NUTRITIONAL AND ENDOCRINE ASPECTS OF THE LACTATION CYCLE OF HOLSTEIN AND JERSEY COWS: NUTRIENT BALANCES, RESPONSE TO SUPPLEMENTAL DIETARY FAT, RIB COMPOSITION AND RIB HISTOLOGY

by

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(ABSTRACT)

Eight mature Holstein and Jersey cows beginning their third or later lactation were used throughout this study to evaluate various aspects of the lactation cycle. The lactation (control) diet consisted of 28.8% corn silage, 22.4% alfalfa haylage, and 48.8% concentrate dry matter. Breeds responded differently to the stress of calving. Jerseys had higher plasma somatotropin on day after calving, but Holsteins had higher glucose on day of and day after calving. Plasma parathyroid hormone did not differ between breeds, but Jerseys had higher 1,25-dihydroxyvitamin D_3 than Holsteins on both days after calving. Plasma total calcium and ionized calcium concentrations were lower for Jerseys on both days. Holsteins and Jerseys had similar concentrations of hormones and calcium at 4 and 8 wk. Ionized calcium as a

percent of total calcium was elevated at calving, as compared to other times in the lactation cycle, in both breeds.

From 9 to 21 wk, 4 of 8 Holsteins and 4 of 8 Jerseys were fed a diet supplemented with tallow. Holsteins fed tallow had lower somatotropin than Holsteins fed control diet at 14 and 18 wk. Plasma glucose, parathyroid hormone, and 1,25-dihydroxyvitamin D_3 were similar between tallow- and control-fed cows in both breeds. Plasma total calcium and ionized calcium were higher at 20 wk for Holsteins and Jerseys fed tallow. Dry matter intake was not influenced by diet in either breed. However, Holsteins, but not Jerseys, fed tallow produced more milk and higher body weights than Holsteins fed the control diet.

Balance trials results indicated dietary tallow addition increased energy intake, energy digestibility, and metabolizable energy, but it decreased partial efficiency of metabolizable energy utilization for lactation. Although digestibility of calcium and magnesium was unchanged, tallow-fed cows were in greater positive calcium and magnesium balance than control-fed cows.

Stage of lactation had little effect on specific gravity, shear stress, percent mineral, and histological measurements of biopsied rib samples. However, Jersey rib had higher specific gravity than Holstein rib. All cows had similar bone histological features throughout lactation.

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CHAPTER 1

INTRODUCTION

The lactation cycle of dairy cattle is characterized by wide fluctuations in dietary and endogenous nutrient partitioning to meet requirements for initiation and continuation of milk synthesis. Initiation of lactation is a severe challenge to calcium homeostasis. Most cows become hypocalcemic for 2 to 3 days through the parturient period (Horst et al., 1978), but only a few cows develop parturient paresis. In conjuction with low plasma calcium at calving, parathyroid hormone (Horst et al., 1978; and Shappell et al., 1987) and 1,25-dihydroxyvitamin D_3 (Goff et al, 1989; and Horst et al., 1978) are elevated. Plasma 1,25-dihydroxyvitamin D_3 stimulates absorption of calcium in the intestine (DeLuca and Schnoes, 1976), parathyroid hormone stimulates reabsorption of calcium from urine (Raisz and Kream, 1983), and both work in concert to mobilize calcium from the bone (DeLuca, 1979). Within a few days after calving, the endocrine mechanisms for calcium regulation stabilize and plasma calcium concentration remains within the normal range (Littledike and Goff, 1987; and Wohlt et al., 1984).

The peak demand for calcium occurs at peak milk yield. Most cows are in negative calcium balance at peak lactation and must mobilize calcium from bone to support calcium

requirements for lactation. Specific gravity and concentrations of calcium and phosphorus in biopsied rib samples were significantly lower in lactating sheep than in non-lactating sheep (Little and McMeniman, 1973).

The metabolic requirements to sustain milk production changes during the lactation cycle. In early lactation, dry matter intake usually does not supply adequate amounts of nutrients for milk synthesis. Therefore, high-producing cows must mobilize large amounts of body tissue to supply the additional nutrients needed for peak milk yield (Bines and Hart, 1982). Metabolic hormones, primarily somatotropin and insulin, are important regulators of nutrient partitioning. Somatotropin promotes mobilization of fatty acids from adipose tissue; whereas insulin directs excess dietary energy toward storage in body tissues. In general, somatotropin decreases as lactation advances and insulin increases as lactation advances (Herbein et al., 1985; Koprowski and Tucker, 1973; Smith et al., 1976; and Vasilatos and Wangsness, 1981). In early lactation, the combination of higher somatotropin and lower insulin promotes nutrient flux toward the mammary gland. In later lactation, cows use dietary energy components to replace body tissue as milk production slowly declines.

Energy intake is not sufficient to meet energy

requirements for high milk yield in early lactation. A practical way to increase intake of energy is to increase the dietary energy density by supplementation of fat. If consumption of dry matter remains constant, then energy intake will increase.

Addition of fat to the diet of dairy cattle may influence nutrient digestibilities. Fat supplementation has been reported to depress fiber digestion (Jenkins and Palmquist, 1982; Kronfeld and Donoghue, 1980; Murphy et al., 1987; Palmquist et al., 1986; and Ward et al., 1957). However, addition of metal cations, especially calcium, may improve fiber digestibility in fat supplemented diets (Jenkins and Palmquist, 1982; Palmquist and Conrad, 1980; and Palmquist et al., 1986) due to formation of calcium soaps that do not inhibit metabolism of rumen microorganisms. Fat supplementation has also been shown to decrease digestibility of calcium and magnesium (Jenkins and Palmquist, 1984; and Kronfeld and Donoghue, 1980) or have no effect on calcium digestibility (Palmquist and Conrad, 1978; and Schauff and Clark, 1989).

Dietary fat supplementation may influence amount and utilization of dietary energy intake. Energy intake was reduced by the addition of soybean oil to a grass silage diet due to reduced dry matter intake (Steele, 1985). In contrast, energy intake and dry matter intake were not

different between control and whole cottonseed diets (Horner et al., 1986). Digestibility of energy was increased with increasing dietary caloric density (Bull et al., 1976). In agreement, addition of protected tallow (Kronfeld and Donoghue, 1980) and whole cottonseed (Smith et al., 1981) increased energy digestibility. In contrast, Schauff and Clark (1989) reported no change in digestibility of energy in fat supplemented diets. If additional energy is absorbed from fat-supplemented diets, it might influence overall efficiency of metabolism. Addition of protected tallow to a basal diet increased the efficiency of metabolizable energy utilization for lactation (Kronfeld et al., 1980). In contrast, van der Honing (1980) reported no alteration in the efficiency due to fat addition.

Supplemental dietary fat may also influence the concentration of somatotropin, insulin, and glucose in plasma. Insulin concentration was decreased when whole cottonseed (Horner et al., 1986) or formaldehyde-treated animal-vegetable fat (Palmquist and Moser, 1981) was added to the diet. On the other hand, basal plasma insulin was increased when whole cottonseed was fed as a supplemental fat (Cummins and Sartin, 1987), but plasma somatotropin was unaltered. Plasma glucose was decreased (Horner et al., 1986; and Palmquist and Moser, 1981), increased (Kronfeld

et al., 1980), or did not change (Palmquist and Conrad, 1978; and Steele, 1985) when fat was added to the diet. The effects of fat supplementation on metabolic hormones and glucose appear to vary considerably.

The objectives of this study were to evaluate the effects of: (1) breed and stage of lactation on concentrations of somatotropin, insulin, parathyroid hormone, 1,25-dihydroxyvitamin D_3, and total and ionized calcium in plasma, milk yield and composition, dry matter intake, and body weight; (2) breed and dietary tallow addition on all of the above parameters; (3) breed and dietary tallow addition on energy, nitrogen, calcium, and magnesium balances; and (4) breed, stage of lactation, and rib location on rib specific gravity, shear stress, percent minerals, and histological characteristics of bone.

REVIEW OF LITERATURE

In early lactation, most dairy cows are in negative energy balance for several weeks because energy intake is not sufficient to meet energy requirements. A practical method for increasing dietary energy density is addition of fats, because fats contain 2.25 times as much energy as the same amount of carbohydrates or protein. Dietary fats can be classified into three groups: (1) unprotected, which include vegetable oils and free tallow; (2) protected, which are fats treated with formaldehyde or calcium salts of long chain fatty acids; and (3) whole seeds, which include soybean and cottonseed.

Effects of fat supplementation on nutrient digestibilities

Fiber digestibility may be affected according to the amount and type of fat included in the diet. Fat supplementation of diets often depresses fiber digestibility in cattle (Boggs et al., 1987; Jenkins, 1987; Jenkins and Palmquist, 1982; Kronfeld and Donoghue, 1980; Murphy et al., 1987; Palmquist et al., 1986; and Ward et al., 1957) and sheep (Brooks et al., 1986; Kowalczyk et al., 1977; and White et al., 1958). Theories to explain lowered fiber digestibility include physical coating of fiber with fat preventing bacteria attachment (Devendra and

Lewis, 1974) and fatty acids binding to the outer membrane of bacteria (Henderson, 1973; Maxcy and Dill, 1967).

Supplementation with unprotected dietary fat causes considerable variation in nutrient digestibilities. Free tallow addition decreased the ratio of acetate to propionate in the rumen (Boggs et al., 1987; Jenkins, 1987; and Palmquist et al., 1986). Evaluating the changes in acetate to propionate ratio indicated that fiber digestion was less affected by animal-vegetable fat than corn oil or tallow fatty acids (Jenkins, 1987). The study by Jenkins (1987) also showed that as fat reduces fiber digestion, acetate to propionate ratio and concentration of total volatile fatty acids are also reduced. Kowalcyzk et al. (1977) observed that feeding increased increments of tallow (0 to 150 g/kg grass in 50 g/kg grass increments) decreased fiber digestibility. In contrast, lactating Jersey cows fed concentrate with 10% tallow did not have decreased digestibility (Palmquist and Conrad, 1980). In agreement, Chalupa et al. (1984) and Storry et al. (1973) observed no change in ruminal fermentation due to tallow addition to the diet. Other authors using unprotected fats reported no alterations in digestibility of diet due to cod-liver oil (Pennington and Davis, 1975) and yellow grease (Zinn, 1988). Oleic acid apparently depresses acetate to propionate ratio by 50-60 %, but

steric acid reduces the ratio by only 20% (Chalupa et al., 1986). The difference between oleic and stearic acid influence on the ratio of acetic to propionic acid was due to the fact that stearic acid was more insoluble in the rumen than oleic acid. Thus, stearic acid addition is less likely to associate with either bacterial cells or feed particles.

The addition of metal cations, primarily calcium, may improve digestibility of fat-supplemented diets (Grainger et al., 1961; Jenkins and Palmquist, 1984; Palmquist and Conrad, 1980; Palmquist et al., 1986; Ward et al., 1957; and White et al., 1958). The improved fiber digestibility is due to fatty acids combining with calcium to form calcium soaps. Formation of calcium soaps limits fatty acid inhibition of ruminal microorganisms. Saturated fatty acids react more readily with metal ions to form insoluble salts (Chalupa et al., 1984; and Jenkins and Palmquist, 1982). The reaction of fatty acids with divalent cations to form insoluble salts is noteworthy because as much as 60 % of tallow fatty acids can form insoluble soaps (Jenkins and Palmquist, 1982). Calcium soaps are inert in the rumen and do not alter nutrient digestion (Chalupa et al., 1984; Chalupa et al., 1986; Grummer, 1988; Kent and Arambel, 1988; Palmquist and Conrad, 1978; and Schauff and Clark, 1989). In general, preformed calcium soaps should not

dissociate in the rumen but should dissociate completely postruminally, making the fatty acids available for absorption and utilization for milk production (Jenkins and Palmquist, 1984). The dissociation constant (pK_a) of calcium salts is between 4 and 5 (Palmquist, 1984). The rumen pH must remain above 6 when feeding calcium salts, because dissociation will begin when pH decreases below 6. Whether feeding preformed calcium soaps or the allowing formation of calcium soaps in the rumen from saturated fatty acids and calcium, the effects of dietary fat on fiber digestibility are minimized. These insoluble complexes provide optimum utilization of high fat diets by ruminants.

In order for ruminants to fully utilize calcium soaps, certain chemical processes are required. First, dissociation of the soap must occur at low pH in the abomasum, then absorption of calcium in an acidic duodenum, followed by absorption of fatty acids in the jejunum and ileum (Davidson and Woods, 1963). If calcium is in excess or not absorbed efficiently, the calcium soaps will reform in the large intestine, with increasing pH, and be excreted in the feces (Grace and Body, 1979).

The source of the calcium is also important for the formation of calcium soaps. Calcium from calcium chloride reacted more quickly than dicalcium phosphate with free

fatty acids to form insoluble soaps (Jenkins and Palmquist, 1982). In another study, percent of total fatty acids in soap was increased by addition of calcium chloride but not dicalcium phosphate or limestone (Palmquist et al., 1986). Thus, the more soluble forms of calcium (ie. calcium chloride) react more rapidly to form calcium soaps than the more insoluble forms (limestone, dicalcium phosphate).

The feeding of protected fat would reduce the possibility of adverse effects of fat on nutrient digestibility in the rumen. Prilled fat addition to the diet did not alter rumen fermentation or apparent total tract digestibilities of dry matter or neutral detergent fiber (Grummer, 1988; and Schauff and Clark, 1989). Fats encapsulated with formaldehyde treated protein can be fed at higher levels without detrimental fermentation effects (Macleod et al., 1977; and Palmquist and Jenkins, 1980). In contrast to the above reports, protected tallow supplementation (18% dry matter) caused digestibility of acid detergent fiber to be decreased by 24 % (Kronfeld and Donoghue, 1980).

Digestibility of nutrients may be affected by feeding of whole seeds in the ration. Whole cottonseed feeding did not influence digestibility of fiber components (Smith et al., 1981). Rapeseed feeding decreased rumen and total dry matter digestibilities and proportion of dry matter

digested in the rumen (Murphy et al., 1987). Rumen digestibility of cellulose was decreased by rapeseed, but hindgut fermentation apparently compensated for this.

Effects of fat supplementation on dry matter intake

Since caloric density of the diet is increased with fat addition, the dry matter intake may be influenced by fat supplementation. Dry matter intake was highest for control diet, intermediate for free fatty acids of tallow and soy diets, and lowest for tallow and soy soap diets (Jenkins and Palmquist, 1984). Consumption of dry matter was depressed when whole cottonseed addition increased from 0 to 30 % of the diet (Coppock et al., 1985). A depression in dry matter intake was not observed in a study (Horner et al., 1986) using 15 % whole cottonseed addition. In agreement, Smith et al. (1981) did not find treatment differences for dry matter intake with 0, 5, 15, or 25 percent whole cottonseed in the diet. Feeding of 2 levels of whole rapeseed did not change dry matter intake compared to no supplement (Murphy et al., 1987). Daily intakes of dry matter were similar between cows fed control and calcium salts of long chain fatty acids (Kent and Arambel,1988). In a similar study (Grummer, 1988), cows fed control, calcium salts of palm oil, and prilled fat had similar dry matter intakes. Addition of ground soybeans or

hydrolyzed fat to the diet did not change intakes compared to controls (Palmquist and Conrad, 1978). Intake of dry matter was not influenced by tallow (Palmquist and Conrad, 1980; and Zinn, 1987) or blended animal-vegetable fat (Palmquist and Conrad, 1980). Added energy in the diet might be expected to reduce dry matter intake. However, a majority of reports concluded no influence on dry matter intake from added fat.

