Effects of alfalfa and grass hay late gestation diets on body weight, condition score, pelvic area, birth weight, calving difficulty, blood metabolite, and steroid hormones
by Bruce D Nisley

Abstract:
An experiment was conducted to compare the effects of alfalfa and grass hay diets on body weight (BW), condition score (CS), pelvic area (PA), calving difficulty score (CDS), calf birth weight (CBW), blood metabolites, and steroid hormone concentrations. Thirty-two crossbred beef heifers, bred to the same sire by A.I., were assigned to either alfalfa or grass hay diets. Heifers were penned in groups of four and assigned to Calan gates to facilitate measurement of individual feed intake. Ninety and 6 days prior to the first expected calving date, BW, CS, and PA were measured. Hay samples were taken for analysis periodically throughout the study. Beginning 20 days prior to each heifer's expected calving date, blood samples were taken daily for metabolite and steroid hormone analysis. At calving, CDS, calf presentation, and CBW were recorded. Dry matter and total digestible nutrient intake were similar (P>. 10) between diets, but crude protein intake was significantly higher (P<.05) for the alfalfa diet. Diet had no significant effect on BW change, CS change, or PA change. Calving difficulty scores were not significantly different between treatments, however CBW was 2.6 kg heavier (P<.10) for calves from heifers on the grass hay diet. Male calves were 3 kg heavier (P<.05) than female calves. Glucose levels increased significantly (P<.05) the day prior to parturition, but were not affected by diet. As CBW increased glucose concentrations decreased (P<.05) for heifers on the grass hay diets; but for heifers fed alfalfa, glucose concentrations were not affected by CBW. Blood urea nitrogen (BUN) levels were higher (Pc.05) for heifers on the alfalfa hay diet, but were not affected by time. Increased CBW caused decreased BUN in grass-hay fed heifers, however, alfalfa-fed heifers demonstrated the opposite trend with increased BUN concentrations as CBW increased. Progesterone (P4) concentrations were not different between diets, however were greatly affected by time with a rapid drop occurring the day prior to parturition. Concentrations of estradiol 17-β (E2) were not significantly (P>.10) affected by diet. Heifers that produced heavier calves had higher (P<.05) E2 concentrations on both diets. In summary, the comparison of alfalfa and grass hay diets did not suggest that alfalfa may increase dystocia or CBW. , It appears that both feeds are acceptable and practical diets for gestating heifers.

EFFECTS OF ALFALFA AND GRASS HAY LATE GESTATION DIETS ON

BODY WEIGHT, CONDITION SCORE, PELVIC AREA, BIRTH WEIGHT,

CALVING DIFFICULTY, BLOOD METABOLITE, AND STEROID HORMONES

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

An experiment was conducted to compare the effects of alfalfa and grass hay diets on body weight (BW), condition score (CS), pelvic area (PA), calving difficulty score (CDS), calf birth weight (CBW), blood metabolites, and steroid hormone concentrations. Thirty-two crossbred beef heifers, bred to the same sire by A.I., were assigned to either alfalfa or grass hay diets. Heifers were penned in groups of four and assigned to Calan gates to facilitate measurement of individual feed intake. Ninety and 6 days prior to the first expected calving date, BW, CS, and PA were measured. Hay samples were taken for analysis periodically throughout the study. Beginning 20 days prior to each heifer's expected calving date, blood samples were taken daily for metabolite and steroid hormone analysis. At calving, CDS, calf presentation, and CBW were recorded. Dry matter and total digestible nutrient intake were similar (P>.10) between diets, but crude protein intake was significantly higher (P<.05) for the alfalfa diet. Diet had no significant effect on BW change, CS change, or PA change. Calving difficulty scores were not significantly different between treatments, however CBW was 2.6 kg heavier (P<.10) for calves from heifers on the grass hay diet. Male calves were 3 kg heavier (P<.05) than female calves. Glucose levels increased significantly (P<.05) the day prior to parturition, but were not affected·by diet. As CBW increased glucose concentrations decreased (P<.05) for heifers on the grass hay diets; but for heifers fed alfalfa, glucose concentrations were not affected by CBW. Blood urea nitrogen (BUN) levels were higher (P<.05) for heifers on the alfalfa hay diet, but were not affected by time. Increased CBW caused decreased BUN in grass-hay fed heifers, however, alfalfa-fed heifers demonstrated the opposite trend with increased BUN concentrations as CBW increased. Progesterone (P_4) concentrations were not different between diets, however were greatly affected by time with a rapid drop occurring the day prior to parturition. Concentrations of estradiol 17-ß (E_2) were not significantly (P>.10) affected by diet. Heifers that produced heavier calves had higher (P<.05) E_2 concentrations on both diets. In summary, the comparison of alfalfa and grass hay diets did not suggest that alfalfa may increase dystocia or CBW. It appears that both feeds are acceptable and practical diets for gestating heifers.

CHAPTER 1

INTRODUCTION

Decreasing the incidence of calving difficulty (dystocia) is of great economic concern to beef producers in Montana and all over the country because dystocia is a major cause of calf death loss. Research at the U.S. Meat Animal Research Center (MARC) Clay Center, Nebraska showed that death loss increased by 12% for calves requiring assistance. (Cundiff et al. 1980). Laster and Gregory (1973) reported that calf mortality was four times greater in calves experiencing dystocia (20.4%) than in those not experiencing dystocia (5.1%). Earlier work by Anderson and Bellows (1967) also showed increased death loss up to 30 days of age for calves experiencing difficulty at birth.

Dystocia is associated with decreased reproductive rate plus increased labor and management cost. Researchers at MARC noted that the number of cows detected in estrus during a 45 day A.I. breeding season was 14% lower for those that had received obstetrical assistance at parturition compared to those which required no assistance. Conception to A.I. was 6% lower in cows experiencing dystocia than those which experienced no dystocia. Pregnancy rates for a 70 day

breeding season were 16% lower in cows that experienced calving difficulty and required assistance in calving than for cows that experienced no dystocia. Laster et al. (1973) reported similar findings. The number of cows showing estrus in a 45 day A.I breeding season and subsequent conception rates were significantly lower.

Doornbos (1978) reported that a 1 minute increase in the duration of labor in two-year-old dams increased postpartum interval by .2 days, lowered conception in a 21 day breeding season by .7% and decreased pregnancy rates in a 45 day breeding period by .6%.

Field observations of extension workers in Montana (Brownson, personal communication) suggest a relationship between calving difficulty and the feeding of alfalfa hay during gestation. The effect of alfalfa hay on the reproductive performance of sheep is well documented. However little research is currently available on the effects of alfalfa on beef cattle parturition.

The objective of this study was to determine if a gestation diet of alfalfa hay could cause increased calving difficulty. In addition, the effects of alfalfa on cow weight, pelvic growth, steroid hormone concentrations, and blood metabolite levels were evaluated.

CHAPTER 2

LITERATURE REVIEW

Dystocia may be classified into two general types. The first is "anatomical dystocia", which deals with physical limitations or disproportional size between the calf and the birth canal of the dam (Erb et al., 1981). Bellows et al. (1971) using eight independent variables (physical measurements) of the dam and calf could account for 46% of the variation in dystocia. This left 54% of the variation unaccounted for. Other researchers have been able to account for from 32% to 45% of the phenotypic variation in dystocia (Rice and Wiltbank 1972, Laster et al. 1974, Rutter et al. 1983, Naazie et al. 1989). The second type of dystocia is termed "physiological dystocia" and includes all dystocia not explained by anatomical relationships of the dam and calf.

Factors Affecting Dystocia

Many factors have been shown to affect both types of dystocia. The complexity of these factors and their interactions may be best outlined by illustrations (Figure 1). Factors related to calving difficulty have been

Figure 1.   Some Currently Known Factors Affecting Dystocia.

reviewed by Price and Wiltbank (1978), Patterson (1979), Meijering (1984), Ritchie (1990).

Age of Dam

Age of dam is a factor that has a major effect on the incidence of dystocia. Research has demonstrated that the greatest risk for dystocia occurs in two-year-old heifers (Bellows et al., 1982). Dystocia in two-year-old heifers may be as high as 54% according to research conducted by MARC in Clay Center, Nebraska. Laster and Gregory (1973) reported dystocia rates of 49.5, 13.5, and 5.0% for two, three, and four plus five-year-old cows, respectively.

Weight of Calf at Birth

Calf birth weight has been shown to be one of the most important factors associated with dystocia in beef cattle. Rice and Wiltbank (1972) found that heifers experiencing dystocia had calves that weighed an average of 2 kg more than the herd average. Bellows et al. (1971) reported correlations between birth weight and dystocia of .54 and .48 for Hereford and Angus herds, respectively.

Short et al. (1979) stated that birth weight is one of the major causes of calving difficulty although many factors are involved. Naazie et al. (1989) reported that the linear effect of calf birth weight was the most important variable used in a model to predict dystocia. Calf birth weight when combined with cows weight accounted for 92% of the variation

explained by the entire model.

