the high affinity hepatic GH receptor in feed restricted animals (Hornick et al., 1998b). Furthermore, elevated concentrations of GH during feed restriction are a result of increased GH pulse amplitude, and not increased GH pulse frequency (Ellenberger et

al. 1989; Breier et al., 1986). Hornick et al. (1998b) also observed a change in GH pulse amplitude in Belgian Blue bulls fed a restricted diet, followed by *ad libitum* realimentation diet high in protein and energy where GH pulse frequency increased.

Circulating GH and IGF-1concentrations are highly related. Growth hormone acts primarily on the liver but also on other tissues to stimulate secretion of IGF-1. Circulating secretions of IGF-1 exert a negative feedback effect on both the hypothalamus and pituitary to inhibit GH secretion (Davis, 1988). Stimulation of IGF-1 secretion is modulated by other factors besides GH secretion, such as nutritional intake (Davis, 1988) and feed restriction is normally associated with decreased circulating IGF-1 concentrations (Breier et al. 1986; Clemmons et al., 1988; Yambayamba et al., 1996). Yambayamba et al. (1996) investigated the endocrine and metabolic changes of beef heifers fed one of the two treatments 1) *ad libitum* intake, or 2) feed restriction for 95d followed by realimentation. The feed restricted heifers had decreased IGF-1 and insulin concentrations compared to the controls. As expected, feed restriction leads to decreased insulin concentrations due to the decreased energy and protein content of the diet. Furthermore, insulin is known to affect synthesis and secretion of IGF-1 (Davis, 1988); thus, it would be expected a drop in IGF-1 concentration may be due to the decrease in insulin.

Metabolic Changes

Animals respond in a number of ways during and after nutritional restriction. Endocrine and body composition changes that occur during nutritional restriction lead to alterations in metabolite concentration. The metabolites most studied during nutritional restriction are non-esterified fatty acids (NEFA), glucose and plasma urea nitrogen (PUN).

Triglycerides are composed of three fatty acids and a glycerol backbone. Hydrolysis of stored triglycerides in adipose tissue by hormone sentitive lipase (HSL) liberates NEFAs and glycerol. Various hormones, such as glucagon, catecholamines, corticosteroids, and growth hormone, stimulate HSL. Circulating NEFAs can be used as an energy source by many tissues, including skeletal muscle and hepatocytes. Circulating NEFAs have been analyzed during feed restriction to develop a better understanding of how mobilization of tissue are necessary to provide energy to meet animal's maintenance and growth requirements. Yelich et al. (1996) fed heifers to either a constant rate of gain (1.36 kg/d) or low rate of gain (0.23 kg/d) for 16 wk followed by an increased rate of gain (1.36 kg/d). The concentrations of NEFA were negatively associated with level of feed intake. Heifers on constant rate of gain had decreased NEFA concentrations compared to heifers exposed to feed restriction. The increase of NEFA concentrations is indicative of negative energy balance (Richards et al., 1989) and fatty acid release from adipocyte (Bines and Hart, 1982) and greater fat mobilization (Blum et al., 1985; Ellenberger et al.,1989). Yelich et al. (1996) suggested that the increase of NEFA in feed restricted heifers is probably due to increases in lipolysis and altered lipogenesis. Hornick et al. (1998b) also observed decreased NEFA concentrations for Belgian Blue bulls fed a diet high in energy and protein compared to bulls fed to gain 0.5 kg/d. During realimentation of the restricted bulls, NEFA concentrations decreased. Blum et al. (1985) observed high concentrations of NEFA in feed restrictedanimals and shortly after the start of realimentation, NEFA concentrations decreased to those of the control group.

Plasma urea nitrogen concentrations are a measure of the amount of nitrogen in the blood in the form of urea. Measuring PUN concentrations is a useful indicator of protein status within a group of animals (Kohn et al., 2005). Elevated PUN concentrations have been observed in feed restricted steers compared to ad-libitum fed steers, and PUN concentrations decline once steers were realimentated (Hayden et al., 1993). Conversely, Hornick et al. (1998b) observed an increase in PUN concentrations during realimentation and suggested that the increase in PUN concentrations when turning from the feed restriction to realimentation reflected a greater hepatic synthesis of urea resulting from enhanced microbial ammonia production in the rumen or greater amino acid degradation in the liver. In contrast, Ellenberger et al. (1989) did not observe any significant difference in PUN concentrations between control and restricted steers, and suggested that dietary restriction was not severe enough to result in amino acid catabolism. In agreement, Yambayamba (1996) did not observe greater PUN concentrations in restricted heifers. During realimentation the author reported an initial decease in PUN concentrations, which was attributed to the increased efficiency of protein and nitrogen utilization in the restricted heifers.

Glucose is a simple sugar used as a source of energy and serves as a metabolic intermediate in cattle. Circulating blood glucose concentrations are positively associated with nutrient intake (Richards et al., 1989; Yelich et al., 1996) and glucose concentrations are frequently evaluated during feed restriction and realimentation studies. Because of the infrequent nature in which many ruminants receive their nutrients, circulating blood glucose supply is often insufficient to meet glucose needs in ruminants; therefore, gluconeogenesis is extremely important in ruminants to maintain

adequate glucose concentrations. Blum et al. (1985) fed restricted steers for 141 d, and reported that glucose concentrations were significantly less compared to the control group during feed restriction. Glucose concentrations became elevated, above those of the control group during the second half of realimentation period. The authors suggested that decreased concentrations of glucose during feed restriction were presumably an expression of insufficient gluconeogenesis. The increase of the glucose concentration after realimentation is probably the result of a higher ruminal production of its precursor, propionic acid, which was associated with greater intake (Hornick et al., 1998). Yelich et al. (1996) also observed a tendency for lower glucose concentrations in feed restricted animals. Ellenberger et al. (1989) also reported that blood glucose was lower for feed restricted steers; however, blood glucose did not increase as rapidly during realimentation period as the feed intake increased. The authors suggested that this may reflect the very high demands for gut and liver hypertrophy at this time. Austin (2009) reported that there was no difference in plasma glucose concentrations between a control group fed round bale silage and DDG for 140 d compared to a low-high group, which were fed only round bale silage for 70 d followed by 70 d of round bale silage and supplemented with DDG. In agreement, Hornick et al. (1998b) reported that steers that were feed restricted for 114 and 243 d did not have lower glucose concentrations during feed restriction compared to non restricted feed steers. However, steers restricted for 419 d had decreased glucose concentrations during feed restriction compared to realimentation period.

In summary, during feed restriction, NEFA concentrations are increased due to the mobilization of triglycerides to meet energy requirements. Plasma urea nitrogen

concentrations are also increased during feed restriction; however, if feed restriction is not severe enough to lead to protein mobilization PUN concentrations do not increase. Glucose concentrations are dependent on feed intake; therefore, they can vary drastically among studies due to variation in diets. The significant variation in blood metabolites concentrations among animals and studies are primarily a result of animals responding differently due to differences in the duration of feed restriction, as well as the variation in the type and quantity of diets fed.

CHAPTER 3
EFFECTS OF TIMING OF GAIN ON GROWTH, BODY COMPOSITION, REPRODUCTIVE PERFORMANCE, AND BLOOD METABOLITES IN BEEF REPLACEMENT HEIFERS

Introduction

Most beef cattle in Florida and in other sub-tropical regions of the world are raised on forage-based systems. Forages can be offered to growing heifers as either grazed or stored forages, such as hay and round bale silage (RBS). Harvesting forage to produce hay or RBS is important to preserve forage for feeding heifers when availability of forage is low. However, neither preservation techniques improve the quality of the stored forage; therefore harvesting high-quality forage is essential (Kunkle, 2003). The quality of sub-tropical forages is usually not enough to meet nutrient requirements for growing heifers, since it is low in CP and TDN compared to forage from temperate regions (Moore et al., 1991a). In order to meet the nutrient requirements of yearling replacement heifers raised on sub-tropical forage diets, either an energy and (or) protein supplement is necessary.

Corn had been used as a diet supplement in cattle for a long time; however, during the past years an increasing amount of corn has been used for ethanol production in the United States causing the price of the corn to increase. Dried distillers grain (DDG), a byproduct from the ethanol industry, has become a common corn substitute in the beef cattle diet (Leupp et al., 2009). Dried distillers grain has an average CP content of 31%, TDN 87%, Fat 11%, ADF 12%, NDF 45%; therefore, DDG may fit well as a supplement for beef cattle raised in forage-based systems because it provides both energy and protein (MacDonald et al., 2004).

In order to optimize the efficiency of a cow-calf operation, the goals of the replacement heifer operation are to decrease feed inputs, and ensure that heifers attain adequate BW to initiate their first estrous cycle before the onset of the breeding season (Lynch et al., 1997). The primary cost of developing replacement heifers is feed cost (33%; Hersom et al., 2010). Therefore, a low cost nutritional program is essential in developing replacement heifers in order to ensure a profitable cow-calf operation. Several researches have shown that nutritional strategies that impose a period of growth restriction followed by a period of rapid gain to take advantage of compensatory growth, have minimal to no effect on age at puberty and subsequent reproductive performance (Grings et al., 1999; Poland and Ringwall, 2001). Compensatory gain, which Park et al. (1987) described as a phenomenon that occurs when animals fed a restricted nutritional diet resulting in minimal BW gains, which is followed by a phase where they are fed a higher nutritional level diet allowing for greater weight gain. During compensatory gain restricted animals have a greater rate of gain due to an increased feed efficiency compared to similar, but continuously fed animals. Clanton et al. (1983) and Lynch et al. (1997) suggested that compensatory gain can be used to reduce feed inputs in heifer development programs; thereby, decreasing total feed cost while heifers are still able to attain the minimum BW required to reach puberty before the breeding season. Although, it is unclear how effective the restricted followed by enhanced growth nutritional strategies are in cattle of *Bos indicus* x *Bos taurus* and *Bos taurus* heifers raised on forage based systems in sub-tropical environments.

Therefore, we hypothesized that if weight gain could be delayed, both yearling Brangus and Angus heifers could be developed with less total feed inputs without

negatively affecting reproductive performance. The objectives of this research were to evaluate the effect of delayed gain on the growth, body composition, reproductive performance, and blood metabolites of Angus and Brangus heifers fed RBS and supplemented with DDG.

Materials and Methods

The experiment was conducted at University of Florida Santa Fe Beef Research Unit, north of Alachua, Florida from October 2008 until June 2009. The experiment was divided into a supplementation and sampling period (d 0 to 140), and the breeding period (d 140 to 240) with d 0 being the start of the experiment and d 70 being the mid point of the supplementation period. The experiment was conducted in accordance with acceptable practices as outlined by Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999) and University of Florida Institutional Animal Care and Use Committee protocol number F065.

Animals and Treatments

Angus (n = 30) and Brangus (n = 30) heifers with initial BW of 276 $\pm$ 9.7 kg and 279 $\pm$ 9.7 kg, respectively were utilized in the experiment. Mean age of the heifers on d 0 was 277 d. A full BW was taken one week prior to the start of the experiment, and heifers were stratified by BW, breed, and age, and allocated to one of twelve 1.2 ha pens with 5 heifers per pen. The pens were composed of a mixture of dormant bahiagrass (*Paspalum notatum*) and bermudagrass (*Cynodon dactylon*). The pastures received no fertilization previous to and during the experiment. The nutritional value of the forage in the pastures for the duration of the experiment was 11.4 % CP, 49.3 %

TDN. The mean forage available per pen at the start of the experiment was estimated at 1,840 DM kg/ha. Heifers remained in the same pen from d 0 to 182 of the experiment.