Effects of fat supplementation on fat digestibility

Addition of fat to the diet will increase caloric density, but digestibility of total lipids may be influenced. A number of reports showed no change in lipid digestibility when supplemental fat was included in the diet. Intestinal fat digestibility was similar when no supplemental fat, yellow grease, or blended animal vegetable fat was fed (Zinn, 1989). The addition of Megalac (Filley et al., 1987), crushed rapeseed (Murphy et al., 1987), and fresh milk (Bauchart et al., 1987) did not significantly change the digestibility of fatty acids in the diet. The feeding of medium or high levels of hydrolyzed animal-vegetable fat or tallow had no affect on ether extract digestibility (Palmquist and Conrad, 1980). Other authors reported increased fat digestibility when different types of fat were added. An early study (Ward et

al., 1957) reported ether extract digestibility was improved by corn oil addition. It was suggested that corn oil reduced dustiness and improved palatability of the ration. Digestibility of fat increased with increasing amounts of added whole cottonseed (Smith et al., 1981). Supplementation of tallow and soybean oil increased digestibility of lipids (Van Der Honing, 1980). Digestibility of ground soybean (59.6%), medium level of added hydrolyzed fat (60.6%), and high level of added hydrolyzed fat (58.2%) had significantly higher fat digestibility than the basal diet (41.9%) (Palmquist and Conrad, 1978). The lower digestibility of the high (10.7% ether extract) versus medium (5.1% ether extract) level of added hydrolyzed fat may indicate the limit of efficient fat utilization was exceeded. Apparent digestibility of lipid was increased when calcium salts of palm oil or prilled fat was added to the control diet (Grummer, 1988). Jenkins and Palmquist (1984) reported that all types of fat added to the control diet increases apparent digestion coefficients of fatty acids. Both the calcium soaps (85%) and free fatty acids of soy (79%) had greater digestibilities than calcium soaps (72%) or free fatty acids of tallow (72%), but all forms of fat were greater than the control diet (60%). In general, addition of fat will either increase or have no effect on total lipid

digestibility. Since all forms of fat are represented in this discussion, more indepth investigation is needed before exact influences of fat on digestibility is established.

Effects of fat supplementation on nitrogen digestibility

Nitrogen balance may be influenced by fat supplementation. In one study (Ward et al., 1957), nitrogen retention was reduced by corn oil addition, but this reduction was overcome when alfalfa ash was added to the corn oil. Nitrogen digestibility was increased in 5, 15, and 25% dry matter whole cottonseed rations over the basal ration (Smith et al., 1981). Digestibility of nitrogen was similar between control and ground soybean diets, but hydrolyzed fat diet increased nitrogen digestibility compared to control diet (Palmquist and Conrad, 1978). Nitrogen digestibility was the highest on the highest fat diet. As kilograms rapeseed fed to cows increased from 0 to 1 to 2 kg, nitrogen digestibility decreased slightly but this difference was not significant (Murphy et al., 1987). Addition of calcium soaps or free fatty acids of tallow or soy had no effect on digestibility of nitrogen (Jenkins and Palmquist, 1984). Neither tallow nor blended animal-vegetable fat in the diet affected nitrogen digestibility (Palmquist and Conrad, 1980).

Feeding 4% yellow grease to steers did not affect digestion of nitrogen (Zinn, 1988).

Effects of fat supplementation on energy parameters

The primary reason for including additional lipid in the diet is to increase energy density. If food consumption remains constant, then energy intake will increase. Energy intake as well as dry matter intake was not different between diets of 0 and 15 percent whole cottonseed (Horner et al., 1986). In a ration using medium-quality silage, the addition of groundnut oil but not tallow increased metabolizable energy intake over no supplement (Steele, 1984). Since dry matter intakes were different, cows ingested more lipid on groundnut oil than tallow diet. The addition of soybean oil to a grass silage ration caused a reduction of energy intake because the soybean oil addition significantly depressed dry matter intake (Steele, 1985). Intracellular oil from crushed soybeans caused a greater reduction than free oil. The form of fat may have an influence on intake. If the goal is to increase energy intake in cows, then dry matter intake must also be monitored. Dry matter intakes were greater for diets of caloric density of .58, .63, and .68 Mcal digestible energy/liter, whereas there was a significant rate of decline as energy concentration increased to .84

and 1.17 Mcal digestible energy/liter in diets (Bull et al., 1976). This shows that animals were regulating energy intake by mechanisms other than digestive fill.

Changes in energy digestibility have been observed following increased energy diets. Energy digestibility increased as dietary caloric densities increased from .58 to .63 to .68 to .84 to 1.17 Mcal digestible energy/liter with the exception of .63 (Bull et al., 1976). Inclusion of protected tallow increased energy digestibility (Kronfeld and Donoghue, 1980). Energy digestibility was increased as increasing amounts of whole cottonseed were added to the basal diet (Smith et al., 1981). Schauff and Clark (1989) reported that digestibility of energy was greater for the diet containing calcium salts of fatty acids than diet containing 680 g/day per cow prilled fats in one experiment. All other comparisons using control, calcium salts of fatty acids, and 2 levels of prilled fat showed no difference in energy digestibility. In experiment 2 using different cows, energy digestibility was not different between any treatments. A study comparing free fatty acids to calcium soaps used the fat forms of fatty acids of tallow and soy, calcium soaps of tallow and soy, and tallow bound to verxite (nonnutritive carrier) added to the control diet (Jenkins and Palmquist, 1984). Digestibility of energy was lowest for the tallow verxite

diet. Energy digestibility did not differ between control and the other diets. Digestibility of energy for tallow soap diet was only 3% lower than the diet containing tallow fatty acids, but there was no difference in energy digestibility between soy diets of fatty acids and calcium soaps.

The energy balance of lactating cows is dependent upon the energy intake and energy output. Most cows are in negative energy balance in early lactation, but these cows can achieve energy balance earlier with higher concentrate feeding (Coppock et al., 1974) or possibly by the addition of fat to the diet. Addition of protected tallow to the diet can increase metabolizable energy and the efficiency of utilization of metabolizable energy for lactation (Kronfeld et al., 1980). In contrast, utilization of metabolizable energy did not differ between diets of basal, tallow, or soybean oil (Van Der Honing, 1980). The energy output in milk (MJ/day) was not different between diets of basal, tallow, and groundnut oil (Steele, 1984). Results from a study comparing levels of soybean oil to the no addition diet (Steele, 1985) indicated energy output in milk was lower for all fat supplemented diets than for the control diet. When no allowances are made for liveweight change in cows (Steele, 1984), basal and tallow diet were used more efficiently for milk production than the

groundnut oil. When changes in liveweight are considered, then efficiences of conversion of dietary energy into milk energy were greater for groundnut oil. In the situation of weight loss, the cows transfer intake energy into milk energy less efficiently than when the cows are gaining weight. In agreement Steele (1985) reported that cows gaining weight were more efficient in the transfer of dietary intake energy to milk energy than cows that were losing weight.

Effects of fat supplementation on body weight

The additional energy provided by dietary fat may be used for deposition of body tissue in addition to milk yield. Both blended animal-vegetable fat and yellow grease fed to growing cattle increased rate of weight gain (Zinn, 1988; and Zinn, 1989). Most of the reports using dairy cattle observed no change in body weight when supplemental fat was fed. Cows receiving protected fat in the form of tallow (Kronfeld et al., 1980), or calcium salts of long chain fatty acids (Kent and Arambel, 1988) did not have different body weights than the cows receiving no addition of fat. When ground soybeans or 2 levels of hydrolyzed fat was fed, the body weights of both Holstein and Jersey cows did not differ due to dietary treatment (Palmquist and Conrad, 1978). A high and medium level of animal vegetable

fat or tallow did not influence body weight in Holstein cows (Palmquist aand Conrad, 1980). Cummins and Sartin (1987) reported that Holstein cows fed 18.5% whole cottonseed had similar body weight to control cows at 50 days of lactation, but these cows tended to have lower body weights than controls at 100 days into lactation. In general, dairy cows do not seem to put on additional body weight with fat supplemented diets.

Fat supplementation effects on milk yield and composition

Addition of fat to the diet of dairy cows had varied effects on milk yield and composition. Some authors reported that fat supplementation increased milk yield (Burgess et al., 1987; Downer et al., 1987; Robb et al., 1987; Rogers et al., 1989; and Schneider et al., 1987) by the addition of calcium salts of fatty acids, but tallow addition decreased milk yield in one study (Palmquist and Conrad, 1980). Most of the research has reported no change in milk yield due to fat addition (Cummins and Sartin, 1987; Dunkley et al., 1977; Grummer, 1988; Horner et al., 1986; Kent and Arambel, 1988; Kwak et al., 1987; Palmquist and Conrad, 1978; Palmquist and Moser, 1981; Pennington and Davis, 1975; Schauff and Clark, 1989; Schneider et al., 1987; Smith et al., 1981; and Yang et al., 1978). As for milk composition, several researchers reported lowered milk

protein percent (Burgess et al., 1987; Downer et al., 1987; Kent and Arambel, 1988; Palmquist and Moser, 1981; and Smith et al., 1981) with fat supplementation. Different trends were reported for fat-feeding effects on milk fat percent. Many reports found no change in milk fat percent (Burgess et al., 1987; Grummer, 1988; Kent and Arambel, 1988; Robb et al., 1987; and Schauff and Clark, 1989), but others found increased (Dunkley et al., 1977; and Palmquist and Moser, 1981) and decreased (Kwak et al., 1987; and Steele, 1985) fat percent in milk. The total production of milk fat may also be increased with the addition of fat to the diet (Dunkley et al., 1977; Palmquist and Moser, 1981; Smith et al., 1981; and Storry et al., 1973).

In a few studies, changes in milk yield occured because of fat addition to the diet. Increases in milk yield were found with the feeding of calcium salts of long chain fatty acids. Addition of calcium salts of long chain fatty acids (Megalac) increased yield of milk and FCM (Burgess et al., 1987; Downer et al., 1987; Robb and Chalupa, 1987; and Schneider et al., 1987) and addition of calcium salts of volatile fatty acids (Rogers et al., 1989) increased milk production. Milk production increased in Jerseys with the high fat diets (6 and 20% hydrolyzed fat), but milk yield of Holsteins was increased only with the 6% hydrolyzed fat diet (Palmquist and Conrad, 1978). Stull et

al. (1957) observed a significant increase (10%) in milk production of mid-lactation cows supplemented with tallow. In contrast, milk production, both actual and 4% FCM, for cows fed tallow (10 % of concentrate) was significantly lower (Palmquist and Conrad, 1980).

A majority of reports indicate that feeding additional fat in the diet does not affect milk production. Reports showing no change in milk production have included a wide variety of fats. In contrast to some reports, feeding of calcium salts of fatty acids did not change milk yield compared to controls (Grummer et al., 1988; Kent and Arambel, 1988; Schauff and Clark, 1989; and Schneider et al., 1987). Schauff and Clark (1989) indicated the lack of increase in milk yield of cows receiving calcium salts of long chain fatty acids was due to the use of medium to low producing cows that were past the peak milk yield. The addition of prilled fat, which is inert in the rumen (Grummer, 1988), did not alter milk production (Grummer, 1988; and Schauff and Clark, 1989). Addition of formaldehyde protected lipid, such as animal-vegetable fat (Palmquist and Moser, 1981), oil seed (Yang et al., 1978), and tallow (Dunkley et al., 1977), did not influence milk yield. Dietary supplementation with unprotected fats such as cod-liver oil (Pennington and Davis, 1975), animal-vegetable fat (Palmquist and Conrad, 1980; and Palmquist

and Moser, 1981), blended hydrolyzed fat (Palmquist and Conrad, 1978), and tallow (Palmquist and Conrad, 1980) did not alter milk production. Palmquist and Conrad (1980) reported a medium level of tallow in the diet did not change milk production, but a high level of added tallow depressed milk yield. Cows fed whole cottonseed at 18.5% dry matter (Cummins and Sartin, 1987), or 15.0% dry matter (Horner et al., 1986), or 5, 15, and 25% dry matter (Smith et al., 1981) in the diet did not produce more milk than cows on the basal diet.

The basic mechanism of milk fat synthesis is directed by the cow's diet and physiological state. In general, about one-half of milk fatty acids are synthesized de novo in the mammary gland from acetate and B-hydroxybutyrate, 40-45% are derived from the diet, and less than 10% are derived from adipose tissue (Palmquist and Mattos, 1978). The short-chain and medium-chain fatty acids in milk fat are synthesized de novo in the gland, but the major part of long-chain fatty acids is absorbed from the blood (Moore and Christie, 1979). Changes in composition of milk lipids may occur after supplementation of dietary fat to lactating cows. Decreases in proportions of short-chain and medium-chain fatty acids with increases in long-chain fatty acids were reported for tallow (Brown et al., 1962; and Storry et al., 1973), soybean oil and crushed soybeans (Steele,

1985), and protected oil seed supplement (Yang et al., 1978). Pennington and Davis (1975) reported lower C18:0 and higher C18:2, C18:3, and C20:0 in milk fat of cows fed cod-liver oil compared to controls. Cows fed tallow and groundnut oil had lower yields of C4 to C14 fatty acids and higher yields of C18 fatty acids in milk fat than cows with no dietary fat supplementation (Steele, 1984). These changes in composition of milk fat demonstrate that feeding various lipids and their subsequent uptake by the mammary gland inhibits de novo synthesis of short-chain fatty acids in the gland. The inhibition is through feedback inhibition on acetyl-CoA carboxylase by increased concentration of long chain acyl CoA in the gland (Storry et al., 1973).

These changes in fat composition may be better understood by investigating the effects of dietary lipids on incubations of mammary gland epithelial cells. The effects of exogenous fatty acids on de novo synthesis of individual fatty acids and their incorporation into triacylglycerols in dispersed ruminant mammary gland epithelial cells were studied (Hansen and Knudsen, 1987; and Hansen and Knudsen, 1987). Using bovine mammary gland epithelial cells (Hansen and Knudsen, 1987), palmitate addition increased synthesis and incorporation of butyrate. Oleic acid strongly inhibited synthesis of all fatty acids

with the exception of butyrate, and lauric acid had no effect. Another study (Hansen and Knudsen, 1987) using epithelial cells from goats reported palmitic acid was stimulatory, whereas stearic and linoleic acid were inhibitory to synthesis and incorporation of fatty acids synthesized de novo into triacylglycerols. The results of these papers indicates that feeding of long chain fatty acids (C18) exerts a direct effect on the mammary gland. Fatty acid synthesized de novo will be dependent upon the amount and composition of exogenous fatty acids supplied.

Addition of fat to the diet may alter ruminal fermentation, as measured by acetate to propionate ratio. Since acetate is a substrate for de novo fatty acid synthesis in the mammary gland, any change in acetate production could lead to a change in short chain fatty acid production in milk. Jenkins and Palmquist (1984) reported that lower digestibility of fiber may lower milk fat content by reducing the ratio of acetic to propionic acids in the rumen and may also lower digestibility of energy for milk yield.

