

DIETARY PROTEIN AND DAIRY CATTLE  
REPRODUCTIVE PERFORMANCE

By

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## CHAPTER I

### INTRODUCTION

Milk production has increased by over two times in United States dairy herds in the past 30 years. With this dramatic increase has come greater requirements for protein and energy components in the diet. Dietary concentrations of protein and energy need to be higher initially to meet the metabolic needs of the mammary system in early lactation. However, it is difficult to provide enough nutrients to high producing cows at this time, as maximum dry matter intake lags behind peak milk flow. Cows rely on the mobilization of body stores of protein and energy in an attempt to make up for nutrient deficiencies during early lactation.

The protein requirements of dairy cattle during the early phase of lactation are of primary interest in this study. High producing cows (30-50 kg milk/day) have dietary protein requirements of 14 to 22% of ration dry matter. Recent limited evidence has indicated that fertility of dairy cattle fed diets with greater than 16% crude protein is reduced. It has been hypothesized that this may occur as a result of biochemical or endocrine modifications. Also,

metabolism of high amounts of dietary protein has been suggested to cause a relative energy deficiency which could have a negative effect of reproduction. In addition there are some indications that the reproductive tract fluid environment is altered by the level of dietary protein. Since fertilization failure and embryonic mortality are major sources of infertility in dairy cattle, any alterations of the female reproductive system could enhance these problems and result in reduced reproductive efficiency.

This study consists of data from the first year of a two year project at the Oklahoma Agricultural Experiment Station considering the influence of dietary crude protein concentrations on reproductive performance. The major objective of this study was to more clearly establish the relationship between dietary crude protein and reproductive performance in dairy cattle.

## CHAPTER II

### REVIEW OF LITERATURE

#### Reproductive Failures in Dairy Cattle

Infertile services in dairy cattle may result from several factors. Anatomical abnormalities, ovulation failure, ovarian adhesions, ovarian cysts and uterine infection cause a modest portion of reproductive failures (Graden et al., 1968; Hawk, 1979). Embryonic mortality (Ayalon, 1978; Hawk, 1979), fertilization failure (Graden et al., 1968; Hawk, 1979), and improper heat detection or timing of insemination (Bozeworth et al., 1972; Rounsaville et al., 1979) represent major losses in reproductive efficiency.

Hawk (1979) summarized several studies and indicated that even under the best of conditions, fertilization failure occurred in approximately 13% of all dairy cattle. This concept that fertilization failure occurs in otherwise normal cows is supported by work done with repeat breeding cows (Tanabe and Casida, 1949; Graden et al, 1968). Tanabe and Casida (1949), using repeat breeding cows with apparently normal reproductive systems, observed a 66.1%

fertilization rate of ova recovered from cows 3 days after insemination. Graden et al. (1968) outlined possible causes for reproductive failure in repeat breeding dairy cows. While they found that a modest proportion of failures were caused by factors such as ovulation failure, infection and anatomical anomalies, 24.7% of repeat breeding cows had fertilization failure for no apparent reason. In beef heifers, fertilization rate after artificial insemination has been reported to range from 84 to 90% (Spitzer et al., 1978; Diskin and Sreenan, 1980). In addition, fertilization failure occurred in 15% of older, multiparous dairy cows that had a three year history of good fertility (Boyd et al., 1969).

Fertilization failure was essentially eliminated when bulls of known high fertility were used to inseminate normal heifers. Kidder et al. (1954) obtained fertilization rates of 100 and 71.9% and Bearden et al. (1956) reported rates of 96.6 and 76.9% for bulls of known high and low fertility, respectively. Fertilization failure due to use of poor fertility bulls can be reduced to a minimum when in heifers.

Of practical importance to reducing fertilization failure is properly timed insemination after a cow is detected in standing heat. Proper detection of estrus is a function of the level of reproductive management practiced in that herd, and represents a major hindrance to the goal of maintaining a 12 month calving interval (Bozeworth et al., 1972; Rounsaville et al., 1979). Cows have the highest

conception rates when inseminated about 12 to 14 hours after the onset of estrus (Trimberger and Davis, 1943). Hoffman et al. (1976) used milk progesterone concentrations to monitor the reproductive cycles of dairy cows, and found 20% of all dairy cows inseminated in the study were bred at times of elevated milk progesterone. This indicated that heat was incorrectly diagnosed in these cows, or that heat detection was not intense enough to identify cows at the onset of behavioral estrus. Improper detection of estrus and related poor timing of insemination are potential sources of infertile services.

Assuming insemination is properly timed, fertilization failure can be attributed to several problems in apparently normal cows. The uterine environment may not be optimal for: proper sperm function in the events that lead to fertilization, effective sperm transport to the ampulla of the oviducts for fertilization to occur, and oocyte function in fertilization (Hawk, 1983).

The other major source of reproductive failure, embryonic mortality, affects about 15% of all dairy cattle (Hawk, 1979). In repeat breeding cows, embryonic mortality can be very high, affecting as many as 65% of these cows (Tanabe and Casida, 1949). Ayalon (1978) noted from his studies that a difference exists between normal cows and repeat breeders as to when embryonic loss occurs. In repeat breeding cows, there was a reduction in percentage of cows with viable embryos from 80% at days 4 to 5, to 42% at 6 to

7 days after breeding. This reduction was only from 88 to 83% in cows with normal breeding histories. Percentage of beef cattle with recoverable embryos was reduced from 93 to 56% between days 8 and 12 of gestation (Diskin and Sreenan, 1983). Maurer and Chenault (1983) reported in beef cattle that 67% of the embryonic mortality had occurred by day 8 of gestation. Thus considerable variation exists among estimates as to when most of the early embryonic death occurs.

Substantial loss of embryos soon after breeding is in contrast to the idea that embryonic mortality may occur late enough in the cycle to lengthen interestrus intervals. Hawk et al. (1955) slaughtered repeat breeding cows at either 16 or 34 days after insemination, and observed a reduction in the proportion of cows with viable embryos from 58% on day 16 to 28% on day 34. No indication was given as to the number of lengthened cycles in this study. It would be expected that estrous cycles should be lengthened since loss of the embryo prior to day 15 resulted in normal cycle length, while embryo removal on days 17 and 19 lengthened the interestrus interval (Northey and French, 1978; 1980). This may be due to maternal recognition of the conceptus on days 15 to 16, that prevents release of the uterine luteolysin (Ginther, 1968; Northey and French, 1980; Knickerbocker et al., 1984).

Bull fertility has been examined as a factor in embryonic mortality. Using bulls of known high and low

fertility, Kidder et al. (1954) observed embryonic mortality rates of 25.5 and 14.9% for bulls of high and low fertility, respectively. However, Bearden et al. (1956) observed embryonic mortality rates of 19.6% with low fertility bulls compared to 10.5% after insemination with high fertility bulls. The variation in embryonic mortality may have been due to factors other than fertility level of the bulls, as embryonic survival did not seem to be clearly related to bull fertility when considering results of these two studies.

The role of hormonal imbalances in embryonic mortality remains unclear. Randel et al. (1971) indicated that estrogen excretion in the urine was greater in fertile cows than in repeat breeders during days 1-9 of the estrous cycle. Ayalon (1978) reported that infertile cows had greater concentrations of estrogen in plasma during days 1-7 of the cycle. However, Erb et al. (1976a) did not observe differences in urinary estrogen excretion for cows with infertile or fertile services. They did find greater asynchrony involving progesterone, luteinizing hormone (LH), and estrogen secretion the day prior to estrus in cows that did not settle. In normal and repeat breeding cows ovariectomized after fertilization, the influence of administration of progesterone or progesterone plus estrone on embryonic survival was studied (Hawk et al., 1963). In cows with normal breeding histories, pregnancy rates of 75 and 71% were observed for cows treated with progesterone and

progesterone plus estrone respectively. However, the proportion of repeat breeding cows maintaining normal pregnancies was 15 and 18% for progesterone and progesterone plus estrone treated cows respectively. Thus, the the endocrine anomalies that influence embryonic mortality are not well understood.

#### Dietary Protein and Reproductive Function in Dairy Cows

High dietary crude protein has been suggested to reduce reproductive efficiency in dairy cattle (Jordan and Swanson, 1979a; Folman et al., 1981; Kaim et al., 1983), possibly by increasing fertilization failure and/or early embryonic death rate. Jordan and Swanson (1979a) fed 45 high producing cows diets of 12.7, 16.3 and 19.3% crude protein. Days to first estrus were significantly shorter for cows on the 19.3% ration when compared to cows on the 12.7 and 16.3% crude protein diets (27 vs. 36 and 45 days). In addition, services per conception (1.47, 1.87 and 2.47) and days open (69, 96 and 106 days), increased with crude protein percentage in the diet. Folman et al. (1981) observed similar results using 3 groups of 20 cows each. The diets consisted of: 1) 16% crude protein using formaldehyde protected soybean meal 2) 16% crude protein using unprotected soybean meal and 3) 20% crude protein using unprotected soybean meal. Intervals to first observed estrus were similar for all three groups of cows. Services

per conception were 1.45, 1.79 and 2.25 for the 16% crude protein-soy protected, 16% and 20% crude protein rations with unprotected soybean meal, respectively. While the results of Folman et al. (1981) were not statistically significant, they do, along with studies of Jordan and Swanson (1979a), suggest a potential reproductive problem in cows fed diets greater than 16% in crude protein.

Kaim et al. (1983) measured conception rates of 250 cows fed either 15-16% or 19-20% crude protein rations. First service conception was significantly greater for cows on 15% crude protein (56.6%) than cows on 20% crude protein (43.1%). First service conception rates for cows in their second and third lactations were 58.2 and 51.5%, for the 15 and 20% crude protein diets. However, cows in their fourth and later lactations had first service conception rates of 52.6 and 28.8% after consuming the 15 and 20% crude protein diets, respectively. Overall pregnancy rates for all services were 79.2 and 70.2% in the younger cows and 76.9 and 51.5% in the older cows, for the medium and high protein rations, respectively. This information suggests that high dietary protein may be detrimental to the reproductive performance of cows in their fourth or later lactations.

Reproductive performance was also characterized in some studies where production responses to added dietary protein received the major emphasis. Edwards et al. (1980) fed cows 13, 15, and 17% crude protein diets and obtained nonsignificant differences in services per conception and

days open. Services per conception were 2.3, 2.6 and 2.7, and days open were 123, 141 and 139 for cows fed 13, 15 and 17% crude protein diets, respectively. Chandler et al. (1976) fed rations of 12.5 and 15.5% crude protein with or without methionine hydroxy analog. The only difference observed was at 12.5% crude protein, where feeding methionine hydroxy analog improved services per conception from 2.88 to 1.82. Roffler and Thacker (1983) reduced crude protein content from 17 to 13.5% after either one or two months of lactation, and observed that reproductive performance was comparable to cows maintained on 17% crude protein diets during the 90 day experimental period. All three groups of cows together averaged 114 days open and 1.9 services per conception. While primary emphasis in these studies was not on reproductive performance, such information suggests that protein concentration in the diet does not influence reproductive efficiency.

#### Possible Role of High Crude Protein in Reducing Fertility

It has been hypothesized that if high dietary protein does depress reproductive performance, it may do so in one of two ways: 1) excess ammonia absorbed from the rumen produces biochemical, hormonal or tissue modifications in the reproductive and/or endocrine systems; or 2) additional metabolized protein alters the balance of net protein and net energy to cause a relative energy deficiency (Chalupa,

1984).

An excess in ammonia load, such as that which may occur with overfeeding nonprotein nitrogen, may tax the detoxifying systems in the liver, and result in reduced animal performance due to alteration of metabolism in the liver and other tissues (Clark and Davis, 1980; Visek, 1984). Though the liver possesses an extremely large capacity to clear ammonia from the portal blood, it has been suggested that if cattle consume diets with a highly soluble protein content, the amount of ammonia reaching the liver may be greater than it is able to detoxify (Symonds, et al., 1981). Increases in dietary protein intake are normally accompanied by increases in rumen ammonia and blood plasma urea concentration (Claypool et al., 1980; Folman et al., 1981; Kaim et al., 1983); which may give a plausible link of ammonia toxicity to reduced reproductive performance in cows fed high crude protein diets.

To look at the possible effects of excessive ammonia on intermediary energy metabolism, several researchers have given ruminants acute doses of urea or ammonia compounds. Barej and Harmeyer (1979) infused ammonium chloride intraruminally in sheep over a six hour period and found that blood plasma glucose was elevated, and immunoreactive insulin concentrations were decreased when large amounts of ammonium chloride were given. Similarly, ammonium chloride infusion of sheep caused elevated plasma glucose, lactate and free fatty acids (Garwacki et al., 1979). Spires and

Clark (1979) obtained comparable results when dairy steers were intraruminally infused with urea. Plasma glucose was elevated by 40 mg/dl over the pre-infusion levels. Chalupa and Opliger (1969) also observed increases in plasma glucose, non-esterified fatty acids, and acetate after oral dosage of urea to sheep. The detrimental effects of ammonia on intermediary metabolism associated with glucose production or utilization, could occur due to decreased glucose utilization or increased glycogenolysis (Spires and Clark, 1979). If high dietary protein feeding causes such modifications in overall energy metabolism, then a net energy deficit could result, that may reduce reproductive performance.

While intermediary metabolism is affected by short term increases in the ammonia load, this represents an acute response. This may not be consistent with alteration in metabolism during more chronic, long term high protein feeding. For example, when considering the associative effects of energy and protein intake, there is little evidence that higher dietary protein hinders energy utilization. Gordon and Forbes (1970) indicated that when feeding 80 or 120% of the dietary requirements for both energy and protein, there was a greater response in milk production to added energy content when the higher dietary protein concentration was fed. This would suggest that the added dietary crude protein actually enhanced energy utilization. Such is supported by results of Moe and Tyrell

(1972) where net energy for lactation was elevated by increasing crude protein from 11.2 to 16.0% of ration dry matter. Moe and Tyrell (1977) also demonstrated that a 14% crude protein ration had a lower metabolizable energy value than diets of 17 and 20% protein content. It appears unlikely that elevated dietary crude protein suppresses reproductive performance by inducing an energy deficiency.