Notter et al. (1978) reported a relationship of high birth weights with calving difficulty. However, several researchers have produced increases in calf birth weight without increasing dystocia (Bellows and Short, 1978; Bellows et al., 1978).

Sex of Calf

Sex of calf has been shown to have significant effects on dystocia. Laster (1974) reported that birth weight and sex of calf had the first and second highest correlations with calving difficulty, respectively. Male calves are characteristically heavier at birth than female calves (Laster et al. 1973; Nelson and Huber, 1971). Bellows et al. (1971) also reported that male calves had a greater frequency for requiring assistance and a higher average calving difficulty score than female calves.

Pelvic Area of Dam

A fourth factor identified as affecting the incidence of dystocia is pelvic area. It is generally agreed that a major cause of dystocia is a disproportion between size of fetus and birth canal of the dam. Bellows et al. (1971), in research conducted with both Hereford and Angus herds, reported that, among factors affecting dystocia attributed to the dam, pelvic area ranked first in Herefords and second for the Angus herd.

Naazie et al. (1989) reported that heifers with higher

dystocia scores had a smaller average pelvic area. Their research indicated that vertical measurements were similar, however, and variation in pelvic area came from horizontal measurements.

Price and Wiltbank (1978) reported that, compared to calf birth weight, pelvic area was the second most important factor in predicting dystocia. Short et al. (1979) in analyses of data representing 529 first-calf heifers, also found that pelvic area ranked second to birth weight in factors accounting for dystocia.

Contrary to many of the other research reports, Laster (1974) indicated that the correlation between pelvic area and dystocia was small and that pelvic area was not an accurate predictor of dystocia. Laster also indicated that cows with larger pelvic areas produced even larger calves thus negating any advantage of a larger pelvic area.

Size of Dam

Cow size also affects the incidence of dystocia. Bellows et al. (1971) using Angus and Hereford herds, reported that heavier heifers in the Angus herd had less dystocia than did lighter heifers. Interestingly, these heavier heifers produced heavier calves with less dystocia. They also reported that heavier heifers had larger pelvic areas. In Hereford heifers, body weight was the factor most closely related to pelvic area. However, Ruttle et al. (1982) found no significant relationship between heifer body weight and

pelvic area, and concluded that heifer weight was of little or no value in predicting dystocia.

Shape of Calf

Shape of calf is a factor some have expected to have an effect on dystocia; however, little research is available to support this. Laster (1974) using five calf measurements (shoulder width, hip width, chest depth, wither height, and body length) found no significant correlation between these measurements and the incidence of dystocia. Laster summarized this research by saying that calf shape measurements independent of birth weight were not related to dystocia. Nugent and Notter (1991) using 7 different body measurements of the calf reported that even though body measurements were different between crossbred calves when adjusted to identical weights calving ease scores were not related to body measurements. Nugent et al. (1991) also reported that body measurements added no additional information for predicting dystocia than those which are observed in birth weight alone. This research is consistent with results reported by Ruttle et al. (1982).

Other researchers have seen a small correlation between calf shape measurements and the incidence of dystocia. Meijering (1984) summarized that the use of body measurements and shape of calf offer little to improve the

accuracy of predicting dystocia over the use of birth weight alone.

Gestation Length

Another factor which has been shown to account for variation in dystocia is gestation length. Burfening et al. (1978) analyzed records from the American Simmental Association and found when gestation length was included in their models as a covariate it had a significant effect on calving ease and birth weight. The regression of percent assisted births on gestation length showed that for each day of increase in gestation length percent calving assistance increased by .70%. However when both birth weight and gestation length were included as covariates, gestation length no longer had a significant effect on calving ease or calving assistance.

Wray et al. (1987) used records from 71,461 Simmental cattle and reported that mature cow dams had a 1.9 day longer gestation than heifers, and gestations were 1.5 days longer for male than for female. However, these workers stated that there was a positive relationship between birth weight and gestation length, which may be due to their both being time-related traits. They concluded that birth weight was a better and more easily recorded predictor of calving difficulty. However gestation length may be a useful tool for selection to shorten gestation length for the entire herd.

Effect of Sire

Several researchers have reported breed of sire effects on calving difficulty. Laster and Gregory (1973) reported significant differences in dystocia and calf mortality between breed groups. Percent dystocia in heifers ranged from 8.7% for Jersey sired calves to 43% for Charolais sired calves. Research conducted by Laster (1974) presented similar findings. Average percentage of dystocia in beef heifers ranged from 22 for Angus sired calves to 70 in Brahman sired calves. Laster stated the sire breed had a significant effect on percentage of dystocia with some indication that pelvic height and condition score influenced dystocia. Most of the variation in dystocia was attributed to breed of calf and breed of sire of dam.

Bellows et al. (1982) noted that calf sire had significant effects on gestation length and calf birth weight but not on dystocia scores. Their data however, represented only heavy and moderate birth weight sires which may not have produced large enough differences in birthing weight to result in significant differences in dystocia.

Breed of Dam

Breed of dam is another factor which may affect the incidence of dystocia. It would appear that the dam effect would be greater than that of the sire because she provides half of the genetic make up of the calf as well as the environment for fetal development.

Laster et al. (1973) using Angus and Hereford cows bred to different breeds of sire reported that Hereford cows had more calving difficulty (P<.05) than did Angus cows (35% vs 27%). Mean calf birth weights were 38 kg for Hereford cows and 35 kg for Angus cows. Smith et al. (1976) also reported that Hereford cows experienced significantly more dystocia than Angus. Gregory et al. (1978), also found significant breed of dam effects on birth weight.

Gregory et al. (1979) reported that in comparing Angus and Hereford dams, Herefords produced significantly heavier calves with significantly longer gestation lengths. They also reported a trend toward a higher percentage of dystocia in the Hereford cows.

Condition Score of Dam

Condition score of dam is another factor which has been examined for its effects on dystocia. Bellows et al. (1971) reported a positive correlation (P<.10) between cow condition and calf birth weight, however the correlation between condition score and calving difficulty was not significant. Arnett et al. (1971) reported that obese cows required more assistance than normal conditioned cows.

Hormonal Effects on Dystocia

Hormonal variation in dams near parturition has been shown to be a significant component of dystocia in beef cattle. Hormonal imbalances have been implicated in causing

physiological dystocia (Erb et al. 1981, Anthony et al. 1986). Several hormones are associated with parturition: ACTH, cortisol, estrogen, prostaglandin, progesterone, oxytocin, and relaxin (Bazer and First, 1983, Ritchie, 1991).

The exact function of the endocrine system of this dam during parturition is not totally clear. Parturition is initiated by the fetus, however hormonal control differs among species. ACTH and cortisol are the principal compounds responsible for the initiation of parturition (Bazer and First, 1983) although other hormones may be involved. Anthony et al. (1986) concluded that endocrine patterns related to parturition change rapidly in preparation for birth. The synchrony and patterns of these changes may be important in assuring normal parturition.

Research conducted to study the effects of relaxin on dystocia has produced inconclusive results. Work by Caldwell et al. (1990), using crossbred beef heifers treated with porcine relaxin, produced no evidence to indicate that relaxin may help to decrease dystocia. However Anderson and Bagna (1991) found that they could significantly reduce the incidence of dystocia by injecting dairy heifers with relaxin 5 to 6 days prior to calving.

Prostaglandins, as explained by Hafez (1987), play a central role in initiating strong uterine contractions in the second stage of labor and aiding in the dilation of the cervix. Prostaglandin release is apparently triggered by

increasing estrogen and decreasing progesterone concentrations in the blood. The complex and dynamic relationships among these hormones suggest that imbalances may lead to or be associated with dystocia.

The function of oxytocin is better defined for pigs than for cattle. It is apparent that it aids in the expulsion of the fetus by accentuating myometrial contraction. Once again, a hormonal balance is required for oxytocin to be able to perform its necessary task. Liggins et al. (1973) reported that response to oxytocin is increased in the presence of estrogen and prostaglandin (PGF2α).

Progesterone plays an important role in the maintenance of pregnancy through depressing myometrial activity. Several researchers have studied progesterone concentration in late gestation through parturition and their results are similar. Peripheral progesterone remains high to help maintain pregnancy (3.7 to 12.0 ng/ml) ; (Donaldson et al., 1970; Schams, 1972; Erb et al., 1982), then begins to decline as early as three weeks prior to parturition. (Donaldson et al., 1970; Schams et al., 1972; Erb et al., 1982). In the two days prior to parturition, there is a significant and rapid decline in progesterone concentration to levels less than 1.0 ng/ml (Donaldson et al., 1970; Edqvist, 1973; Comline et al., 1974; Robertson, 1974; Agathe and Kolm, 1975; Erb et al., 1982;).

O'Brien and Stott (1977) reported that heifers experiencing dystocia had higher peripheral plasma

progesterone concentration from 23 to 12 days prior to parturition. Erb et al. (1981) found that heifers experiencing physiological dystocia had higher concentrations of progesterone from one day prior to parturition until one day after.