The secretion of fatty acids into milk is influenced by dietary fats or oils. Investigations of the effects of dietary lipids on secretion of milk fat have had varied conclusions. Increases in milk fat percent have been reported when protected lipid was fed. Fat percent was

greater for Jerseys receiving a protected form of animal vegetable fat (5.7%) than Jerseys on the control diet (5.0%) (Palmquist and Moser, 1981). The feeding of protected tallow (Dunkley et al., 1977), and whole cottonseed (Horner et al., 1986) resulted in higher percent fat. A report by Steele (1984) observed milk fat percent was higher for cows fed tallow (4.5%) than for cows fed control (3.9%) or groundnut oil (3.6%) diets. Increased milk fat was observed with dietary fat addition where average fat percent of low and high fat diets was 2.7 and 3.4 percent (Palmquist and Conrad, 1978). Feeding a medium level (6.4% dry matter) of animal-vegetable fat or tallow to cows produced a higher fat percent than low (3.0% dry matter) or high fat (8.5% dry matter) diets (Palmquist and Conrad, 1980). Some authors observed increases in yield of milk fat, with or without the corresponding increases in milk fat percent. Yield and percent of milk fat was greater when cows were supplemented with protected tallow (Dunkley et al., 1977), protected lipid supplement (Palmquist and Moser, 1981) and unprotected tallow (Steele, 1984). Dunkley et al. (1977) explained the increased yield of fat was due to cows transfering dietary fatty acids directly to milk fat. The feeding of protected lipid supplement may increase efficiency of transfer of dietary energy to milk energy. Even though fat percent in milk was

increased, the total fat yield was not changed by the addition of whole cottonseed (Horner et al., 1986) in the diet. In contrast to this, whole cottonseed feeding increased yields of milk fat, but it did not increase percent fat (Smith et al., 1981). Synthesis of fatty acids in mammary gland were depressed by cottonseed, but the transfer of dietary long chain fatty acids to milk resulted in greater yield of total fat. Storry et al. (1973) also found a change in milk composition when feeding tallow, but the total yield of fat was not changed by tallow addition. In contrast, milk fat production was 12.4% higher for protected tallow-fed cows than cows receiving no addition (Kronfeld et al., 1980).

Dietary lipid supplementation may alter milk fat percent negatively. Addition of cod-liver oil decreased fat percent in milk (Brumby et al., 1972; Pennington and Davis, 1975; and Varman et al., 1968). Milk fat percent was depressed when cod-liver oil was infused into the rumen or abomasum, but the extent of depression on milk fat was reduced with infusion in the abomasum (Pennington and Davis, 1975). High level of soybean oil in the diet (3.7%) decreased milk fat percent compared to crushed soybeans (4.3%) or control (4.0%) diet (Steele, 1985). Secretion of milk fat was reduced on free oil diets compared to those containing crushed seeds of groundnuts (Steele, 1984).

Polyunsaturated oils that are protected apparently do not depress of milk fat percent (Cook et al., 1972; and Pan et al., 1972). Percent milk fat was decreased by the addition of calcium stearate, tallow, and whole cottonseed to the diet (Kwak et al., 1987). With the exception of one study (Kwak et al., 1987), free oils appear to be more detrimental to the secretion of milk fat than other types of dietary fat.

Other studies have observed no change in milk fat percent when dietary lipids were included. When ground raw soybeans or blended hydrolyzed fat was fed, fat percent was not different in either Holstein or Jersey cows (Palmquist and Conrad, 1978). Feeding of calcium salts of long chain fatty acids did not alter milk fat percent between treatments (Burgess et al., 1987; Grummer, 1988; Kent and Arambel, 1988; Robb and Chalupa, 1987; and Schauff and Clark, 1989). The lack of alteration of milk fat percent by calcium salts could be attributed to the observation that preformed salts do not alter fermentation in the rumen (Chalupa et al., 1984; Chalupa et al., 1986; and Jenkins and Palmquist, 1984). Prilled fatty acids added to the diet did not change percent of fat in milk (Grummer, 1988; and Schauff and Clark, 1989) because prilled fat is inert in the rumen like calcium salts of fatty acids. The protected forms of fat, calcium salts and prilled fats,

seem to have less of a influence on milk fat than the other forms of fat.

Fat addition to diets may also influence milk protein content. Whole cottonseed feeding decreased milk protein percentage (Anderson et al., 1979; Horner et al., 1986; and Smith et al., 1981). The feeding of protected fat in the form of tallow (Dunkley et al., 1977) or animal-vegetable fat (Palmquist and Moser, 1981) depressed percent milk protein. Crushed soybeans also decreased milk protein percent (Steele, 1985), but soybean oil did not. Percent protein in milk was lower for cows fed diets containing calcium salts of long chain fatty acids (Grummer, 1988; and Kent and Arambel, 1988). Reduced content of milk protein was attributed to decreases in the casein fraction (Dunkley et al., 1977). The mechanism involved in the negative effect of dietary fat on milk protein is not known, but both glucose and insulin have been implicated (Palmquist and Moser, 1981; and Schmidt, 1966). Kent and Arambel (1988) reported the decline in milk protein percent may be due either to impairment of transport of amino acids to the mammary gland or reduction of milk protein synthesis due to insulin resistance (Palmquist and Moser, 1981).

All reports on fat supplementation in the diet do not report influences on milk protein percent. In general, protected forms of fat seemed to affect milk protein more

than other forms of fats. Cows fed animal-vegetable fat or tallow did not have different protein percent than cows without fat addition (Palmquist and Conrad, 1980). Neither Holstein or Jersey had altered milk protein content when fed ground raw soybeans or hydrolyzed fat (Palmquist and Conrad, 1978). In two reports (Grummer, 1988; and Schauff and Clark, 1989), milk protein content was not influenced by prilled fat addition to the diet. The exact influence of added fat on milk protein content is not understood, but the type of fat fed seems to be important.

Fat supplementation effects on calcium metabolism

The degree to which calcium-fat interactions interfere with calcium metabolism is not known. Early studies indicated that calcium addition to a high fat diet would alleviate the depressing effects of fat on fiber digestibility (Ward et al., 1957; and White et al., 1958). The calcium may restore fiber digestibility in the rumen, but how does the calcium-fat interaction effect the availability, absorption, and retention of calcium. From sources of literature, fat will either depress or have no effect on calcium metabolism. When corn oil was added to the diet, apparent and true digestibility as well as retention of dietary calcium were reduced in sheep (Tillman and Brethour, 1958). Digestibilities for calcium and

magnesium were decreased 76% and 5% when protected tallow was fed to Guernsey heifers (Kronfeld and Donoghue, 1980). The decreased digestibility suggests that insoluble soaps may be formed in the intestine. Feeding of free oil, whether soybean (Steele, 1985) or groundnut (Steele, 1984) reduced plasma concentrations of calcium and oil also reduced plasma magnesium (Steele, 1984). Increasing recommended dietary calcium and magnesium by 50% prevented recurrence of lowered plasma calcium and magnesium. Even though calcium digestibility was reduced by tallow addition, the digestibility was reduced the least when tallow was fed as fatty acids and was reduced the most by tallow soaps (Jenkins and Palmquist, 1984). Also, magnesium digestibility was decreased by tallow.

Fat addition does not always affect calcium digestibility or plasma concentration. Digestibility of calcium was not affected by prilled fat (Schauff and Clark, 1989), Megalac (Filley et al., 1987), or hydrolyzed fat (Palmquist and Conrad, 1978), but magnesium digestibility was reduced when hydrolyzed fat was fed (Palmquist and Conrad, 1978). Net absorption of calcium and magnesium was not different between cottonseed and control diets in Holstein cows (Smith et al., 1981). Addition of protected tallow did not influence plasma concentration of calcium and magnesium (Kronfeld et al., 1980). In a study using

growing Holstein calves fed low or high fat (5% emulsified animal fat) diet, fat did not significantly affect calcium uptake, retention, or metabolism. Since different fats have inconsistent effects on calcium metabolism, fat influences from the calcium-fat interaction are unclear.

Diet may also influence the concentration of ionized calcium in the rumen. Addition of 10% fancy bleachable tallow doubled long chain fatty acid and calcium soap concentration, but it halved ionized calcium concentration (Palmquist et al., 1986). The type of calcium supplement also affected ionized calcium levels in rumen. Insoluble calcium addition (dicalcium phosphate and limestone) did not change ionized calcium concentration or rumen insoluble soap content whereas soluble calcium (calcium chloride) addition tripled ionized calcium and rumen insoluble soaps. Also, concentration of ionized calcium in the rumen was inversely related to pH, but proportion of long chain fatty acids into soap was directly related to pH.

Fat supplementation effects on blood hormones and constituents

Supplementation of fat may have influences on the blood metabolic hormones and other components. Investigations of dietary fat effects on blood glucose and insulin concentrations have drawn varied conculsions. Cows

on whole cottonseed diet had lower glucose and insulin in blood plasma (Horner et al., 1986). In agreement, concentration of total lipids in plasma was increased whereas glucose and insulin were reduced by a protected animal-vegetable fat in the diet of Jerseys (Palmquist and Moser, 1981). In order to explain their findings, Palmquist and Moser (1981) concluded from the linear negative relationship between glucose utilization rate and insulin release that dietary fat may impair both amino acid transport into the mammary gland and milk protein synthesis by inducing insulin resistance. Apparent insulin resistance (i.e. inability of insulin to stimulate tissue glucose utilization) in adipose tissue has been demonstrated in rats fed high fat diets (Ip et al., 1976; and Ip et al., 1977). Smith et al. (1978) suggested that excess dietary fat might alter glucose metabolism by altering ruminal fermentation and decreasing production of glucose precursors. Other reports have shown increased plasma insulin or glucose concentration. Basal plasma insulin and insulin:glucagon ratio were increased in cows fed high fat diet (18.5% dry matter from whole cottonseed) but somatotropin remained unchanged compared to control Holsteins (Cummins and Sartin, 1987). Mean plasma glucose was increased with the addition of tallow in the diet (Kronfeld et al., 1980). The elevated glucose could be

explained in that extra utilization of fat spares utilization of glucose. Palmquist and Moser (1981) reported that basal glucose and insulin concentrations were increased by fat feeding. This is in contrast to the other part of their experiment where lower blood glucose and insulin were found. The reason for the difference between the two experiments is not known, but the reason could be attributed to the type of fat fed (unprotected versus protected form). In an incubation study using both adipose and mammary tissue slices, glucose uptake and utilization, and glucose conversion to lipid was lower in cows fed whole cottonseed diet (Cummins and Russell, 1985). Feeding a high fat diet appears to affect glucose metabolism in both mammary gland and adipose tissue in lactating cows. The effects on glucose metabolism might be explained by an increased availability of free fatty acids.

Several studies reported no effects on plasma glucose from the addition of fat to the diet. The feeding of crushed or free oil of groundnuts (Steele, 1984) or soybeans (Steele, 1985) did not affect plasma glucose concentration. Glucose was not different between treatments when ground soybeans or hydrolyzed fat was included (Palmquist and Conrad, 1978). Protected oil seed addition to the diet also did not affect concentration of plasma glucose (Yang et al., 1978). Since different forms

of fat affected plasma insulin and glucose in different ways, the mechanism of influence is unclear. The type, quanity, degree of saturation, form of protection, and metabolic status of the cow may all play crucial roles in the mechanism by which additional dietary fat will affect metabolic parameters.

Factors influencing metabolic hormones and glucose

In lactating dairy cows, hormonal regulation of nutrient partitioning is of vital importance to metabolism. Dietary energy requirements are the highest during early lactation when milk synthesis is at its peak. Conversely, maximum feed intake does not occur until after peak production. The cow must mobilize body tissues to supply the energy required for high milk production. The metabolic actions of somatotropin (ST) and insulin (INS) are important during this critical period of early lactation. Somatotropin promotes mobilization of adipose tissue and INS regulates nutrient utilization. The high-yielding cow mobilizes large amounts of fat, as much as 50 kg, to supply additional energy needed at peak production (Bines and Hart, 1982). Insulin is associated with metabolic processes that divert energy away from milk synthesis and toward body tissue. The combination of higher ST and lower INS plasma concentrations in early

lactation allows greater milk yield.

Stage of lactation has a significant influence on plasma ST. As gestation progressed, serum ST increased (Koprowski and Tucker, 1973). Concentration of plasma ST decreased as lactation progressed (Herbein et al., 1985; Koprowski and Tucker, 1973; Sartin et al., 1985; Smith et al., 1976; and Vasilatos and Wangsness, 1981). Smith et al. (1976) found higher ST in the first 20 days of lactation than in 21 to 40 or 41 to 56 days of lactation. At 30 days postpartum, plasma ST was higher (13 ng/ml) than at 90 days (10 ng/ml) (Vasilatos and Wangness, 1981). They attributed the greater concentration of ST to greater magnitude of secretory spikes instead of more frequent ST secretion. In contrast, Reynolds (1989) found similar concentrations of ST at 4 and 8 weeks postpartum.

The genetic potential of milk production is related to the concentration of plasma ST. Barnes et al. (1985) observed greater ST in dairy cattle of different ages that were sired by bulls with high predicted difference for milk. This greater blood concentration of ST was present by 6 months of age. Greater ST levels continued in Holstein yearlings, bred heifers, and primiparous cows. A study by Hart et al. (1978) was in agreement with these findings. Throughout lactation, plasma concentrations of ST were greater in high-yielding cows than in the low-

yielding group. The increased ST is uniformly associated with increased milk yield. This has been concluded in several reports because exogenous ST administration will increase milk yield (Eppard et al., 1985; Peel et al., 1981; and Pocius and Herbein, 1986).

Factors concerning nutrition may affect concentration of plasma ST. Prepartum concentrate and forage feeding did not have an effect on plasma ST in dairy cows (Smith et al., 1976). In sheep, no differences were detected between diets containing different levels of energy (Trenkle, 1971). Neither feeding nor fasting for 72 hours had any effect on plasma ST. In contrast, Barnes et al. (1985) observed increased ST after feeding. The level of nutritive feeding may affect plasma ST. Cows fed inadequate dietary nutrients had twice the concentration of plasma ST as cows recieving an adequate diet (Hove and Blom, 1973). Plasma ST was elevated in these underfed cows that were losing body weight.

Another factor, age, may have an influence of ST. Plasma ST differed overall with age (Barnes et al., 1985). Greater ST was reported for 6- and 24-month-old animals than for 12- or 18-month-old heifers. In contrast, neither age or breed of beef cattle influenced plasma ST in either males or females (Irvin and Trenkle, 1971). The plasma ST was similar at 18, 204, and 371 days of age. Another study

using dairy cattle reported that age was not a significant source of variation in determining ST concentration (Herbein et al., 1985).

Stage of lactation effects on INS are opposite of those for ST. Concentration of INS increased with advancing days in milk (Herbein et al., 1985; Koprowski and Tucker, 1973; Reynolds et al., 1989; Smith et al., 1989; and Vasilatos and Wangsness, 1981). Plasma INS was higher at 14 days prepartum then decreased to a third of prepartum value by 5 days postpartum (Sartin et al., 1985). Concentration of INS gradually increased from the period of 1 to 20, to 21 to 40, to 41 to 56 days postpartum (Smith et al., 1976). Insulin content was higher at 90 days (29 uU/ml) than at 30 days postpartum (17 uU/ml) (Vasilatos and Wangsness, 1981). This increase was due to both greater secretory spike frequency and spike magnitude. In contrast to the above reports, Denbow et al. (1986) observed no significant changes in INS for early, middle, or late lactation cows.

Season of the year may affect the concentration of plasma INS. Insulin concentration was lowest in the summer months (Denbow et al., 1986; and Herbein et al., 1985). Concentration of INS was higher for winter (.87 ng/ml) and spring (.89 ng/ml) than for summer (.69 ng/ml) (Denbow et al., 1986). They suggested that the lower concentration of

INS during the summer may be due to decreased volatile fatty acids, which are stimulators of insulin secretion in ruminants. In agreement, INS concentration was higher in winter and spring, intermediate in fall, and lower in summer (Herbein et al., 1985). The temperature in summer was 15 to 23 degrees higher than temperatures in the other 3 seasons while only 8 degrees separated the seasons of fall, winter, and spring.