Reproductive performance in cows fed urea, a nitrogen source that is rapidly degraded to ammonia in the rumen, has been studied. Erb et al. (1976b) observed that when urea was fed to Holstein females during the first two years of their productive lives, progesterone concentrations were increased as urea was added to the diet. This suggests that ovarian activity or clearance of progesterone was modified in some fashion by including urea in the diet. However, estrous cycles, intervals between inseminations, intervals to first postpartum ovulation, intervals between ovulations or gross measures of ovarian activity were not impaired in these same Holstein heifers (Erb et al., 1976c). In addition, no differences in services per conception or days open were observed in cows fed urea-supplemented diets (Wohlt and Clark, 1978).

Reproductive endocrine function may also be altered in cows fed high protein diets. Jordan and Swanson (1979b) fed cows 12.7, 16.3 and 19.3% crude protein diets, and observed that concentrations of progesterone in plasma were greater on day 14 of the first observed estrous cycle and the

conception cycle in cows fed the lower protein diet. Concentrations of luteinizing hormone (LH) were also reduced on days 2 and 14 of both the preconception and conception cycle in cows fed a 12.7% crude protein diet in comparison to cows fed 16.3 or 19.3% crude protein diets. Release of LH after gonadotropin releasing hormone (GnRH) challenge tended to be higher in ovariectomized cows on a 15.9% protein diet when compared to cows fed 23.9% crude protein rations (Blauwiekel et al., 1984). There were no differences in anterior pituitary GnRH receptor concentrations between cows on 15.9 and 23.9% crude protein rations. The anterior pituitary may be less responsive to GnRH when high protein diets are fed. While the endocrine system may be altered by high protein or urea feeding, the relationship remains unclear as to how or if this may be related to impaired fertility.

Recent evidence indicates that the uterine environment may be changed so that the incidence of fertilization failure or embryonic death could increase. Since ammonia is readily diffusable across tissues (Visek, 1984), rapid uptake could affect the uterine environment, as the reproductive tract is in close proximity to the rumen. Along with the rise in plasma urea, urea concentrations have been shown to increase in uterine/vaginal flushings of cows (Jordan et al., 1983; Duby et al., 1984;), and oviducal and vaginal fluids of ewes (Duby et al., 1984) when high protein diets are fed. Jordan et al. (1983) also demonstrated that

the mineral composition of uterine fluids in cows fed high protein rations was modified. Zinc increased during the entire estrous cycle, while magnesium was lower. During the luteal phase of the cycle, high protein feeding decreased potassium and phosphorous in uterine flushings.

Increased ammonia in the reproductive organs could also directly affect their function. When bovine oviducal tissue was cultured in the presence of either ammonium sulphate or jack bean urease to increase ammonia concentrations, the incidence of ureaplasma epithelial lesions increased (Stallheim and Gallagher, 1976). With scanning electron microscopy, substantial ciliostasis and deciliation was evident. If the environment of the female reproductive tract was altered in such a way to enhance the breakdown of urea to ammonia, tissue damage in the female reproductive tract could occur. With altered tract function and modification of the uterine fluid environment, sperm transport, sperm and oocyte function at fertilization, and/or early embryonic development may be affected (Ayalon, 1978; Hawk, 1983).

#### Relationship Between Dietary Protein and Milk Production

In the past 30 years, milk production in the U.S. has increased dramatically. With this has come the need to increase both quality and quantity of feed consumed to realize this potential for high milk production. Since

maximum dry matter intake lags behind peak of lactation by four to eight weeks (Clark and Davis, 1980), the protein and energy components of the ration must be greater in the initial part of lactation to meet the metabolic demands of the mammary system.

In some studies, milk production was enhanced when greater amounts of crude protein were fed. Van Horn et al. (1979) increased crude protein content from 13.5 to 16.3%, using cottonseed meal, soybean meal, and peanut meal. Milk production increased from 15.3 to 17.0 kg milk per day, but only in the cottonseed meal supplemented diets. Grieve et al. (1974) fed diets of 14, 16 and 18% crude protein and production increased from 23.4 to 26.0 and 28.9 kg/day, respectively. In another experiment, cows were fed 13.5, 15.5 and 17.5% crude protein diets and milk production was 25.4, 27.0 and 28.8 kg/day for the three diets (Sparrow et al., 1973). When diets of 11, 13, and 15% crude protein were fed, milk production increased from 19 to 20.6 and 21.3 kg/day, respectively (Van Horn and Jacobson, 1971). Soybean meal was the major protein source. However, when Van Horn and Jacobson (1971) fed these amounts of crude protein using urea as the primary nitrogen source, no increases in production were observed.

Van Horn et al. (1979), used data from several studies with cows of different producing abilities to devise a mathematical model for predicting changes in production with soybean meal supplemented diets. Crude protein was

increased in increments of 1% from 9 to 17%. Increasing crude protein from 9 to 10% resulted in 7.9% more milk and 3.9% greater dry matter intake. Further increases in crude protein intake resulted in successively lower increases in production. Increasing crude protein from 16 to 17% resulted in only 0.7% more milk and 0.1% greater dry matter intake. In addition, increasing crude protein enhanced organic matter digestibility, which contributed to some of the increase in milk production. The limiting factor in some instances may be the genetic ability of those cows to respond to added protein with more milk production.

Best production responses to added dietary protein may be in the first half of early lactation. In cows fed 13, 15 and 17% crude protein rations with soybean meal as the major source of protein, milk production was monitored from calving to 42 weeks postpartum (Edwards et al., 1980). Over the duration of the study, cows on the 13% crude protein diet produced less milk than cows fed rations containing 15 and 17% crude protein (19.9, 22.9 and 23.2 kg/day, respectively). This difference in production between cows on the 13% diet and those on 15 and 17% crude protein diets existed during the first 35 weeks of lactation. In another experiment, milk production was 32.7 to 40.3 kg/day during weeks four to five of lactation and 29.0 to 35.8 kg/day during weeks 11 and 12 of lactation for cows fed 12.4 and 17.7% crude protein rations, respectively (Cressman et al., 1979). Roffler and Thacker (1983) fed three groups of cows

a 17% crude protein diet immediately following parturition. One group was reduced from 17 to 13.5% crude protein at one month of lactation, another reduced to 13.5% crude protein at two months, while the third group was held at 17% during the entire 90 day feeding period. Daily milk yield was not affected in first calf heifers. Multiparous cows whose diet was reduced to 13.5% at one month of lactation produced less milk per day than cows fed the 17% crude protein diet during the first two or three months of lactation. In addition, production did not differ between groups of cows fed 17% crude protein through either two or three months of lactation. Feeding the greater percentage of crude protein seemed beneficial for production in these cows through two months, but not three months of lactation.

Increased crude protein in the diet has not always been demonstrated to benefit milk production. Cows fed rations of 12.7, 16.3 and 19.3 crude protein, using mainly soybean meal as the protein source, experienced no significant increase in four percent fat-corrected milk production (Claypool et al., 1980). There was a linear tendency for increased production, averaging 29.2, 30.9 and 32.2 kg/day for cows fed increasing percentages of crude protein. In another study, dairy cows were fed three diets that consisted of: 16% crude protein, formaldehyde protected soybean meal; 16% crude protein, unprotected soybean meal; 20% crude protein, unprotected soybean meal (Folman et al., 1981). Average 122 day milk yield was 40.4, 38.9 and 38.4

kg/day. Though these levels of production were not significant, the tendency of the cows fed protected soybean meal to have greater milk production may indicate that more protein in these cows escaped ruminal degradation and was available for production. Kaim et al. (1983) observed little benefit of increasing dietary protein from 15 to 20% of ration dry matter. Cows in their second and third lactation averaged 35.3 and 35.7 kg/day for 15 and 20% crude protein diets, whereas cows in their fourth and later lactations averaged 39.4 and 38.0 kg/day. Chandler et al. (1976) fed 12.5 and 15.5% crude protein rations with soybean meal as the major plant protein source, and observed no difference in milk production. In another study, dairy cows were fed to meet 75 or 100% of the Agricultural Research Council recommendations for crude protein (about 13.5 and 18.0% of ration dry matter), and averaged 22.3 and 23.3 kg of milk per day (Treacher et al., 1976). This difference was also not significant.

Variable responses in milk production to added crude protein may be due to three factors: 1) the additional protein must be in a form that is utilizable for milk production; 2) higher dietary protein may be more important in early lactation; 3) the cows must have the genetic ability to utilize the added increments of crude protein for milk production. Based on research describing the production response of dairy cows to increased dietary protein concentrations, the National Research Council has

estimated their crude protein needs to range from 14 to 22% of ration dry matter (NRC, 1978). For example, a 650 kg cow in her second lactation, producing 30 kg of 3.5% fat milk per day may need a diet with 15% crude protein, while a similar cow producing 60 kg of milk per day could potentially require a diet that contains 22% crude protein.

#### Cost of Impaired Fertility in Dairy Cattle

If feeding diets with a large percentage of crude protein cause a reduction in reproductive efficiency, then there are economic aspects to consider as well. Low reproductive efficiency represents a major economic loss to U.S. dairymen (Spalding et al., 1975). These losses are not accrued as direct cash outlays, but rather as losses in the form of opportunity costs (Britt, 1976). Not only is there a sacrifice in income from production, but there are added costs, such as breeding fees, veterinary services, and replacement heifer losses. In addition, by increasing the number of cows culled for reproductive failure, culling based on milk production is decreased. This represents the hidden cost associated with decreased genetic progress.

Call (1976) summarized data of Bonowitz and Dunham (1975) from Kansas DHI records in which low production and poor reproductive performance accounted for large proportions of the total cows culled (36 and 23% respectively). He indicated however, that the trend was

towards an increasing percentage of cows leaving dairy herds for reproductive problems. In another study , culling for reproductive failure accounted for 51.1% of all culls, whereas 26.7% was based on production (Oltenacu et al., 1984). While these data seem to overemphasize the trends that Call (1976) indicated, they illustrate the impact that poor reproductive performance can have on the level of voluntary culling based on production.

The inability of U.S. dairymen to maintain calving intervals of around 365 days is quite costly. Gerrits et al. (1979) identified areas to potentially increase income in the nation's dairy herds by improving reproductive efficiency. In general, for the nation's approximately 11.5 million dairy cattle, if the average calving interval could be reduced from 13.5 to 12 months, an increase of \$135 million in income could be realized. Other areas of improved reproductive management and their economic value to potentially increase farm income are: reduce sterile cows from five to two percent, \$57 million; reduce calf losses from 10 to four percent, \$85 million; reduce services per conception from 2.0 to 1.5, \$20 million; reduce infertile bulls from seven to three percent, \$1 million. The total potential for increased income from improved reproductive management is: \$298 million. An earlier report (Pelissier, 1972) summarized some estimated costs of low fertility in U.S. dairy herds, based on 1970 figures. Even more dramatic losses in potential income are apparent (Table I). These

TABLE I  
ESTIMATED COST OF LOW FERTILITY  
IN DAIRY HERDS-1970

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Milk production and calf losses	\$394,033,500
Replacement losses	93,817,500
Veterinary services and medication	37,527,000
Additional breeding fees	14,570,126
Total	\$539,948,126

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Source: Pelissier, 1972.

cost estimates are associated with loss in potential income incurred by extending the calving interval from 12 to 13.5 months. While both of these reports show some marked differences in losses due to reproductive inefficiency, it must be realized that these are only estimated costs. Even though the estimates may not reflect current trends and prices, they serve as examples of the magnitude of a problem that is too often overlooked.

Extending the calving interval beyond 12 months results in losses in milk production and returns over feed cost (Speicher and Meadows, 1967; Speicher and Brown, 1971). Depression in production or monetary returns were not substantial until calving intervals were extended past 13 months (Speicher and Meadows, 1967; Table II). Cows that remained open beyond 86 but less than 116 days postpartum resulted in a \$0.50 decrease in returns over feed costs per day open past 86 days. Cows that remained open for more than 116 days after calving showed a decrease in income over feed costs of \$0.78 per day open after day 86. Similarly, Louca and Legates (1968) estimated losses at \$0.25 to \$0.71 per day open. Milk production per day was not hindered greatly unless calving intervals exceeded 13 months (Speicher and Brown, 1971; Table III). When calving interval was lengthened from 12 to 15 months, returns over feed costs were \$67 less per year, or about \$0.74 less per day open beyond day 85 post calving.

In recent years, computer analyses have allowed more

TABLE II  
EFFECT OF CALVING INTERVAL ON  
MILK YIELD AND PROFITS

Calving interval (months)	305 day Milk yield (kg)	Annual return over feed costs (\$)
12	6351	384
12-13	6315	383
13-14	6096	365
14	5840	350

Source: Speicher and Meadows, 1967.

TABLE III  
AVERAGE DAILY MILK PRODUCTION AS AFFECTED  
BY CALVING INTERVAL AND  
PRODUCTIVE ABILITY

Calving Interval (months)	Production ability (kg/305 days, mature equivalent)			
	<5455	5455- 6363	6364- 7272	>7272
	kg milk/day			
12	14.5	16.4	18.3	20.5
13	14.4	16.4	17.9	20.4
14	14.1	15.7	17.2	19.7
15	13.4	15.0	16.4	19.0

Source: Speicher and Brown, 1971.

refined estimates of costs of decreased reproductive efficiency by integrating several production inputs to arrive at losses due to calving intervals of various lengths (Lineweaver, 1975; Olds et al., 1979; Holmann et al., 1984). Based on an average of 130 days open, and taking into consideration production loss, breeding costs, veterinary costs, calf losses and replacement costs, Lineweaver (1975) estimated a loss of \$91.74 per cow as compared with cows that calved at yearly intervals. Such translates into a loss per day open past 85 days postpartum of \$2.04, which is somewhat higher than previous estimates. Olds et al. (1979) estimated losses of \$0.71 and \$1.18 per day open for first lactation heifers and multiparous cows respectively, for each day open from 40 to 140 days after calving. Holmann et al. (1984) calculated income over feed costs for cows of different producing abilities and calving intervals, utilizing milk prices, feed costs, and nutrient recommendations of the National Research Council (Table IV). Extending the calving interval from 12 to 13 months actually resulted in an increase in income over feed costs of \$0.21 to \$0.40 depending on the level of milk production. When the calving interval was increased from 12 to 15 months, income over feed costs was essentially zero, indicating that the increase from 12 to 13 months was offset by the decrease in income associated with extending the calving interval from 13 to 15 months (Table IV).