Anthony et al. (1986) working with 59 crossbred heifers found that heifers requiring no calving assistance had a curvilinear progesterone profile, with progesterone being high at 7 days prior to parturition and decreasing rapidly to a lower level at parturition. Heifers that required assistance had lower progesterone levels at day 7 prior to parturition, but did not decrease to as low a level as seen in those requiring no assistance.

Estrogen also plays an important role in parturition. According to Hoffman et al. (1973) interaction of estrogens with glucocorticoids may be involved in the induction of parturition. Serum estrogens increase slowly beginning 20 to 10 days prepartum and rise rapidly 4 to 1 days before parturition (Hunter. et al., 1977; Robertson, 1974; Comline et al., 1974).

The importance of estrogen in parturition goes beyond parturition initiation. Gap junctions which are responsible for uterine contractions are increased in the presence of estrogen (Bazer and First. 1983). Increased estrogen levels are apparently necessary for preparation for parturition.

Work by Osigna (1978), using twin Freisian heifers with

one twin being bred to a Charolais bull and the other to a Beef Freisian bull, was conducted to determine the relationship between estrogen levels and dystocia. Urinary estrogen levels were found to be lower in the Charolais x Freisian calves. This information was used to postulate a relationship between dystocia and estrogen level because of Charolais x Freisian cross calves have a history of dystocia. O'Brien and Stott (1977) also reported that heifers exhibiting dystocia showed a significantly lower estradiol 17-ß concentration 20-12 days prepartum than did heifers that experienced no dystocia.

In research conducted to observe hormonal differences associated with dystocia, Erb et al. (1981) distinguished between physiological and anatomical dystocia by using calf birth weight as a percentage of the dam's weight. These researchers formed two groups, those with low calf birth weight percentage and those with high calf birth weight percentages. Their results indicated that increases in dystocia with high calf birth weight percentages were, in most cases, due to anatomical dystocia (disproportionate size of calf and birth canal). However, in 76% of the dystocia cases involving low calf birth weight percentage, abnormal patterns were found in two or more hormones. Estradiol-17ß was found to be significantly lower for heifers that experienced dystocia with high birth weight percentage calves.

Anthony et al. (1986) also reported that estrogen

concentrations were different for heifers with calving difficulty scores (CDS) of 2 verses 3 (calving difficulty was reported as 1 being no difficulty to 4, extreme difficulty or cesarean section). Heifers with CDS3 had significantly lower estrogen concentrations prepartum than heifers recorded as having a CDS2.

Lower estrogen levels or decreased estrogen production appear to influence the incidence of dystocia. Estrogen levels that are in balance with other hormonal parameters prepartum are required for normal parturition.

Nutritional Effects on Dystocia

A large amount of research has been conducted to determine the effects of nutrition on the incidence of dystocia. However results have been contradictory, leaving producers without conclusive evidence upon which to build management practices.

Dunn et al. (1969) fed Angus x Hereford heifers receiving 3.6 kg or 2.0 kg of TDN per day to determine how dystocia was effected. Heifers which received 3.6 kg of TDN gained 54.5 to 68.2 kg in the 120 day period as compared to 5.9 to 15.9 kg for the group receiving 2.0 kg of TDN. Calves from the heifers receiving lower TDN levels weighed 6 to 7 lbs less at birth than the higher TDN groups. Heifers receiving higher levels of TDN experienced slightly more dystocia over a two year period.

Bellows and Short (1978) conducted two studies to

determine the effects of varying levels of TDN (6.2 to 6.4 and 3.2 to 3.4 kg TDN daily) for 90 days prior to gestation on birth weight, dystocia and subsequent fertility. Dams receiving higher levels of TDN produced significantly heavier calves but no difference was observed in either the incidence or severity of calving difficulty. Further work by Bellows et al. (1982) feeding two levels of TDN (3.6 or 6.8 kg per cow daily) showed no significant differences in dystocia or calf birth weight between diet groups.

The effects of dietary gross energy on dystocia and calf birth weight have also been investigated. Corah et al. (1975) conducted two experiments, one with 59 first-calf heifers and one with 43 second-calf cows. Groups received 100% or 65% of NRC requirements in experiment 1 or 100% and 50% of NRC requirements in experiment 2. Heifers in experiment 1 on the restricted gross-energy diet produced calves which were an average of 5.8 kg lighter at birth than calves produced by heifers that received their requirement for gross-energy. However, no difference was observed in dystocia. Results were similar in experiment 2, with cows receiving restricted energy producing lighter calves (3.7 kg) with no effect on dystocia.

Feeding differing levels of digestible energy to gestating cattle produced little effect on fetal weight. This research dystocia did not report scores (Prior et.al.1979). Prior et al. concluded the restrictions of energy intake had little effect on the fetal development although maternal

metabolism may have been altered.

Research conducted to determine the effect of crude protein on dystocia and calf birth weight has produced varying results. Bellows et al. (1978) reported that heifers receiving 138% of their daily recommended NRC requirements for crude protein in isocaloric diets for 82 days prepartum produced significantly heavier calves (5 kg average) than heifers that received 79% of the NRC recommended level for crude protein. Interestingly, however, even though there was a significant difference in calf birth weight no significant differences were found in calving difficulty score.

Further work on the effect of crude protein on dystocia and birth weight was conducted by Anthony et al. (1986). In their research 59 crossbred heifers were fed isocaloric diets containing 81 or 141% of recommended NRC requirements for crude protein for 75 days prepartum. They reported no significant differences in calf birth weight or calving difficulty score.

Effects of nutrition on calf birth weight and dystocia are still unclear. This lack of clarity may be due to the effect of nutrition on steroid hormone parameters in gestating cattle. Research conducted to study the effects of nutrition on these parameters indicates that only in cases of severe nutritional stress are blood hormone levels altered by nutrition (Prior and Laster, 1979; Corah et al., 1974,). However, others have reported that nutrition may have an

effect on steroid hormones in late gestation. Boyd et al. (1987) fed moderate and high levels of dietary energy to 37 Angus cows and collected blood samples 50, 30 and 10 days prior to parturition. Estrone, estrogen sulfate and progesterone levels were not different among diets at parturition, but significant differences were found prior to parturition. Boyd et al. concluded that there is a relationship among prepartum nutrition, altered steroid secretion and calf birth weight.

Killen et al. (1989) reported that available energy is an important component in the mediation of GnRH induced LH release during pregnancy. They reported that even though LH release was affected by nutritional restriction, blood progesterone and estrogen concentrations were not significantly different.

Anthony et al. (1986), comparing levels of protein in late gestation diets, reported that heifers receiving low protein diets (.56 kg, 81% NRC) had higher progesterone concentrations (5.94 vs. 4.26 ng/ml) than heifers receiving high protein (141% of NRC or .93 kg CP per day) diets. However, these researchers reported no significant difference in estrogen concentration. These results are similar to those reported by Donaldson et al. (1970) using undernourished cows.

Effects of Geographical Location on Dystocia

Another factor that affects calf birth weight and dystocia is geographic location (Burfening et al., 1982;

Buchanan and Nielsen, 1979; and Butts et al., 1971). This research indicates that heat may cause calves born in southern regions to be lighter at birth.

The Effects of Alfalfa on Hormone Levels

Although alfalfa hay is often fed to cattle throughout the United States, little research has been conducted to investigate what effect it may have on the endocrine or reproductive systems of beef cattle. However considerable research has been conducted to study the effect of feeding alfalfa on the reproductive performance and the endocrine levels in sheep. Early researchers reported that feeding of alfalfa as compared to grass hay diets decreased conception rates and twinning rates (Coop and Clark 1960; Smith et al., 1980; Goodlett et al., 1984). Barberan (1990) reported that the estrogenic effect of alfalfa diets caused glandular edema and enlargement of the reproductive tract.

Montgomery (1985) found that feeding low and high levels of phyto-estrogenic coumestrol to ewes in the breeding season produced differing levels of LH. Ewes receiving higher levels of coumestrol had lower levels of LH.

Possibly even more pertinent to the effects of feeding alfalfa to beef cattle was a study conducted by Newsome and Kitts (1977). They stated that alfalfa contained phyto-estrogens which are similar in chemical structure and biological activity to that of estradiol-17β. Their research showed that ewes receiving alfalfa had higher levels of phyto-

estrogen in their plasma and lower levels of endogenous estrogens than ewes receiving orchard grass. This suggests that gonadotropin stimulation of the ovary is reduced by the presence of phyto-estrogen in the plasma. Research performed thus far on sheep indicates that alfalfa may affect endogenous estrogen production which is important in the process of parturition.

CHAPTER 3

MATERIALS AND METHODS

Beginning 115 days prior to their first expected calving date, 32 crossbred (varying percentages of Hereford, Angus and Tarentaise) 2-year-old first calf heifers were randomly assigned to grass or alfalfa hay diets. All heifers had been bred by artificial insemination within a 17 day period to the same Angus sire.