Pens were randomly assigned to one of the two treatments: 1) Heifers were fed costal bermudagrass (*Cynodon dactylon)* round bale silage (RBS) and supplemented dried distillers grain (DDG) three times per wk from d 0 to 140 (CON); 2) Heifers were fed costal bermudagrass RBS from d 0 to 140 followed by supplementation with DDG three times a wk from d 70 to 140 (LH). Heifers were adapted to treatments from d -14 to -1. The RBS and DDG were offered to allow CON heifers to gain 0.84 kg/d throughout the experiment and to allow LH heifers to gain 0.28 kg/d from d 0 to 70 followed by 1.4 kg/d from d 70 to 140 of the experiment. The gain was based on Beef Cattle NRC (2001) for growing yearling beef heifers. The quantity of supplement offered to heifers was adjusted every two wk based on animal performance to achieve the targeted ADG. Heifers were offered water and custom-made mineral-vitamin mix (see appendix A) *ad libitum* throughout the experiment. The total amount of DDG offered from d 0 to 140 of the experiment to LH and CON heifers was 162 kg/heifer and 228 kg/heifer, respectively. Amount of DDG offered throughout the experiment to CON and LH heifers is presented on Figure 3-1. The total amount of RBS offered to LH and CON heifers from d 0 to 140 of the experiment was 1,091 kg/heifer and 1,122 kg/heifer, respectively.

Feed sampling

Samples of RBS were collected from each bale throughout the experiment and DDG samples were collected monthly. Pasture samples were also obtained monthly from each pen to estimate forage quantity and quality by hand clipping three 0.25 m^2 areas and compositing the samples. Pasture and RBS samples were dried at 60°C in

forced air oven for approximately 72 h. Dried samples were ground to pass through a 1-mm screen in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA, USA). After grinding, RBS samples were pooled monthly for laboratory analysis. Pasture and RBS samples were analyzed for CP, OM, and in vitro dry matter digestibility (IVDMD). Total nitrogen was determined using macro elemental N analyzer (Elementar, vario MAX CN, Elementar Americas, Mount Laurel, NJ, USA) and used to determine CP (CP = N x 6.25). In vitro dry matter digestibility of samples was determined by the procedure of Tiley and Terry (1963), as modified by Marten and Barnes (1980) with filtration on filter paper. Monthly DDG samples were analyzed by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Nutritional composition for DDG, pasture, and RBS are presented in Table 3-2.

Sampling and Analysis

Heifers were weighed biweekly from d 0 to 70 and weekly from d 70 to 140 and BW measurements were used to calculate ADG. Hip height (HH) and body condition score (BSC; 1 = severely emaciated; 5 = moderate; 9= very obese; Wagner et al., 1988) were obtained every 28 d. Pelvic area (PA = pelvic width x pelvic height) was measured on d 0, 70 and 140 with a Rice Pelvimeter (Lane Mfg., Denver, CO). Ultrasound measurements of *longissimus dorsi* area (REA) at the 13^{th} rib, 13^{th} rib fat thickness (RIBFT), rump fat thickness (RMPFT), and intramuscular fat of the REA (IMF) were taken on d 0, 70, and 140. The REA and BW measurements were used to calculate REA/cwt, which adjusted REA per 100 kg of BW.

Blood samples were collected via jugular venipuncture into 7.5 mL polypropylene syringes containing 1.6 mg potassium EDTA as an anticoagulant (Monovette, Sarstedt Inc., Newton, NC) biweekly from d 0 to 70 and weekly from d 70 to 140 during the

sampling period of the experiment. Additional blood samples were taken on d -14, -7 and 63 for determination of blood progesterone concentrations for estrous cycling status. Samples were placed on ice immediately after collection and transported to the lab for further processing. Blood samples were centrifuged at 2165 x *g* for 15 min at 5°C to obtain plasma, which was placed in sample vials and frozen at -20°C for subsequent analysis.

Concentrations of plasma progesterone in samples were analyzed by radioimmunoassay using Coat-A-Count kit (Diagnostic Products Corp., Los Angeles, CA; Seals et al., 1998). Assay tubes for the standard curve contained 0.01, 0.025, 0.05, 0.2, 0.5, 1, 2, and 4 ng/tube. Assay sensitivity for a 100 μl sample was 0.1 ng/ml. The intra- and inter-assay coefficients of variation were 9.4% and 8.3%, respectively. Angus heifers were considered pubertal when progesterone concentrations were ≥ 1.0 ng/mL followed by 1 of 2 samples in the next two weekly blood samples ≥ 1.0 ng/ml. Brangus heifers were considered pubertal when progesterone concentrations were ≥ 1.5 ng/mL followed by 1 of 2 samples in the next two weekly blood samples ≥ 1.5 ng/ml (Cooke and Arthington, 2009). Date of puberty was defined as the first progesterone concentration ≥ 1.0 ng/mL for Angus and ≥ 1.5 ng/mL for Brangus

Plasma urea nitrogen concentrations were determined using BioAssay Systems QuantiChrom™ Urea Assay Kit series DIUR-500 kit (BioAssays Systems, Hayward, CA). Plasma glucose concentrations were determined using Cayman Chemical Co. Glucose Analysis kit (Cayman Chemical Co., Ann Arbor, MI). Plasma NEFA concentrations were determined using Wako HR Series NEFA-HR kit (Wako Diagnostics, Richmond, VA). See Appendix for assay protocols. The intra- and inter-

assay coefficients of variation were 4.9% and 4.3% for NEFA, 7.6% and 10.4% for PUN, and 4.3% and 20.6% for glucose, respectively.

Breeding

On d 140 of the experiment, heifers received 100 μg (i.m) of GnRH (Cystorelin, Merial, Inc., Duluth, GA) and a progesterone device was inserted into the vagina (EAZI-BREED CIDR®; Pzifer Animal Health, New York, NY). Seven days later, heifers received 25 mg (i.m.) of prostaglandin $F_{2\alpha}$ (PG; Lutalyse® Sterile Solution, Pfizer Animal Health, New York, NY) and the CIDR were removed. Estrus was detected for 72 h after CIDR removal using Estrotec™ estrous detection patches (Rockway, Inc., Spring Valley, WI). Heifers were AI 8 to 12 h after the onset of estrus by single AI technician. Heifers not exhibiting estrus by 72 h were administered 100 μg (i.m.) GnRH and timed-AI at 72 to 76 h after PG. Estrus detection continued for 32 d after timed-AI using radiotelemetric estrous detection devices (HeatWatch®, Cow Chips, Denver, CO; Dransfield et al., 1998). After a 32 d AI period, heifers were grouped by breed and each breed group was pasture exposed for an additional 28 d to a bull of similar breed type. Pregnancy was detected on d 182, 210 and 240 of the experiment by transrectal ultrasonography, using a real-time, B-mode ultrasound (Aloka 500v, Corometrics Medical Systems, Wallingford, CT) equipped with 5.0 MHz transducer. Response variables to the synchronization treatment included: estrous response (number of heifers that exhibited estrus during the 3 d after PG divided by the total treated), conception rate (number of heifers that became pregnant to the AI divided by the total that exhibited estrus), timed-AI (number of heifers that failed to exhibit estrus and became pregnant to timed-AI divided by the total number of heifers timed-AI),

synchronized pregnancy rate (total number of heifers pregnant divided by the total number of heifers treated), thirty-day pregnancy rate (total number of heifers pregnant in the first 30-d of the breeding season divided by the total number of heifers treated), and final pregnancy rate (total number of heifers pregnant during a 60-d breeding season divided by the total number of heifers treated)

Statistical Analysis

Heifer growth performance, body composition, and blood metabolite data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). The model statement used for BW, BCS, HH, PA, ADG, IMF, REA, REA/cwt, RIBFT and RMPFT contained the effect of treatment, breed, time, and all appropriate interactions. Data were analyzed as repeated measures using pen(treatment) as the random variable. The model statement used for glucose, NEFA, and PUN, contained the effects of breed, treatment, time, and all appropriate interactions. Data were analyzed as repeated measures using heifer(pen) and pen(treatment) as random variables. The appropriate covariance structure of the data was chosen for each analysis from the structures of autoregressive one (AR(1)), heterogeneous (ARH(1)), and Heterogeneous TOEP (TOEPH), using Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC). Mean comparisons were made using the PDIFF function associated with generation of least squares means. Results are reported as least square means.

Reproductive data were analyzed using GLIMMIX procedure of SAS. The model statement used for puberty, estrous response, conception rate, timed-AI pregnancy rate, synchronized pregnancy rate, and final pregnancy rate contained the effect of treatment, breed, pen, and all appropriate interactions. Data were analyzed as binary measures using either heifer(treatment) or pen(treatment) as the random variable for

comparison purpose. No difference was observed between the models using the different random variables. The results reported were from the statistical model using heifer(treatment) as random variable. For all analysis, significance was set at $P \leq 0.05$, tendencies were determined if $P > 0.05$ and ≤ 0.10.

All correlations coefficients reported for this experiment were calculated using PROC CORR procedure of SAS with the standard Pearson correlation formula.

Results and Discussion

Growth Performance and Body Composition

At the initiation of the experiment (d 0) BW, BCS, and PA were similar ($P > 0.05$) between treatments and breeds (Table 3-2). There were no treatment, breed, and treatment x breed effects on BW, BCS, and PA on d 0 of the experiment. However, there was a breed effect on HH as Brangus heifers were taller ($P < 0.05$; 116 ± 0.9 cm) compared to Angus (111 ± 0.9 cm) heifers.

At the mid-point of the trial (d 70) CON heifers were 29.1 kg heavier ($P < 0.05$; Figure 3-2), had greater ($P < 0.05$) BCS, and tended ($P = 0.08$) to have larger PA compared to LH heifers (Table 3-2). The HH were similar ($P > 0.05$) between treatment on d 70 (Table 3-2); however, there was a breed effect ($P < 0.05$) as Brangus (119 ± 0.8 cm) heifers were taller compared to Angus (115 ± 0.8 cm) heifers.

The difference between treatments for the aforementioned growth traits can be further explained by evaluating ADG from d 0 to 70 of the experiment (Figure 3-3). During the first 70 d of the experiment, CON heifers (0.52 kg/d) had a greater ($P < 0.05$) ADG compared to LH heifers (0.16 kg/d) but there was no breed effect ($P > 0.05$) on ADG during this period. Because LH heifers were not supplemented with DDG during

the first 70 d of the experiment they were expected to have lower ADG compared to CON heifers. However, the ADG for both treatments were considerably less than the ADG predicted by Beef Cattle NRC at the start of the experiment, which was 0.84 kg/d for CON and 0.28 kg/d for LH heifers. There are several possible reasons for the decreased gain observed for both treatments. The decreased ADG observed for the LH heifers could be due to gut fill, which could also negatively affect DMI. Gut fill can account for 12-22 % of the BW (Freking, 2000). Even though, weights were taken at the same time and day of the week; gut fill could cause bias errors in weights (Freking, 2000) leading to variation in ADG. Furthermore, heifers were still growing and their rumen-reticulum had not reached full capacity yet (Allen, 1996). The reticulo-rumen and possibly the abomasum have stretch and touch receptors in their walls that negatively impact DMI as the weight and volume of digesta accumulate (Allen, 1996). In addition, the RBS used in this experiment was long stem RBS and not of the chopped variety; therefore, it is expected that long stem RBS would result in greater gastrointestinal tract fill compared to RBS of similar digestibility in the chopped form (Montgomery et al., 1965; Jaster and Murphy, 1982). Even further, LH heifers were able to attain the predicted ADG from d 0 to 42 of the experiment (0.40 kg/d) but they had negative ADG from d 42 - 70 of the experiment (Figure 3-1). Therefore, the negative ADG for LH heifers from d 42 to 70 of the experiment suggests that other factors besides gut fill could be affecting heifer performance. The ambient temperature from d 42 to70 of the experiment was colder than normal with temperatures below 0°C (FAWN; Alachua Station) for 4 d. During cold weather, cattle during cold weather grow slower because less energy is available for production due to the necessity to increase heat production

to maintain homeothermy (Young, 1981). Furthermore, *Bos indicus* and *Bos taurus x Bos indicus* breeds are not well adapted for cool weather; therefore, the necessity to increase heat production is even greater (Gregory et al., 1979). Maintenance energy requirements for heifers under extreme environmental conditions could be over four times NRC recommendation values (Fox et al., 1988). Therefore, during this experimental period heifers were probably under cold stress and increased their maintenance requirements to maintain homeothermy, which may have resulted in decreased heifer performance.