Maintenance of a 12 to 13 month calving interval seems

TABLE IV  
 VALUE OF DAYS OPEN BY ALTERNATIVE CALVING  
 INTERVAL AND MILK PRODUCING  
 ABILITY

Calving interval (months)	Milk producing ability (kg/300 days)			
	5900	6800	7700	8600
12 to 13	.36	.21	.40	.36
12 to 15	.12	-.07	.07	.00
13 to 15	-.04	-.23	-.12	-.20

Value = Income over feed cost (\$) per additional day open

Source: Holmann et al., 1984.

to be optimal (Speicher and Meadows, 1967; Speicher and Brown, 1971; Holmann et al., 1984). Depending on current economic conditions, strict adherence to a 365 day calving interval may not result in maximum returns over feed costs of the current lactation. Not only would there be losses in production per day of that lactation due to extending the calving interval beyond 12 to 13 months, but there is also a decrease in lifetime production by not allowing that cow to go through a maximum number of lactations during her productive life. One must also consider losses due to added breeding and veterinary costs associated with reduced reproductive efficiency. Finally, by not allowing for a maximum number of replacement heifers, there are losses in opportunity costs in terms of decreased selection intensity. The economic value of lost genetic progress is very important, though it is hard to estimate.

## CHAPTER III

### DIETARY PROTEIN AND DAIRY CATTLE REPRODUCTIVE PERFORMANCE

#### Summary

Eighty-two Holstein and Ayrshire cows of second or later lactation were randomly assigned to receive either a moderate (15%) or high (20%) crude protein diet beginning one to two weeks postpartum, for 19 to 20 weeks of lactation, respectively. Diets were fed to individual cows as an isocaloric, complete mixed ration. Dry matter intake was increased slightly in cows fed the high crude protein diet. Protein intake, expressed as a percentage of NRC recommendations, increased from 78 to 122% and from 98 to 167% for cows on the moderate and high crude protein diets, respectively. Cows on the high protein diet had greater milk production, particularly at the peak of lactation. Milk fat and protein percentages were similar for cows on the two diets. Changes in body weight and condition score during the experiment were small and similar for cows on the two levels of dietary protein. Services per conception (1.6 vs. 1.3), days to first estrus (41.1 vs. 36.3), days to

onset of ovarian activity (36.3 vs. 32.7), days open (85.3 vs. 81.3), and percentage pregnant (85.7 vs. 88.6) were not different for cows on the moderate and high crude protein diets respectively. The crude protein concentration in the diet did not adversely affect reproductive performance in normal dairy cows.

(Key Words: Dairy Cattle, Dietary Protein, Fertility, Days Open, Body Condition).

### Introduction

The dietary crude protein requirements for high producing dairy cattle (30-50 kg milk/day) range from 14 to 22% of ration dry matter (NRC, 1978). For example, a 650 kg cow producing 30 kg of 3.5% fat milk per day requires a 14-15% crude protein diet, whereas a 650 kg cow producing 50 kg 3.5% fat milk per day requires a 19-20% crude protein diet. With the dramatic increase in milk production that has taken place in the past 30 years, a trend that will likely continue, the recommendations for dietary protein should be even greater in future years in an attempt to meet the metabolic needs of high producing cows.

Recently, limited evidence has suggested that a conflict exists between dietary protein requirements and reproductive performance of high producing cows. Services per conception were increased in cows fed diets with greater than 16% crude

protein (Jordan and Swanson, 1979a; Folman et al., 1981). Kaim et al. (1983) indicated that increased dietary protein mainly affected fertility of cows in their fourth and later lactations.

It has been suggested that reduced reproductive performance is caused by the increased ammonia concentrations in cows fed high protein rations. Increased ammonia that is associated with highly soluble protein sources may overwhelm the detoxifying system of the liver (Symonds et al., 1981). A high ammonia load can cause biochemical, endocrinological, or local tissue modifications (Chalupa, 1984; Visek, 1984), possibly affecting general metabolism and reproductive function.

Recent experiments suggest some direct effects of dietary protein concentration on the female reproductive system. Jordan and Swanson (1979b) reported that progesterone and luteinizing hormone (LH) secretion may be altered by the protein percentage in the diet. Blauwiekel et al. (1984) indicated that pituitary function may be impaired by high dietary crude protein. Feeding cows high protein diets also changes the fluid environment of the female tract (Jordan et al., 1983; Duby et al., 1984). Bovine oviducal tissue cultured in media having elevated ammonia concentrations developed substantial ciliostasis and deciliation (Stallheim and Gallagher, 1976).

High protein diets have been proposed to affect reproductive function by changing the balance of protein

and energy, causing a relative energy deficiency (Chalupa, 1984). However, there is little evidence to support this concept. For example, increasing crude protein percentage in the ration has a positive associative effect on organic matter digestibility (Van Horn et al., 1979), metabolizable energy (Moe and Tyrell, 1977), and net energy for lactation (Moe and Tyrell, 1972).

Other research questions the negative effect of high crude protein feeding on reproductive performance. In some milk production studies, reproductive performance was also characterized. Increasing the crude protein percentage from 12.5 to 15.5% (Chandler et al., 1976), 9.2 to 18.1% (Wohlt and Clark, 1978), or 11 to 15% (Edwards et al., 1980) did not reveal a relationship between dietary protein concentrations and reproduction. Along with these reports, previous studies concerning dietary protein affects on reproduction are also due criticism concerning the limited number of cows used per treatment and/or control of other factors, such as variation in semen fertility and individual cow feeding that can also bias reproductive performance and ration effects.

As the potential conflict between dietary protein and reproduction is important to the management of high producing cows, the objective of this study was to investigate the influence of two concentrations of dietary crude protein on reproductive performance in normal dairy cows in as controlled a manner as possible.

## Materials and Methods

### Animals and Experimental Design

Sixty Holstein and 22 Ayrshire cows in their second or later lactation were randomly assigned, based on breed and lactation group, within period of the year, to receive either a moderate (15%) or high (20%) crude protein diet. Feeding began one to two weeks postpartum, and extended through the first 19 to 20 weeks of lactation. Two lactation groups were created: cows in their second or third lactation; or cows in their fourth or later lactation. Based on date of calving, the year was divided into five to six week periods (6 total periods). This was done primarily to account for the potential seasonal variation in fertility. Cows started on the study in August of 1983, with the last cows completing the experiment in August of 1984.

### Intake and Nutritional Responses

Diets consisted of isocaloric, complete mixed rations, of 45% sorghum silage and 55% corn grain-soybean meal concentrate (Table V) on a dry matter basis. Cows were fed individually to the limit of consumption at 0300, 1100 and 1900 hours. Net energy for lactation and crude fiber were

TABLE V  
PERCENTAGE COMPOSITION (AS FED) OF GRAIN/  
SOYBEAN MEAL CONCENTRATE

Ingredients	% Crude Protein	
	15%	20%
Soybean Meal	23.5	53.0
Wheat Middlings	29.5	----
Corn, Ground	27.0	27.0
Oats, Crimped	5.0	5.0
Cottonseed Hulls	5.0	5.0
Molasses, Liquid	5.0	5.0
Limestone	1.5	1.5
Dicalcium Phosphate	1.3	1.3
Sodium Bicarbonate	1.0	1.0
Salt	0.7	0.7
Magnesium Oxide	0.5	0.5

calculated as 1.47 and 1.48 Mcal/kg and 16.0 and 15.7% for the 15 and 20% crude protein diets, respectively. Daily feed intake and feed refusals were recorded, with refusals mixed into a weekly composite for each cow. Dry matter and crude protein percentages were determined on feed samples and refusal composites to calculate daily dry matter and crude protein intake. Plasma urea concentrations were measured to indicate differences in protein degradation and metabolism in cows on the two diets. Weekly blood samples were taken via the tail vein using evacuated blood tubes 2.5 hr after the 1100 hr feeding. Samples were immediately chilled on ice and transported to the physiology laboratory. Blood was centrifuged within 2 h of collection at 4000 rpm for 20 minutes at 5 C. Plasma was decanted into vials and frozen at -20 C until analysis. Concentration of urea in plasma was determined weekly for the first five weeks of the study and every other week from 7 to 19 weeks. Modified procedures of Fawcett and Scott (1960) and Searcy et al., (1961) were used to quantify concentrations of urea in plasma. Body weight and body condition scores were determined every other week. Body weight was averaged to estimate weights during the weeks that weight was not recorded. This was necessary to calculate protein intake expressed as a percentage of NRC recommendations. Body condition was evaluated using a 1 to 9 scale where 1=extremely thin and 9=over conditioned (Aalseth et al., 1983; Appendix Table VII).

### Lactational Responses

Cows were milked twice daily at 0500 and 1700 hr, with milk production recorded at both milkings. Daily production was summarized on a weekly basis. Milk samples were taken on four consecutive milkings for each cow, and a weekly milk protein and fat percentage was estimated. Protein and fat percentages were measured by the Oklahoma State Dairy Herd Improvement laboratory, using a Foss Pro-Milk I to determine milk protein percentage and Foss Milko-tester Mark III to measure milk fat percentage. Instruments were calibrated using standards from D & F Control Systems, Inc., San Francisco, CA.

### Reproductive Management

Cows were observed for estrus twice daily at 0700 and 2100 hours. Cows were also observed for estrous activity throughout the day, especially prior to and after handling for feeding and milking. Weekly palpation was performed to follow uterine involution by estimating cervical and uterine horn diameters. Uterine infections were treated when necessary. Ovarian size was estimated in three dimensions, and diameters of corpora lutea and follicles were also recorded. Ovarian cysts detected by palpation were treated if abnormal estrous activity was also observed. Cows were

not palpated after insemination, except for pregnancy determination. Pregnancy status was diagnosed 35 to 40 days following the most recent insemination. A second palpation was conducted two weeks later to confirm the previous diagnosis. Weekly plasma progesterone concentrations were determined to ascertain the onset of cyclic ovarian activity. This was defined as the first elevation in progesterone greater than one ng/ml of plasma for at least two consecutive weeks. Sampling and processing of blood was as described earlier for plasma urea analysis. Plasma progesterone was determined by single antibody radioimmunoassay as described by Lusby et al. (1981). Cows were artificially inseminated at the first heat 55 or more days postpartum, utilizing primarily one inseminator. Cows in each breed were inseminated with cryopreserved semen frozen as one batch, from one sire for that breed. This eliminated variation in semen fertility within each breed.

#### Statistical Analysis

Data were analyzed using General Linear Models procedures least squares analysis of variance. Included in the model for analysis of reproductive parameters were period, lactation group, diet and breed, and all possible two- and three-way interactions. To examine changes in various productive and nutritional parameters taken over weeks of the study, a similar model was fit with

interactions between the above stated main effects (and two and three factor interactions) and the linear, quadratic, and cubic effects of week. Responses were considered different if one or more of the polynomial responses were significant ( $P < 0.05$ ). This latter model was reduced by excluding interactions that included period, as each of the parameters, when compared over weeks of the study in each period, showed consistent responses across periods. In addition, there were certain periods in which treatment combinations were unequally distributed, with some combinations missing from particular periods altogether.

## Results

### Dietary Responses

Actual crude protein percentages during the study were 14.6 and 19.5%, respectively, for the moderate and high protein diets. Protein intake, expressed as a percentage of National Research Council Recommendations (NRC, 1978), increased from 78 to 122% and from 98 to 167% for cows fed the 15 and 20% crude protein diets, respectively (Figure 1). Cows on the 15% crude protein diet were fed to meet their requirements for crude protein, whereas cows fed the 20% diet were overfed crude protein during the experiment.