Insulin may be influenced by other factors such as age of animal and nutrition. Age effects on INS have shown varied results. Somatotropin and INS showed a inverse relationship with regard to age in Barnes et al. (1985). Plasma INS was greatest in 18-month-old heifers and lowest in 24-month-old animals. In contrast, age did not contribute to differences in plasma INS in Holsteins (Herbein et al.,1985) or in 3 breeds of beef cattle (Irvin and Trenkle, 1971). Dietary nutrient intake may influence INS content in plasma. Insulin increases in response to feeding (Hove and Blom, 1973; and Bassett, 1978). However, a significant trend was not found for feeding in Holsteins of different ages (Barnes et al., 1985). Insulin was similar between groups of cows fed diets containing adequate nutrients or inadequate nutrients (Hove and Blom, 1973). Since ST is elevated in high-producing cows, the opposite trends might be expected for INS in these cows.

The low-yielding cows in Hart et al. (1978) had higher INS than their higher-producing herdmates. The inverse relationship between ST and INS was observed in these 2 groups of cows during lactation except for the dry period. In contrast to this report, another study found INS was not different between cows with different genetic potentials for milk (Barnes et al., 1985).

Influences of stage of lactation on plasma glucose concentration has shown varied results. Some studies showed concentration of glucose increased as lactation increased (Herbein et al., 1985; and Smith et al., 1976). Herbein et al. (1985) reported a steady increase in glucose through 200 days of lactation then glucose leveled off. Glucose concentration increased by 3 mg/100 ml blood between the first 20 days to the second 20 days of lactation and from the second 20 days to the third 20 days of lactation (Smith et al., 1976). Other studies did not find significant differences in glucose due to lactation cycle (Denbow et al., 1986; and Vasilatos and Wangsness, 1981).

Other physiological factors may influence blood levels of glucose. Dietary treatment may alter glucose. Barnes et al. (1985) found similar concentrations of glucose before and after feeding. However, blood glucose was depressed in sheep that were fasted for 72 hours (Trenkle,

1971). On the other hand, plasma glucose was similar between cows fed adequate and inadequate dietary nutrients (Hove and Blom, 1973). While INS and ST were inversely related in high versus low producing cows, concentration of glucose was not different between groups (Hart et al., 1078). In agreement, cows with high genetic potential for milk had similar plasma glucose to control herdmates (Barnes et al., 1985). Also, season of the year may exert an effect of plasma glucose. Results indicated that plasma glucose was higher in summer and spring (Herbein et al., 1985). However, Denbow et al. (1986) did not find differences between winter, spring, and summer seasons.

Factors influencing calcium metabolism

Calcium is a crucial component of the normal functioning of a wide variety of physiological processes in the body. These processes include bone, blood clotting, nerve transmission, and muscle contraction. Intracellular calcium, whether directly or indirectly, regulates activity of many enzymes, generation of ATP, and release of hormones and neurotransmitters (Rasmussen, 1986). Since calcium is required for all these processes, an elaborate system of homeostatic controls maintains normal blood concentrations of calcium of 9-11 mg/100 ml plasma (Bloom and Fawcett, 1986; and Littledike and Goff, 1987). A balance of inputs,

outputs, and nutrient recycling make up the homeostatic mechanisms of calcium regulation. Lactation, especially the onset of lactation, puts a serious stress on the calcium homeostasis system. A cow can adapt to this increased demand for calcium, but some cows will subcome to metabolic disorders, such as hypocalcemia and parturient paresis, during the adaptation period following parturition. Dairy cows would benefit from our greater understanding of how the endocrine system, prepartum feeding and postpartum feeding of calcium affects the homeostatic control of calcium regulation.

Calcium entry into and exit from the extracellular plasma pool is governed by the endocrine system. Bone, kidney, and intestine are considered as control subsystems (Hurwitz et al., 1983), whereas parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D (1,25-VitD) and calcitonin are the regulating hormones. First, PTH is stimulated by hypocalcemic conditions and enhances calcium entry into the extracellular plasma pool. Parathyroid hormone stimulates reabsorption of calcium in the kidney and decreases excretion of calcium in the kidneys. The 1,25-VitD increases the efficiency of intestinal calcium absorption and works in conjunction with PTH to mobilize calcium from the bone. The third hormone, calcitonin, works opposite of

the other two hormones. Calcitonin lowers plasma calcium by stimulating bone mineral deposition and increasing excretion of calcium via urine.

Plasma total calcium is comprised of three factions. First, approximately 45% of total calcium is bound calcium. This calcium is bound to the plasma proteins, primarily albumin. Second, there is 5% of total calcium that are calcium ions bound to complexing compounds such as lactate, citrate, and bicarbonate. The rest of the calcium, around 50%, is ionized calcium (Littledike and Goff, 1987). This calcium is "free" as it is immediately available for biological use. Concentration of calcium ions from both complexed and ionized fraction are dependent upon blood pH. The pH of the blood primarily depends on CO_2-bicarbonate equilibrium (Watson and Anbar, 1985). The concentration of ionized calcium will decrease as CO_2 decreases. As blood pH increases because of loss of CO_2 to air when sampled, then the equilibrium favors the protein-bound form of calcium in blood. The total calcium concentration may give an overall indication of calcium statis, but the ionized calcium concentration may be a better estimate of what is actually available to the animal's system.

Stage of lactation influences on plasma calcium are most apparent at parturition. At calving, plasma calcium concentration is the lowest than at any other point during

the lactation cycle (Littledike and Goff, 1987; Shappell et al., 1987; and Wohlt et al., 1984). Plasma calcium concentration starts to decline right before calving and usually is the lowest on the day of calving (Littledike and Goff, 1987; and Shappell et al., 1987). Then plasma calcium will gradually increase over the next few days and be normal by 4 days postpartum (Littledike and Goff, 1987; and Wohlt et al., 1984). After the sudden stress of onset of lactation is over, the homeostatic control of calcium regulation is primed and maintains normal calcium levels. By 14 days into lactation, serum calcium had stabilized at prepartum values in Holstein heifers (Shappell et al., 1987). Wohlt et al. (1984) found similar plasma calcium during lactation points of 4 to 151 days of lactation for both young and older dairy cows.

Many dairy cows experience hypocalcemia with the initiation of lactation. The sudden stress of colostrum synthesis and lactation decreases the available calcium from the plasma pool. When plasma calcium decreases to 5 mg/100 ml plasma, parturient paresis (milk fever) develops (Goings et al., 1974). The exact cause of this disorder that only effects some dairy cows is not known. The cause of parturient paresis is not an insufficiency of PTH or 1,25-VitD (Horst et al., 1978) because paretic cows have high concentrations of these hormones. Age does influence

the incidence of milk fever with older cows being more susceptible than younger ones (Horst et al., 1978; Shappell et al., 1987; and Wohlt et al., 1984). One-half of the aged cows (> third lactation) and none of the young cows developed severe hypocalcemia (4.95 mg/100 ml plasma) at parturition (Horst et al., 1978). Increased concentration of PTH and 1,25-VitD were associated with the severe hypocalcemia.

Dietary calcium intake during the dry period will influence the calcium homeostasis at calving. The calcium regulating hormones, PTH and 1,25-VitD, need to be primed during the dry period. Increasing concentrations of these hormones before calving will aid in alleviating the extremely low concentrations of plasma calcium at calving. Feeding a high-calcium diet prepartum only increased parturient paresis (Manston, 1967). In agreement, cows, but not heifers, recieving a high-calcium ration prepartum exhibited extreme hypocalcemia for three days (Shappell et al., 1987). Both Boda and Cole (1954) and Goings et al. (1974) prevented parturient paresis in dairy cattle by feeding a calcium-deficient diet prepartum. Initially the plasma calcium fell in the cows on calcium-deficient diet, but normal calcium levels returned within 4 days (Goings et al., 1974). During the period of decreased plasma calcium, the PTH concentration increased. In agreement, a low-

calcium diet stimulated prepartal production of PTH and 1,25-VitD and initiates bone resorption and intestinal calcium transport systems (Green et al., 1981). The feeding of a calcium deficient diet prepartum seemed to sensitize the homeostatic mechanisms and ready these for the onset of lactation.

In addition to being more prone to develop milk fever, the age of the dairy cow has an affect on the other parts of calcium metabolism. Ramberg et al. (1976) found a decline in calcium clearance, lower plasma PTH concentration, and decrease of calcium transport into the extracellular plasma pool with age. In short, the age of the animal had a major influence on the calcium kinetics and plasma PTH. As the animal increases with age, less of the bone calcium is available for exchange with blood calcium (Hansard et al., 1954; and Jacobson et al., 1972). It should be noted that age of the animal did not have an effect upon the calcium content in soft tissues once calcium content reached a maximum in 18-month-old cattle (Hansard et al., 1954). Also, older animals take longer to adapt to a low-calcium diet than younger animals (Jacobson et al., 1972).

The amount and source of dietary calcium will affect plasma calcium. The source of calcium in the diet can influence the amount of calcium absorbed from the diet.

Data from Wohlt et al. (1986) suggest that dietary calcium should be fed at higher amounts when using a slow reacting (coarser) inorganic calcium source. Also these researchers found calcium retention was increased when .9 versus .6% calcium was fed in the diet. In agreement, cows fed .9 versus .6% dietary calcium had higher serum calcium (Wohlt et al., 1984). Diets containing more than 1% dietary calcium have not improved calcium availability and utilization because dry matter intake may be reduced and milk yield was not increased (Kincaid et al., 1981; and Nocek et al., 1983). In contrast, Ramberg et al. (1976) observed that calcium intake (.05 to 1.4%) did not influence plasma concentration of calcium or PTH in nonlactating dairy cattle. They explained their observations in that as calcium intake increased, calcium absorption increased, but calcium removal from bone decreased. The total calcium transport into the exchangeable pool remained fairly constant for all levels of calcium intake. Interpretation of these results should be cautioned because these cows did not experience the stress of lactation. Level of dietary intakes of calcium has an influence on calcium absorption in rats also (Sammon et al., 1970). Compared to normal rats, parathyroidectomized rats on high intake of calcium had lower calcium absorption. This was not observed for parathyroidectomized

rats on low calcium diet. Parathyroidectomy of these rats led to lower plasma calcium and rate of calcium in and removal from bone. Without normal levels of PTH, the calcium homeostasis was not maintained properly. Modulation of intestinal calcium absorption because of dietary calcium intake is mediated by the parathyroid glands and renal biogenesis of 1,25-VitD (Ribovich and DeLuca, 1978). If dietary calcium intake is not sufficient, the animal will adapt to the reduced calcium supply by reducing fecal excretion and increasing efficiency of intestinal absorption (Jacobson et al., 1972). At the onset of lactation, calcium absorption increased and rate of calcium outflow to bone and feces decreased (Ramberg et al., 1970). Also, they noted that it was several weeks until increases in removal of calcium from bone was observed.

Another influence on serum calcium is the blood sampling site. Wohlt et al. (1984) found significant differences due to sampling site. Serum calcium was highest in blood from the jugular vein, followed by blood taken from internal iliac vein, and lowest in blood from mammary vein. This trend makes physiological sense because a large amount of calcium would be taken out of the blood by the mammary gland to use in milk synthesis. Since most studies obtain blood from the jugular vein, this sampling

site will be assumed unless otherwise noted.

Regulation of calcium homeostasis by hormones

The complex regulation of calcium homeostasis is dependent upon the combination of plasma calcium, PTH, and 1,25-VitD. Parathyroid hormone is secreted by the parathyroid gland in response to low plasma calcium (DeLuca, 1979; Goff et al., 1986). Parathyroid hormone increases concentration of 1,25-VitD when hypocalcemic conditions exist. Both PTH and 1,25-VitD work in conjuction to mobilize calcium from bone, but each also works separately. In the kidney, PTH stimulates reabsorption of calcium so less calcium is excreted (Sutton and Dirks, 1978). There are two ways that PTH stimulates bone mineral mobilization. The first course of action of PTH on bone mobilization is to stimulate the osteocyte-osteoblast pump (Littledike and Goff, 1987). This pump moves calcium from the bone fluid compartment surrounding the osteocytes across the endosteal lining cells into the extracellular fluid compartment. This supplies needed calcium quickly. Parathyroid hormone also stimulates osteoclastic resorption of bone (Bloom and Fawcett, 1986). This process takes much longer to stimulate than the first, but it is capable of releasing much more calcium from bone (Capen and Martin, 1983).

The calcium metabolism is also affected by the vitamin D system. In review, vitamin D enters the blood from the gut or through irradiation of the skin. In the liver, vitamin D is converted to 25-hydroxyvitamin D (Ponchon et al., 1969). This form is the major form of circulating vitamin D and serves as precusor to the other vitamin D metabolites (Horst and Littledike, 1982). After being transported by the blood from the liver to the kidney, 25-hydroxyvitamin D is converted to 1,25-dihydroxyvitamin D by the enzyme 25-hydroxyvitamin D - 1∝-hydroxylase. The kidney is the sole site of production of 1,25-VitD (DeLuca, 1979). The most biologically active form of vitamin D in its effects on calcium metabolism is 1,25-VitD (Tanaka et al., 1973). Parathyroid hormone increases the production of 1,25-VitD by stimulating the 1∝-hydroxylase enzyme (Kremer and Goltzman, 1982). Once PTH has stimulated increased production, 1,25-VitD can work alone or with PTH to elevate plasma calcium. Acting separately, 1,25-VitD stimulates calcium transport across the epithelial brush border of the small intestine (DeLuca and Schnoes, 1976; and Wasserman and Taylor, 1976). In the bone, PTH works in conjunction with 1,25-VitD to mobilize calcium from the bone fluid compartment (DeLuca, 1979). While PTH is required for initiation, 1,25-VitD enhances mechanisms of bone resorption. Whether working alone or together, the

major role of PTH and 1,25-VitD is to maintain normal plasma calcium concentration. This is accomplished through a series of complex processes in the kidney, intestine, and bone.

A question exists on what is the active form or forms of PTH. Parathyroid hormone is synthesized as a 115 amino acid molecule which is converted to pro-PTH (90 amino acids). Then the Golgi apparatus cleaves the peptide to PTH (1-84). The intact molecule of PTH (1-84) is fully active on renal tissue (Littledike and Goff, 1987). In agreement, Both 1-84 and 1-34 PTH stimulated enzyme activity in mammalian kidney (Kremer and Goltzman, 1982). Only a small fraction of circulating PTH is PTH (1-34), which is the form taken up and stimulates bone (Slatopolsky et al., 1981). Administration of synthetic PTH (1-34) increased plasma calcium and prevented parturient paresis in dairy cows (Goff et al., 1986; and Goff et al., 1989). Increasing evidence suggests that PTH (1-84) must be cleaved to PTH (1-34) before it will stimulate bone resorption (Martin et al., 1978).