As mentioned above, the ADG of CON heifers from d 0 to 70 was also considerably less compared to the ADG predicted by Beef Cattle NRC at the start of the experiment. In contrast to LH heifers, CON heifers never attained the predicted ADG from d 0 to 70 and their ADG decreased for every weigh period from d 0 - 70 (Figure 3-3) even though the absolute amount of DDG offered was increased (Figure 3-1). All DDG offered for CON heifers during this period was consumed; therefore, heifers probably were not able to consume the amount of RBS expected to meet their requirements for the target ADG. Gut fill probably had an even more important role on CON heifers performance, since DDG was also being accounted for in gut fill. In contrast with the present study, Morris et al. (2005) evaluated the effect of increasing levels of DDG on forage intake and heifer performance. Heifers were fed either a low-quality forage (smooth brome grass hay), or a high-quality forage (alfalfa hay and sorghum silage mix) and supplemented with 0, 0.75, 1.35, 2.05 or 2.75 kg•heifer•d^{-1} of DDG. The authors reported that ADG increased linearly with increased DDG supplementation while forage intake decreased. The amount of DDG offered for CON

heifers during this period increased from 0.86 to 1.77 kg• heifer^{-1}•d^{-1}; therefore, an increase in ADG should probably have been observed in the present study. As with the LH heifers, the ADG results observed for CON heifers could also be influenced by the weather and its potential negative effects on CON heifer performance.

The ADG from d 70 to 140 of the experiment was 0.74 kg/d for CON heifers and 0.77 kg/d for LH heifers. The increase in ADG for the LH heifers is commonly observed in cattle that had a restricted feed period (Yambayamba et al., 1996). The faster growth rates could be attributed to increased feed efficiency and increased feed intake due compensatory growth (Carstens et al., 1991). The LH heifers reached an ADG peak of 1.26 kg/d by the end of the first 28 d of supplementation, which was close to the predicted ADG of 1.40 kg/d for the period. However, after 28 d of supplementation the ADG of LH heifers decreased to 0.62 kg/d. Yambayamba et al. (1996) exposed heifers to a 95 d feed restriction period, where heifers gained on average 0.07 kg/d, followed by a realimentation period, and observed that heifers exposed to the restriction period gained up to 2 kg/d during realimentation. However, the heifers were not grazing but were kept indoors and the heifers were offered a weight amount of feed. Compensatory growth for grazing cattle is variable and hard to predict due to factors that can influence the degree of compensation, such as days of restriction, ADG, and quality of the diet during re-alimentation (Ryan et al., 1993; Klopfenstein, et al.,1999). Previous reports were unable to induce complete compensatory growth in cattle (Drori et al., 1974; Horton and Holmes, 1978). Ryan et al. (1993) suggested that most of the experiments that were unable to induce cattle to fully compensate during realimentation, the cattle either maintained weight or gained weight slowly during the restriction period. Similarly,

LH heifers in the present study were gaining weight from d 0 to 42 of the experiment and even though they lost weight for 28 d before the supplementation period started, the duration of the feed restriction was probably not enough to allow heifers to fully compensate. Furthermore, weather could also be playing an important role on heifer's performance during this time as there were 9 d where the temperatures were below 0°C. In addition, from d 98 to 112 of the experiment, where the drastic drop in ADG occurred, there were 4 consecutive days below 0°C. The negative effects that decreases in ambient temperature have on animal performance have been previously described.

The mean ADG for CON heifers increased from 0.52 kg/d from d 0 to 70 of the experiment compared to 0.74 kg/d for d 70 to 140 of the experiment. However, the CON heifers were still not able to attain the target ADG (1.40 kg/d) again from d 70 to 140, even though the amount of DDG offered was increased. A possible factor affecting CON heifers performance during this period could be the amount of fat in the DDG. The amount of DDG offered for CON heifers increased from 1.86 kg/d to 2.36 kg/d from d 70 to 140 of the experiment (Figure 3-1). Fat supplementation often leads to a decrease in fiber digestion (Kowalczyk et al., 1977; Doreau et al., 1997; Williams et al., 2000) due to alteration of some rumen microorganisms, particularly cellulolytic bacteria (Kowalczyk et al., 1977), leading one to believe that RBS fiber digestion during this period could be affected by fat supplementation resulting in decreased heifer performance. In contrast, Morris et al. (2005) observed that ADG increase linearly with a linear increase (0, 0.75, 1.35, 2.05 or 2.75 kg•heifer•d^{-1}) in the amount of DDG in the diet while intake of both low and high quality forages decreased. The less than expected ADG of both the CON

and LH heifers from d 92 to 140 could be due to several factors including decreased fiber digestion and forage intake due to the increased levels of fat coming from the DDG supplementation and possibly the negative effects of decreased temperature on heifer performance.

In addition, Angus heifers (0.80 kg/d) tended ($P = 0.06$) to have greater ADG compared to Brangus heifers (0.70 kg/d) from d 70 to 140 of the experiment. When fed a high quality forage or forage plus supplement, *Bos taurus* breeds consume more feed relative to their maintenance requirements (Moran, 1976), thereby gaining faster and more efficiently compared to *Bos indicus* cattle (Krehbiel et al., 2000).

Control heifers had greater ($P < 0.05$) BCS and tended to have greater BW ($P = 0.06$) compared to LH heifers at the end of the experiment (d 140), but no difference ($P > 0.05$) was observed for PA and HH. Angus and Brangus heifers had similar ($P > 0.05$) BW, BCS and PA on d 140. However, Brangus (123 ± 0.8 cm) heifers had greater HH ($P < 0.05$) on d 140 compared to Angus (120 ± 0.8 cm) heifers. Therefore, LH heifers fed RBS only for the first 70 d had their BCS altered and tended to have decreased BW compared to CON heifers, suggesting that heifers were not able to fully compensate during the realimentation period and return to a body composition similar to CON heifers. Although, skeletal growth was not affected by nutritional treatment as evidenced by the similar HH and PA measurements between the CON and LH treatments on day 140 of the experiment.

Ultrasound measurements taken on d 0, 70 and 140 of the experiment are presented on Table 3-3. The REA/cwt was calculated to adjust REA per 100 kg of BW to account for differences in REA due to differences in heifers BW. The REA, REA/cwt, IMF, RIBFT

and RMPFT did not differ ($P > 0.05$) between treatments on d 0 of the experiment. There was also no breed effect ($P > 0.05$) for REA, REA/cwt, RMPFT and RIBFT, but there was a breed ($P < 0.05$) effect for IMF (Figure 3-4) as Angus heifers had greater ($P < 0.05$) percentage of IMF on d 0 of the experiment compared to Brangus heifers. For the entire 140 d experiment, there were time and treatment x time effects ($P < 0.05$) for RMPFT, RIBFT, and REA. And for IMF, there were time, treatment x time, and treatment x breed x time effects ($P < 0.05$; Figure 3-5).

At the end of the first half of the experiment (d 70) LH heifers had smaller ($P < 0.05$) REA compared to CON heifers, but when expressed per 100 kg of BW there was no difference ($P > 0.05$) between CON and LH heifers. The REA/cwt measurement is a better indicator of lean:bone in the animal compared to REA (Huffman, 1991). Whereas, the REA measurement is more closely related to BW and animals that are heavier have greater REA (Huffman, 1991). Huffman (1991) analyzed REA adjusted for BW in steers and heifers and concluded that this might be the best method of muscling indicator across various frame sizes and breed groups. Therefore, the fact that REA/cwt was similar between CON and LH heifers likely indicates that the LH heifers were not mobilizing a significant amount of muscle protein as a source of energy. Furthermore, PUN concentrations for LH heifers were not elevated compared to CON heifers during the first 70 d of the experiment, reinforcing the observation that LH heifers were not mobilizing a significant amount of muscle protein as an energy source. However, the small decrease of REA in LH heifers compared to the CON should not be ignored. The reduction in REA of LH heifers could be due to factors that influence ultrasound images, which in turn could affect ultrasound accuracy. Ultrasound is more accurate at

predicting fat thickness, a one-dimensional measurement, than at predicting REA (Perkins et al., 1992; Houghton and Turlington, 1992). Furthermore, ultrasound measurement could be altered by location of the transducer, the angle the transducer was held to obtain a measurement, and the amount of hair coat on the animal (Houghton and Turlington, 1992).

Heifers on the LH treatment had less ($P < 0.05$) RIBFT on d 70 compared to CON heifers. The decreases ($P < 0.05$) observed in RIBFT and RMPFT (Table 3-3) from d 0 to d 70 in the LH heifers indicate that there was mobilization of fat reserves during the first half of the experiment when LH heifers were fed RBS only. The loss of RIBFT and RMPFT is supported by decreased BCS by d 70, which was due to the decreased ADG experienced by these animals during d 42 to 70 of the experiment. The positive correlation between BCS and RIBFT ($r = 0.29$; $P < 0.01$) or BCS and RMPFT ($r = 0.37$; $P < 0.01$) indicates that BCS accurately reflects the amount of subcutaneous fat similar to reports in dairy (0.36 to 0.86; Domecq et al., 1995) and beef cattle 0.45 to 0.96 (Houghton and Turlington, 1992). The fact that the correlations on the present study were not greater could be due the fact that heifers had decreased amounts of fat reserve compared to most studies conducted in feedlot cattle with greater amounts of fat being measured (Houghton and Turlington, 1992). The NEFA concentrations for LH heifers were greater ($P < 0.05$) compared to CON heifers during d 42 to 70 of the experiment, reinforcing the fact that LH heifers were mobilizing fat during this period. When animals are fed at maintenance levels, muscle growth is close to zero and fat mobilization is increased, leading to altered body composition (Hornick et al., 2000).

Angus LH heifers had similar IMF as Angus and Brangus CON heifers on d 70. After d 70, LH and CON Angus heifers maintained greater IMF compared to Brangus LH, while Brangus CON heifers decreased the IMF percentage (Figure 3-5). This result agrees with numerous researchers that have reported lower marbling scores when straightbred Brahman or Brahman crossbred cattle were compared to cattle of *Bos taurus* breeding (Adams et al., 1982; Kock et al., 1982; Huffman et al., 1990; DeRouen et al., 2000).

At d 140 of the experiment, REA, RIBFT and RMPFT for LH heifers were greater ($P < 0.05$) compared to d 70. The increase in fat and muscle reserve for LH heifers is due to the DDG supplementation from d 70 to 140. The supplement provided more protein and energy for LH heifers during the second period of the trial. These results are further supported by the increases in ADG and BCS for LH heifers from d 70 to 140. There was no difference ($P > 0.05$) on RIBFT, IMF and REA/cwt at d 140 of the experiment between CON and LH heifers. However, REA and RMPFT were greater ($P < 0.05$) for CON compared to LH heifers. These results suggest that LH heifers were not able to fully compensate to reach the same body composition as CON heifers at d 140 of the experiment.

In summary, it is hard to predict how effective compensatory gain will be for cattle fed stored forages as appears to be the case in the present experiment. The LH heifers were not able to fully compensate since there was a tendency for LH heifers to weigh less and have significantly decreased BCS at d 140 of the experiment compared to CON heifers. Although, skeletal growth was not affected by treatment as evidenced by similar HH and PA measurements between treatments suggests that the nutrient

restriction was not very severe since skeletal mass is usually the last tissue that is negatively affected by nutrient restriction. Hence, most of the difference between treatments was due to fat mobilization and possible muscle mobilization. During this experiment factors such as weather and gut fill likely played a major role on heifer's performance. Therefore, implementation of nutritional management strategy relying on compensatory gain can be risk for beef cattle producers raising cattle on a forage-based diet.

Blood Metabolites

Glucose concentrations are presented in Figure 3-6. There were breed and time ($P < 0.05$) effects on glucose concentrations; however, there were no treatment, treatment x breed, breed x time or treatment x breed x time effects ($P > 0.05$). Additionally, there was a tendency ($P = 0.08$) for a treatment x time effect on glucose concentrations. Mean plasma glucose concentrations were greater for Brangus heifers (71.4 mg/dL) compared to Angus (67.8 mg/dL) heifers. Numerous researchers have also observed greater plasma glucose concentrations for Brahman compared to Angus cattle (Alvarez et al., 2000; Williams et al., 2002; Obeidat et al., 2002). The difference in glucose concentrations between Angus and Brangus cattle could be due the difference in grazing behavior between these breeds (Forbes et al., 1998), as well as physiological differences between *Bos indicus* and *Bos taurus* cattle, such as rumen retention time and fermentation rate (Warwick and Cobb, 1976; Howes et al., 1963; Frisch and Vercoe., 1969; Ikhatua et al., 1985).