There was a significant diet by breed interaction over time for dry matter intake (Figure 2; Appendix Table VIII;

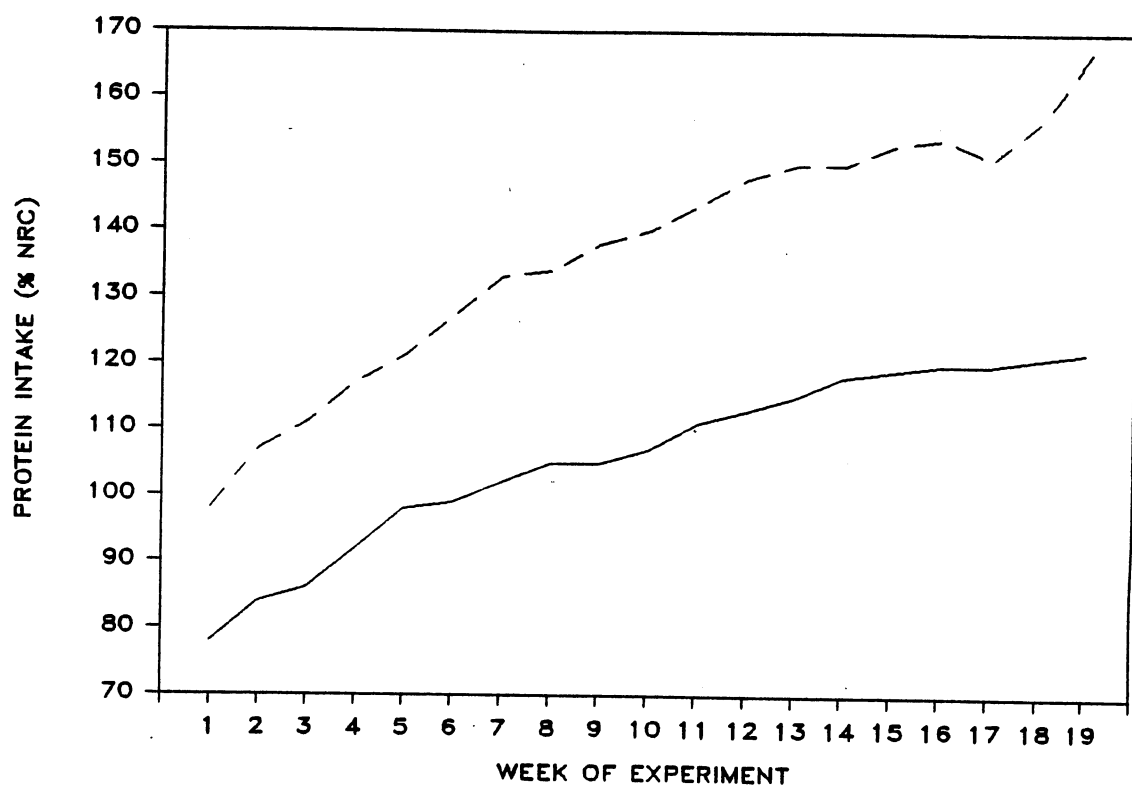


Figure 1. Daily Protein Intake (% of NRC Requirements) for Dairy Cows fed 15 (—) and 20% (---) Crude Protein Diets.

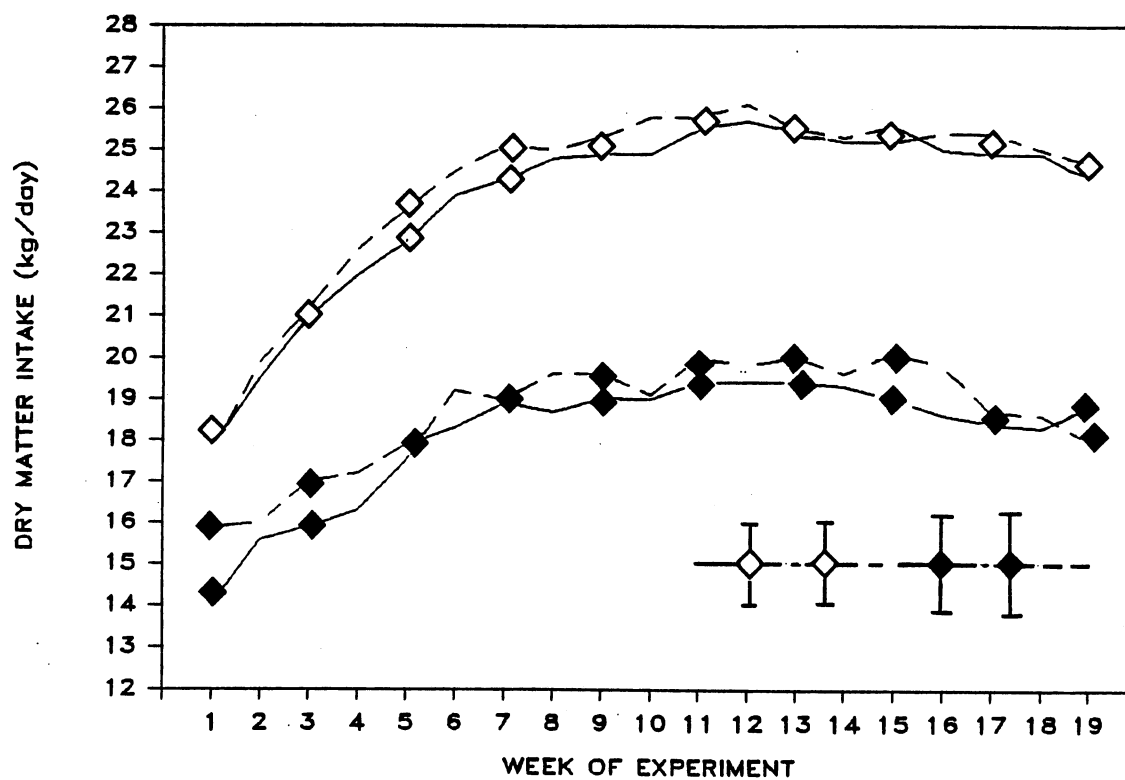


Figure 2. Daily Dry Matter Intake for Holstein (◇) and Ayrshire Cows (◼) fed 15 (—) and 20% (---) Crude Protein Diets. Homogeneous S.E.M. are Illustrated.

$P < 0.001$ ). There was a strong influence of breed, as Holsteins consumed more dry matter than the Ayrshires. Within each breed, cows on the 20% crude protein diet tended to consume more dry matter than cows fed the 15% protein ration. In addition, there was a significant interaction over weeks of the study between lactation group and diet for dry matter intake (Figure 3; Appendix Table VIII;  $P < 0.05$ ). Within each lactation group cows on the high protein diet tended to consume more feed, particularly after week four of the experiment. There was little difference in dry matter intake between lactation groups. For all treatment combinations, dry matter intake increased through the first 8 to 10 weeks of the study.

The effects of diet, breed and lactation group on plasma urea are illustrated in Figure 4, as an interaction existed among these parameters over time (Appendix Table IX;  $P < 0.05$ ). Concentrations of urea in plasma tended to be greater for cows fed the 20% versus the 15% crude protein diet, regardless of lactation group or breed. The interaction was due mainly to the irregular response of the Ayrshire cows in their fourth or later lactation fed the high protein diet. However, there were a limited number of cows in this group ( $n=2$ ). By week five of the experiment, plasma urea of cows on the 20% crude protein diet stabilized about 12 mg/dl above concentrations of cows fed 15% crude protein.

Cow weight as affected by diet, breed and lactation

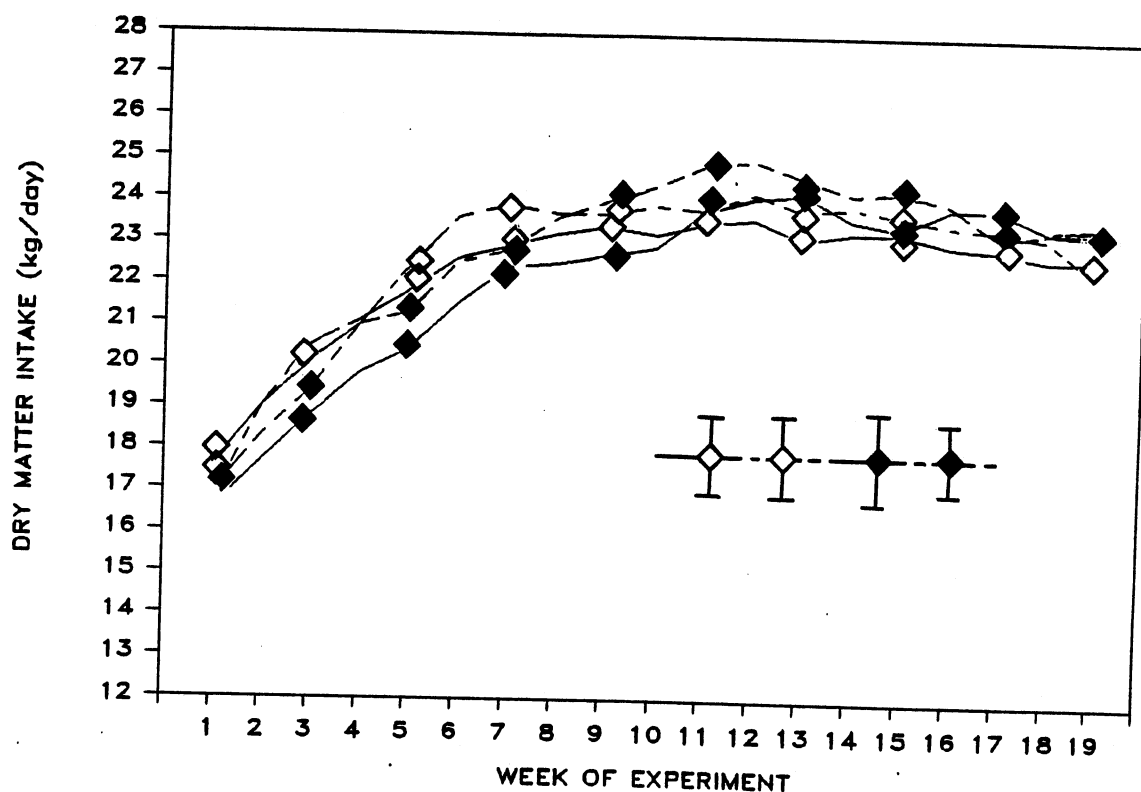
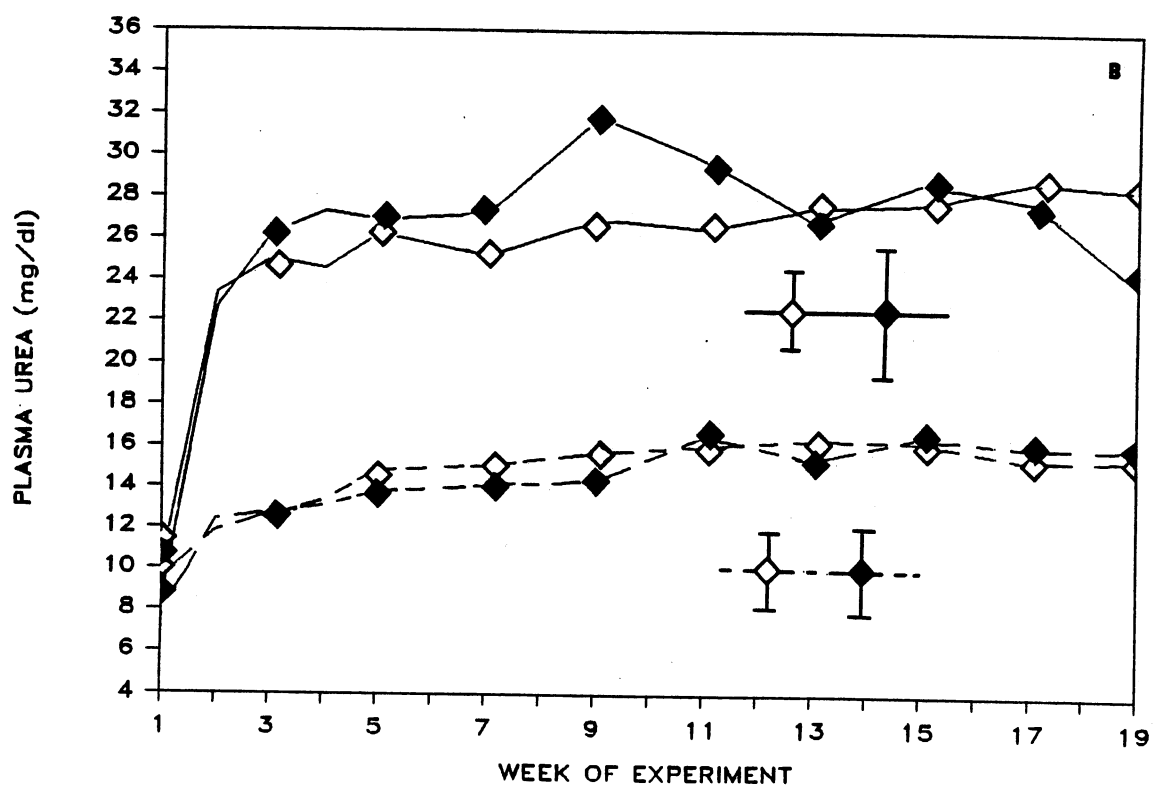
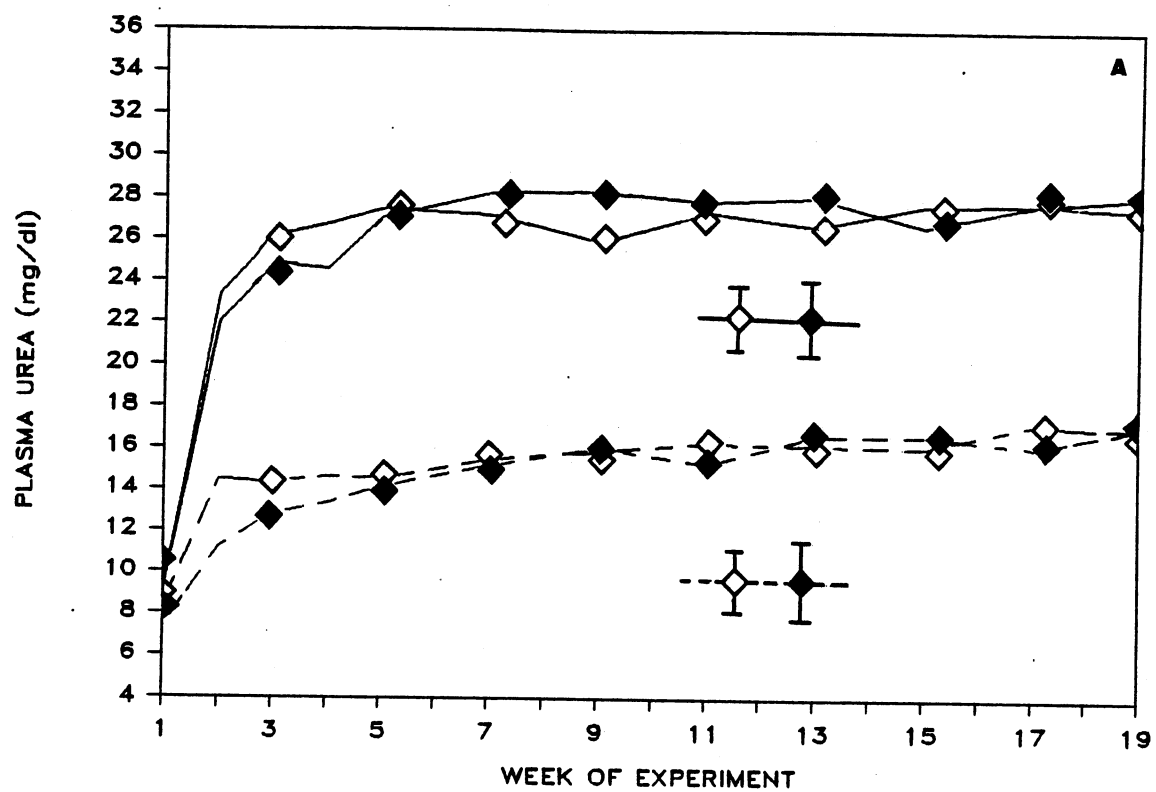


Figure 3. Daily Dry Matter Intake for Dairy Cows of Either Second and Third ( $\diamond$ ), or Fourth and Later ( $\blacklozenge$ ) Lactations, fed 15 (—) and 20% (---) Crude Protein Diets. Homogeneous S.E.M. are Illustrated.