Parathyroid hormone adminstration has a direct effect on calcium metabolism. An early study using parathyroid gland extract (Todd et al., 1962) found increased plasma calcium with this treatment. Infusions of synthetic PTH (1-34) in calves caused an increase in plasma calcium (Hove

et al., 1984). Plasma 1,25-VitD levels decreased after the start of the PTH infusion. Two parathyroidectomized lactating goats also received PTH infusion. The infused PTH corrected the hypocalcemia and raised the levels of plasma 1,25-VitD. The authors concluded that calcium concentration in the plasma influences the concentration of plasma 1,25-VitD in ruminants. In the case of the calves, continual infusion of PTH increased concentration of calcium to adequent levels and decreased 1,25-VitD because 1,25-VitD was not needed to stimulate calcium absorption. Another study infused synthetic bovine PTH (1-34) in pregnant dairy cows (Goff etal., 1986). A 96-hour intravenous infusion of PTH increased plasma 1,25-VitD within 16 hours and calcium within 48 hours. The cows were hypercalcemic (15 mg/100 ml plasma) at the end of the infusion period. Even in the presence of hypercalcemia, 1,25-VitD concentration was twice it was before infusion. Hydroxyproline was also monitored as an indication of bone resorption (Dull and Henneman, 1963). Since concentration of hydroxyproline was elevated within this time period, the results suggest that 48 hours of PTH stimulation is necessary for initiation of bone resorption. Another part of this same experiment used 8 periparturient cows that were fed a high-calcium diet prepartum. Four cows recieved PTH infusion prior to parturition. All of the other 4 cows

that did not recieve PTH developed parturient paresis at calving. Cows recieving PTH infusion for greater than 60 hours before calving had normal levels of calcium at calving while cows recieving PTH for less than 24 hours before calving became hypocalcemic but not paretic. These results indicate that exogenous PTH administration can prevent parturient paresis if given at least 60 hours before calving.

Studies using infusions may be more similar to the physiology of the cow, but infusions will present a major problem to the management of a dairy herd. Intramuscular injection is a more practical approach to the problem. Parathyroid hormone was given intramuscularly to dairy cows for about 6 days before and continued several days after calving (Goff et al., 1989). All the cows on the study recieved a high-calcium diet prepartum, but only half the cows were injected with PTH. All the control cows exhibited hypocalcemia within 24 hours after calving. The plasma calcium of the PTH-treated cows were normal or slightly elevated after calving. Injections of PTH increased both 1,25-VitD and hydroxyproline prior to parturition. Research results show that PTH administration will prevent parturient paresis in dairy cows due to PTH increasing 1,25-VitD and in turn increasing plasma calcium prior to calving. The remaining problem will be to develop

a practical method of delivery of the required PTH dose over the required period of time.

While PTH affects plasma calcium concentration, a modified blood calcium will influence PTH concentration. Serum calcium was altered in canine and bovine species plus man by infusing either calcium chloride or EDTA (D'Amour et al., 1986). Parathyroid hormone levels were measured to determine the response to conditions of hypercalcemia and hypocalcemia. Hypercalcium, induced by calcium chloride infusion, only decreased PTH by 40% of the pre-infusion value. These results indicate that even in the condition of excess calcium, a certain level of secretion of PTH is maintained (Littledike and Goff, 1987). On the other hand, hypocalcemia, induced by EDTA infusion, increased PTH by 300-450% over the basal concentration of PTH. The rapid elevation of PTH stimulated by hypocalcemia indicates that PTH is immediately secreted in order to correct the imbalance in calcium stasis. Even the elevation of PTH, induced by hypocalcemic conditions, has limits to its secretion.

Effects on bone metabolism

Bone is a specialized connective tissue. The unique feature of bone is the mineralization of the intercellular matrix. The major mineral is calcium, in the form of

hydroxyapatite crystals [$Ca_{10}(PO_4)_6(OH)_2$]. This mineral matrix makes an extremely hard tissue ideally suited for the supportive and protective role of skeletal bone. The other major functions of bone include the blood-forming elements of bone marrow and a mobilizable store of calcium.

Bone has a combination of physical properties. The high tensile and compressive strength comes from the mineral matrix. The elasticity of the bone comes from the organic matrix (primarily collagen). Therefore, the hardness of bone depends on the inorganic constituents while great toughness and resilience reside in organic matrix. Despite its strength and hardness, bone is a dynamic living material that is constantly being remodeled throughout the lifetime of the animal.

In compact bone, the basic unit is the haversian system or osteon. These vary in size and are made up of 4-20 lamellae (layers). If the bone is cut in cross-section, the haversian system appears as concentric rings around a circular opening, the haversian canal.

There are four cell types in the organic matrix of bone (Bloom and Fawcett, 1986). The first is the osteoprogenitor cell which is a precusor to the osteoblast and is only visibly distinguishable in actively growing or repairing of bones. Second, the osteoblast cell is responsible for formation of bone matrix. Third, the

osteocytes are the principal cells of fully formed bone that reside in the lacunar spaces within the calcified interstitial substance. Lastly, osteoclasts are giant multinucleated cells which are responsible for bone resorption. The first three cell types described are different functional states of the same cell type. Osteoclasts arise from fusion of blood mononuclear cells. These cell types are valuable histological measurements of the remodeling state of bone. Large numbers of osteoclasts would indicate active bone mineral resorption, while prevalent osteoblasts would indicate formation of bone.

Mechanical properties of bone

The physical properties such as strength of bone have been used by nutritionists to assess the mineralization status of bones. The mechanical properties of bone are determined by the same methods used in studying similar materials of metals and woods (Baker and Haugh, 1979). These methods are based on certain fundamental principles of mechanics.

The three most common tests of bone strength are the shear test, three-point bending test, and the torsion test. Shear test works well on bones of varying shape and size (Harner and Wilson, 1986). Three-point bending test should be used on bones that are straight and have a length to

diameter ratio greater than 10. The torsion test should be used when the bone is straight and has a symetrical cross section.

The decision of using the shear or three-point bending test will depend upon the bone itself. Wilson et al. (1984) reported that shear deflection can influence the amount of force measured in the three-point bending test. When the ratio of length (L) to diameter (D) is less than 10, shear becomes part of the measured force for the bending test. Shear accounts for 50% of the total deflection when L/D ratio is approximately 5. When L/D ratio is greater than 10, shear deflection is approximately zero. The authors recommended that a pure shear test be used if the ratio of L/D is less than 10.

Force is a measure of the amount of load a bone can withstand until breakage (Crenshaw et al., 1981b). A bone that is larger or more mineralized would be expected to withstand a greater force. Force does not account for the differences in size and shape of the bone. This measurement alone could lead to misinterpretation of results. On the other hand, another property of bone, stress, could be used to evaluate changes in the degree of mineralization. Stress is the calculation of the amount of force a bone can withstand per unit area of bone (Crenshaw et al., 1981b). Differences in size and shape of bones

would be accounted for in the stress calculation.

Factors influencing measurements of bone strength

The moisture content of the bones can influence the breaking strength. Both Crenshaw et al. (1981a) and Kornegay et al. (1981) reported that changes in moisture content of bone can alter the mechanical properties of bone, including the amount of force it takes to fracture the bone. Crenshaw et al. (1981a) reported that just 10 minutes of air exposure could dry the bones enough to detect differences in mechanical strength. Lott et al. (1980) observed only minor differences between fresh and frozen bones. However, drying bones decreased their strength by about 50%. In agreement, Sedlin (1965) reported that freezing of wet bones did not change the mechanical properties as long as the bone did not go through dessication during the freezing period.

Even though the literature contains many reports on accessing bone strength in swine and poultry, research in this area in bovine species has been largely ingored. Any discussion of influences from diet on mechanical properties of bone will use data from swine or poultry. Increasing dietary calcium and phosphorus increased bone breaking strengths in poultry (Rowland et al., 1967; and Ruff and Hughes, 1985) and swine (Crenshaw et al., 1981b; Cromwell

et al.,1972; and Lepine et al., 1985). Crenshaw et al. (1981b) reported that the femur, humerus, and rib of two-month-old pigs fed .8, .8% calcium and phosphorus in diet could withstand a greater stress than the same bones of pigs fed .4, .4% dietary calcium and phosphorus.

A limited amount of reports studying bone strengths included rib. Granik and Stein (1973) selected rib as a bone of choice in studying diseases that pathologically demineralize bones. The rib bone was compared with metacarpals and metatarsals for bending moment and shear stress in pigs (Crenshaw et al., 1981b). Results showed that rib had a much lower bending moment than metacarpals and metatarsals, but when expressed as force per unit area, the shear stress of the rib was greater than that of the metacarpals and metatarsals. Since only a small amount of bending occurred in the rib, they considered the rib to be a very brittle bone.

The rib has been used in a few studies in ruminants and even more studies in humans to quantitatively and qualitatively evaluate changes in bone imposed by nutrition or endocrine disorders. Most studies obtain rib samples at autopsy in humans (Follis, 1952; and Granik and Stein, 1973) or at sacrifice in animals (Hansard et al., 1954). In older literature, three studies using ruminants (Little, 1972; Little and McMeniman, 1973; and Little et al., 1978)

and one study with deer (Banks et al., 1968) obtained rib samples by biopsy. All the ruminant studies using rib biopsies were performed in Australia and chose rib because bones of axial skeleton and ribs are more sensitive to resorption and rehabilitation than the long bones (Hill, 1962).

Factors influencing bone density or specific gravity

The density or specific gravity can be determined using an isolated piece of rib or other type bone. These parameters will be influenced by the calcium state of the animal. In cattle, specific gravity of rib was significantly lower in cattle on phosprorus deficient ration (1.58 g/ml) than in cattle fed adequate phosphorus (1.68 g/ml) (Little, 1972). Specific gravity of rib samples in sheep that were lactating had a significantly lower value (1.47 g/ml) than sheep that were not lactating (1.63 g/ml) (Little and McMeniman, 1973). Little et al. (1978) observed lower specific gravity of rib in lactating sheep, but not non-lactating sheep, that were placed on a phosphorus deficient diet. Lactating and non-lactating sheep on adequate phorphorus diet did not have different specific gravity, but lactating sheep did have non-significantly lower specific gravity. In the lactating sheep, rib specific gravity decreased by 11% during the

phosphorus deficient period, while bone phosphorus decreased by 22% during this time. The authors concluded that the level of phosphorus supplementation, and of lactation and level of lactation, were all reflected by changes in rib composition. Another physiological calcium challenge in animals is the growth of antlers in male deer. Calcium was mobilized from the skeleton during antler growth (Banks et al., 1968b). During the peak of antler growth, density of biopsied rib was the lowest in these deer. Since bone densities decreased and high levels of calcium, phosphorus, and magnesium in bone were mobilized during antler growth which indicated loss of bone mass, the authors concluded that these data supported the morphological observation of a cyclic physiological osteoporosis in the mule deer. From these studies in cattle, lactating sheep, and deer, changes in rib density or specific gravity was due to substantial loss of bone mineral because of the demand for calcium to support lactation or antler growth.

Factors influencing mineral concentrations in bones

Evaluation of bone mineral concentration changes, in addition to density or specific gravity measurements, can provide valuable information on the calcium status of the animal. A study using biopsied rib from cattle (Little,

1972) showed both concentration of calcium and phosphorus and total ash expressed as mg/cc fresh bone and percent fresh bone were significantly lower after 6 weeks on a phosphorus deficient diet. After the phosphorus depletion period, a period of increasing supplements of phosphorus were administered and another bone biopsy was obtained. Non-significant increases had occurred during this period. Since increases were apparent after a short period of phosphorus repletion, the authors concluded that the changes in bone composition during the phosphorus depletion period were due to the failure of normal remodelling process, in that newly formed osteoid tissue remains poorly calcified. Similar results have been found in sheep that had rib biopsies. Concentrations (mg/cc) of calcium, phorphorus, and ash were significantly lower in lactating sheep than in non-lactating sheep (Little and McMeniman, 1973) while no differences were detected for blood phosphorus. Another study in sheep (Little et al., 1978) found lactating sheep were more sensitive to a phosphorus deficient diet than non-lactating sheep. Lactating sheep had significantly lower bone phosphorus (mg/cc) after the period of dietary phosphorus deficiency than before, but in non-lactating sheep the reduction of bone phosphorus was not as severe. Both lactating and non-lactating sheep recieving adequate dietary phosphorus did not have

differences in bone composition between before and after the experimental period. These data indicate that bone phosphorus was mobilized to maintain adequate phosphorus levels in plasma in the dietary phosphorus deficient animals, but the greater mobilization occurred in lactating sheep because of the additional stress of lactation. Data from rib biopsies in deer showed mineral concentration differences due to stage of antler growth cycle (Banks et al., 1968b). Even though the molar calcium:phosphorus ratio of the ash remained constant throughout the growth period, chemical composition per unit volume (mM/ml) of calcium, phosphorus, and magnesium were lower in bone during the rapid antler growth period. In addition to mineral concentration determinations in the rib, histological evaluations were also made (Banks et al., 1968a). Numerous resorption spaces were apparent in osteonal and nonosteonal bone during antler growth. The bone porosity became more pronounced during peak of antler growth and declined after antlers ceased to grow. During the period of antler growth, both refilling osteons (bone formation) and resorbing surfaces in the ribs indicated that both processes of bone accretion and resorption were continually going on. Both functions were increased as antlers were being made and this elevated remodeling process probably made mobilized minerals more available for

antler growth. Even though both formation and resorption mechanisms were increased during antler growth, there was a net reduction in the amount of bone by the time antlers had attained peak growth. The decreased amount of bone was reflected in lower densities, mineral concentrations, and altered histological measurements.

Endocrine hormone influences on calcium metabolism in deer

Studies in calcium kinetics have been conducted in deer because of the unique calcium stress during a major part of the year. When male deer are actively growing antlers, calcium is mobilized from the skeleton and used to make antlers (Chao et al., 1984; Cowan et al., 1968; and Stephenson and Brown, 1984). Chao et al. (1984) studied serum PTH and calcitonin concentration in relation to antler growing cycles. Serum PTH increased during velvet initiation in April-May then PTH levels significantly elevated during post-antler casting. On the other hand, calcitonin increased during the rapid antler growth period. The authors suggested that increased PTH levels during velvet initiation is responsible for calcium absorption and/or mobilization. Plus increasing PTH concentration are related to final mineralization of antlers post-velvet shedding. Also, the higher levels of calcitonin during rapid antler growth may have prevented excessive bone

resorption. Another study investigated calcium and phosphorus metabolism during pregnancy and lactation in white-tailed deer (Chao et al., 1985). Plasma calcium tended to increase during pregnancy while plasma PTH and estradiol, but not calcitonin, was highest during the last trimester of pregnancy. Plasma calcitonin was higher during lactation and post-weaning than during pregnancy. The authors believed that increased PTH in late pregnancy may be responsible for increased calcium absorption and mobilization, whereas increased plasma estradiol may inhibit excessive bone resorption. After parturition, increased PTH may enhance calcium absorption and mobilization, low concentration of estradiol may allow bone resorption to proceed, and elevated calcitonin may protect the maternal skeleton from excessive bone resorption.

Factors influencing calcium metabolism in rats

Influences on bone metabolism have been studied more extensively in rats. Sinha et al. (1988) developed an in vivo bone model using implants of mineralized or demineralized bone powder to examine bone formation and resorption. In one experiment, enzyme, mineral, and histological assessment showed bone formation in demineralized implants and bone resorption in mineralized implants. Another experiment used dietary calcium and

vitamin D intakes as effects on bone parameters. Both low dietary calcium (.2%) and 1,25-VitD stimulated resorption in mineralized implants. This in vivo bone model system mimicked the physiological processes of bone formation and resorption and this could be used as a valuable tool in studying alterations in bone metabolism. A different study with rats investigated the effects of physiological concentrations of PTH and calcitonin on acid phosphatase activity (bone resorption) in cultured rat bone (Braidman et al., 1986). It is known that PTH increases osteoclastic bone resorption but the exact mechanism is incompletely understood. Increasing evidence suggests that PTH stimulates osteoclastic bone resorption indirectly through the osteoblasts. Isolated bone cells were characterized as C cells (having properties similar to osteoclasts) or B cells (osteoblast-like cells). Acid phosphatase was used as a marker for C cells. Results showed that calcitonin decreased C cells acid phosphatase, but it did not influence B cells at all. Administration of PTH increased enzyme activity in C cells when they were cultured with B cells. PTH even increased enzyme activity when C cells and B cells shared the same medium but were not in contact with each other. The authors indicated that PTH may increase acid phosphatase activity in C cells through a humoral factor produced by cells present in B cell cultures. More

research is needed to fully understand the mechanism of PTH stimulation on osteoclasts.