As previously indicated, glucose concentrations had a tendency to be decreased in LH compared to CON heifers on d 28, 42 and 56 of the experiment (Figure 3-6), which is probably due to the DDG supplementation that the CON heifers are receiving

compared to no supplement in the LH heifers. Glucose concentrations in feed restricted cattle are decreased compared to non-restricted animals, as glucose concentrations are positively associated with increased nutrient intake (Yelich et al., 1996; Yambayamba et al., 1996). The fact that glucose concentrations for LH heifers were similar to CON heifers on d 14 of the experiment could be due to the low amount of DDG being offered to CON heifers during that period compared to d 28, 42 and 56 of the experiment. Therefore, the nutrient intake on CON heifers was probably not significant enough to elevate glucose concentrations. Furthermore, the lack of difference in glucose concentrations between CON and LH on d 14 of the experiment could be due the timing of sampling. Blood samples were taken 48 h after supplementation of CON heifers. This length of time could allow the glucose concentrations of CON heifers to decrease and reach a similar concentration as LH heifers. Austin et al. (2010) supplemented heifers daily or three times a week and observed that glucose concentrations were greater in daily supplemented heifers for the first 16 h after supplementation. However, after 16 h the concentrations of glucose did not differ between treatments, which could be explained by the fact that ruminants can control plasma glucose concentrations through gluconeogenesis. Gluconeogenesis is a continual metabolic pathway extremely important for ruminants, which results in the generation of glucose mainly from propionic acid (Young, 1977). Therefore, ruminants are able to produce glucose from non-carbohydrate sources and maintain glucose concentrations relatively constant throughout and across the days. From d 84 to 140 of the experiment, glucose concentrations were similar between the CON and LH heifers with the exception of a tendency for greater glucose concentrations on LH heifers on d

112 of the experiment. Since glucose concentrations are positively correlated with DMI, the increased glucose concentrations for LH heifers for the last 70d of the experiment compared to the first 70 is directly related to the LH heifers being supplemented with DDG similar to the CON heifers.

Plasma urea nitrogen concentrations are presented in Figure 3-7. There were no ($P > 0.05$), breed, breed x time, breed x treatment, treatment x breed x time effects on PUN concentrations. However, there was a treatment x time effect ($P < 0.05$) on PUN concentrations. The CON and LH heifers had similar ($P > 0.05$) PUN concentrations on d 0 of the experiment. The LH heifers had greater ($P < 0.05$) PUN concentrations on d 14 and 70 of the experiment compared to CON heifers and the LH heifers had numerically greater PUN concentrations on days 14, 42, and 56 of the experiment compared to CON heifers. The PUN concentrations of LH heifers decreased after d 14, reaching the lowest concentration (15.79 mg/dL) at d 70 of the experiment. Hayden et al. (1993) and Cole and Hallford (1994) have reported greater PUN concentrations in feed-restricted animals compared to *ad libitum* access to feed. In contrast, Yambayamba et al. (1996) and Ellenberger et al. (1989) did not observe elevated concentrations of PUN for restricted fed animals. They suggested that dietary restriction was probably not enough to reach a catabolic state for protein during the restriction period. In agreement, heifers in the current study were probably not feed restricted enough to cause mobilization of protein as evidenced by similar PUN concentrations from d 70 to 140 between LH and CON heifers. Furthermore, tissues are mobilized sequentially during feed restriction. Fat is mobilized first whereas the protein pool is conserved as much as possible (Hornick et al., 2000). Protein mobilization occurs when

lean-animals are feed-restricted and muscle constitutes the main source of energy. However, this does not appear to be the case in this experiment, reinforcing the observations that LH heifers were not mobilizing significant amounts of muscle as an energy source. Heifers in this current study were probably not feed restricted enough to cause mobilization of protein as evidenced by the PUN concentrations and the positive BW gain that occurred between d 0 to 42 for the LH heifers. Furthermore, PUN concentrations account for all the nitrogen available in the blood plasma of the animal; therefore, there is no distinction between nitrogen coming from the diet or protein mobilization. More specific analyses would have to be performed to know the origin of the nitrogen. Therefore, the lack of difference between CON and LH heifers from d 0 to 70 could be due to the fact that CON heifers were being fed DDG, which is high in protein, and would lead to increased PUN concentrations for CON heifers as well.

There was no difference ($P > 0.05$) in PUN concentrations between treatments from d 70 to 140 of the experiment. The PUN concentrations increased in LH heifers after DDG supplementation reaching a peak of 23.8 mg/dL on d 126. The increased PUN concentrations of LH heifers after d 70 are probably due to the DDG supplementation. During the second half of the experiment LH heifers received an average of 2.38 kg•heifer•d^{-1} of DDG, which equates to 0.75 kg of CP•heifer•d^{-1} from the DDG compared to CON heifers which received an average of 2.25 kg•heifer•d^{-1} of DDG, which equates to 0.71 CP•heifer•d^{-1}. The supplementation of high levels of rumen undegradable protein (RUP) has been shown to increase PUN concentrations primarily due to deamination of excess metabolizable protein (MP) that is fed (Dhuyvetter et al.,1993). The fact that DDG is high in RUP is probably the reason for the increase in

PUN concentrations of LH heifers from d 70 to 140 of the experiment. Furthermore, heifers in both groups were probably over supplied protein, as the PUN concentrations exceed those suggested by Byers and Moxon (1980) of 11 to 15 mg/dL and Hammond et al. (1994) of 8 to 12 mg/dL to be sufficient for animal performance.

Mean NEFA concentrations were not different ($P > 0.05$) between the LH (353.5 mEq/ml) and CON (319.6 mEq/ml) heifers from d 0 to d 140 of the experiment. There were no treatment x breed and breed x time effects ($P > 0.05$). However, there were time, breed and treatment x time effects ($P < 0.05$) on NEFA concentrations, which are presented in Figure 3-8. The NEFA concentrations were similar between treatments from d 0 to 28 of the experiment. However, from d 28 to 70, NEFA concentrations were greater ($P < 0.05$) for LH heifers compared to CON heifers. The NEFA concentrations for the LH heifers reached a peak ($P < 0.05$) of 526.4 mEq/ml on d 70, while NEFA concentrations for CON heifers during the same period were never greater than 347.0 mEq/ml. As cattle lose BW, fat is mobilized first as an energy source (Hornick et al., 2000). Metabolism of fat leads to increased NEFA concentrations (DiMarco et al., 1981), which is typically observedin feed restricted heifers (Yambayamba et al., 1996) during the restriction period. Increased NEFA concentrations are generally indicative of negative energy balance, which can result in decreased BW due to negative ADG. In the present study, a negative ADG was observed from d 42 to 70 of the experiment, when NEFA concentrations were increased for LH heifers. The increased NEFA concentrations for LH heifers were also associated with decreases of BCS, RIBFT and RMPFT of these heifers observed during the same experimental period, reinforcing that fat mobilization occurred during the first 70 d of the experiment for LH heifers.

Brangus heifers (356.9 mEq/ml) had greater ($P < 0.05$) mean NEFA concentrations compared to Angus heifers (316.0 mEq/ml). Obeidat et al. (2000) observed greater NEFA concentrations for Brahman cattle and suggested that a different mechanism may exist between Angus and Brahman cows in regard to maintenance and mobilization of adipose tissue. *Bos indicus* cattle have been shown to have greater lipoprotein lipase (LPL) and hormone-sensitive lipase (HSL) activity compared to *Bos taurus* breeds (Sprinkle et al., 1998). The enzymes, LPL and HSL are both involved with the hydrolysis of triglycerides to fatty acids. Glucose and insulin concentrations are greater in *Bos indicus* breeds, and insulin has been implicated in the synthesis (Speake et al., 1986), and possibly in the transport (Hollenberg, 1990), of LPL to the adipose tissue cell surface endothelium where it is active. The difference in NEFA concentrations between treatments was diminished within 14 d of supplementation for LH heifers. Yambayamba et al. (1996) also observed that NEFA concentrations decreased after realimentation, and by d 10 of realimentation no differences between treatments were apparent. DiMarco et al. (1981) reported that realimentation periodof approximately 8 d was necessary to decrease the NEFA concentrations to a basal level of fed-restricted animals. Yambayamba et al., (1996) suggested that the metabolic shift takes several days, and while this happening, there is a considerable increase in efficiency of energy utilization because maintenance requirements for animals that were under feed restriction are still low.

Reproduction Performance

There were four Angus heifers (2 CON, 2 LH) that were estrous cycling before the beginning of the experimental period and they were excluded from the puberty analysis. There were no treatment, breed, or treatment x breed effects ($P > 0.05$) on the

percentage of heifers that attained puberty during the first half of the experiment (d 0 to 70; Figure 3-9), as well as by d 140 of the experiment. Even though the LH heifers had a decreased BCS and tended to be lighter at d 140 of the experiment compared to CON heifers, the LH heifers were able to reach a target BW of 55% of their potential mature BW. The onset of puberty is highly correlated to heifer BW and BW gain (Warnick et al., 1956) and it is an important trait on the attainment of puberty. Yelich et al. (1996), fed heifers either a high gain diet (1.36 kg/d) or low-high gain (0.23 kg/d for 16 wk, then fed 1.36 kg/d), and observed that heifers on different treatments had similar BW at puberty. However, heifers in the aforementioned study were fed an energy pellet plus soybean hull mix ration compared to the forage based in the present study and low-high gain heifers were able to fully compensate to similar BW as full fed heifers in the Yelich et al. (1996) study.

There were no treatment, breed, or treatment x breed effects ($P > 0.05$) on estrous response. Although, numerically 15% more CON heifers exhibited estrus compared to LH heifers. A similar estrous response ($P > 0.05$) between CON and LH heifers is mainly because the CIDR induced many heifers to show estrus in both LH and CON heifers. There were a greater number of prepubertal heifers (n = 43) at the start of the synchronization protocol compared to the number of pubertal heifers (n = 17). However, estrous response was similar ($P > 0.05$) between heifers that were estrous cycling (78.2%) and non-estrous cycling (71.6%) heifers at the start of the breeding season, suggesting that the progestin used in the synchronization protocol induced a visable estrus in 30 out of 43 prepubertal heifers. Progestins are known to mimic hormonal changes that occur around puberty, and induce puberty in prepubertal heifers

(Gonzalez-Padilla et al., 1975; Patterson et al., 1989). The non-estrous cycling heifers had a mean age of 415 d and BW of 352 kg at the start of the synchronization treatment and they were evidently close to attaining puberty by d 140 of the experiment as evidences by the progestogen treatment inability to induce puberty.

The fact that most of the heifers were not cycling at d 140 of the experiment is intriguing. For both the CON and LH heifers, they were at least 55% of their mature BW at the start of the breeding season suggesting that heifers weighed enough to attain puberty. Additionally, Angus heifers are known to reach puberty at a younger age compared to Brangus heifers (Reynolds et al., 1963). However, there was not a breed effect on percent of heifers pubertal by d 140 of the experiment. Non-estrous cycling Angus heifers in the present experiment had a mean age of 421 d, while non-estrous cycling Brangus heifers were 410 d of age at the start of the breeding season. The estrous cycling heifers had numerically more 0.54 cm of RMPFT and 0.46 cm of RIBFT, while non-cycling heifers had 0.50 cm of RMPFT and 0.40 cm of RIBFT, leading to believe that probably cycling heifers had a better BCS compared to non-cycling heifers. Hopper et al. (1993) suggested that there is a critical threshold level of fat necessary for occurrence of puberty. Furthermore, Richards et al. (1989) reported the importance of body fat for maintenance of estrous cycles.