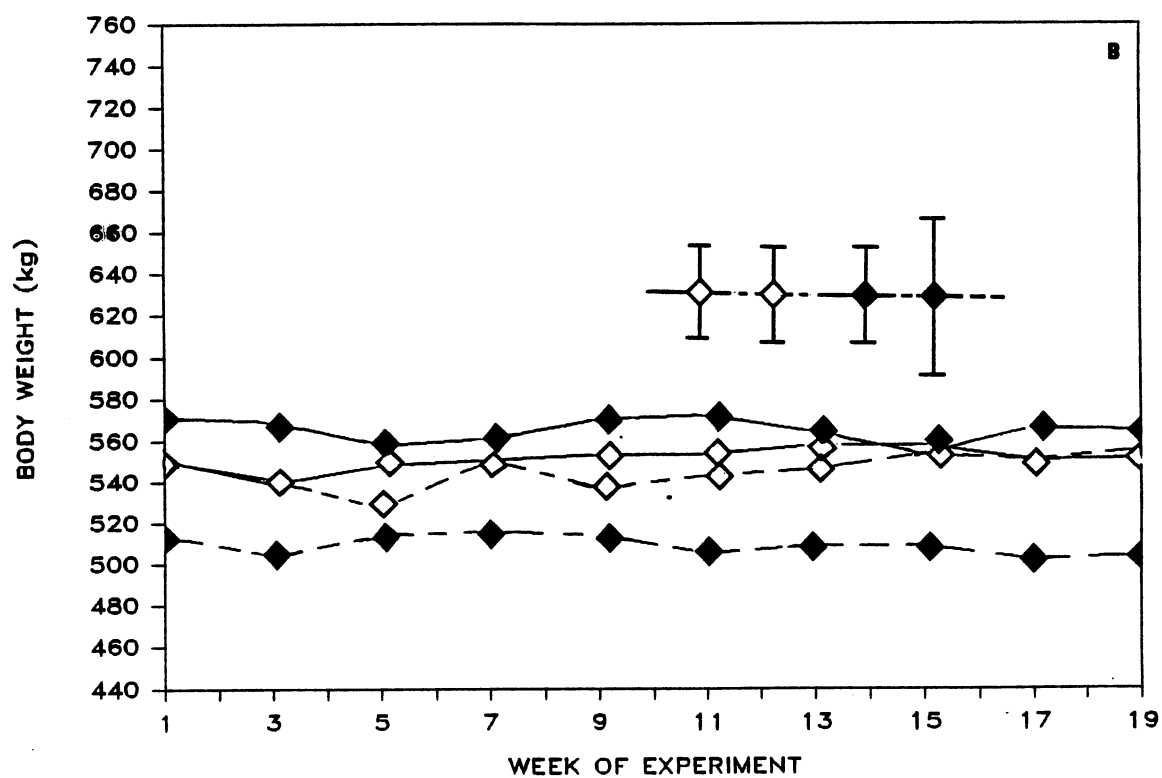
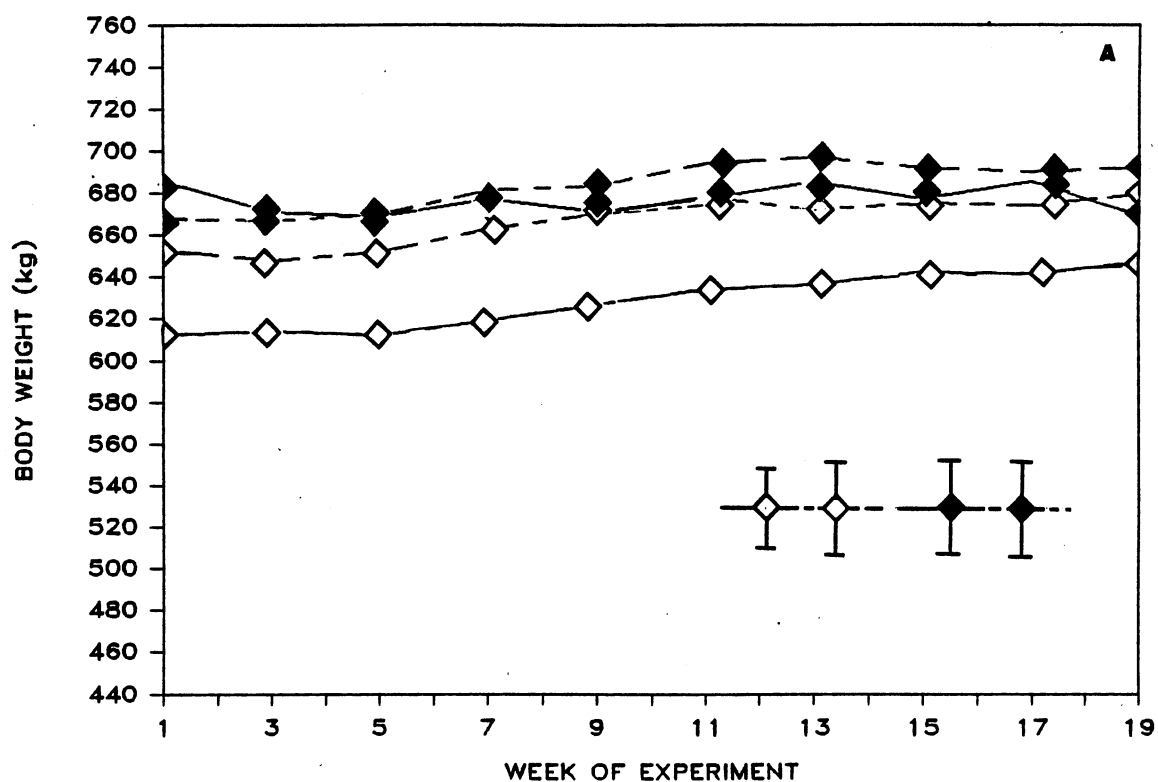
Figure 4. Plasma Urea Concentrations for Holstein (A) and Ayrshire (B) Cows, of Either Second and Third (◊), or Fourth and Later (◆) Lactations, fed 15 (---) and 20% (—) Crude Protein Diets. Homogeneous S.E.M. are Illustrated.



group over weeks of the experiment is presented in Figure 5. There was a significant interaction among these main effects over weeks of the study (Appendix Table X;  $P < 0.001$ ). Ayrshire cows had minor fluctuations in body weight. However, Holsteins in their second or third lactation fed both diets, and Holsteins in their fourth or later lactation fed the high protein diet, had a tendency to gain weight in the latter part of the experimental period. Regardless of diet, breed or lactation group, cows experienced little weight loss during the experiment.

There was a significant diet by breed interaction over time for body condition score (Figure 6; Appendix Table XI;  $P < 0.001$ ). Holsteins on the 15% crude protein diet lost more condition than Holsteins on the 20% crude protein diet, whereas Ayrshires fed both diets had similar changes in body condition score. Ayrshire cows were also in better condition than Holsteins. Over weeks of the study, there was also a significant interaction between lactation group and diet for body condition score (Figure 7; Appendix Table XI;  $P < 0.001$ ). Cows in their fourth or later lactation fed the 15% crude protein diet lost more body condition than cows in the other lactation group, diet combinations. Regardless of diet, breed or lactation group, all cows lost a minor amount of body condition in the first third of the experiment. Cows regained initial body condition by the end of the experiment.

Figure 5. Cow Weight for Holstein (A) and Ayrshire (B)  
Cows of Either Second and Third (◊), or  
Fourth and Later (◆) Lactations, fed 15 (—) and 20% (—) Crude Protein Diets. Homogeneous S.E.M. are Illustrated.



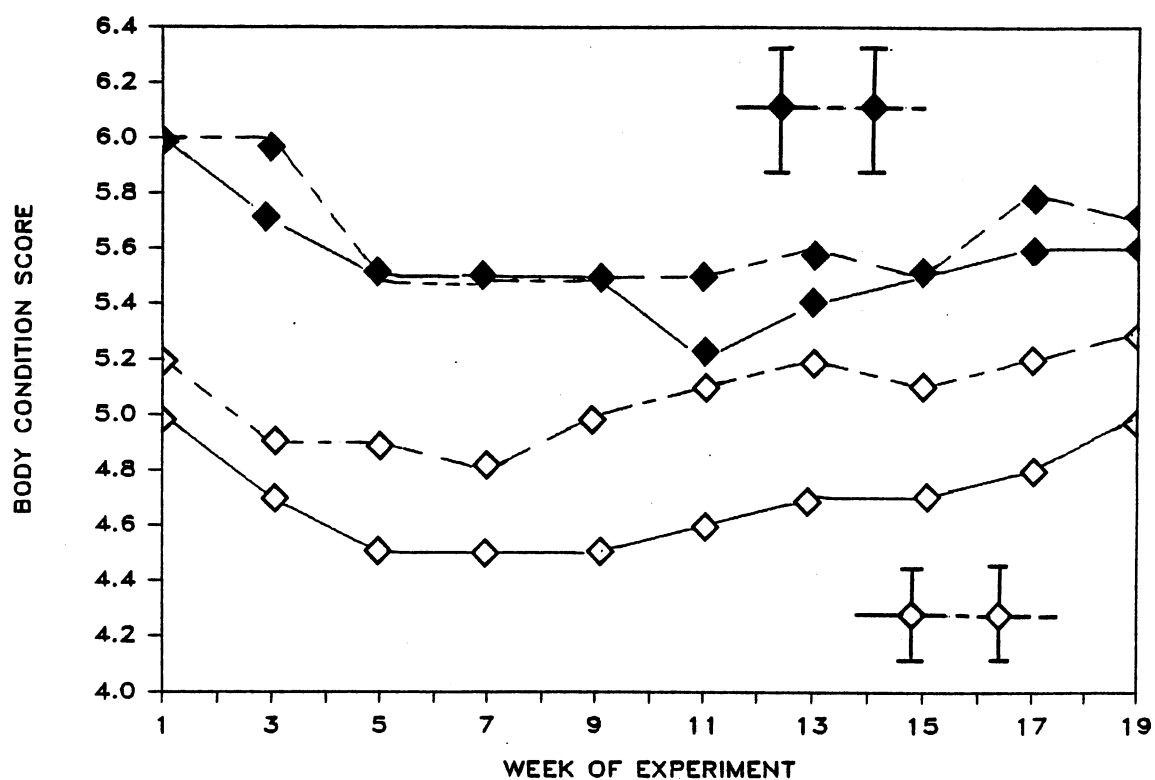


Figure 6. Body Condition Score for Holstein (◇) and Ayrshire (◆) Cows fed 15 (—) and 20% (---) Crude Protein Diets. Homogeneous S.E.M. are Illustrated.