Changes in bone composition due to pregnancy and lactation have also been studied in rats. Pregnancy and lactation have distinct influences on bone chemistry and morphology. Data from Miller et al. (1986) showed that there were significant increases in bone weight, ash weight, calcium content, and femoral cross-sectional area by late pregnancy. During lactation, all of these parameters plus bone mass had decreased. The results also indicated that during pregnancy there are increases in bone formation rates to increase skeletal mass. During lactation, the reductions in skeletal mass are accompanied by increases in bone turnover, especially in trabecular bone. It seems that these rats increased skeletal bone mass during late pregnancy to ready the bone for increased mineral loss that occurs during lactation. In a similar lactation study, Brommage (1989) determined the milk transfer rate for calcium and phosphorus from their dams to their pups. From calculations, he determined that 19% of calcium transferred to milk was derived from the maternal skeleton with the maternal diet supplying the rest. This shows that approximately 20% of dietary calcium intakes in pups is being taken from the dam's skeletal reserves. This amount of mobilized calcium (24 mg/d) could be a serious

drain on the maternal system. Vitamin D also has an influence on mineral metabolism in lactating rats. Skeletal changes were determined in vitamin D-deficient mother rats and their litters at the end of lactation (Cancela et al., 1985). At the beginning of lactation, half the rats were given oral supplement of vitamin D but the other half remained deficient. Results showed that all vitamin D-deficient dams and pups had increased osteoclastic bone resorption and severe osteomalacia as indicated by decreased calcification rate and increased endosteal osteoid surface, volume, and thickness. The rats recieving vitamin D supplement had normal histomorphometric features in bone. However, the pups of these rats still showed rickets and osteomalacia, and had raised 1,25-VitD levels in plasma. The authors concluded that osteomalacia was produced in vitamin D-deficient rat mothers and pups and that osteomalacia was still present in pups of dams that recieved vitamin D supplement for 20 days. Similar results were cited in a study by Marie et al. (1986). This study also used vitamin D-depleted and vitamin D-repleted lactating rats. In all rats, lactation stimulated bone resorption and reduced the trabecular bone volume and decreased ash weight and calcium content. However, vitamin D-depleted rats lost twofold more calcified bone than vitamin D-repleted rats during lactation. Also, rats

recieving vitamin D had increased bone formation rates during lactation, but the other rats did not. This study, along with others, indicates that vitamin D is required for normal growth of bone (mostly endosteal bone formation) that occurs during lactation. The role of calcitonin on bone mineralization during lactation has also been explored in rats. From reports by both Lewis et al. (1971) and Taylor et al. (1975), results suggested that increased levels of calcitonin during lactation may have a special role to protect the maternal skeleton from excess demineralization. In contrast, more recent findings by Hirsch and Hagaman (1986) did not support this proposed claim.

CHAPTER 3

CALCIUM-REGULATING AND METABOLIC HORMONES AND PLASMA CALCIUM DURING THE LACTATION CYCLE OF HOLSTEIN AND JERSEY COWS

INTRODUCTION

Onset of lactation presents a challenge to the endocrine control of calcium homeostasis. Most dairy cows become hypocalcemic after parturition for 2 to 3 days, but only a few cows will develop parturient paresis. The exact cause of parturient paresis is not completely understood. Predisposing factors, such as increasing age (Horst et al., 1978; and Wohlt et al., 1984) and high calcium, prepartum diets (Manston, 1967; and Shappell et al., 1987) have been associated with increased incidence of parturient paresis at calving. Failure to maintain normal plasma calcium was not due to insufficiency of parathyroid hormone (PTH) or 1,25-dihydroxyvitamin D_3 (1,25-VitD) secretion (Horst et al., 1978). Both PTH and 1,25-VitD elevate plasma calcium through their actions on intestine, kidney, and bone.

The metabolic demands for milk production change throughout lactation. In early lactation, dairy cows experience a period of negative energy balance because dry matter intake is below the requirement for milk yield. Tissue energy stores are mobilized to supply energy needed

for milk synthesis (Bines and Hart, 1982). Both somatotropin (ST) and insulin (INS) are important metabolic regulators during early lactation. Somatotropin stimulates mobilization of free fatty acids from adipose tissue and INS regulates nutrient partitioning toward body tissues. The combination of high ST concentration and low INS concentration in early lactation facilitates increasing milk yield during negative energy balance. In later lactation, ST decreases (Herbein et al., 1985; and Smith et al., 1976) and INS increases (Herbein et al., 1985; and Vasilatos and Wangsness, 1981) and intake energy is diverted toward deposition of body tissue in addition to milk production.

Change in concentrations of calcium-regulating and metabolic hormones of dairy cattle have been determined. However, a breed comparison at different stages of lactation has not been reported. The objective of this study was to evaluate the effects of stage of lactation on plasma concentrations of total calcium (TCA), ionized calcium (ICA), ST, INS, glucose (GLU), PTH, and 1,25-VitD in Holstein and Jersey cows. Also, changes in milk production and composition, dry matter intake (DMI), and body weight (BW) were monitored.

MATERIAL AND METHODS

Eight Holstein and 8 Jersey cows were randomly selected to obtain blood samples during the lactation cycle. All animals were mature cows beginning their third or later lactation. The cows were placed into a tie-stall barn approximately 4 wk before parturition.

Blood samples were obtained every 2 wk beginning approximately 4 wk before calving and continued through 8 wk of lactation. On each sampling day, the individual samples (0800 h, 1600 h, and 0000 h) were pooled for a daily composite after plasma calcium concentration had been determined. Only samples at 2 wk before calving (-2 wk), day of calving (day 0), day after calving (day 1), 4, and 8 wk into lactation were used for hormone analyses with the exception of 1,25-VitD which was not determined at 8 wk.

Blood samples were collected by jugular venipuncture. Blood was placed in heparinized tubes, put on ice, and immediately transported to the laboratory. Plasma was harvested by centrifugation, plasma calcium concentration determined, then remaining plasma was frozen for later analyses.

Plasma calcium was determined using a Nova 7 Total calcium/ Ionized calcium Analyzer[1] which measures TCA, ICA,

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[1] Nova Biomedical

pH, and normalized ionized calcium (NORMCA). Normalized calcium ion concentration is a calculation of ionized calcium corrected to pH 7.4 (Watson and Anbar, 1985). The equation for calculating NORMCA is given below:

$$\log[Ca^{++}]_{7.4} = \log[Ca^{++}]_x - 0.24(7.4-X)$$

where:

x is measured pH of sample

$[Ca^{++}]_x$ is ionized Ca concentration in test solution

$[Ca^{++}]_{7.4}$ is normalized concentration of ionized Ca at pH=7.4

The technology to determine plasma TCA and ICA, has only become available within the past few years. Therefore, bovine ICA data has not been reported previously.

Plasma GLU was measured using the glucose oxidase method (Sigma Technical Bulletin No. 510, 1973). Plasma ST, INS, and PTH were quantified by standard homologous double antibody radioimmunoassay procedures (Yalow and Berson, 1960). Parathyroid hormone was assayed using purified canine PTH, I^{125}-labeled canine PTH and canine specific PTH antisera (Cambridge Medical Research Lab, Cambridge, MA). Concentrations of standards were linear over a range of .1 to 6.4 ng INS, .5 to 32 ng ST and .5 to 17 ng PTH. All samples were assayed in triplicate. Plasma 1,25-VitD was determined by double antibody radioimmunoassay procedures (Hollis, 1986) in the

laboratory of Dr. Bruce Hollis, Medical University of South Carolina.

Daily milk yields were used to determine a weekly average. Body weights were determined at 2 wk intervals. Two consecutive milk samples were obtained every other wk and the analysis of milk fat, protein, and solids-non-fat (SNF) percent was carried out utilizing an Infrared milk analyzer.

The diet was fed during the prepartum period consisted of 80% chopped orchardgrass hay and 20% corn silage (dry matter). Each cow recieved 1.14 kg for every 45 kg of live body weight. The diet contained .25% calcium.

After calving, all cows were fed a diet containing 28.8% corn silage, 22.4% alfalfa haylage, and 48.8% concentrate (dry matter). Chemical composition of the diet was 16.4% CP, 23.3% ADF, and 1.82% ether extract (for more details see Tables 7 and 8, Chapter 4). The diet contained .50% calcium, .39% phosphorus, and .24% magnesium.

Statistical Analysis. Stage of lactation and breed effects on all parameters were measured using the model below:

$$Y_{ijkl} = u + B_i + C_{(i)j} + S_k + (BS)_{ik} + E_{ijkl}$$

where:

Y_{ijkl} is observed dependent variable

u is mean of Y

B_i is fixed effect of i^{th} breed; i=1,2

$C_{(i)j}$ is random effect of j^{th} cow within i^{th} breed; j=1,8

S_k is fixed effect of k^{th} day, k=1,7

$(BS)_{ik}$ is the interaction between breed and day

E_{ijkl} is random residual.

Breed was tested for significance using cow(breed) mean square error. Day and breed by day interaction were tested for significance using residual error. Orthogonal contrasts were used to evaluate breed differences on day 0 and day 1 for all parameters. All analyses were performed using the general linear models in SAS (SAS, 1985) and level of significance was $P<.05$. Comparisons among means were by Tukey's multiple comparison test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Stage of lactation influenced all variables, but most of the variation was found at calving. Breed effects were apparent for several variables at calving. Least-square means for plasma ST, INS, ratio of ST:INS, GLU, PTH, and 1,25-VitD are presented in Table 1. Plasma ST concentrations were higher in both breeds 2 wk before calving compared to day of calving. Plasma ST was similar between breeds on day 0, but by day 1 Jerseys had higher ST than Holsteins. In contrast, INS declined from prepartum to calving. Both breeds had similar INS on day 0 and day 1.

TABLE 1. Least-square means for plasma somatotropin (ST) (ng/ml), insulin (INS) (ng/ml), ST:INS ratio, glucose (GLU) (mg/dl), parathyroid hormone (PTH) (ng/ml), and 1,25-dihydroxyvitamin D (1,25-VitD) (pg/ml).

Breed[a]	Day	ST	INS	ST:INS	GLU	PTH	1,25-VitD
H	-2 wk	11.4	.61	18.5	62.2	3.8	31.3
J	-2 wk	11.4	.72	16.4	57.3	4.6	34.6
H	0	22.6	.59	38.6	64.2*	16.0	71.8
J	0	21.4	.59	41.9	59.8	18.0	89.5*
H	1	18.7	.59	32.3	56.2*	8.2	85.8
J	1	30.2*	.55	60.2*	49.3	10.3	99.2*
SE		3.1	.08	5.5	2.0	2.3	5.2

[a] Breed: H = Holstein and J = Jersey.

* Breed difference ($P<.05$) on day indicated.

The ratio of ST:INS followed the same trend as ST. Both breeds had a higher ratio at calving compared to prepartum. Jerseys had a higher ST:INS ratio than Holsteins on day 1. In agreement with these findings, Sartin et al. (1985) reported that INS decreased by 30% from 14 days prepartum to 5 d postpartum, but ST was similar during this period.

Plasma GLU was lower on day 1 in both breeds, when compared to prepartum and day 0 observations. Holsteins had higher GLU than Jerseys on both days after calving. In Jerseys, plasma GLU concentration was different between day 0 and day 1 with higher GLU occurring on day 0. Sartin et al. (1985) reported similar changes in Holstein cows.

Plasma PTH increased 4-fold from prepartum to day of calving. There were no breed differences for PTH at calving. Levels of 1,25-VitD were elevated 2-fold from prepartum to day 0 in both breeds. Jerseys had higher 1,25-VitD than Holsteins on both day 0 and day 1. Shappell et al. (1987) reported that plasma PTH increased 2- to 4-fold from 14 d before calving to the time of calving in Holstein cows fed either a low or high calcium diet. Plasma 1,25-VitD was elevated 3-fold from 2 wk prepartum to day after parturition in Holsteins (Goff et al., 1989). Both Green et al. (1981) and Horst et al. (1978) found increased plasma 1,25-VitD from a few days before parturition to parturition.

Plasma calcium concentration (Table 2) declined from prepartum to calving. The degree of hypocalcemia was greater in Jersey cows. For the first 2 days postpartum, Jerseys had an average of 7.47 mg TCA/dl and Holsteins averaged 8.10 mg TCA/dl. Ionized calcium and NORMCA followed the same trend as plasma TCA. Two Jerseys had TCA below 5.0 mg/dl at calving and had to be treated for parturient paresis. Blood pH was similar for days and breed. In agreement with these findings, total calcium was the lowest at calving than at any other point during the lactation cycle (Littledike and Goff, 1987; and Wohlt et al., 1984). Plasma TCA usually starts to decline 2 to 3 d before calving (Littledike and Goff., 1987; and Shappell et al., 1987). Ionized calcium also decreased at calving in this study, but there are no published reports to compare.

The greater degree of hypocalcemia in Jerseys was accompanied by higher 1,25-VitD concentrations, when compared to Holsteins. Both breeds had elevated PTH at calving, but breed differences were not significant. Both breeds were fed the low calcium, prepartum diet, but 25% of Jersey cows developed parturient paresis. Littledike (1974) reported the incidence of parturient paresis in the Jersey breed is 12.4 to 30% versus 2.1 to 3.9% in the Holstein breed. The parturient paresis occurance rate of Jerseys in this study was within this range.

TABLE 2. Least-quare means for total calcium (TCA), ionized calcium (ICA), and normalized calcium (NORMCA) in mg/dl and pH in plasma.

Breed[a]	Day	TCA	ICA	NORMCA	pH
H	-4 wk	9.40	4.67	5.23	7.62
J	-4 wk	9.05	4.44	4.92	7.60
H	-2 wk	9.71	4.80	5.37	7.63
J	-2 wk	9.55	4.70	5.27	7.63
H	0	8.10 *	4.12 *	4.62 *	7.63
J	0	7.45	3.73	4.19	7.64
H	1	8.10 *	4.20 *	4.70 *	7.63
J	1	7.49	3.82	4.30	7.61
SE		.18	.08	.10	.01

[a] Breed: H = Holstein and J = Jersey.

* Breed difference (P<.05) on day 0 or day 1.

Plasma ST, INS, ST:INS ratio, PTH and 1,25-VitD least-square means for 4 and 8 wk are presented in Table 3. Plasma ST and ST:INS ratio declined from parturition (Table 1) to 4 wk. In contrast, INS increased as lactation advanced. In agreement, similar changes have been reported by others (Herbein et al., 1985; Smith et al., 1976; and Vasilatos and Wangsness, 1981). In Jerseys, plasma GLU was different between day 0 and 4 wk with higher GLU occuring on day 0. Also, plasma GLU was different between day 1 and 8 wk in Jerseys only with higher GLU found at 8 wk.

Plasma PTH and 1,25-VitD concentrations were lower at 4 wk postpartum, as compared to concentrations at calving. In contrast, Shappell et al. (1987) found that cows fed a low calcium (.52%), prepartum diet had similar PTH concentrations at 3 wk postpartum and calving. However, cows fed a high calcium (1.16%), prepartum diet had lower PTH 3 wk of lactation. Goff et al. (1989) reported that plasma 1,25-VitD decreased from 64 pg/ml at calving to (21 pg/ml) 4 wk postpartum.