Heifers were penned in groups of four. Each heifer was assigned to a Calan gate to facilitate measurement of daily fed intake. A 25 day acclimation period was allowed before intake and other data were collected. Heifers were allowed free-choice access to their assigned diet with excess feed weighed back daily. Heifers were provided with free choice mineral to meet NRC (1984) requirements for calcium and phosphorous. Hay samples were taken for analysis every 28 days (Table 1).

Table 1. Average Nutrient Composition of Alfalfa
          and Grass Diets·

	Alfalfa Hay	Grass Hay
Dry matter, %	90	91
Crude Protein, %	16.3	7.5
TDN, %	59.2	55.3
Neutral detergent fiber, %	49.4	59.5
Acid detergent fiber,%	38.7	42.3

Ninety days prior to the first expected calving date initial body weights, pelvic dimensions, and condition scores were determined. Condition scores represented the average of two technicians and were recorded on a scale from 1 (thinnest or emaciated) to 9 (very fat or obese). Six days prior to the first expected calving date final weights, body condition scores and pelvic measurements were recorded.

Blood samples were taken daily on each heifer beginning 20 days prior to her predicted calving date. Each 27 ml sample was collected from the jugular vein for determination of glucose, blood urea nitrogen, progesterone, and estradiol concentrations. Blood samples were allowed to clot and centrifuged at 1000 x g at 4° C for 30 minutes. Serum was decanted and stored at -25° C until analyzed.

Calf birth weight was recorded within 12 hours after birth. Calving difficulty was scored in one of four categories:

    1 = unassisted;

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2 = easy pull, minimal assistance;
3 =  difficult   pull,   requiring   mechanical
    assistance;
4 = surgical intervention, caesarean;
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Calf presentation was recorded in one of six categories:

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1 = normal (feet and nose first), anterior
    delivery;
2 = anterior delivery, head back;
3 = anterior delivery, foreleg(s) back;
4 = posterior (hind legs first) and breech (tail
    first) delivery;
5 = posterior with other complications;
6 = other;
```

Blood Sample Analyses

Upon completion of calving, blood serum samples were sorted and the final 10 days prior to calving were selected for analysis. Glucose analysis was conducted at the Marsh Veterinary Research Laboratories using the hexokinase enzymatic method with a commercially available kit produced by Sigma Diagnostics[1]. Blood urea nitrogen levels were also analyzed with commercially produced kits at the Marsh Veterinary Research Laboratories using a coupled enzyme reaction involving urease and glutamate dehydrogenase[1].

Progesterone assays were conducted at the MSU Animal and Range Sciences Department of Physiology Laboratories. Concentrations were determined by solid-phase radioimmunoassay (RIA) provided by commercially available kits[2]. The procedure

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[1]Sigma Diagnostic, St. Louis, MO.

[2]Diagnostic Products Corporation, Los Angeles, CA.

for the progesterone assay was modified by using standards prepared in ovariectomized dexamethasone-blocked cow serum. Intra- and inter- assay coefficients of variation were 2.07 and 6.34% for a sample containing 34.2 ng/ml and 1.37 and 5.06 for a sample containing .694 ng/ml.

Estradiol 17-ß assays were conducted at the MSU Animal and Range Sciences Department of Physiology Laboratories. A modified estradiol, double-antibody radioimmunoassay technique was used (Diagnostics Products Corporation[2]). Samples were extracted with 2 ml of ethyl acetate. Extracts were dried under nitrogen and reconstituted in 200 μl assay buffer. Extraction recovery was 98%. Intraassay and interassay coefficients of variation were 4.21% and 9.45% respectively for the high pool (198 pg/ml) and 3.46% and 4.80% respectively for the low pool (2.92 pg/ml).

Statistical Analysis

Data were analyzed by least-squares analyses of variance using the General Linear Model Procedures of SAS (1988). Two approaches were used in the analysis of each variable. First, each variable was analyzed using a "base model" that simply reflected the experimental design plus any blocking factors beyond experimental control, such as sex of calf. Second, for each variable, an attempt was made to identify the statistical model that best fit the data. These expanded models included covariates and interaction terms involving the other potential

response variables and were used to aid in the interpretation of the observed responses.

The base model for feed intake and changes in cow weight, pelvic area and condition score was a one-way analysis of variance. The expanded model for feed intake included the main effect of treatment plus covariates for initial weight and initial body condition score. Expanded models for weight, pelvic area and body condition score changes included treatment as the main effect, initial weight and feed intake as covariates, plus two-way interactions. Terms that were not significant (P>.10), with the exception of treatment, were eliminated from the model and reduced models were fit to the data.

Base models for calf traits included the main effects of treatment and calf sex. Expanded models considered covariates for cow weight, cow weight change, pelvic area, feed intake, cow condition score and appropriate two-way interactions. All terms that were not significant (P>.10), except for treatment, were dropped from the model and reduced models were fit to the data.

Steroid hormone and blood metabolite concentrations were analyzed using repeated measures analysis of variance techniques (SAS, 1988). The base model included treatment as the whole plot factor, with time (i.e., days prior to parturition) and time x treatment interaction as the sub-plot factors. Expanded models evaluated the whole-plot factors of

calf sex, calving difficulty score, covariates for cow weight, cow weight change, feed intake, calf birth weight, and appropriate two-way interactions. Sub-plot factors always included time and interactions between time and whole-plot factors. Added terms that were not significant (P>.10) were deleted from the model and reduced models were fit to the data with the exception that non-significant factors were maintained if interactions involving these factors were significant.

Comparisons among least squares means were made by single degree of freedom contrasts.

CHAPTER 4

RESULTS AND DISCUSSION

Two heifers (one on each diet) calved 21 or more days after their expected calving date and were not included in any analysis because of uncertainty regarding sire of calf.

Results from the base model for the analyses of body weight, body condition score, and pelvic area changes are summarized in Table 2. The expanded models determined for these variables were the same as the base models; thus Table 2 presents the results of both the expanded and the base models. Body weight change, condition score change, and pelvic area did not differ (P>.05) between diets.

In the analysis of feed intake the base model and expanded model were also the same. Daily dry matter intake was calculated by dividing gross intake by days on feed for each heifer, and nutrient intake was calculated using daily dry matter intake and diet nutritive value. These values were then compared to NRC (1984) requirements for each heifer's average body weight for the trial (Table 3). Dry matter intake and TDN intake were not different between diets (P>.05), however heifers on the alfalfa diets received more (P<.05) crude protein than heifers receiving the grass hay diet.