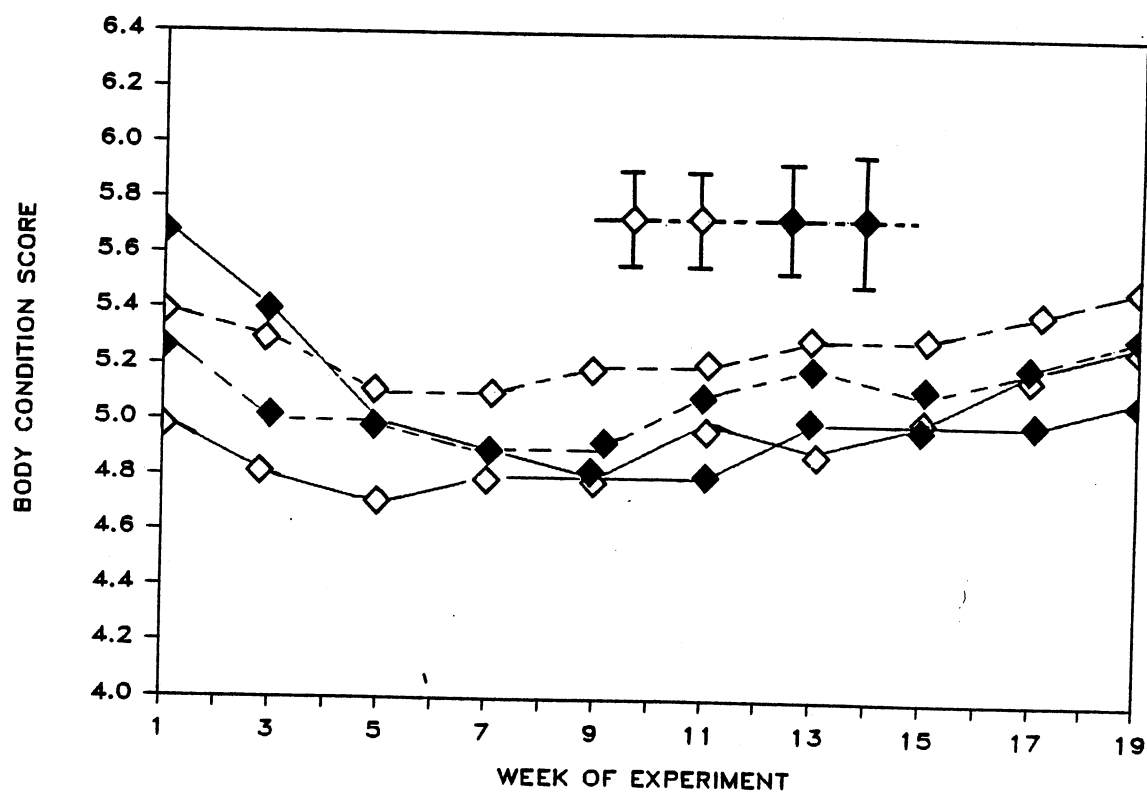


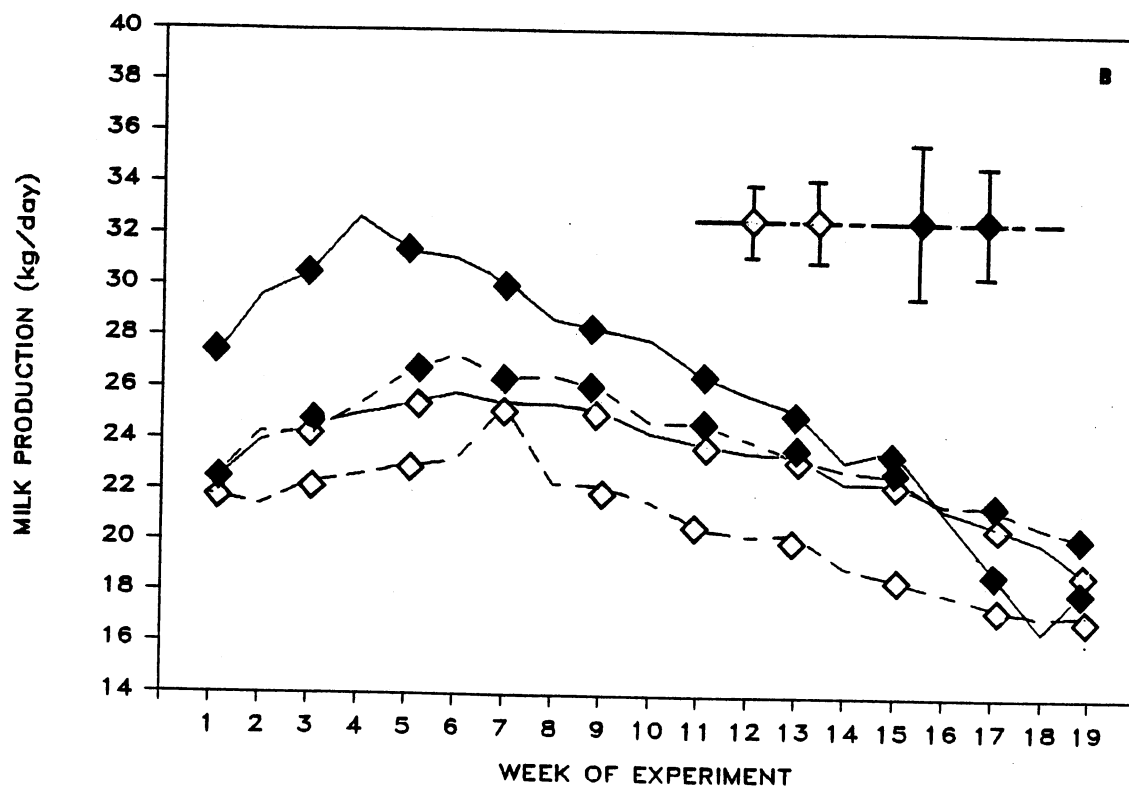
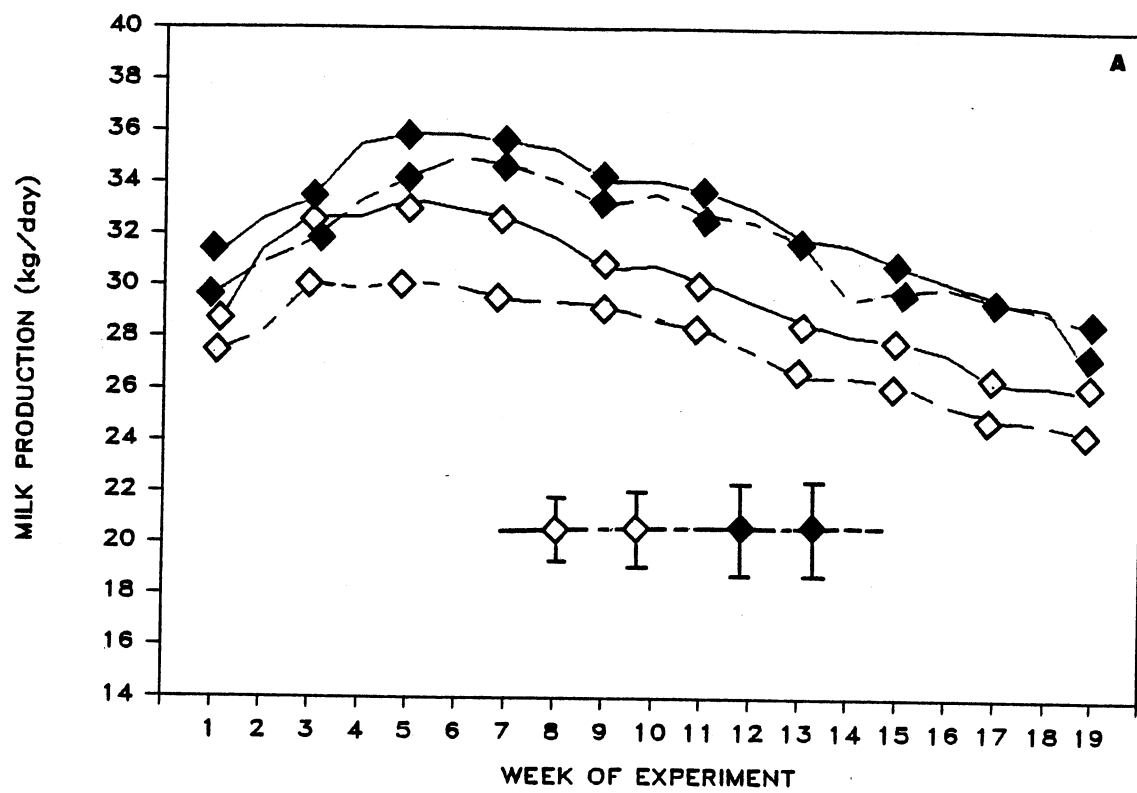
Figure 7. Body Condition Score for Dairy Cows of Either Second and Third (◇), or Fourth and Later (◆) Lactations, fed 15 (—) and 20% (---) Crude Protein Diets. Homogeneous S.E.M. are Illustrated.

### Lactational Responses

For daily milk production, there was a significant interaction over weeks of the study among lactation group, diet, and breed (Figure 8; Appendix Table XII;  $P < 0.001$ ). The high protein diet increased production for each breed, lactation group combination. However, the response was minimal for Holstein cows in their fourth or later lactation, though a major response occurred in early lactation for Ayrshire cows in this same lactation group. Milk production was at its greatest five to six weeks after the beginning of the study.

There was a significant diet by breed interaction over time for milk fat percentage (Figure 9; Appendix Table XIII;  $P < 0.001$ ). Milk fat percentage was similar for all cows, except those Ayrshires fed the 15% crude protein diet. These cows had fat percentages greater than cows in the other diet, breed combinations. There was also a significant lactation group by diet interaction for milk fat percentage over time (Figure 10; Appendix Table XIII;  $P < 0.001$ ). In the first week of the experiment, second and third lactation cows on both diets had similar milk fat percentages, whereas cows of fourth and later lactations fed the 15% diet had a higher milk fat percentage than cows on the 20% crude protein diet. Second and third lactation cows tended to have greater milk fat percentage after week six of the experiment. In general, milk fat percentages were

Figure 8. Daily Milk Production for Holstein (A) and Ayrshire (B) Cows of Either Second and Third ( $\diamond$ ), or Fourth and Later ( $\blacklozenge$ ) Lactations, fed 15 (--) and 20% (—) Crude Protein Diets. Homogeneous S.E.M. are Illustrated.



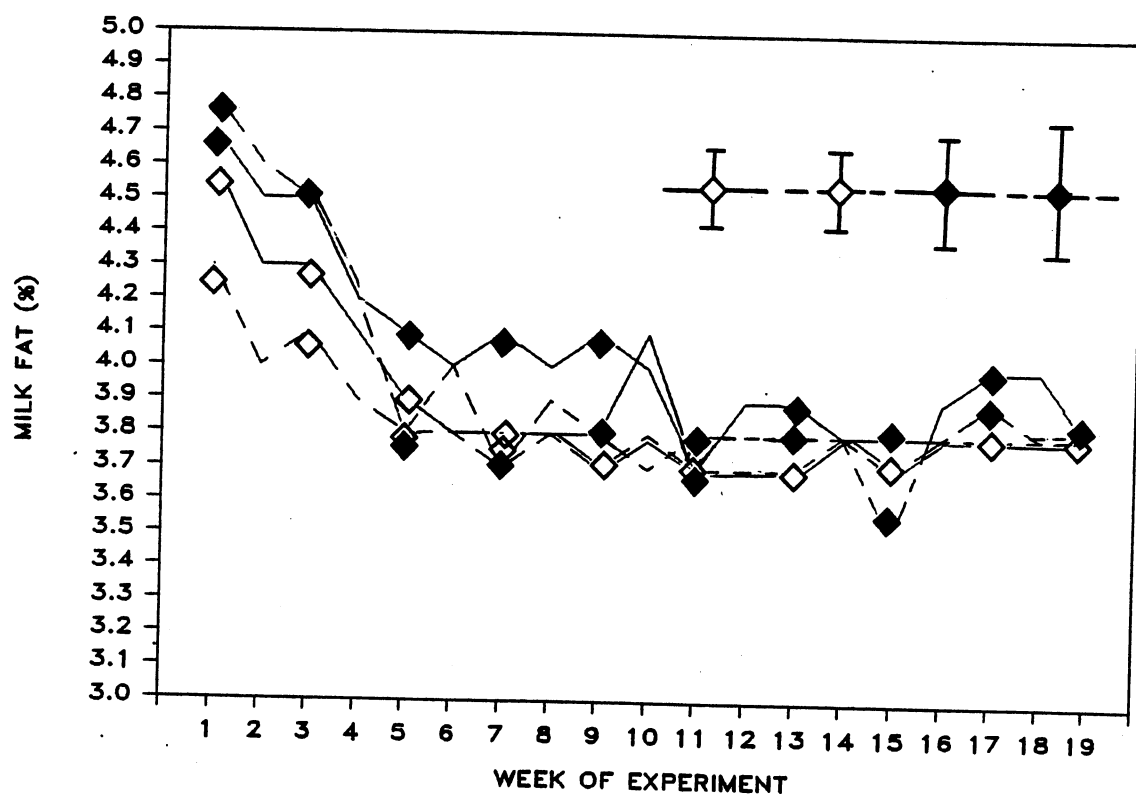


Figure 9. Milk Fat Percentage for Holstein (◇) and Ayrshire (◆) Cows fed 15 (—) and 20% (---) Crude Protein Diets. Homogeneous S.E.M. are Illustrated.

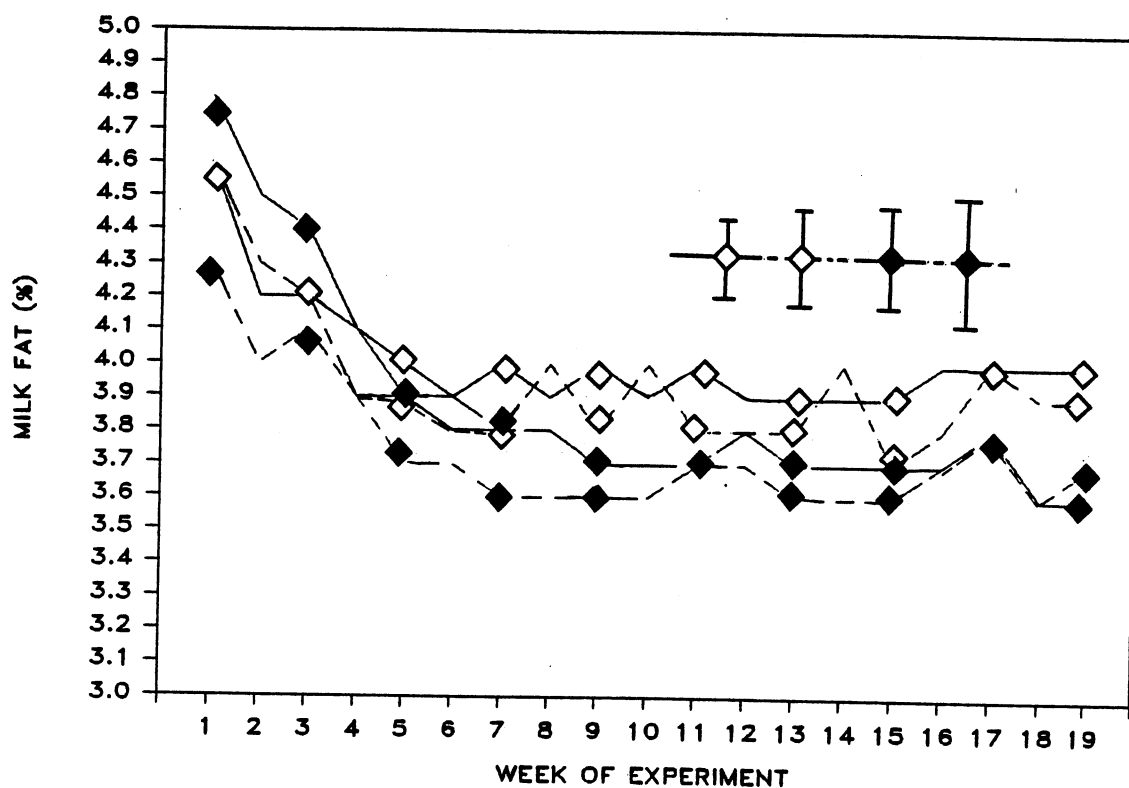


Figure 10. Milk Fat Percentage for Dairy Cows of Either Second and Third ( $\diamond$ ), or Fourth and Later ( $\blacklozenge$ ) Lactations, fed 15 (—) and 20% (---) Crude Protein Diets. Homogeneous S.E.M. are Illustrated.

highest initially and decreased until week five.