Table 4 contains least-square means for TCA, ICA, NORMCA, and pH for 2 through 8 wk of lactation. Plasma calcium concentrations had increased and were normal by 2 wk postpartum. Total calcium, ICA and NORMCA were similar between breeds at 2, 4, 6, and 8 wk. Even though breed differences were detected at calving for plasma calcium

TABLE 3. Least-square means for somatotropin (ST) (ng/ml), insulin (INS) (ng/ml), ST:INS ratio, glucose (GLU) (mg/dl), parathyroid hormone (PTH) (ng/ml), and 1,25-dihydroxyvitamin D (1,25-VitD) (pg/ml) in plasma.

Breed[a]	Week	ST	INS	ST:INS ratio	GLU	PTH	1,25-VitD
H	4	12.4	.85	15.1	58.9	6.52	36.2
J	4	11.1	.76	15.5	51.2	4.15	37.7
H	8	17.2	.91	17.7	58.8	6.22	-
J	8	9.7	1.08	9.5	58.4	4.34	-
SE		3.1	.08	5.5	1.9	2.3	5.2

[a] Breed: H = Holstein and J = Jersey.

TABLE 4. Least-square means for total calcium (TCA), ionized calcium calcium (ICA), normalized calcium (NORMCA) in mg/dl and pH in plasma by breed at 2, 4, 6, amd 8 weeks of lactation.

Breed[a]	Week	TCA	ICA	NORMCA	pH
H	2	9.12	4.64	5.23	7.64
J	2	8.94	4.53	5.09	7.64
H	4	9.16	4.58	5.10	7.62
J	4	9.03	4.57	5.06	7.61
H	6	9.60	4.68	5.18	7.60
J	6	9.34	4.62	5.08	7.59
H	8	9.33	4.54	5.00	7.60
J	8	8.97	4.38	4.84	7.58
SE		.18	.08	.10	.01

[a] Breed: H = Holstein and J = Jersey.

concentrations, the calcium concentrations were similar between Holsteins and Jerseys after the initial stress of lactation onset was over. Plasma TCA usually returns to normal within 4 d after calving (Littledike and Goff, 1987; and Wohlt et al., 1984). In agreement with our findings, Goff et al. (1989) found normal plasma TCA (> 8.0 mg/dl) at 2 wk after calving.

The effects of stage of lactation and breed on ICA as a percent of TCA (ICA/TCA) were also evaluated (Table 5). Stage of lactation significantly influenced ICA/TCA, but breeds did not differ at any point in lactation. The percent ICA/TCA increased from prepartum to calving with the higher ICA/TCA occuring at calving. The higher percent occurred in conjunction with higher plasma PTH and 1,25-VitD concentration. Enhanced mobilzation from bone due to increased plasma PTH and 1,25-VitD at calving would increase available calcium, that is ionized calcium. After calving, ICA/TCA gradually decreased through 8 wk of lactation. The changes in ICA/TCA are numerically small, but statistically significant. These small changes in ICA, which is the available calcium, may be physiologically important. The low ICA/TCA levels in early lactation coincide with the period of negative energy balance and negative calcium balance as evidenced by balance trial results (see chapter 5). The lowest ICA/TCA, 48.7%,

TABLE 5. Least-square means for ionized calcium as a percent of total calcium (ICA/TCA) for all lactation points (week) for all cows.

Week	ICA/TCA[a]
-4	49.4
-2	49.3
Day 0	51.0
2	50.8
4	50.3
6	49.1
8	48.7
SE	.4

[a] Significant week difference ($P<.05$) between day 0 and 8 wk.

occurred at 8 wk which was peak lactation for most cows. The lower ICA/TCA at peak lactation may be attributed to the severe drain of calcium from the blood to mammary gland to support milk synthesis. These results indicate that ICA as a percent of TCA is influenced by stage of lactation. Studying ICA concentration, as well as TCA, may provide additional information on the calcium homeostatic system.

There were obvious breed differences between Holsteins and Jerseys for milk yield and composition, DMI, and BW. Jerseys had higher milk fat %, protein %, and SNF %. Milk yield, DMI, and BW were greater for Holsteins. Percent BW change was greater for Jerseys than Holsteins in early lactation where Jerseys lost more weight as a percent of original weight.

In order to evaluate intake and production measures, several ratios were calculated. Milk (kg) per kg of DMI, DMI (kg) per kg of metabolic BW (MBW), and milk (kg) per kg MBW are presented in Table 6. Milk produced per unit of DMI was higher for Holsteins. Dry matter intake and milk yield per kg of MBW also were higher for Holsteins. Thus, Holsteins seem to have a higher capacity for feed intake and appear to be more efficient in the conversion of DMI to milk. Even though the volume of milk was lower in Jerseys, they had significantly higher milk fat, protein, and SNF % in their milk. Therefore, breeds were compared on the basis

TABLE 6. Least-square means for ratios[a] of M/DMI, DMI/MBW, M/MBW, and ME/NE_L.

Breed[b]	Week	M/DMI	DMI/MBW	M/MBW	ME/NE_L
H	2	1.88 *	.146	.273 *	.833
J	2	1.56	.155	.237	.828
H	4	1.83 *	.178	.322 *	.818
J	4	1.66	.167	.279	.858
H	6	1.74	.187 *	.322 *	.760
J	6	1.70	.163	.280	.900 *
H	8	1.69 *	.193 *	.326 *	.718
J	8	1.54	.172	.265	.819 *
SE		.06	.006	.008	.03

[a] Ratios designation are M = milk yield (kg), DMI = dry matter intake (kg), MBW = metabolic body weight, ME = milk energy output (Mcal), and NE_L = net energy intake (Mcal).

[b] Breed: H = Holstein and J = Jersey.

* Significant difference ($P<.05$) between breeds at week indicated.

of milk energy output per unit of NE_L intake. The ratio was similar at 2, 4, and 8 wk of lactation. Jerseys had a higher ratio of conversion at 6 wk than Holsteins. The Jerseys had a lower ratio of DMI/MBW than Holsteins but they had a similar ratio of M/DMI to Holsteins at 6 wk. Jerseys were consuming less dry matter on a MBW basis, but they were producing the same amount of milk on a MBW basis. Jerseys were more efficient at converting NE_L intake into milk at 6 wk of lactation.

In conclusion, stage of lactation and breed effects were apparent at calving. Jerseys had higher ST and 1,25-VitD, but lower GLU. Plasma PTH was similar between breeds at calving. Also, plasma TCA, ICA, and NORMCA were lower in Jerseys. After the stress of parturition and onset of lactation, breeds had similar plasma hormone and calcium profiles. Ionized calcium concentration as a percent of TCA was influenced by stage of lactation. This change could be physiologically important in that it may be a better estimate of calcium availability for milk synthesis during early lactation than total plasma calcium.

CHAPTER 4

METABOLIC AND CALCIUM-REGULATING HORMONES AND PLASMA CALCIUM IN LACTATING HOLSTEIN AND JERSEY COWS FED SUPPLEMENTAL DIETARY FAT

INTRODUCTION

Energy intake of dairy cows can be increased by the addition of fat to the diet. The greater energy density as a result of fat can increase milk production, especially by high-producing cows and cows in early lactation (Bines and Hart, 1982; Kronfeld et al., 1980; and Palmquist and Jenkins, 1980). Dietary fat supplemention may spare excessive mobilization of body tissue reserves. Supplementation of dietary fat, however, may influence digestibilities of other nutrients. Additional fat may lower fiber digestibility in the rumen (Chalupa, 1984; and Kowalczyk et al., 1977) by interfering with bacterial metabolism and attachment to feed particles. Saturated fatty acids are less likely to alter fermentation than unsaturated fatty acids, because saturated fatty acids are less soluble in the rumen (Chalupa et al., 1984). Saturated fatty acids combine more readily with metal ions to form insoluble soaps of fatty acids (Chalupa et al., 1982; and Jenkins and Palmquist, 1982). Insoluble soap formation decreases the negative effects of fat on fiber digestion by rumen bacteria (Jenkins and Palmquist, 1984;

and Schauff and Clark, 1989). Possible disadvantages of soap formation include reduced digestibility of fat and minerals.

Composition of milk may be altered by the addition of fat to the diet. Lower digestibility of fiber may lower milk fat percent by reducing the ratio of acetate to propionate (Jenkins and Palmquist, 1984). Milk fat percent has been reported to increase, decrease, or remain unchanged depending on the type of dietary fat. Milk protein percent was decreased with the addition of protected fat (Dunkley et al., 1977; and Palmquist and Moser, 1981) and calcium salts of long chain fatty acids (Grummer, 1988; and Kent and Arambel, 1988). Other reports have shown no influence of fat on milk protein percent (Palmquist and Conrad, 1978; Palmquist and Conrad, 1980; and Schauff and Clark, 1989).

Dietary fat addition also may influence the endocrine system. It has been suggested that additional fat may alter glucose metabolism by decreasing production of glucose precusors through changes in ruminal fermentation (Smith et al., 1978). In contrast, mean plasma glucose was increased by addition of tallow to the diet (Kronfeld et al., 1980). Palmquist and Moser (1981) observed a 25% decrease in plasma insulin when protected tallow was fed to dairy cows, but in a second experiment the trend was not

seen. Plasma insulin was increased in cows fed 7.5% fat from whole cottonseed diet, while somatotropin remained unchanged (Cummins and Sartin, 1987).

The objective of this study was to evaluate the effects of dietary tallow on plasma concentrations of somatotropin (ST), insulin (INS), ratio of ST:INS, glucose (GLU), parathyroid hormone (PTH), 1,25-dihydroxyvitamin D_3 (1,25-VitD), total calcium (TCA), and ionized calcium (ICA) in Holstein and Jersey cows. Milk production and composition, dry matter intake (DMI), and body weight (BW) responses are also presented.

MATERIAL AND METHODS

Eight Holstein and 8 Jersey cows were fed a control diet from calving through 8 wk of lactation. From 9 wk through 21 wk, 4 cows of each breed were fed a diet supplemented with tallow. The control and tallow diets contained 28.8% corn silage, 22.4% alfalfa haylage, and 48.8% concentrate dry matter. Composition of the control and tallow concentrates are given in Table 7. The ingredients in the concentrates were the same, except tallow was substituted for corn grain at 4% of the total concentrate to obtain a diet with approximately 2% supplemental tallow. The tallow used in this diet was a mixture of rendered animal fats. Chemical analysis for the

Table 7. Composition of ingredients in the control and tallow concentrate.

	%	
Ingredient	Control	Tallow
Corn grain	59.10	55.10
Tallow	-	4.00
Soybean meal (44% CP)	26.92	26.92
Dried brewer's grains	11.90	11.90
Trace mineral salt	.80	.80
Limestone	.42	.42
Sodium bicarbonate	.35	.35
Dicalcium phosphate	.32	.32
Magnesium oxide	.17	.17
Vitamins A,D, and E[1]	.02	.02

[1] Concentrates contain 6608 IU/kg vitamin A, 617 IU/kg vitamin D and 27 mg/kg vitamin E.

control and tallow diets are given in Table 8.

Blood samples were collected by the procedures detailed in Chapter 3. Samples were obtained at wk 8, 12, 14, 16, 18, and 20 of lactation. Plasma GLU, and PTH concentrations were determined at 8, 12, 16, and 20 wk of lactation. Plasma 1,25-Vit D concentration was determined at 4, 12, 16, and 20 wk. All other parameters were determined at all weeks. Plasma hormone analyses and calcium determination were measured according to procedures listed in Chapter 3.

Statistical analysis. The effects of dietary tallow on all variables were analyzed using the repeated option in General Linear Models (SAS,1985). Observations at 8 wk of lactation were used as covariate in the model for all parameters expect 1,25-VitD which used observation at 4 wk as covariate. The observations during the dietary treatment period (12, 14, 16, 18, and 20 wk) were tested for effects from breed and diet. Each week observation was analyzed separately with the covariate in the model. The model is given below:

$$Y_{ijk} = u + B_i + D_j + (BD)_{ij} + b(X_{(ij)k} - \bar{X}_{...}) + e_{(ij)k}$$

where:

Y_{ijk} is observation of k^{th} cow of i^{th} breed fed j^{th} diet

u is mean of Y

B_i is fixed effect of i^{th} breed; i=1,2

Table 8. Chemical composition of the control and tallow diet.

	%	
Component	Control	Tallow
DM	57.6	57.5
CP	16.4	16.7
ADF	23.3	23.7
EE	1.82	3.55
Ca	.50	.52
P	.39	.38
Mg	.24	.24

D_j is fixed effect of j^{th} diet; j=1,2

$(BD)_{ij}$ is the interaction between breed and diet

$b(X_{(ij)k} - X_{...})$ is effect explained by the difference of $X_{(ij)k}$ minus $\bar{X}_{...}$ (covariant)

$e_{(ij)k}$ is residual error.

RESULTS AND DISCUSSION

Least-squares means from the analysis with the covariant for ST are given in Table 9. The means at 8 wk for each group are included in the table for comparison, but all Holstein and Jersey cows were fed the control diet at 8 wk. Significant breed effects (without consideration of diet) were found at 4 of the 5 wk sampled during the fat-feeding period. Holsteins had higher ST throughout this treatment period with significant breed differences occurring at 14, 16, 18, and 20 wk of lactation. Diet (without consideration of breed) affected ST during the fat-feeding period. Cows fed the tallow diet had lower ST at 14 and 18 wk with 16 wk approaching significance (P=.11). Holsteins fed tallow diet had lower ST during this period. In contrast, Cummins and Sartin (1987) reported no differences in ST between control diet cows or cows fed 7.5% fat from whole cottonseed diet.

Table 10 contains the least-squares means for INS for breed and diet. Plasma INS was similar between Holsteins

Table 9. Least-squares means for somatotropin concentration (ng/ml) in plasma.

Breed[ab]	Diet[c]	Week of lactation					
		8[d]	12	14	16	18	20
H	C	10.6	7.97	8.11*	7.57	6.56*	8.66
H	T	11.7	7.91	5.51	6.95	4.88	6.60
J	C	10.0	8.25	5.09	6.13	4.45	4.89
J	T	7.4	5.73	4.85	4.38	4.16	4.27
SE			1.17	.56	.68	.35	1.28

[a] Breed: H = Holstein and J = Jersey.

[b] Significant breed difference (P<.05) at 14, 16, 18, and 20 wk.

[c] Diet: C = control and T = tallow.

[d] Means which are covariate for each diet group.

* Significant difference (P<.05) due to diet within breed.

Table 10. Least-squares means for insulin concentration (ng/ml) in plasma.

		Week of lactation					
Breed[a]	Diet[bc]	8[d]	12	14	16	18	20
H	C	.88	1.05	1.13	1.08	1.12	1.04
H	T	.94	.97	1.30	1.06	1.20	1.00
J	C	.91	1.11	1.29	1.03	1.30	1.34
J	T	.99	1.14	1.14	1.20	1.30	.90
SE			.10	.11	.12	.10	.10

[a] Breed: H = Holstein and J = Jersey.

[b] Diet: C = control and T = tallow.

[c] Significant diet difference (P<.05) for 20 wk.

[d] Means which are covariate for each diet group.