Conception rate was not different ($P > 0.05$) between treatments, breeds, nor was there a treatment x breed effect (Table 3-4). Furthermore, conception rate tended ($P = 0.09$) to be greater for estrous cycling heifers (69.2%) compared to non-estrous cycling (40.0%) heifers. The conception rate of the present study was less compared to Martin et al. (2007), who reported a conception rate of 75% for heifers fed DDG and

synchronized with two doses of prostaglandin. However, Lynch et al. (1997) also observed conception rate of 55.5% for heifers developed on constant rate of gain (0.45 kg/d) for 159 d, synchronized with two doses of prostaglandin 14 d apart. In agreement with the results of the present study, there was no difference on conception rate for heifers developed on constant rate of gain or even-late gain, which were developed on 0.11 kg/d from d 0 to 112, followed by 0.91 kg/d from d 112 to 159 (Lynch et al., 1997).

Timed-AI pregnancy rates were not different ($P > 0.05$) between treatments, breeds, and treatment x breed (Table 3-4). Timed-AI pregnancy rates were similar ($P > 0.05$) between non-estrous cycling (56.3%) and estrous cycling heifers (53.8%). Furthermore, synchronized pregnancy rates were not different between, treatment, breeds and treatment x breed ($P > 0.05$: Table 3-4). Synchronized pregnancy rate for CON heifers was 56% compared to 43% of LH heifers ($P > 0.05$). In addition, synchronized pregnancy rates were also similar ($P > 0.05$) between non-estrous cycling (44.2%) and estrous cycling heifers (64.7%). Final pregnancy rates were similar ($P > 0.05$) between CON and LH heifers as well as between estrous cycling (94.1%) and non-estrous cycling (79.1%) heifers. Other studies (Clanton et al., 1983; Lynch et al., 1997) have shown that delaying the gain can be used as management tool to decrease feed intake without having a negative effect on final pregnancy rates. Based on the reproductive performance results of this experiment, the supplementation strategy of delayed gain should be carefully implemented on heifers development program. The synchronization protocol utilized induced estrus in a high percentage of non-estrous cycling heifers, which resulted in similar pregnancy rates to the synchronized AI in both

the CON and LH heifers. Additionally, final pregnancy rates were similar between treatments.

In summary, feeding RBS only for the first 70 d altered BCS and tended to decrease BW of Angus and Brangus heifers. The body composition of LH heifers was altered due to mobilization of fat during the first 70 d of the experiment as evidenced by a decrease in RMPFT and RIBFT, which was supported by the increased NEFA concentrations of LH compared to CON heifers during the first 70 d of the experiment. Skeletal growth was not significantly affected by treatment as LH and CON heifers had similar HH and PA on d 70 and 140 of the experiment. There was a slight decrease in REA of LH heifers at the end of the restriction phase of the experiment compared to CON heifers; however, REA/cwt was not different between treatments suggesting that there was no significant protein mobilization in the LH heifers. There tended to be a treatment ×time effect on glucose concentrations with CON heifers tending to have greater glucose concentrations on day 28, 42, and 56 of the experiment but it returned to similar concentrations once LH heifers began receiving their supplement. There was a significant treatment × time effect on PUN concentrations as the LH heifers had decreased PUN concentrations from d 0 to 84 of the experiment but they were of similar concentrations to CON heifers from d 94 to 140 of the experiment

Delaying the weight gain of the LH heifers did not significantly alter the percentage of heifers that were pubertal heifers at the start of breeding season compared to CON heifers. Furthermore, the CON heifers and LH heifers had similar synchronized pregnancy rates as well as final breeding season pregnancy rates.

Implications

Heifers supplemented with DDG for only 70 d prior to the breeding season were only able to partially compensate and body composition was altered by d 140 of the experiment. These results suggest that DDG supplementation for beef heifers only during the 70 d prior to breeding do not negatively affect pubertal status and final pregnancy rates. Final pregnancy rates were similar between treatments; as well as synchronized pregnancy, suggesting that the supplementation strategy could be implemented to decrease feed input for beef replacement heifers without negatively affecting the reproductive performance.

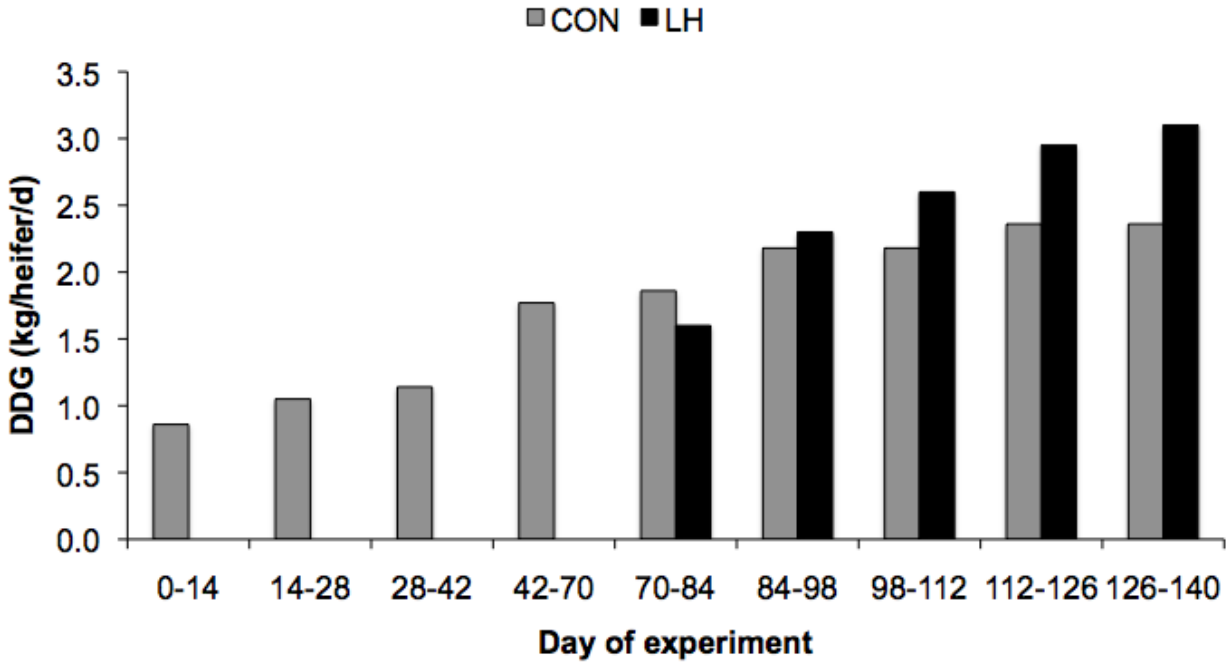

Figure 3-1. Dried distillers grain (DDG) offered to Brangus and Angus heifers fed to gain either at a constant (CON) or low-high (LH) rate of gain from d 0 to 140 of the experiment. The CON heifers were fed round bale silage (RBS) and DDG from d 0 to 140 of the experiment and LH heifers were fed RBS from d 0 to 70 followed by RBS and DDG from d 70 to 140.

Table 3-1. Nutritional value of round bale silage (RBS), dried distillers grain (DDG) and pasture offered to yearling Angus and Brangus beef heifers throughout the experiment.

Item	RBS	DDG	Pasture[1]
DM %	55.5	87.7	70.5
DM basis			
CP, %	15.0	31.6	11.4
IVDMD , %	52.2	-	33.4
TDN, %	62.0	81.7	49.3
Sulfur, %	0.28	0.65	-

[1] Dormant mixture of bahiagrass and bermudagrass forage.

Table 3-2. Growth characteristics on d 0, 70 and 140 of the experiment of yearling Angus and Brangus heifers consuming round bale silage (RBS) and supplemented with dried distillers grain (DDG) to gain at either a constant (CON) or low-high (LH) rate of gain (LS means ± SE).

	Day of Experiment		
Item	0	70	140
Body Weight, kg			
CON [1]	279.1 ± 9.6 [x]	315.7 ± 9.9 [a, y]	367.2 ± 10.1 [c, z]
LH [2]	275.5 ± 9.6 [x]	286.6 ± 9.9 [b, y]	340.6 ± 10.1 [d, z]
BCS [3]			
CON	5.3 ± 0.07 [x]	5.3 ± 0.08 [a, x]	5.6 ± 0.07 [a, y]
LH	5.2 ± 0.07 [x]	4.8 ± 0.08 [b, y]	5.2 ± 0.07 [b, x]
Hip Height, cm			
CON	114.3 ± 0.87 [x]	118.8 ± 0.92 [y]	122.6 ± 0.82 [z]
LH	114.6 ± 0.87 [x]	117.6 ± 0.92 [y]	121.4 ± 0.82 [z]
Pelvic Area [4], cm^2			
CON	126.4 ± 4.62 [x]	166.9 ± 3.97 [c, y]	170.9 ± 3.99 [y]
LH	121.0 ± 4.62 [x]	156.9 ± 3.97 [d, y]	165.6 ± 3.99 [z]

[a,b] Treatment means in a column within the same item with different superscript differ ($P < 0.05$)
[c,d] Treatment means in a column within the same item with different superscript tended to differ (PA; $P = 0.07$ and BW; $P = 0.06$)
[x,y,z] Time means in the same item and treatment with different superscript differ ($P < 0.05$)
No significant interaction ($P>0.05$) for treatment x breed was observed for all reported values.
[1] CON: heifers fed RBS and DDG from d 0 to 140 of the experiment
[2] LH: heifers fed RBS from d 0 to 70 followed by RBS and DDG supplementation from d 70 to 140
[3] BCS: 1= severely emaciated; 5=moderate; 9= very obese.
[4] PA = pelvic height x pelvic width

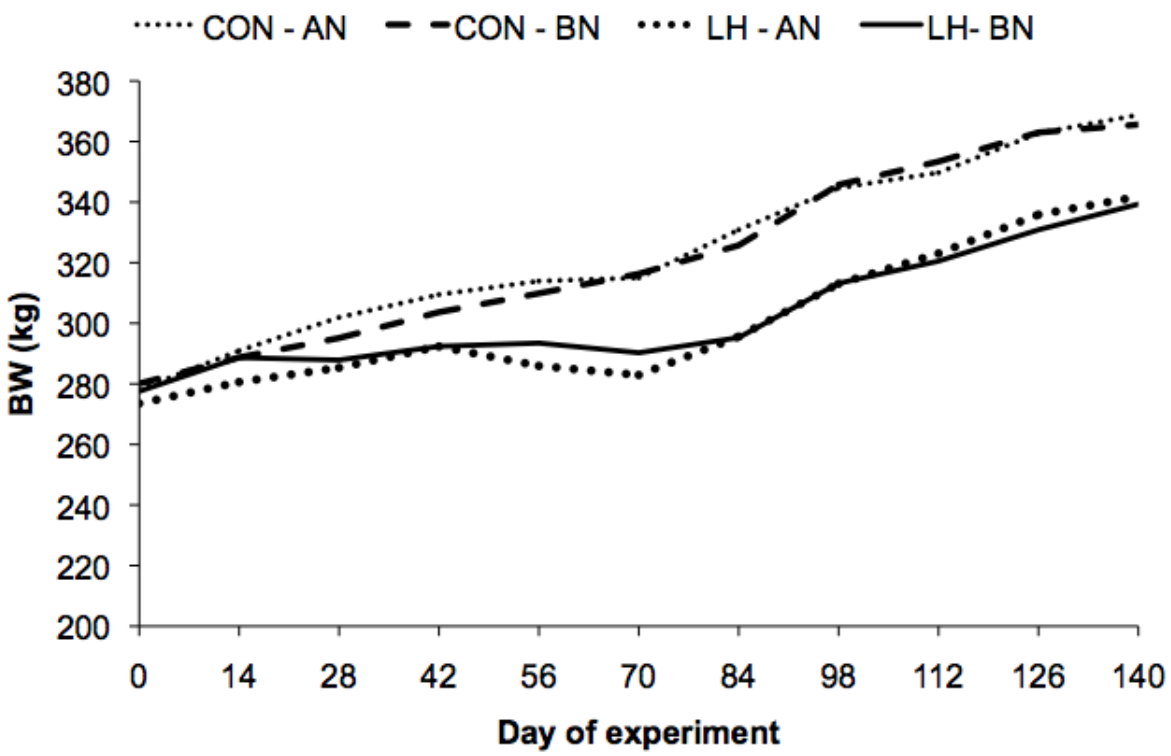

Figure 3-2. Body weight (BW) by treatment, and day for Angus (AN) and Brangus (BN) heifers consuming round bale silage and supplemented with dried distillers grain to gain at either a constant (CON) or low-high (LH) rate of gain. Treatment x time and breed x time ($P < 0.05$).