Table 2. Means for Body Weight, Condition Score, and Pelvic Area[a].

	Alfalfa	Grass	SE
Number of heifers	15	15	
Initial data:			
Body weight, kg	446.91	462.58	5.41
Condition score	5.03	5.00	.05
Pelvic area, cm^2	158.03	167.28	4.55
Change over 84 day period:			
Body weight, kg	77.27	69.40	3.13
Condition score	.07	-.13	.09
Pelvic area, cm^2	81.85	73.00	4.76

[a] Values for base and expanded models were the same.

Table 3. Average Daily Nutrient Intake.[a]

	Hay diet	
	Alfalfa	Grass
Average daily intake (kg/day)	11.67	12.52
CP intake (% of NRC Requirement)[b]	207.3[c]	109.9[d]
TDN daily intake (% of NRC Requirement)[a]	135.0	136.0

[a]Values for base and expanded model were the same.
[b]Based on average weight during trial.
[cd]Means within rows with different superscripts differ
P<.05.

All calves were born in the normal front-feet-first, anterior presentation, so calf presentation was not analyzed statistically.   The F-statistics from the analysis of variance using the base model for calf traits are summarized in Table 4. Neither diet nor calf sex had significant effects

on calving difficulty score. The effect of diet on calf birth weight approached significance (P<.10). Calf sex had a significant effect (P<.05) on calf birth weight.

Table 4. F-statistics for Calving Difficulty Score (CDS) and Calf Birth Weight (CBW) -Base Model.

		CDS	CBW
Source	df	F	F
Diet	1	.11	2.93[**]
Calf sex	1	1.73	5.99[*]
Error mean square	27	.506	53.59
R^2		.069	.227

[*]P<.05
[**]P<.10

A summary of the F-statistics from the analysis using the expanded models for calving difficulty score and calf birth weight are shown in Table 5.

The final model that best fit the data for calving difficulty score included treatment as a main effect and calf birth weight as a covariate (Table 5). Calf birth weight was positively correlated with calving difficulty score; for each kg increase in calf birth weight dystocia score increased .077 points. The best-fit model for calf birth weight was identical to the base model (Table 5).

Table 5. F-statistics for Calving Difficulty Score (CDS) and Calf Birth Weight (CBW) - Expanded Model.

		CDS	CBW
Source	df	F	F
Diet	1	1.07	2.93**
CBW	1	4.70**	--
Calf sex	--	--	5.99*
Error mean square	27	.4584	53.595
R^2		.156	.227

*P<.05

**P<.10

There were no significant effects of diet on calving difficulty score or calf birth weight. Male calves were heavier (P<.05) than female calves (36.5 kg for male vs 33.5 kg for female calves). Calving data are reported in Table 6.

Table 6. Least Squares Means for Calving Difficulty Score and Calf Birth Weight - Expanded Model.

	Hay diet		
	Alfalfa	Grass	SE
Calf birth weight (kg)	33.96	36.06	.87
Calving difficulty score	1.77	1.57	.17

Results of this experiment for the effect of diet on calf birth weight and calving difficulty score are similar to those of Anthony et al. (1986). Even though this experiment was not designed to compare the effects of the amount of protein as was Anthony et al. the fact that TDN intakes were similar

between diets and protein intakes were different gives the comparison validity. These researchers fed late gestation diets to heifers which were isocaloric, but contained two levels of crude protein. The low protein group received 81% of NRC requirements for crude protein while the high protein group received diets which contained 141% of NRC requirement for crude protein. They found that the late-gestation protein level had no significant effect (P>.05) on calf birth weight or calving difficulty score.

Bellows et al. (1978) also fed heifers isocaloric diets with low and high levels of protein (78% and 138% of NRC requirements), and received contradicting results. These researchers reported a significant difference (P<.05) in calf birth weight (5 kg) between treatments, however, no significant difference in calving difficulty score was observed. In our study, absorbed crude protein was apparently sufficient to meet all maternal and fetal requirements in both diets so no differences in birth weight or calving difficulty score were detected.

The experiment was designed to collect blood samples beginning 20 days prior to each heifers expected calving date to ensure samples for 10 days prior to parturition. However, only 8 samples were received on one heifer prior to calving. To provide balanced data across the experiment only the final 8 days prior to parturition were included in the statistical analysis.

Table 7. F-Statistics from Repeated Measures Analysis of Variance for Glucose (GLU), Blood Urea Nitrogen (BUN), Progesterone (P_4), and Estradiol 17-β (E_2) - Base Moel.

Source	df	GLU F	BUN F	P_4 F	E_2 F
Diet[a]	1	.00	46.14*	1.51	2.70
Error A[a]	28	501.8	63.87	7.04	30408
Time (T)[b]	7	15.81*	5.01*	87.6*	10.32*
(T) * Diet[b]	7	.88	1.81	.74	2.19**

[a]Error A = Error mean square for whole-plot factors.
[b]Error term for F-statistics for sub-plot factors was the sum of squares and cross products matrix (not shown).
*P<.05
**P<.10

The F-statistics for the analysis of variance of the steroid hormones and blood metabolites using the base model are reported in Table 7. Least squares means by diet for the base model over time (i.e., days prior to parturition) are illustrated in Figures 2 - 5. The F-statistics for the expanded models are presented in Table 8.

The analysis of glucose using the base model showed that diet had no significant effect on glucose concentration over time (Fig 2). The effect of time on glucose was significant with a marked increase occurring on the day prior to parturition.
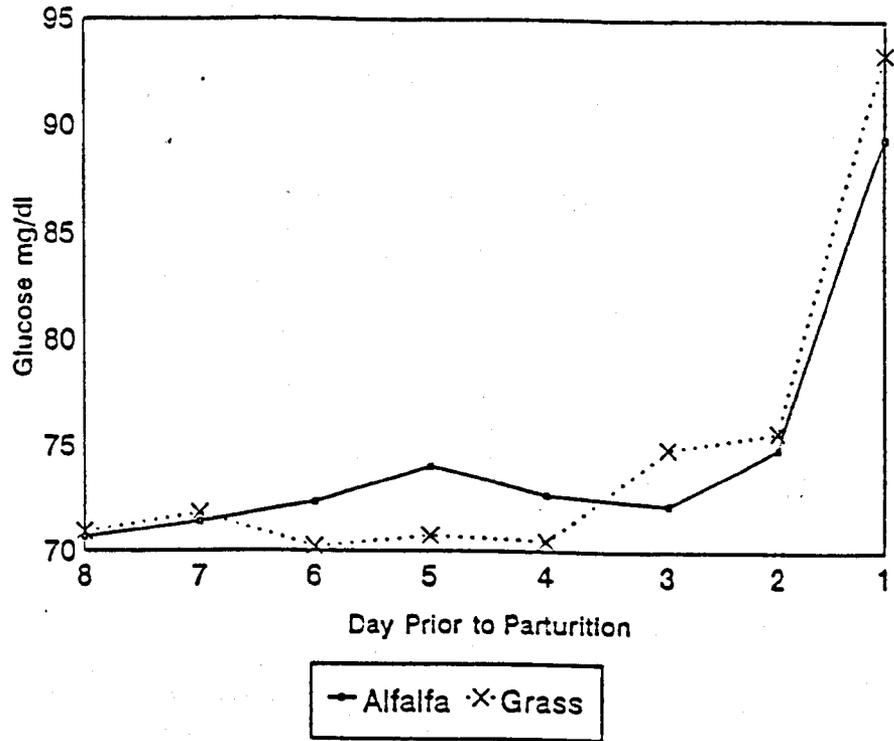
Figure 2. Least Squares Means for Glucose - Base Model.
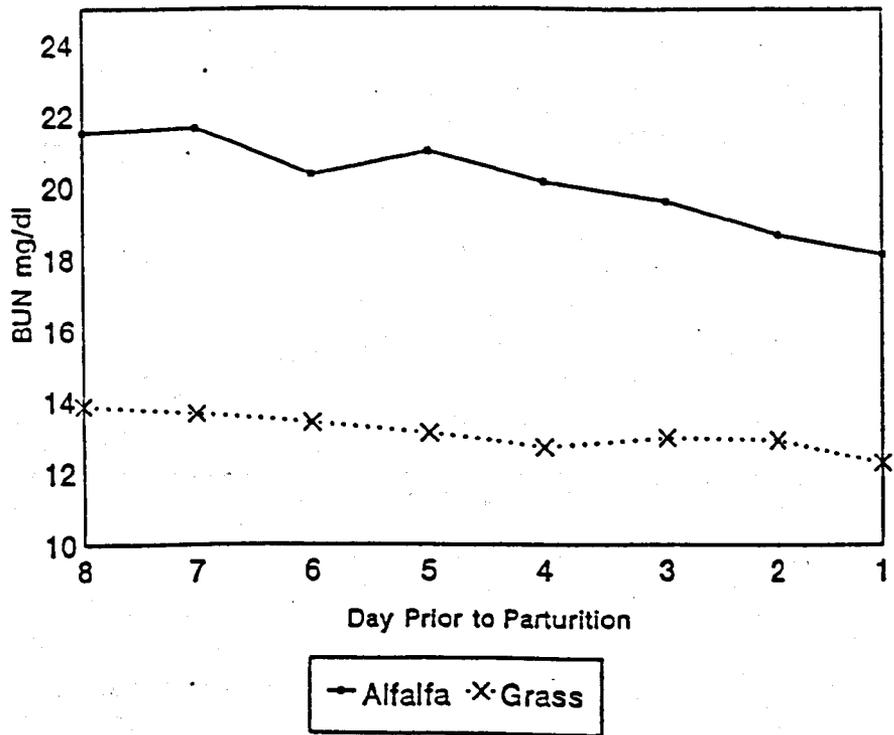