Over weeks of the experiment, milk protein percentage was significantly different for cows on the two diets (Figure 11; Appendix Table XIV;  $P < 0.05$ ). Cows fed the 15% crude protein diet tended to have a greater protein percentage at one and two weeks of the study. However, at various subsequent weeks cows on the 20% crude protein diet tended to have a greater milk protein percentage. Milk protein averaged 3.45% initially, decreased to its lowest percentage by weeks three to four, and increased linearly from four weeks through the end of the experiment (3.55%).

#### Reproductive Performance

Reproductive data are summarized in Table VI. Cows with abnormal uterine involution were removed from analysis of reproductive data. In addition, cows treated for ovarian cysts were eliminated from analysis of estrus and ovarian activity data. Elimination of problem cows from the analysis of the study was not related to the percentage of crude protein in the diet. Cows on both diets were similar for days to first observed estrus and to the onset of cyclic ovarian activity (Appendix Tables XV and XVI). As some cows showed cyclic patterns of progesterone secretion before they were observed in standing estrus, there were fewer days to the onset of ovarian activity than to first observed estrus.

Total services divided by total conceptions were 2.0 and

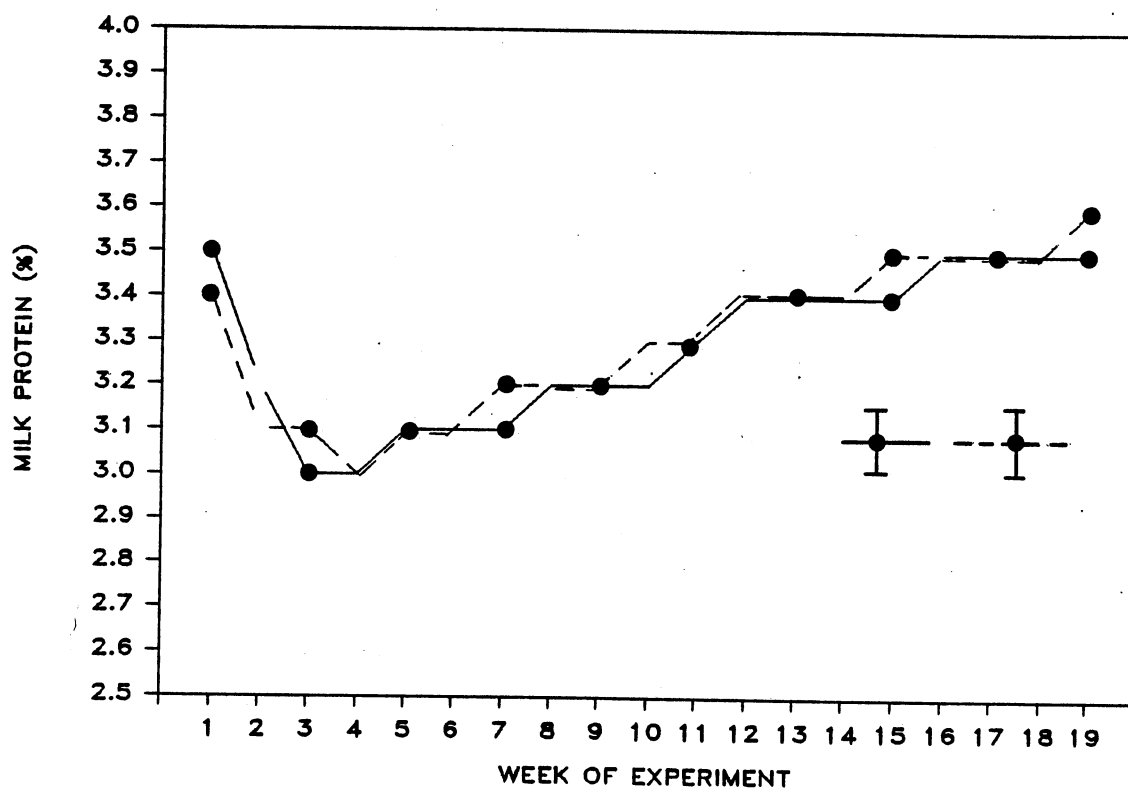


Figure 11. Milk Protein Percentage for Dairy Cows fed 15 (—) and 20% (---) Crude Protein Diets. Homogeneous S.E.M. are Illustrated.

TABLE VI  
REPRODUCTIVE PERFORMANCE FOR DAIRY CATTLE FED  
15 AND 20% CRUDE PROTEIN (CP)

	% Crude Protein	
	15%	20%
Cows per Treatment	42	35
Interval to First Estrus(days) <sup>a</sup>	41.1±3.0 <sup>c</sup>	36.3±3.4
Onset of Ovarian Activity(days) <sup>a</sup>	36.3±2.9	32.7±3.2
Days Open	85.3±4.7	81.3±4.8
<u>Services per Conception</u>		
Cows Conceiving <sup>b</sup>	1.6±0.2	1.3±0.2
Total Services/Total Conceptions	2.0	1.7
Percent Pregnant	85.7	88.6

<sup>a</sup> n=40 for 15% CP and n=34 for 20% CP

<sup>b</sup> n=36 for 15% CP and n=31 for 20% CP

<sup>c</sup> ±SE

1.7 for cows on the 15 and 20% crude protein diets, respectively (table VI). However, these values include fertility data from four high protein and six moderate protein cows that failed to conceive during the experiment. These ten cows were inseminated from three to eight total times during and after the 90 day breeding phase of the experiment. Three finally conceived, and the remainder were reproductive culls. To alleviate the potential bias that might occur if problem breeding cows were included in the analysis, the principal fertility parameter was services per conception for cows that conceived during the experiment. Cows on the high protein diet maintained their 0.3 service per conception advantage over cows fed the 15% crude protein diet (table VI), though this difference was not significant (Appendix Table XVII). Furthermore, lactation group and breed had no significant effect on services per conception.

Days open and percent pregnant were not different for cows on the 15 and 20% crude protein diets (table VI; Appendix Tables XVIII and XIX).

### Discussion

Protein intake expressed as a percentage of NRC recommendations, was fed to meet the requirements of cows on the 15% protein diet. Cows fed 20% crude protein were overfed protein during the entire experimental period. It should also be emphasized, that for higher producing cows

than these, a 20% crude protein diet would be even closer to meeting their dietary protein requirements.

Dry matter intake was typical for cows on the two levels of crude protein. Van Horn et al. (1979) demonstrated that increasing crude protein concentrations, up to 16 or 17%, increased organic matter digestibility and also dry matter intake. At the higher levels of dietary protein, they showed very small increases of intake, consistent with responses for feed intake in this study.

Plasma urea concentrations increased dramatically in the first week and stabilized about 15 and 27 mg/dl for cows on the 15 and 20% crude protein diets by week five of the experiment. Plasma urea for cows on the 15 and 20% crude protein diets was about 8 and 10 mg/dl respectively, above concentrations reported in other studies feeding comparable levels of crude protein (Claypool et al., 1980; Folman et al., 1981; Kaim et al., 1983). This suggests that the source of protein in this study may be more readily degraded and metabolized to ammonia in the rumen. If the increased urea and ammonia associated with high protein feeding causes alterations in the uterine environment that is detrimental to reproduction of dairy cows (Jordan et al., 1983; Duby et al., 1984), it should have been elucidated in this study.

Changes in body weight and condition score indicated that the degree of nutritional stress was small and not different for cows on the two diets. This was consistent

with other studies for weight change (Claypool et al., 1980; Folman et al., 1981; Kaim et al., 1983), suggesting that a postpartum energy deficiency was not accentuated by the high protein diet. In addition, there should not have been sufficient nutritional stress to affect reproductive performance.

Cows on the high protein diet had the typical increase in milk production associated with higher levels of dietary crude protein (Van Horn and Jacobson, 1971; Sparrow et al., 1973; Van Horn et al., 1979; Cressman et al., 1979; Edwards et al., 1980). The dietary protein fed was in a utilizable form for production, and cows were genetically able to respond to the added increment of crude protein. Milk protein and fat percentages were normal for cows on both rations. Typical fat tests for both diets suggests that there was sufficient effective crude fiber content, and thus an adequate acetate to propionate ratio to maintain optimal milk fat synthesis (Van Soest and Allen, 1959; Emery and Brown, 1961; Bietz and Davis, 1964; Huber et al., 1969).

Jordan and Swanson (1979b) indicated that the endocrine system of reproduction was influenced by feeding 19.3 and 16.3 versus 12.7% crude protein. They found that the concentrations of progesterone in plasma were greater on day 14 of both the first observed estrous cycle and the conception cycle in cows fed lower level of crude protein. Cows fed the lower protein diet had less LH on days 2 and 14 of the preconception and conception cycles. LH release was

also less after a gonadotropin releasing hormone (GnRH) challenge. In contrast, Blauwiekel et al (1984) reported that ovariectomized cows fed 23.9% dietary protein tended to have less LH release after GnRH challenge than did cows fed a 15.9% crude protein diet. However, pituitary GnRH receptor concentrations were not different for cows fed either diet, suggesting the responsiveness of the anterior pituitary to GnRH was altered. Our results demonstrate that ovarian function was probably not altered by high protein feeding, regardless of any potential hormonal changes. Onset of cyclic ovarian activity and days to first estrus were similar for cows on both diets, and within the normal range for postpartum dairy cows (Casida et al., 1968; Casida, 1971).

Days open, an indicator of overall reproductive performance, was not altered by the level of crude protein fed in our study, in contrast to other reports (Jordan and Swanson, 1979a; Folman et al., 1981; Kaim et al., 1983). These studies suggested that fertility was depressed which in turn increased days open. Jordan and Swanson (1979) demonstrated an advantage of 1.0 service per conception for cow fed a 12.7 versus a 19.3% crude protein diet. Folman et al. (1981) reported 0.8 service per conception more for cows fed 20% crude protein instead of 16%. In contrast to another report (Kaim et al., 1983), there was no significant difference in fertility between younger (second and third lactation) and older (fourth and greater lactation) cows.

Our results, while not significant, showed a 0.3 service per conception advantage for cows on the high protein diet. Although this study was conducted with a limited number of cows, we have attempted to overcome some of the limitations of other studies. We reduced sources of variation in fertility that were independent of the possible crude protein influences on reproduction. This was done by using semen frozen as a single batch from one sire to inseminate all cows in each breed, using primarily one inseminator, feeding cows individually for more consistent protein intake, increasing cow numbers over those of some other studies, and eliminating potential problem breeding cows. Reproductive performance in normal dairy cows in this experiment was not impaired when cows were fed the high protein diet.

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APPENDIX

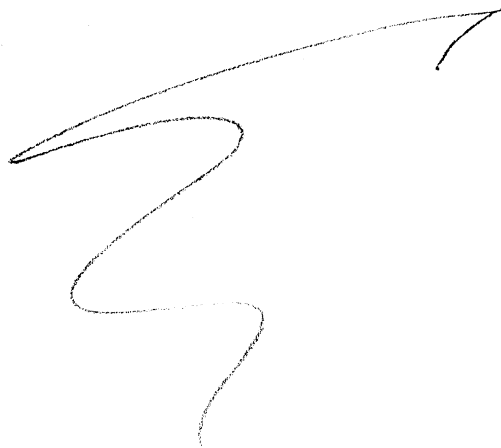


TABLE VII  
BODY CONDITION CHARACTERISTICS FOR EACH  
UNIT OF THE DAIRY COW CONDITION  
SCORING SYSTEM

Score	Body Condition Characteristics
1	EXTREMELY THIN - Prominent ribs, transverse processes, hip bones and obviously thin thighs with skin stretched tightly over the skeleton with no visible subcutaneous fat.
2	THIN - Visible ribs, transverse processes, slight amount of fleshing between the hooks and pins, adequate tissue on hind legs, and chine-shoulder area is thin. there is a slight amount of fat cover over the lower ribs.
3	MODERATELY THIN - Some flesh covering over the ribs (especially over lower ribs) and transverse processes with minimum fleshing between hooks and pins and in chine-shoulder area.
4	LOW MODERATE - Some fleshing over the ribs and transverse processes, yet they are still visible. Sufficient fleshing between hooks and pins such that there is only a moderate depression in this area. Light fat covering over the loin and chine-shoulder area.
5	MODERATE - Lower ribs are not visible. Upper rear ribs are distinguishable, evident fleshing along sides of chine and in loin area with transverse processes barely visible. Evident fat cover over hooks and pins with a small depression in the rump area. The brisket area is thin.

TABLE VII (Continued)

Score	Body Condition Characteristics
6	EXCELLENT CONDITION - Obvious flesh covering over the shoulder-chine area extending down top line; the hooks, pin bones and transverse processes have rounded appearance, the ribs are not visible, thighs are moderately thick; only a slight depression exists between hooks and pins and there is some fat deposition in the brisket.
7	FAT - Smooth but moderate fat covering over entire body with crop and chine area filled with fat, patchy fat around the tailhead and pin bones; thighs are obviously thick. The brisket is moderately full of fat.
8	EXTREMELY FAT -Excessive fat covering over entire body with patchy fat deposition over most bone structures.
9	OVERCONDITIONED - Extremely thick fat cover over entire body, especially in the neck, shoulders, loin, hind quarters and lower barrel. Dewlap is not easily distinguishable.