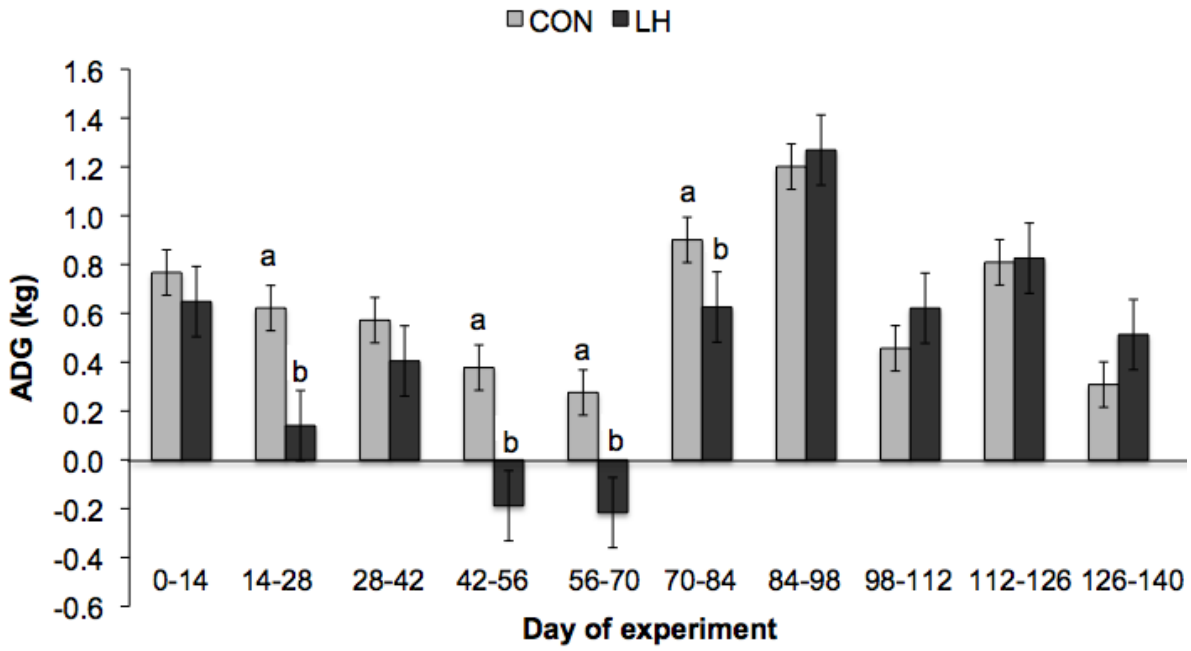

Figure 3-3. Average daily gain (ADG) by 14 d period by treatment for heifers consuming round bale silage and supplemented with dried distillers grain to gain at either a constant (CON) or low-high (LH) rate of gain. Treatments means within a period with different superscript differ ($P < 0.05$).Treatment, treatment x time, treatment x breed ($P < 0.05$).

Table 3-3. Body ultrasound measurements on d 0, 70 and 140 of the experiment for yearling Angus and Brangus heifers consuming round bale silage and supplemented with dried distillers grain to gain at either a constant (CON) or low-high (LH) rate of gain (LS means ± SE).

	Day of Experiment		
Item	0	70	140
IMF [1], %			
CON	2.64 ± 0.11 [x]	3.77 ± 0.15 [y]	3.75 ± 0.17 [y]
LH	2.39 ± 0.11 [x]	3.50 ± 0.15 [y]	3.70 ± 0.17 [y]
RIBFT [2], cm			
CON	0.49 ± 0.02	0.47 ± 0.02 [a]	0.47 ± 0.02
LH	0.46 ± 0.02 [x]	0.37 ± 0.02 [b, y]	0.42 ± 0.02 [z]
RMPFT [3], cm			
CON	0.52 ± 0.02 [x]	0.48 ± 0.02 [y]	0.55 ± 0.03 [a, x]
LH	0.51 ± 0.02 [x]	0.41 ± 0.02 [y]	0.45 ± 0.03 [b, z]
REA [4], cm^2			
CON	46.69 ± 1.60 [x]	47.74 ± 1.57 [a, x]	51.42 ± 1.65 [a, y]
LH	45.77 ± 1.58 [x]	43.35 ± 1.55 [b, y]	48.22 ± 1.63 [b, z]
REA/cwt [5], cm^2			
CON	16.68 ± 0.32 [x]	15.10 ± 0.28 [y]	14.09 ± 0.29 [z]
LH	16.68 ± 0.32 [x]	15.16 ± 0.27 [y]	14.19 ± 0.29 [z]

[a,b] Treatment means in a column within the same item with different superscript differ ($P < 0.05$).
[x, y, z] Time means in the same item and treatment with different superscript differ ($P < 0.05$).
[1] IMF: intramuscular fat in *longissimus dorsi* muscle
[2] RIBFT: 13th rib subcutaneous fat thickness
[3] RMPFT: rump subcutaneous fat thickness
[4] REA: l*ongissimus dorsi* area
[5] REA/cwt: ribeye area adjusted for 100 kg of BW

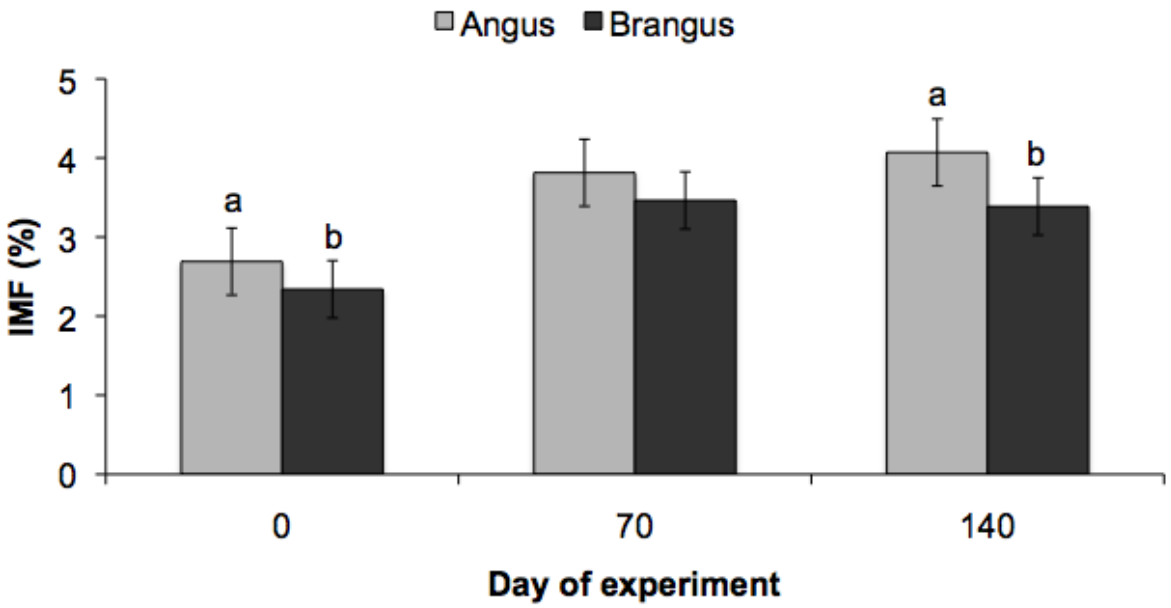

Figure 3-4. Intra muscular fat (IMF) by breed and time for yearling Angus and Brangus heifers consuming round bale silage and supplemented with dried distillers grain to gain a constant or low-high rate of gain. Means within a period with different superscript differ ($P < 0.05$). Breed and treatment x time x breed ($P < 0.05$).Treatment, treatment x breed, treatment x time, and breed x time ($P > 0.05$)

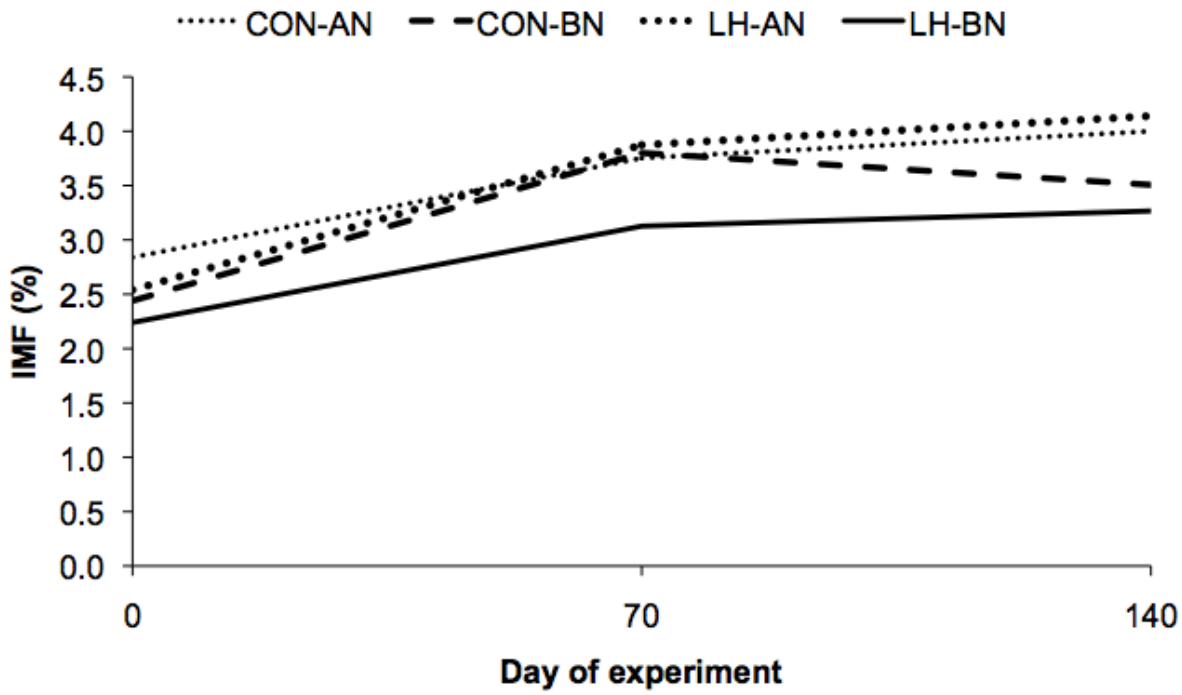

Figure 3-5. Intra muscular fat (IMF) by breed x treatment x time for yearling Angus and Brangus heifers consuming round bale silage and supplemented with dried distillers grain to gain at either a constant (CON) or low-high (LH) rate of gain.Treatment, treatment x breed, treatment x time, and breed x time ($P > 0.05$). Breed and time x breed x treatment ($P < 0.05$).