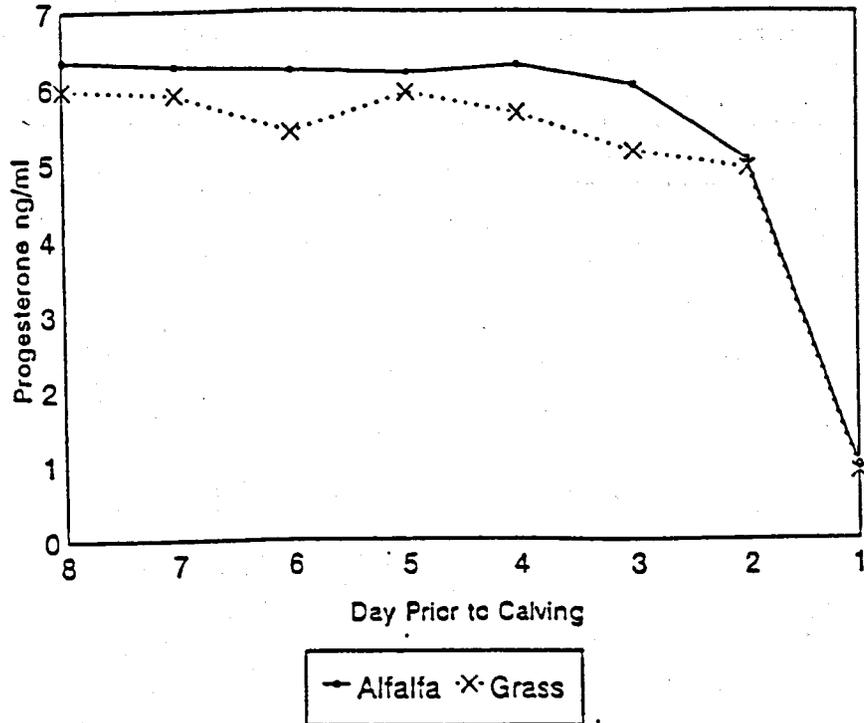Figure 3. Least Squares Means for BUN - Base Model.

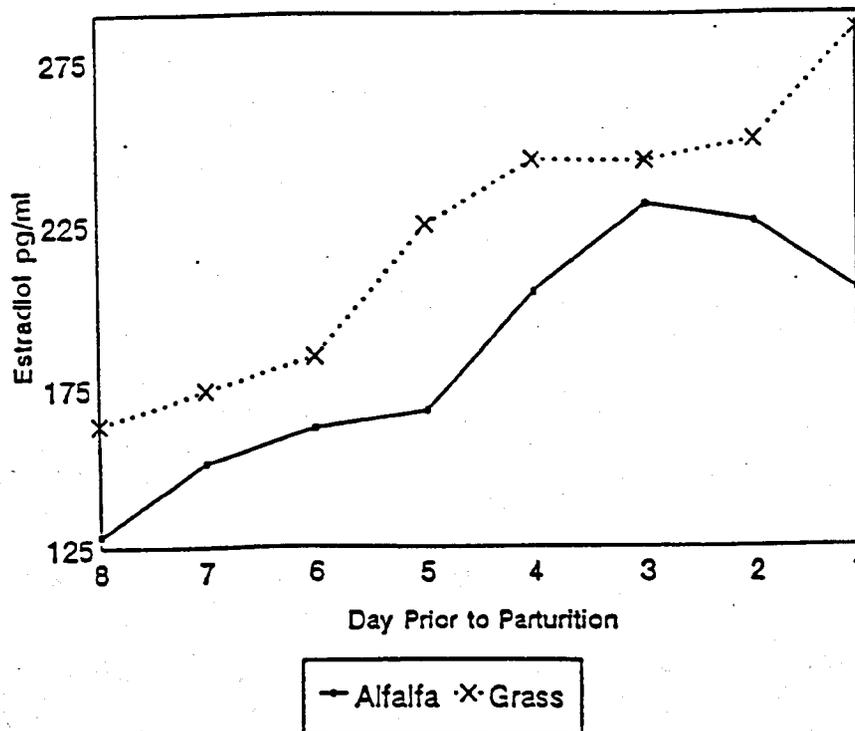Figure 4. Least Squares Means for Progesterone - Base Model.

Figure 5. Least Squares Means for Estradiol 17-ß - Base
           Model.

Table 8. F-statistics from Repeated Analysis of Variance for Glucose (GLU), Blood Urea Nitrogen (BUN), Progesterone (P_4), and Estradiol 17-ß (E_2) - Expanded Model.

Source	GLU df	GLU F	BUN df	BUN F	P_4 df	P_4 F	E_2 df	E_2 F
Diet[a]	1	5.00*	1	6.56*	1	1.51	1	1.17
Calf Birth Weight(CBW)[a]	1	2.06	1	0.02	--	--	1	10.45*
Heifer weight Change(HWC)[a]	--	--	1	4.80*	--	--	--	--
CDS[a]	--	--	2	2.11	--	--	--	--
Diet*CBW[a]	1	4.87*	1	11.4*	--	--	--	--
Diet*CDS[a]	--	--	2	4.35*	--	--	--	--
Error A[a]	26	419.5	21	41.98	28	7.03	27	22735
Time (T)[b]	7	2.51*	7	.35	7	87.6	7	.98
T*Diet[b]	7	.31	7	.82	7	.74	7	1.83
T*CBW[b]	7	2.77*	7	.21	--	--	7	.83
T*HWC[b]	--	--	7	.91	--	--	--	--
T*CDS[b]	--	--	14	.56	--	--	--	--
T*Diet*CBW[b]	7	.40	7	.67	--	--	--	--
T*Diet*CDS[b]	--	--	14	.62	--	--	--	--

[a]Error A = Error mean square for F-statistics for whole-plot factors.
[b]Error term for F-statistics for sub-plot factors was the sum of squares and cross products matrix (not shown).
*P<.05
**P<.10

The model which best fit the data for glucose included diet as a main effect, calf birth weight and the interaction of diet x calf birth weight as covariates (Table 8). Least

squares means of serum glucose concentration were not different among diets (P>.10) (Figure 6). There was a significant effect (P<.05) of time (i.e,. day prior to parturition) for glucose concentration (Figure 6).

The treatment x calf birth weight interaction is illustrated in Figure 7. This interaction might be that the effect of excess protein in the alfalfa hay diet was utilized as a glucose precursor. Heifers on the grass hay diet had less protein available for gluconeogenesis, therefore the additional demand of heavier calves resulted in a lower blood glucose concentration.

Blood glucose levels in this experiment were within normal limits (Kaneko, 1980,) except for the high levels on the last day prior to parturition which was likely caused by epinephrine and norepinephrine release prior to parturition. No effects of treatment on glucose level were found in this experiment due to the similar energy content of the diets, and because glucose is also a highly regulated metabolite in ruminants. It might require a larger difference in nutritional intake to produce significant effects on glucose concentrations.

Base-model least squares means for blood urea nitrogen (BUN) were different (P<.05) between diets on all days prior to parturition (Figure 3). The repeated measures analysis of variance for the base model showed a significant effect
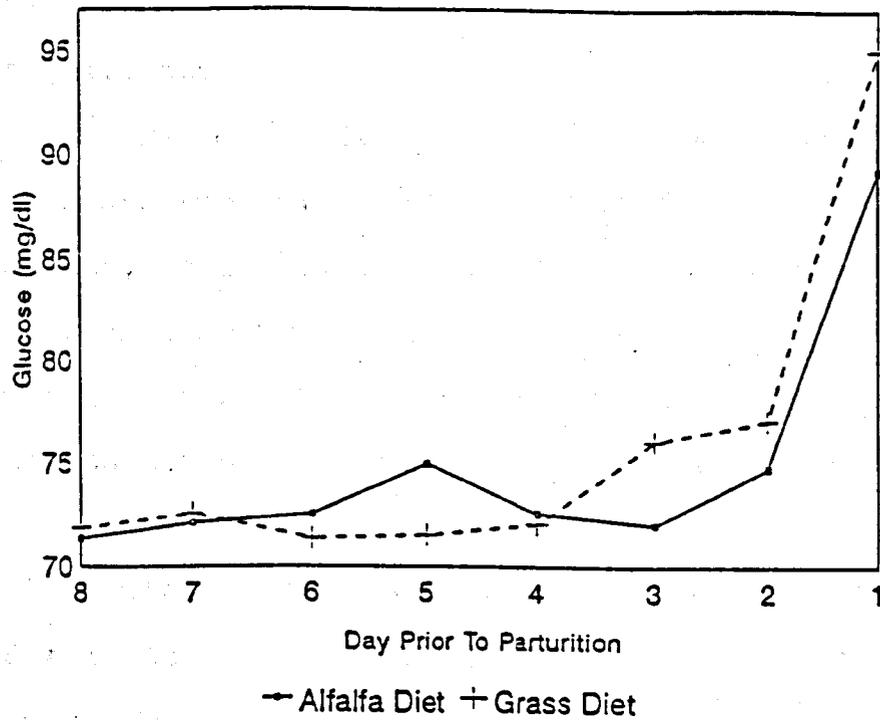
Figure 6. Least Squares Means for Glucose - Expanded Model.

Alfalfa diet y = 29.4118 + .2932x
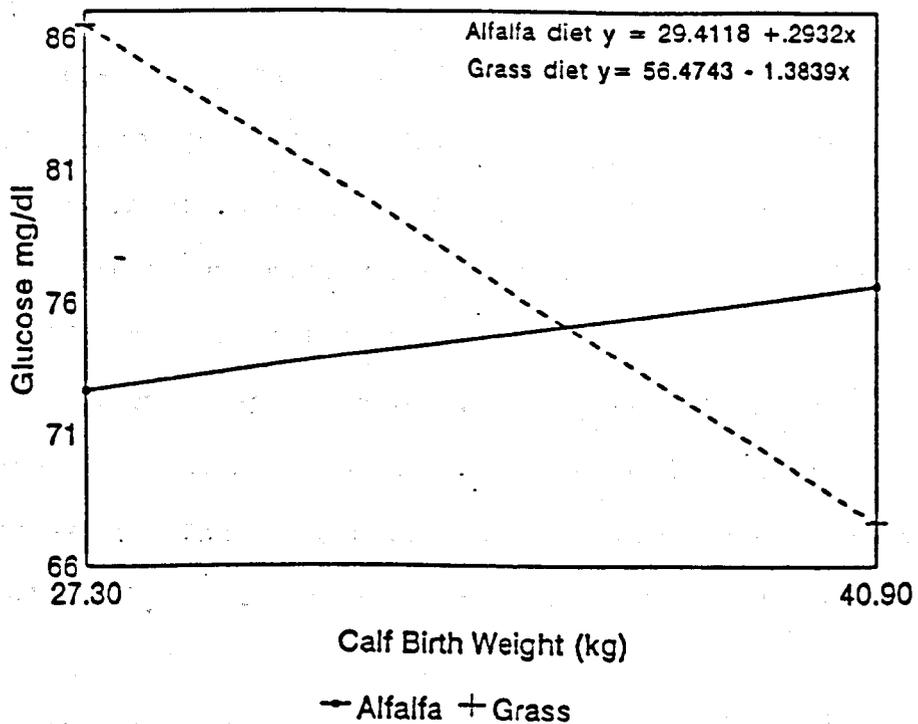
Grass diet y = 56.4743 - 1.3839x

Figure 7. Regression of Glucose on Calf Birth Weight by Diet.

of time on BUN concentration, but the diet x time interaction was not significant.

The expanded model for BUN included diet and calving difficulty score as main effects, with calf birth weight, cow weight change, calf birth weight x diet, and calving difficulty scores x diet interactions included as covariates.

In the expanded model the effect of time (i.e., day prior to calving) on BUN was not significant ($P>.10$). Interaction of time with diet, calf birth weight, heifer weight change, calving difficulty score, diet x calf birth weight and diet x calving difficulty score were not significant ($P>.05$).

In the whole-plot analysis of variance the effect of diet was significant ($P<.05$) which might be expected because of the different amount of protein in the two diets. Least square means for diet (Figure 7) were significantly different over all eight time periods. This is consistent with other research (Treacher et al., 1976; Chew et al., 1984; Anthony et al., 1986) which reported that cattle on lower protein diets had lower BUN concentrations.

Heifer body weight change and BUN also had a significant relationship. Heifers that gained more weight had higher BUN concentrations. This might be due to heifers that gained more having slightly higher intakes although not significant ($P>.10$).

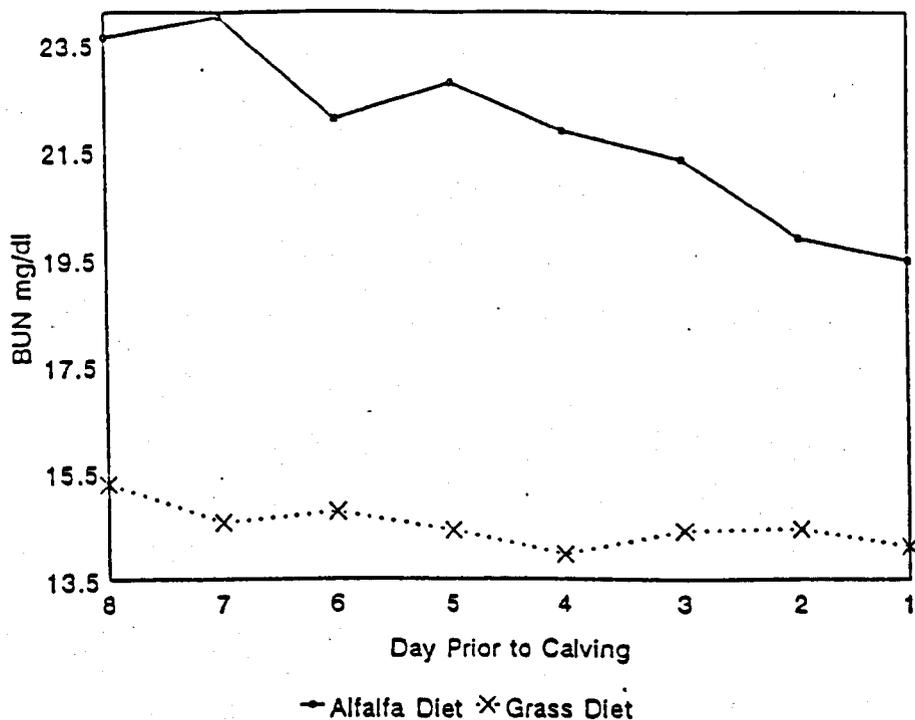Diet x calf birth weight and diet x calving difficulty
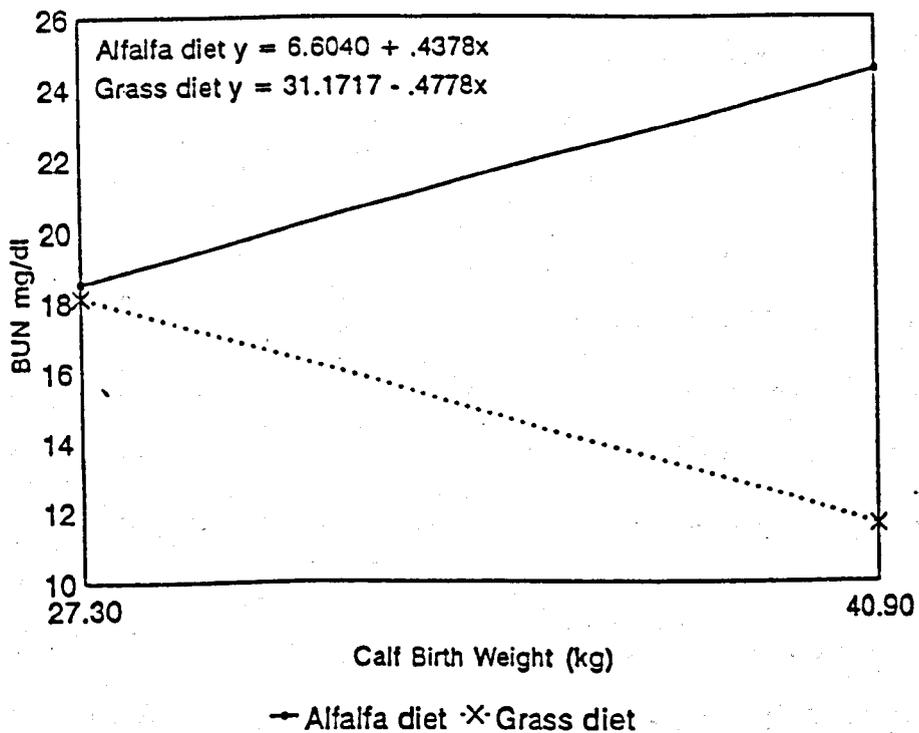
Figure 8. Least Squares Means for BUN – Expanded Model.

Figure 9. Regression of BUN on Calf Birth Weight by Diet.

score interactions were also significant (P<.05). These interactions are not readily explained. The diet by calf birth weight interaction (Figure 8) is apparently related to the fact that heifers on grass hay diets were close to their protein requirement and larger calves, having a greater demand for protein, thus decreased the BUN concentration in the dam. The increase of BUN concentration with increased calf birth weight for heifers on the alfalfa hay diet seems to be without logical explanation. The diet x calving difficulty score interaction (Figure 10) is shown but no physiological explanation is offered.

In the analysis of Progesterone (P_4) the base model and best fit model were identical. Diet had no significant effect on P_4 (Figure 4). Repeated measures analysis of variance indicated that there was a significant effect of time on P^4 (ie. day prior to parturition), however, a time x diet interaction was not observed (Table 8).