Source: Aalseth et al., 1983.

TABLE VIII  
LEAST SQUARES ANALYSIS OF VARIANCE  
FOR DRY MATTER INTAKE

Source	df	Mean Square
Period (P)	5	116.7344
Lactation Group (L)	1	31.3371
Diet (D)	1	66.6908
Breed (B)	1	9671.2062***
L X D	1	126.9556
L X B	1	0.9583
D X B	1	14.2951
L X D X B	1	30.4682
Error A	72	60.0688
Total A	84	-----
Week (W)	1	3111.9304***
W X W	1	2467.0562***
W X W X W	1	143.8672***
W X L	1	150.5538***
W X W X L	1	0.4081
W X W X W X L	1	23.3356**
W X D	1	0.0044
W X W X D	1	42.5467***
W X W X W X D	1	1.4453
W X B	1	142.7361***
W X W X B	1	57.4795***
W X W X W X B	1	28.5103**
W X D X B	1	55.8713***
W X W X D X B	1	5.5407
W X W X W X D X B	1	2.7026
W X L X D	1	23.5431**
W X W X L X D	1	0.6216
W X W X W X L X D	1	2.3326
W X L X B	1	44.6128***
W X W X L X B	1	20.8107**
W X W X W X L X B	1	2.3158
W X L X D X B	1	1.7265
W X W X L X D X B	1	1.0918
W X W X W X L X D X B	1	0.4780
Error B	1449	2.9651
Total	1557	-----

\*P&lt;0.05

\*\*P&lt;0.01

\*\*\*P&lt;0.001

TABLE IX  
LEAST SQUARES ANALYSIS OF VARIANCE  
FOR PLASMA UREA LEVELS

Source	df	Mean Square
Period (P)	5	501.5574***
Lactation Group (L)	1	330.4052*
Diet (D)	1	25954.4394***
Breed (B)	1	0.0218
L X D	1	23.9558
L X B	1	3.0026
D X B	1	22.1379
L X D X B	1	6.9466
Error A	72	55.5199
Total A	84	-----
Week (W)	1	5065.0178***
W X W	1	2696.0410***
W X W X W	1	1869.4029***
W X L	1	24.3511
W X W X L	1	36.5044*
W X W X W X L	1	0.0215
W X D	1	252.4085***
W X W X D	1	517.8760***
W X W X W X D	1	644.6992***
W X B	1	1.3436
W X W X B	1	0.0070
W X W X W X B	1	36.7870*
W X D X B	1	9.7627
W X W X D X B	1	3.9342
W X W X W X D X B	1	0.6935
W X L X D	1	5.3669
W X W X L X D	1	19.1631
W X W X W X L X D	1	0.1922
W X L X B	1	15.2334
W X W X L X B	1	1.0483
W X W X W X L X B	1	0.3872
W X L X D X B	1	8.5659
W X W X L X D X B	1	50.0050*
W X W X W X L X D X B	1	0.0373
Error B	871	8.8741
Total	979	-----

\*P&lt;0.05

\*\*P&lt;0.01

\*\*\*P&lt;0.001

TABLE X  
LEAST SQUARES ANALYSIS OF VARIANCE  
FOR COW WEIGHT

Source	df	Mean Square
Period (P)	5	43271.3840
Lactation Group (L)	1	119944.6944
Diet (D)	1	86003.8688
Breed (B)	1	2089775.9825***
L X D	1	26630.3644
L X B	1	10220.0931
D X B	1	92238.0663
L X D X B	1	7528.1146
Error A	70	32816.5620
Total A	82	-----
Week (W)	1	37848.1652***
W X W	1	220.9380
W X W X W	1	4535.0159***
W X L	1	5961.2452***
W X W X L	1	165.6761
W X W X W X L	1	147.1908
W X D	1	870.9527
W X W X D	1	281.7698
W X W X W X D	1	103.9463
W X B	1	8533.4599***
W X W X B	1	617.0168
W X W X W X B	1	69.0699
W X D X B	1	100.4510
W X W X D X B	1	1286.3249
W X W X W X D X B	1	178.4829
W X L X D	1	3147.1225**
W X W X L X D	1	650.4258
W X W X W X L X D	1	81.2799
W X L X B	1	199.8670
W X W X L X B	1	0.1932
W X W X W X L X B	1	773.7897
W X L X D X B	1	1725.9229***
W X W X L X D X B	1	205.6840
W X W X W X L X D X B	1	2.4525
Error B	713	378.9549
Total	819	-----

\*P&lt;0.05

\*\*P&lt;0.01

\*\*\*P&lt;0.001

TABLE XI  
LEAST SQUARES ANALYSIS OF VARIANCE  
FOR BODY CONDITION SCORE

Source	df	Mean Square
Period (P)	5	12.1512*
Lactation Group (L)	1	0.0000
Diet (D)	1	12.7840
Breed (B)	1	74.9594***
L X D	1	0.8495
L X B	1	0.3952
D X B	1	11.2050
L X D X B	1	4.7919
Error A	70	4.7466
Total A	82	-----
Week (W)	1	0.7210*
W X W	1	13.1850***
W X W X W	1	2.3378***
W X L	1	3.6007***
W X W X L	1	0.3784
W X W X W X L	1	0.2547
W X D	1	0.1534
W X W X D	1	0.3888
W X W X W X D	1	0.0010
W X B	1	2.7506***
W X W X B	1	0.1012
W X W X W X B	1	0.0333
W X D X B	1	2.1559***
W X W X D X B	1	0.6062*
W X W X W X D X B	1	0.0360
W X L X D	1	3.2644***
W X W X L X D	1	0.7503*
W X W X W X L X D	1	0.0104
W X L X B	1	0.4338
W X W X L X B	1	0.1456
W X W X W X L X B	1	0.0003
W X L X D X B	1	0.1546
W X W X L X D X B	1	0.1738
W X W X W X L X D X B	1	0.0016
Error B	1449	0.1450
Total	1557	-----

\*P&lt;0.05

\*\*P&lt;0.01

\*\*\*P&lt;0.001

TABLE XII  
LEAST SQUARES ANALYSIS OF VARIANCE FOR  
ACTUAL DAILY MILK PRODUCTION

Source	df	Mean Square
Period (P)	5	1637.3182***
Lactation Group (L)	1	2579.0979***
Diet (D)	1	1466.4068***
Breed (B)	1	14189.8104***
L X D	1	70.5942
L X B	1	24.9682
D X B	1	160.7387
L X D X B	1	26.9977
Error A	72	223.9419
Total A	84	-----
Week (W)	1	4445.2723***
W X W	1	2187.4304***
W X W X W	1	660.8708***
W X L	1	11.1376
W X W X L	1	70.4125***
W X W X W X L	1	0.0292
W X D	1	90.7152***
W X W X D	1	49.3426***
W X W X W X D	1	6.6246
W X B	1	2.3118
W X W X B	1	1.8532
W X W X W X B	1	10.7938
W X D X B	1	1.1901
W X W X D X B	1	22.3757*
W X W X W X D X B	1	0.0001
W X L X D	1	55.5396***
W X W X L X D	1	0.2606
W X W X W X L X D	1	16.6515
W X L X B	1	62.0796***
W X W X L X B	1	9.1186
W X W X W X L X B	1	0.0027
W X L X D X B	1	114.5180***
W X W X L X D X B	1	0.0127
W X W X W X L X D X B	1	10.4778
Error B	1449	4.8657
Total	1557	-----

\*P&lt;0.05

\*\*P&lt;0.01

\*\*\*P&lt;0.001

TABLE XIII  
LEAST SQUARES ANALYSIS OF VARIANCE  
FOR MILK FAT %

Source	df	Mean Square
Period (P)	5	6.2843**
Lactation Group (L)	1	7.8902*
Diet (D)	1	2.4027
Breed (B)	1	10.2605*
L X D	1	0.3903
L X B	1	0.0883
D X B	1	1.1372
L X D X B	1	0.7693
Error A	72	1.6965
Total A	84	-----
Week (W)	1	28.2475***
W X W	1	25.9298***
W X W X W	1	6.7587***
W X L	1	2.9196***
W X W X L	1	0.1759
W X W X W X L	1	0.0811
W X D	1	0.5025
W X W X D	1	0.0286
W X W X W X D	1	0.0076
W X B	1	0.6436*
W X W X B	1	0.0048
W X W X W X B	1	0.0232
W X D X B	1	2.7331***
W X W X D X B	1	1.5459**
W X W X W X D X B	1	0.1777
W X L X D	1	0.9483*
W X W X L X D	1	0.2964
W X W X W X L X D	1	0.1537
W X L X B	1	0.1612
W X W X L X B	1	0.6896*
W X W X W X L X B	1	0.1257
W X L X D X B	1	0.0178
W X W X L X D X B	1	0.1560
W X W X W X L X D X B	1	0.4225
Error B	1449	0.1509
Total	1557	-----

\*P&lt;0.05

\*\*P&lt;0.01

\*\*\*P&lt;0.001

TABLE XIV  
LEAST SQUARES ANALYSIS OF VARIANCE  
FOR MILK PROTEIN %

Source	df	Mean Square
Period (P)	5	4.2316***
Lactation Group (L)	1	10.6787**
Diet (D)	1	0.5646
Breed (B)	1	3.3902
L X D	1	0.2012
L X B	1	0.0096
D X B	1	2.3490
L X D X B	1	2.6932
Error A	72	0.8825
Total A	84	-----
Week (W)	1	29.4075***
W X W	1	4.0289***
W X W X W	1	8.1166***
W X L	1	0.6481***
W X W X L	1	0.1188
W X W X W X L	1	0.0382
W X D	1	0.1126***
W X W X D	1	0.2336*
W X W X W X D	1	0.0603
W X B	1	0.4479**
W X W X B	1	0.2917**
W X W X W X B	1	0.0172
W X D X B	1	0.0830
W X W X D X B	1	0.1222
W X W X W X D X B	1	0.0533
W X L X D	1	0.0086
W X W X L X D	1	0.0194
W X W X W X L X D	1	0.0034
W X L X B	1	0.6839***
W X W X L X B	1	0.0810
W X W X W X L X B	1	0.0449
W X L X D X B	1	0.0385
W X W X L X D X B	1	0.0300
W X W X W X L X D X B	1	0.0006
Error B	871	0.0386
Total	979	-----

\*P&lt;0.05

\*\*P&lt;0.01

\*\*\*P&lt;0.001

TABLE XV  
LEAST SQUARES ANALYSIS OF VARIANCE  
FOR DAYS TO FIRST ESTRUS

Source	df	Mean Square
Period (P)	5	1084.0041*
Lactation Group (L)	1	5.4224
Diet (D)	1	150.1431
Breed (B)	1	14.4464
P X L	5	176.0090
P X B	4	109.7599
P X D	5	525.9767
L X D	1	41.9029
L X B	1	38.6954
D X B	1	26.9306
P X L X D	4	163.7613
P X L X B	2	56.6989
L X D X B	1	21.1853
P X D X B	3	63.5645
Residual	39	311.0931

\*P<0.05

TABLE XVI  
LEAST SQUARES ANALYSIS OF VARIANCE FOR  
ONSET OF OVARIAN ACTIVITY

Source	df	Mean Square
Period (P)	5	731.3217*
Lactation Group (L)	1	0.7363
Diet (D)	1	79.8413
Breed (B)	1	4.9569
P X L	5	295.9237
P X B	4	230.9196
P X D	5	486.9063
L X D	1	82.1950
L X B	1	227.2536
D X B	1	100.1653
P X L X D	4	21.8229
P X L X B	2	9.0353
L X D X B	1	9.0353
P X D X B	3	84.3290
Residual	39	221.0650

\*P<0.05

TABLE XVII  
LEAST SQUARES ANALYSIS OF VARIANCE FOR  
SERVICES PER CONCEPTION

Source	df	Mean Square
Period (P)	5	0.4537
Lactation Group (L)	1	0.0017
Diet (D)	1	0.0089
Breed (B)	1	0.1280
P X L	5	0.4801
P X B	4	0.0278
P X D	5	0.2369
L X D	1	0.1407
L X B	1	0.0097
D X B	1	0.0832
P X L X D	3	0.0259
P X L X B	2	0.1405
L X D X B	1	0.0514
P X D X B	2	0.9021
Residual	32	0.8755

TABLE XVIII  
LEAST SQUARES ANALYSIS OF VARIANCE  
FOR DAYS OPEN

Source	df	Mean Square
Period (P)	5	616.0842
Lactation Group (L)	1	6.1595
Diet (D)	1	9.3800
Breed (B)	1	0.2324
P X L	5	169.0465
P X B	4	120.9580
P X D	5	159.5935
L X D	1	593.9572
L X B	1	16.2999
D X B	1	23.2455
P X L X D	4	480.1412
P X L X B	2	349.1120
L X D X B	1	1.7743
P X D X B	2	648.8286
Residual	32	755.2089

TABLE XIX  
LEAST SQUARES ANALYSIS OF VARIANCE FOR  
% PREGNANT ON EXPERIMENT

Source	df	Mean Square
Period (P)	5	0.0837
Lactation Group (L)	1	0.0230
Diet (D)	1	0.0801
Breed (B)	1	0.0042
P X L	5	0.0364
P X B	4	0.2596
P X D	5	0.0264
L X D	1	0.0447
L X B	1	0.0230
D X B	1	0.2241
P X L X D	4	0.2064
P X L X B	2	0.0322
L X D X B	1	0.2206
P X D X B	3	0.0828
Residual	41	0.1220

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