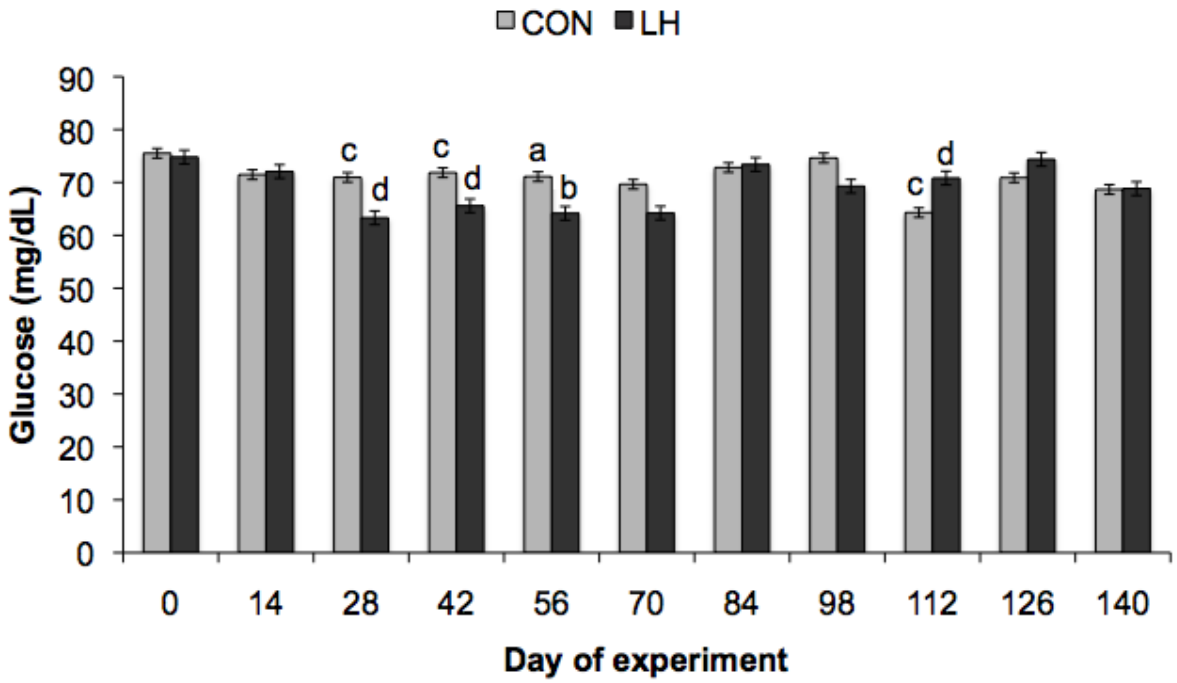

Figure 3-6. Glucose concentrations by treatment and day of the experiment for yearling Angus and Brangus heifers consuming round bale silage and supplemented with dried distillers grain to gain a constant (CON) or low-high (LH) rate of gain.Treatment x breed and breed x time ($P > 0.05$). Treatment x time ($P = 0.08$). Means within a day with different letters (a,b) differ ($P < 0.05$). Means within a day with different letters (c,d) tended to differ ($P < 0.10$).

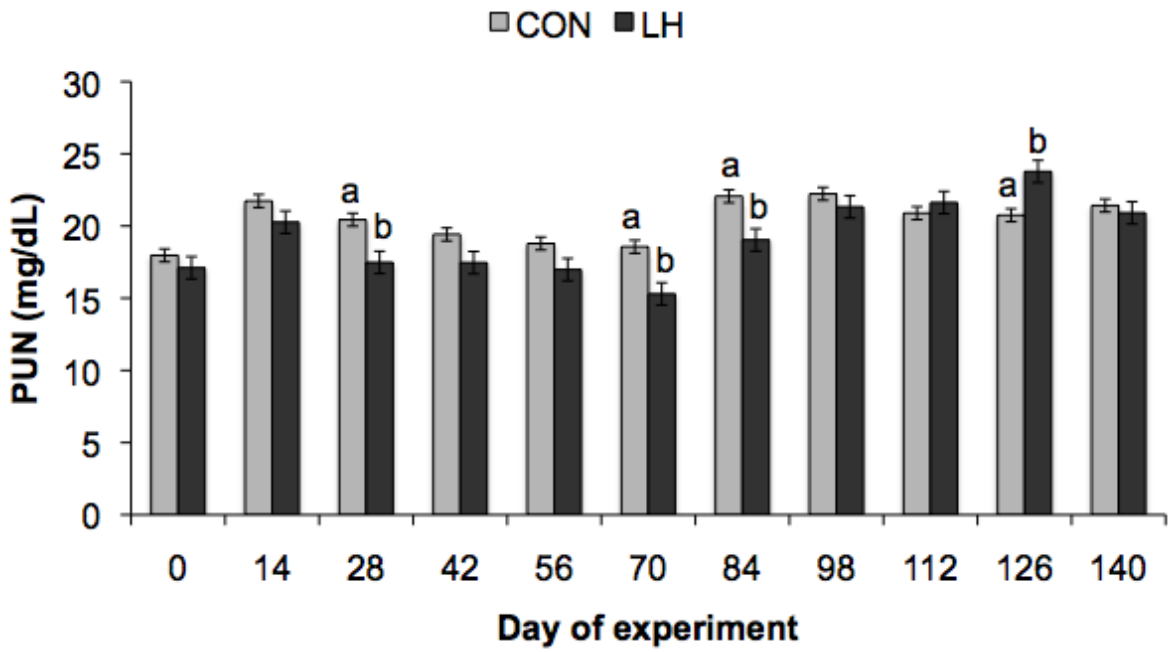

Figure 3-7. Plasma urea nitrogen (PUN) concentrations by treatment and day of the experiment for yearling Angus and Brangus heifers consuming round bale silage and supplemented with dried distillers grain to gain at either a constant (CON) or low-high (LH) rate of gain. Treatment x time ($P < 0.05$). Means within a day with different letters differ ($P < 0.05$).

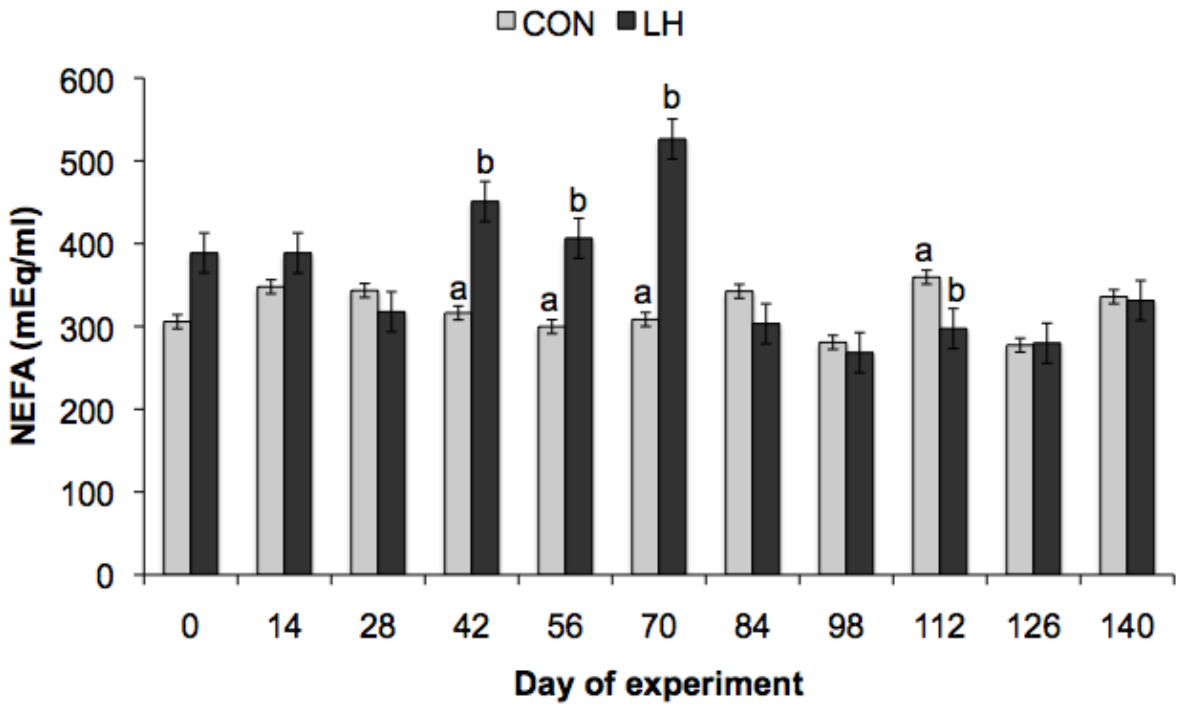

Figure 3-8. NEFA concentrations by treatment and day of the experiment for yearling Angus and Brangus heifers consuming round bale silage and supplemented with dried distillers grain to gain at either a constant (CON) or low-high (LH) rate of gain. Breed and treatment x time ($P < 0.05$). Means within a day with different letters differ ($P < 0.05$).

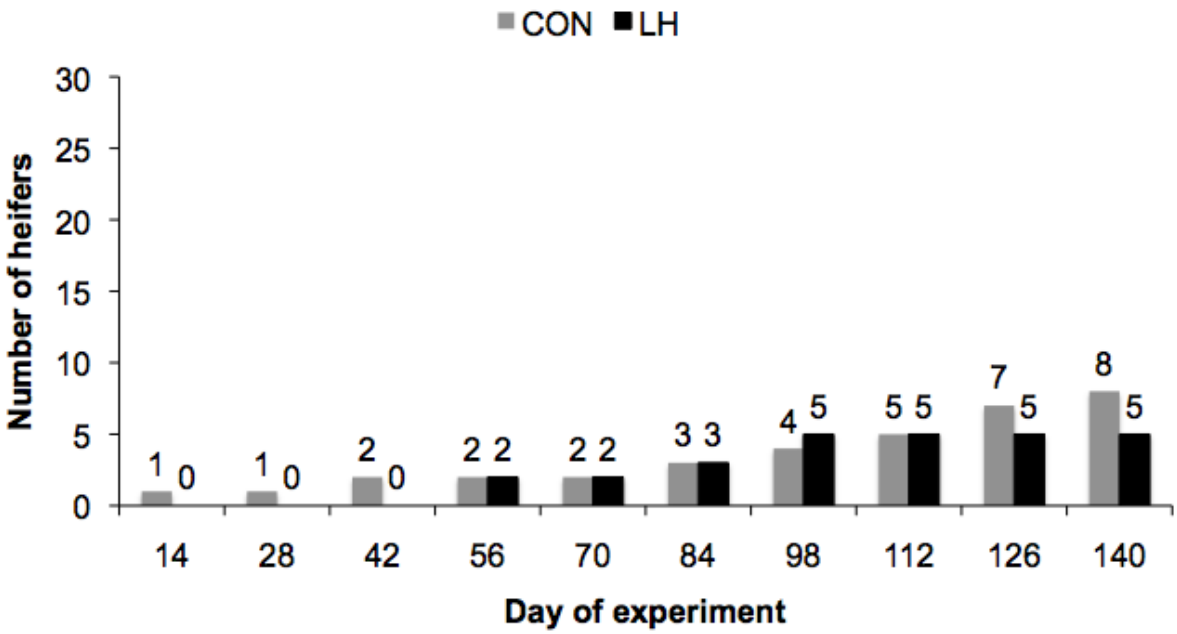

Figure 3-9. Cumulative number of total pubertal heifers throughout the experimental period for yearling Angus and Brangus heifers consuming round bale silage (RBS) and supplemented dried distillers grain (DDG) to gain at a constant (CON) or low followed by a high (LH) rate of gain. Treatment, treatment x breed, treatment x time, and treatment x breed x time ($P > 0.05$)

Table 3-4. Estrous response, conception rate, timed-AI, synchronized pregnancy rate, and overall pregnancy rate of yearling Angus and Brangus heifers consuming round bale silage and supplemented with dried distillers grains to gain at either a constant (CON) or low followed by high (LH) rate of gain (Means are reported as LS means).

Treatments	Estrous response, % [a]	Conception rate,% [b]	Timed-AI pregnancy rate, % [c]	Synchronized pregnancy rate,% [d]	Overall pregnancy rate,% [e]
CON	80 (24/30)	54 (13/24)	66 (4/6)	56 (17/30)	81 (24/30)
Angus	80 (12/15)	50 (6/12)	100 (3/3)	60 (9/15)	87 (13/15)
Brangus	80 (12/15)	58 (7/12)	33 (1/3)	53 (8/15)	73 (11/15)
LH	65 (19/30)	39 (8/19)	52 (5/11)	43 (13/30)	88 (26/30)
Angus	80 (12/15)	50 (6/12)	66 (2/3)	53 (8/15)	93 (14/15)
Brangus	47 (7/15)	29 (2/7)	37 (3/8)	33 (5/15)	80 (12/15)
P-value					
Treatment	0.22	0.34	0.98	0.30	0.46
Breed	0.22	0.66	0.97	0.30	0.18
Treatment x Breed	0.22	0.34	0.98	0.60	0.80

[a] Percentage of heifers displaying estrus three d after $PGF_{2\alpha}$
[b] Percentage of heifers pregnant to AI of the total that exhibited estrus.
[c] Percentage of heifers pregnant to timed-AI.
[d] Percentage of heifers pregnant to the synchronized breeding system.
[e] Percentage of heifers pregnant during the entire 60 d breeding season.