Donaldson et al. (1970) reported greater P4 concentrations in undernourished cows during late gestation. However, in this study both dietary groups received more than their nutrient requirement for crude protein and TDN and no significant differences were seen in P4 levels. Corah et al. (1974) fed two groups of heifers isonitrogenous anisocaloric diets and could not detect differences in P4 concentration. However, Anthony et al. (1986) fed anisonitrogenous diets and found different (P<.05) mean concentrations between low and
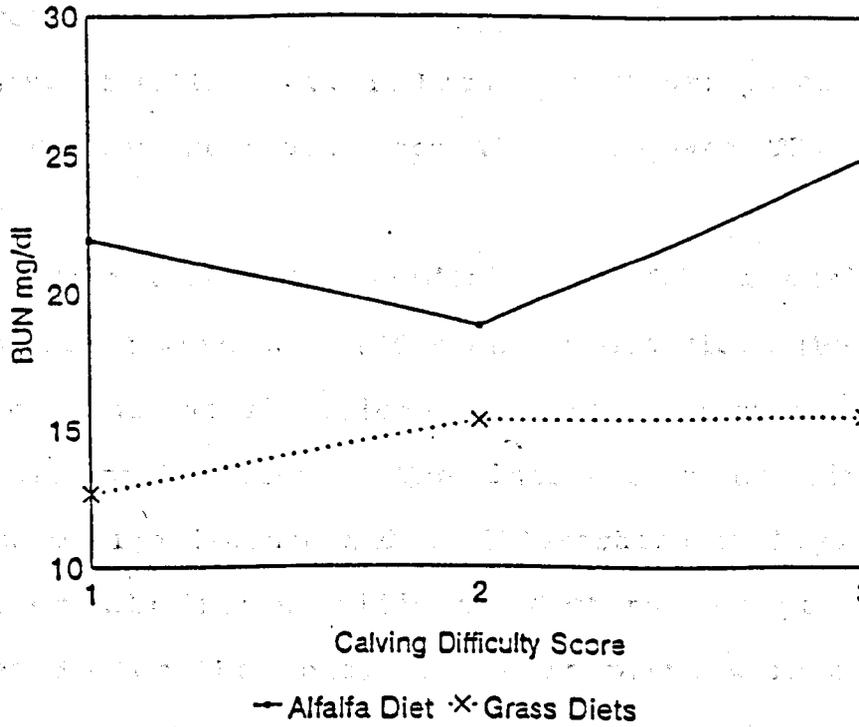
Figure 10. Effects of Diet and Calving Difficulty Score on BUN.

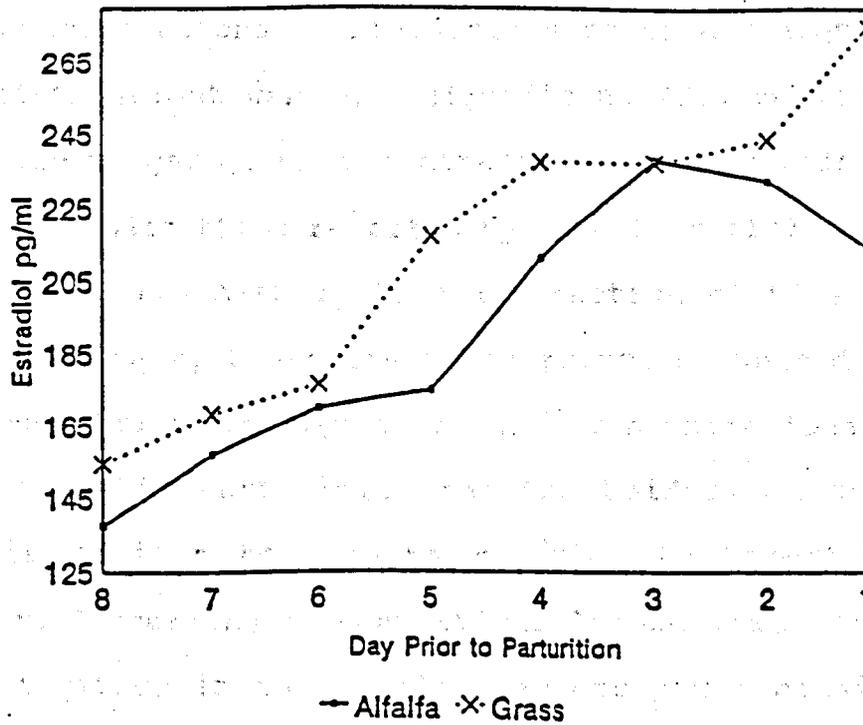
Figure 11. Least Squares Means of Estradiol 17-ß - Expanded Model.

high protein diets. It would appear that our results differ from those of Anthony et.al. because both groups of heifers in our experiment received more than adequate TDN and crude protein.

Concentrations of Estradiol 17-ß (E_2) as analyzed with the base model were not different between diets (P>.10). The effect of time was significant (P<.05), E_2 increasing as cows approached parturition. The interaction of time x diet approached significance and is illustrated in Figure 5.

After all non-significant factors except diet were eliminated from the model only calf birth weight remained. Diet did not affect E_2, but calf birth weight effect was highly significant (Table 8). Time had no significant effect on E_2 concentrations. Interactions of time x diet and time x calf birth weight were not significant (Figure 11).

The ranges of E_2 concentrations found in this study were consistent with those reported by Robertson (1974) and Anthony et al. (1986). Although the interaction of time x diet was not significant, there was a divergence between diets in the last day prior to parturition. Concentrations increased steadily until parturition for the heifers on grass diets. This is similar to results reported by Robertson (1974). However, decreasing concentrations for the last two days prior to parturition in the alfalfa hay group was consistent with results reported by Smith et al. (1973). Hoffman et al. (1973) and Edqvist et al. (1973) described E_2 concentrations

at constant plateau levels for the last 3 days before birth.

The effect of calf birth weight on E_2 concentration suggest that placental estrogen production may be a function of placental mass (Figure 12). Eley et al. (1978) reported a significant positive correlation between calf weight and the sum of placenta and membrane weight. Hafez (1987) stated that the placenta is a transient endocrine organ and like the corpus luteum, it secretes both trophic and steroid hormones that are released into the fetal and maternal circulations. Hoffman et al. (1976) reported that the placenta is capable of producing large quantities of estrogen. This understanding of the placenta's function as a endocrine organ and the relationship of fetal size to placental weight may explain why heavier calves had increased E_2 concentrations.

Anthony et al. (1986) and Chew et al. (1978) reported that calf sex was a significant source of variation for E_2 concentration. In the present study calf sex was only significant in absence of calf birth weight. Since, when calf birth weight was included in the model calf sex was no longer significant. In our data the variation in E_2 concentration was best explained by calf birth weight, and its inclusion in the model accounts for much of the variance that may be seen in using calf sex.
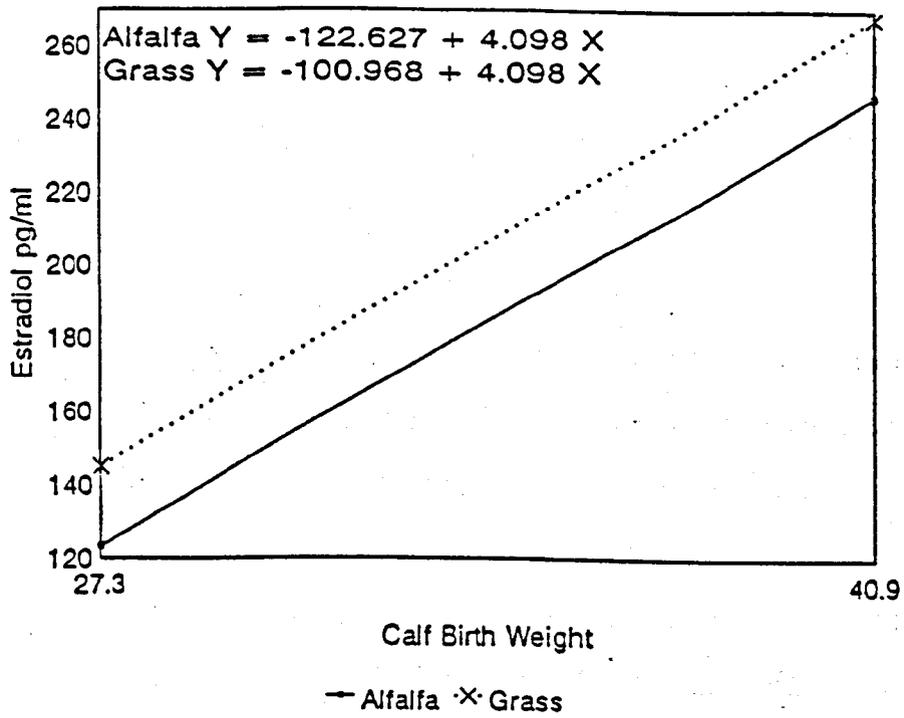
Figure 12.   Regression of Estradiol on Calf Birth Weight.

CHAPTER 5

CONCLUSIONS

Results from this experiment indicate that alfalfa and grass hay diets when fed free choice are very similar in their effects on weight change, condition score change, and pelvic area growth, even though heifers on the alfalfa diet received twice their requirement for crude protein (NRC, 1984). The effect of diet on calving difficulty was not significant, although heifers on the grass diet produced slightly heavier calves (2.6 kg). Bull calves in the study had a mean birth weight 3 kg heavier than the heifers.

Glucose and blood urea nitrogen levels were within normal limits for both diets. Diet had no significant effect on glucose concentration. A significant interaction between diet and calf birth weight showed that in the grass hay diet group, as calf birth weight increased glucose concentration increased. This was likely due to the difference in protein level between the diets. The alfalfa hay fed heifers had more gluconeogenic substrate in the form of excess protein than did the grass hay fed heifers, therefore heavier calves depressed glucose levels in the grass hay group.

Blood urea nitrogen was significantly higher on all days

prior to parturition in the alfalfa fed heifers. The interaction of diet x calf birth weight was significant. In the grass hay group BUN decreased as calf birth weight increased, however, BUN concentration in the alfalfa diet increased as calf birth weight increased.

Progesterone levels in this experiment were not affected by diet, but showed a marked drop prior to parturition.

The hypothesis that alfalfa affects calving difficulty by causing decreased E_2 concentration was not substantiated in our study. Heifers that produced heavier calves had higher (p<.05) E_2 concentrations, which was possibly related to greater placental mass from the heavier calves secreting larger amounts of E_2.

The comparison of alfalfa and grass hay in our study gave no indication that feeding alfalfa produces any increased risk in the incidence of dystocia or increased calf birth weights. Both diets were similar in their effect on weight change, condition score change, and pelvic area change. From this research it appears that both diets are useful and practical feeds for gestating beef cattle.

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