APPENDIX A
NUTRITIONAL COMPOSITION OF MINERAL-VITAMIN MIX OFFERED TO YEARLING ANGUS AND BRANGUS BEEF HEIFERS THROUGHOUT THE EXPERIMENT.

Item		Amount
Calcium (Ca)	Max	14.00%
Calcium (Ca)	Min	12.00%
Phosphorus (P)	Min	6.00%
Salt (NaCl)	Max	21.00%
Salt (NaCl)	Min	19.00%
Potassium (K)	Min	0.8%
Magnesium (Mg)	Min	1.00%
Sulfur (S)	Min	0.40%
Iron (Fe)	Min	0.40%
Copper (Cu)	Min	2,000 ppm
Cobalt (Co)	Min	200 ppm
Manganese (Mn)	Min	2,200 ppm
Iodine (I)	Min	175 cpm
Selenium (Se)	Min	48 ppm
Zinc (Zn)	Min	9,500 ppm
Fluorine (F)	Max	800 ppm
Vitamin A	Min	100,000 IU/lb
Vitamin D3	Min	20,000 IU/lb

APPENDIX B
GLUCOSE ASSAY PROTOCOL

Materials:

- Microcentrifuge tubes
- Rack for microcentrifuge tubes
- 12 x 75 mm test tubes
- 18 x 150 mm test tubes
- Ultra Pure water
- Control sample (pool of plasma samples)
- Rack for test tubes
- Incubator
- Plate reader
- 10μl Pipette
- 1000μl Pipette
- 200μl Pipette
- Pipette tips
- Cayman Glucose Kit
  - Glucose Assay Standard
  - Glucose Assay Buffer
  - Glucose Enzyme Mixture
  - Two 96-well plates

Before start the assay:

- Glucose assay buffer should be thawed and equilibrated to 4°C.
- All reagents and ultra pure water should be equilibrated to 4°C before the start of the assay.

Standard preparation:

- Take eight 12 x 75 mm test tubes and label them A- H. Place tubes in a rack.
- Add the amount of glucose standard and assay buffer to each tube as described in the table below.

Tube	**Glucose Standard (**μl)	**Assay Buffer (**μl)	**Glucose Concentration (mg/dl)**
A	0	200	0
B	2.5	197.5	12.5
C	5	195	25
D	10	190	50
E	20	180	100
F	30	170	150
G	40	160	200
H	50	150	250

Performing the assay part I:

- Label first 8 microcentrifuge tubes from A-H where the standards are going to be placed. The following microcentrifuge tubes are labeled according to samples ID. Label the last microcentrifuge tube of each well-plate as control.
- Add 5 μl of plasma sample, control sample and standard to labeled microcentrifuge tube.

Enzyme mixture preparation:

One vial of the enzyme is sufficient to evaluate 48 well plates.

- Add 500 μl of 4°C UltraPure water to the enzyme vial.
- Transfer the reconstituted solution to an 18 x 150 mm test tube.
- Add 12 ml of assay buffer to the reconstituted solution and mix well.
  NOTE: A portion of the 12 ml should be used to rinse any residual solution from the vial.
- This reconstituted solution is now ready to be used in the assay.
  NOTE: The reconstituted solution is stable for at least one hour when stored at 4°C.

Performing the assay part II:

- Add 500 μl of the enzyme mixture forcefully down the side of each tube (standard and samples). Tap tubes a couple of times to mix thoroughly.
- Place tubes in a 37°C incubator for 10 minutes.
- After 10 minutes, remove tubes from incubator.
- Load 150 μl (in duplicate) from each tube to the 96-well plate.
- Read the absorbance at 500-520 nm using a plate reader.

Plate reader:

- Turn on plate reader.
- Open plate reader program. Select glucose protocol and the run.
- Values are reported as final glucose concentrations.

APPENDIX C
NON-ESTERIFIED FATTY ACIDS (NEFA) ASSAY PROTOCOL

Materials:

- Color reagent A
- Solvent A
- Color reagent B
- Solvent B
- Serum samples
- Control sample (Pool sample)
- Kit standards
- Saline
- Deionized water
- 10 μl pipette
- 1000 μl pipette
- Pipette tips
- 96 well plates with lids
- Colored paper
- Plate reader

Saline Solution:
Prepare 0.9% saline solution before the start of the assay.
Procedure: Mix 0.9g of NaCl with 100 ml of deionized water.

Assay procedure:

- Sort samples and prepare assay sheet. Randomly select samples and place in rack, recording the order on your assay sheet. Each plate allows for 42 unknown samples.
- Obtain plates. Wells 1A and 2A are blanks. Wells 3A – 12A are standards. Wells B1 and B2 are pools. Remaining wells are duplicate samples. Repeat blanks, standards and pool for each plate.
- Prepare reagents: Add one bottle of Solvent A to one vial of Color Reagent A. Add one bottle of Solvent B to one vial of Color Reagent B. Mix gently by inverting the vials until contents are completely dissolved.
- Prepare the standard curve dilutions in test tubes and transfer 5 μl into the following wells.

Standard (mEq/ml)	*Saline Solution (*µl)	*Standard Solution (*µl)
0	500	0
200	400	200
400	300	400
600	200	600
800	100	800
1000	0	1000

- Pipette 5µl of Control (pool) in wells 1B and 2B.
- Pipette 5µl of serum sample in duplicate as per the order on your assay sheet.
- Pipette 200µl Color Reagent A Solution to all wells.
- Turn on plate reader (Dr.Ealy's lab). Turn on computer and open the program for the plate reader. Press turn on button for temperature on the plate reader. Once the temperature reached 37°C place well-plate in the plate reader and incubate for 5 minutes. After 5 minutes, press read on the computer.
- Measure and record absorbance of each well at 560 nm (sub: 660nm) for Abs 1 (only with reagent A).
- Remove well-plate from the plate reader and add 100µl of Color Reagent B Solution to all wells.
- Place well-plate in the plate reader and incubate for 5 minutes again. After 5 minutes, press read on the computer.
- Measure and record absorbance of each well at 560nm (sub:660nm) for Abs 2 (reagent A and reagent B).

Calculations:

- The results obtained from the plate reader are not the final results.
- Calculation of final absorbance:
  The absorbance 1 from the first measurement should be multiplied by a factor (F) in order to correct for changes in volume, as follows:

  F = (Sample volume + Solution A volume) / (Sample volume + Solution A volume + Solution B volume)

  F = (4 + 200) / (4 + 200 + 100) = 0.67

  Therefore:

  Final sample absorbance = Absorbance 2 – (Absorbance 1 * 0.67)

- Plot absorbance vs. concentration of standards to generate a linear regression curve and equation. The equation will be used to calculate the final results
- Use the excel template to calculate final results.

APPENDIX D
BLOOD UREA NITROGEN (PUN) ASSAY PROTOCOL

Materials:

- Reagent A
- Reagent B
- Urea standard
- 1000μl pipette
- Pipette tips
- 96 well plates
- Serum or plasma samples
- Control sample (pool sample)
- Plate reader

Assay procedure:

- Reagent preparation: Equilibrate reagents to room temperature. Prepare enough working reagent by combining equal volumes of Reagent A and Reagent B, shortly prior to assay. Use working reagent within 20 minutes after mixing.
- Add 5μl water (blank) to wells 1 and 2.
- Add 5μl standard (50mg/dL) to wells 3 and 4.
- Add 5μl of control sample to wells 5 and 6.
- Add 5μl samples in duplicate into following wells.
- Add 200μl working reagent and tap tightly to mix.
- Incubate for 20 minutes on room temperature.
- Turn on plate reader. Turn on computer and open the program for the plate reader. Select PUN protocol.
- Read optical density at 520nm.
- The results obtained are not the final results since it is based on urea concentrations. The following conversion is necessary.
  Conversion formula: PUN (mg/dL) = [Urea] / 2.14

APPENDIX E
PROGESTERONE ASSAY PROTOCOL

Materials:

- 100μl pipette
- Pipette tip
- Clear polypropylene Tubes
- Tube rack
- Count-A-Count Kit

Assay set up:

- Label the standard curve tubes and place on a rack. Tubes 1 – 4 are clear polypropylene tubes. The following tubes are pink coated tubes. Tubes 5 – 22 are standards. Tubes 23-26 are controls.
- Tube 27 begins unknown samples. Repeat control tubes at the end of the assay. Place unknown tubes in a different rack.
- Cover racks with plastic and refrigerate until use. If there is any left over tubes put in the bag and refrigerate it.
- Cover the bench space where you plan to pipette.
- Double-cover bench space in the hot room.

Assay procedure:

- Pipette 100 μl of unknown sample into corresponding coated tube per order on your assay sheet.
  Use a new pipette tip for each unknown sample
- Pipette controls into tubes at the end of the assay.
- Cover unknown and control samples with plastic and place in the refrigerator.

Standard curve preparation:

- Pipette the standards using a new pipette for each calibrator.
- The standard curve dilution is shown on the table below.

Tubes	*Concentration (ng/ml)*	*Calibrator*	*Calibrator Volume (µl)*
3-4	0	A	100
5-6	0	A	100
7-8	0.1	B	100
9-10	0.25	A + C	50 + 50
11-12	0.50	C	100
13-14	1	A + D	50 + 50
15-16	2	D	100
17-18	5	A + E	50 + 50
19-20	10	E	100
21-22	20	F	100

Addition of I^{125}

- In the hot room, prepare the repeat pipette and combi-tip to dispense 1 ml.
- Pour content of I^{125} bottle into a beaker.
- Use the repeat pipette to dispense 1 ml of I^{125} progesterone to ALL tubes. This step must be completed within 10 minutes.
- Cover tube racks with plastic and vortex briefly.

Incubation and Decating

- Afetr adding I^{125} progesterone, allow tubes to incubate for 3 hours at room temperature.
- Remove tubes 1 and 2 from rack and store separately.
- Transfer all other tubes to foam racks, maintaining the same tube order.
- Under the hood, quickly pour tube contents into the waste container. DO NOT pour off contents of tubes 1 and 2.
- Place inverted racks on the doubled bench paper and gently tap to remove all drops and bubbles.
- Allow tubes to dry on the bench paper for approximately 15-20 minutes.
- Transfer ALL tubes from the foam racks to the gamma counter racks.

Counting gamma counter

- Put the racks in order in the counter.

Blank rack/background (with clip band #30 attached to the left side of the rack)
First rack (with clip band #7)
Rest of the racks
Stop rack (after the last sample)

- Insert a low disk into the A drive and press F2 (next protocol). Counter will start read at a rate of 5 tubes / min.

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BIOGRAPHICAL SKETCH

Aline Monari was born in São Bernardo do Campo, São Paulo, Brazil in 1985. She is the youngest daughter of Julia Martins Monari and Antonio Jair Monari. After graduating from high school in 2002, she attended the University of São Paulo (USP-FZEA) where received her Bachelor's degree in Animal Sciences. The University of São Paulo is ranked as the best university of Brazil and is ranked among the top universities in the world.

In 2006, during her senior year at University of São Paulo, she was honored with the CAPES-FIPSE Scholarship, where she had the opportunity to complete a part of her bachelor's degree and senior internship program at Cornell University, Ithaca, New York. At Cornell, Aline had the opportunity to help in a research focused on enzyme combinations to improve digestibility of soy-containing milk replacers for neonatal calves.

After completing her exchange program at Cornell University, she initiated her master's degree with Dr. Joel Yelich at University of Florida, Gainesville, Florida, in 2008. Aline's master research at University of Florida was focused on the effects of dried distillers grain supplementation on two rates of gain for replacement beef heifer consuming round bale silage. During her time at University of Florida, Aline was also involved with other projects focusing on reproductive physiology and the nutritional management of beef cattle.