* Significant difference (P<.05) due to diet within breed.

and Jerseys. Dietary influences were found at wk 20 only where cows fed tallow diet had significantly lower INS at this wk. Looking more closely, plasma INS was lower in the Jerseys only at wk 20. We do not attach any physiological significance to the lower INS observation at 20 wk because only 1 observation out of 5 showed any difference. Horner et al. (1986) reported that INS tended to be lower in Holstein cows fed a diet containing 5% total fat from whole cottonseed diet. Palmquist and Moser (1981) found conflicting results with INS when fat was fed to Jersey cows in 2 experiments. In experiment 1, plasma INS concentration was increased by fat feeding, but INS concentration was depressed by fat supplementation in experiment 2. The reason for the difference is unknown, but a possible explanation may reside in the type of fat fed to the cows. Unprotected fat was fed in the first experiment, but protected (formaldehyde-treated) lipid supplement was fed in the second experiment. Another study reported higher plasma INS when cows were fed a high fat diet (whole cottonseed) at 7 wk of lactation, but decreased plasma INS at 14 wk of lactation (Cummins and Sartin, 1987). Fat supplementation apparently causes inconsistent responses, dependent upon type of dietary fat and length of feeding period.

The ST:INS ratio followed the same trends as ST. Significant breed effects were found at wk 14, 18, and 20 of lactation where Holsteins had a higher ratio than Jerseys. Holstein, but not Jersey, cows fed the control diet had a significantly higher ratio of ST:INS at 14 and 18 wk than Holsteins fed tallow diet (Table 11).

Least-squares means for plasma GLU concentration by breed and diet are given in Table 12. Neither breed nor diet had any influence on GLU during the fat-feeding period. These results are in agreement with a number of reports. Addition of groundnuts (Steele, 1984), soybeans (Palmquist and Conrad, 1978; and Steele, 1985), hydrolyzed fat (Palmquist and Conrad, 1978), and protected oil seed (Yang et al., 1978) did not influence plasma GLU. Other reports have found effects of fat addition on GLU concentration. Kronfeld et al. (1980) reported that mean plasma GLU was increased in tallow-fed Holsteins during 6 through 26 wk of lactation. Palmquist and Moser, 1981 reported a similar response. Kronfeld et al. (1980) suggested the elevated GLU was possibly due to utilization of dietary fat as a cellular energy substrate, sparing utilization of GLU. In contrast, Horner et al. (1986) found lower GLU in cows fed whole cottonseed and Palmquist and Moser (1982) found lower GLU in Jerseys fed a mixture of animal-vegetable fat. Smith et al. (1978) suggested that

Table 11. Least-squares means for ratio of somatotropin to insulin.

Breed[ab]	Diet[c]	Week of lactation					
		8[d]	12	14	16	18	20
H	C	13.3	7.33	7.57*	7.01	5.93*	8.04
H	T	12.4	8.43	4.47	6.72	4.19	6.82
J	C	11.5	7.77	4.41	6.58	3.52	3.96
J	T	7.4	5.39	4.08	3.75	3.01	4.60
SE			1.24	.97	1.11	.55	1.22

[a] Breed: H = Holstein and J = Jersey.

[b] Significant breed difference (P<.05) at 14, 18, and 20 wk.

[c] Diet: C = control and T = tallow.

[d] Means which are covariate for each diet group.

* Significant difference (P<.10) due to diet within breed.

Table 12. Least-squares means for glucose concentration (mg/dl) in plasma.

		Week of lactation			
Breed[a]	Diet[b]	8[d]	12	16	20
H	C	59.0	57.8	62.3	63.0
H	T	58.7	58.9	63.3	60.6
J	C	59.0	54.0	60.0	61.0
J	T	57.7	60.7	60.8	59.0
SE			2.9	2.2	2.7

[a] Breed: H = Holstein and J = Jersey.

[b] Diet: C = control and T = tallow.

[d] Means which are covariate for each diet group.

excess dietary fat might alter GLU metabolism by decreasing production of GLU precursors during ruminal fermentation.

Table 13 contains least-squares means for PTH and 1,25-VitD concentrations in plasma by breed and diet. Both breeds had similar PTH during the fat-feeding period. Plasma 1,25-VitD also was similar between breeds. Dietary tallow did not influence either PTH or 1,25-VitD concentration.

Least-squares means for TCA by breed and diet are given in Table 14. Breed did not affect TCA. However, diet significantly ($P<.05$) influenced TCA concentration at 20 wk lactation, with observations at wk 12 ($P=.13$) and 18 wk ($P=.11$) approaching significance. At wk 20, both Holstein and Jersey cows fed the tallow diet had higher plasma TCA, despite the lack of change in the calcium-regulating hormones. Part of the experiment not reported in this section was a nutrient balance trial at 21 wk of lactation. Balance trial results (see Chapter 5) indicate higher plasma calcium in tallow fed cows was due to greater digested calcium and lower urinary calcium excretion. In contrast to our results, addition of free oils of soybeans (Steele, 1985) or groundnuts (Steele, 1984) reduced plasma calcium concentration. Kronfeld et al. (1980) reported that addition of protected tallow in diets of Holsteins did not influence plasma calcium. In agreement, blood calcium

Table 13. Least-squares means for parathyroid hormone (PTH) (ng/ml) and 1,25-dihydroxyvitamin D (1,25-VitD) (pg/ml) concentration in plasma by breed (B) and diet (D).

		Week of lactation							
		8		12		16		20	
B[a]	D[b]	PTH[c]	1,25-VitD[d]	PTH	1,25-VitD	PTH	1,25-VitD	PTH	1,25-VitD
H	C	6.44	38.1	9.49	40.0	5.25	32.7	5.87	44.0
H	T	6.00	34.4	8.34	46.2	8.63	35.3	4.68	40.0
J	C	4.54	39.7	9.68	45.0	5.30	36.9	4.51	35.9
J	T	4.15	35.7	5.70	33.4	5.78	36.8	4.92	37.7
SE				3.90	7.2	1.61	5.2	1.08	3.1

[a] Breed: H = Holstein and J = Jersey.

[b] Diet: C = control and T = tallow.

[c] Means which are covariate for each diet group.

[d] Means which are covariate foe each diet group.

Table 14. Least-squares means for total calcium concentration (mg/dl) in plasma.

		Week of lactation					
Breed[a]	Diet[b]	8[c]	12	14	16	18	20
H	C	9.23	9.05	9.23	8.94	9.12	9.16
H	T	9.45	9.20	8.87	9.02	9.56	9.55*
J	C	8.83	8.49	9.05	8.88	9.06	9.16
J	T	9.08	9.49	9.60	8.98	9.21	9.58*
SE			.36	.15	.24	.17	.19

[a] Breed: H = Holstein and J = Jersey.

[b] Diet: C = control and T = tallow.

[c] Means which are covariate for each diet group.

* Significant difference (P<.05) due to diet.

was not altered by addion of emulsified animal fat to diets of Holstein calves (Fielding et al., 1985).

Plasma ICA (Table 15) followed the same trends as TCA. Breed effects were not detected. In general, both Holstein and Jersey cows recieving tallow diet tended to have higher ICA. Diet effects were apparent at 20 wk ($P<.05$) and at 12 wk ($P=.12$) and wk 14 ($P=.14$), the dietary differences approached significance. Despite differences in TCA and ICA due to diet, diet did not influence ICA as a percent of TCA (Table 16). A breed difference was found at 18 wk where Jerseys had a higher percent (50.7) than Holsteins (48.6).

Least-squares means for dry matter intake are given in Table 17. Analysis of dry matter intake was conducted separately for each breed. Holsteins consumed more DM than Jerseys. Within breed, DMI was similar between cows fed control and cows fed tallow diet. Due to greater energy density in the tallow diet, all cows fed the tallow diet had greater energy intake than respective controls. Increased energy density might be expected to reduce DMI, but most reports concluded no influence of dietary fat on DMI. Addition of tallow (Palmquist and Conrad, 1980), blended animal-vegetable fat (Palmquist and Conrad, 1978), or whole cottonseed (Horner et al., 1986; and Smith et. al., 1981) in the diet did not alter DMI. However, DMI was

Table 15. Least-squares means for ionized calcium concentration (mg/dl) in plasma.

		Week of lactation					
Breed[a]	Diet[b]	8[c]	12	14	16	18	20
H	C	4.53	4.43	4.48	4.49	4.47	4.47
H	T	4.54	4.68	4.66	4.55	4.60	4.69*
J	C	4.33	4.21	4.56	4.49	4.59	4.56
J	T	4.41	4.48	4.66	4.61	4.66	4.67*
SE			.15	.08	.11	.08	.06

[a] Breed: H = Holstein and J = Jersey.

[b] Diet: C = control and T = tallow.

[c] Means which are covariate for each diet group.

* Significant difference (P<.01) due to diet.

Table 16. Least-squares means for ionized calcium as a percent of total calcium.

		Week of lactation					
Breed[a]	Diet[b]	8[c]	12	14	16	18	20
H	C	48.3	49.1	48.8	50.6	49.1	49.2
H	T	48.9	50.7	52.3	50.1	48.0	48.9
J	C	48.7	49.7	50.3	50.4	50.7	49.3
J	T	49.1	47.4	48.9	51.8	50.8	49.1
SE			1.1	.7	1.6	.9	.7

[a] Breed: H = Holstein and J = Jersey.

[b] Diet: C = control and T = tallow.

[c] Means which are covariate for each diet group.

Table 17. Dry matter intake (kg) least-squares means.

		Week of lactation					
Breed[a]	Diet[b]	8[c]	12	14	16	18	20
H	C	24.2	23.6	23.8	25.9	26.5	25.7
H	T	23.4	26.9	26.4	26.3	25.5	23.8
SE			1.4	1.3	1.1	1.0	1.1
J	C	16.8	16.4	16.6	17.1	16.6	17.4
J	T	15.3	17.5	18.4	18.5	18.9	18.4
SE			1.3	1.0	1.5	1.2	.7

[a] Breed: H = Holstein and J = Jersey.

[b] Diet: C = control and T = tallow.

[c] Means which are covariate for each diet group.

lower for cows fed tallow soaps or tallow fatty acids compared to cows fed a control diet (Jenkins and Palmquist, 1984).

Least-squares means for BW are given in Table 18. Holsteins had obviously higher BW than Jerseys. Body weight differences due to diet were apparent in Holsteins, but not Jerseys. Holsteins fed the tallow diet had significantly higher BW at 14, 16, and 20 wk of lactation and dietary differences approached significance (P=.11) at 12 wk. Most reports indicate that BW is not affected by the addition of fat to the diet. Body weights were not influenced by addition of tallow (Kronfeld et al., 1980; and Palmquist and Conrad, 1980), hydrolyzed fat (Palmquist and Conrad, 1978), or calcium salts of long chain fatty acids (Kent and Arambel, 1988) to diets of dairy cows.

Milk yield least-squares means are listed in Table 19. Holsteins fed tallow produced significantly more milk throughout the fat-feeding period. However, milk yield was similar between control and tallow-fed Jerseys at all points. The higher milk yield of the Holsteins fed tallow does not coincide with the lower plasma ST concentration of this group. Stull et al. (1957) observed a significant 10% increase in milk production by mid-lactation cows supplemented with dietary tallow. In contrast, other authors reported no change in milk production when cows

Table 18. Body weight (kg) least-squares means.

Breed[a]	Diet[b]	Week of lactation					
		8[c]	12	14	16	18	20
H	C	601	598	613	624	633	637
H	T	596	624	646*	647*	642	661*
SE			8	6	6	7	9
J	C	413	418	428	431	432	434
J	T	416	422	428	433	438	444
SE			7	6	6	7	4

[a] Breed: H = Holstein and J = Jersey.

[b] Diet: C = control and T = tallow.

[c] Means which are covariate for each diet group.

* Significant difference (P<.05) due to diet.

Table 19. Milk yield (kg) least-square means.

Breed[a]	Diet[b]	Week of lactation					
		8[c]	12	14	16	18	20
H	C	41.0	35.7	36.3	36.5	35.8	35.3
H	T	39.4	42.8*	42.7*	40.9*	39.9*	39.5*
SE			.8	1.0	1.0	.9	1.1
J	C	26.4	22.0	21.6	22.4	21.4	20.1
J	T	22.8	21.9	23.2	21.9	22.7	22.4
SE			1.9	1.4	1.4	.9	.9

[a] Breed: H = Holstein and J = Jersey.

[b] Diet: C = control and T = tallow.

[c] Means which are covariate for each diet group.

* Significant difference ($P<.05$) due to diet.

were fed dietary tallow (Dunkley et al., 1977; and Palmquist and Conrad, 1980). A majority of published reports indicate that milk yield is not influenced by addition of fat in the form of whole cottonseed (Cummins et al., 1987; Horner et al., 1986; and Smith et al., 1981) or calcium salts of fatty acids (Grummer, 1988; Kent and Arambel, 1988; and Schauff and Clark, 1989).

Least-squares means for milk fat %, protein %, and SNF % by breed and diet are listed in Appendix Tables 41, 42, and 43. Jerseys had higher milk fat, protein, and SNF % than Holsteins at all lactation points. Cows fed the tallow diet had similar fat % to cows fed the control diet of both breeds. As for protein %, Holsteins fed tallow had lower protein % at 18 ($P<.01$) and 20 ($P<.05$) wk. On either dietary treatment, Jerseys had similar protein % during the treatment period. At 20 wk of lactation, Holsteins fed the tallow diet had lower SNF % than Holsteins fed control diet. All of the other observations were similar between tallow- and control-fed cows in both breeds. Several authors have found no change in milk fat % when fat was added to the diet (Grummer, 1988; Kent and Arambel, 1988; and Schauff and Clark, 1989). In agreement with our findings at 18 and 20 wk, milk protein % was lower in cows fed fat diets (Kent and Arambel, 1988; and Palmquist and Moser, 1981). Feeding protected tallow decreased milk

protein % (Dunkley et al., 1977) and reduced protein content was attributed to a decrease in the casein fraction. Smith et al., (1981) reported reduced milk protein % and SNF % in cows fed whole cottonseed diets containing 6% total fat in the diet.

In summary, fat supplementation influenced several variables. Supplemental energy provided by dietary tallow was utilized for additional milk yield and body weight gain in Holsteins, but not Jerseys. Plasma ST and ratio of ST:INS was lower in tallow fed cows in Holsteins. Both plasma TCA and ICA concentration was higher in cows fed tallow of both breeds. Dietary tallow did not affect ICA as a percent of TCA. Plasma GLU, PTH, and 1,25-VitD were not influenced by dietary treatment in either breed.

CHAPTER 5

ENERGY, NITROGEN, CALCIUM, AND MAGNESIUM BALANCE OF LACTATING HOLSTEIN AND JERSEY COWS FED SUPPLEMENTAL DIETARY FAT

INTRODUCTION

Although fat usually comprises a small percent in the ruminant diet, ruminants depend more on non-glucose sources for energy than do nonruminants. In early lactation, output of fatty acids in milk usually exceeds dietary fatty acid intake, resulting in body weight loss. Lipid metabolism, therefore, plays an important role in the utilization of energy during early lactation in dairy cows.

The primary reason for including supplemental lipid in the diet is to increase the energy density. As long as dry matter intake remains constant, dietary energy intake will increase. Supplemental dietary lipid, however, may affect dry matter intake and digestible energy intake. Calculated net energy intake was increased by whole cottonseed (Horner et al., 1986) and metabolizable energy intake was increased by groundnut oil (Steele, 1984) addition to the diet. In contrast, addition of tallow (Steele, 1984) did not alter energy intake and addition of soybean oil (Steele, 1985) caused an apparent reduction in energy intake, because dry matter intake was decreased.

Energy digestibility, however, can be increased with increasing caloric densities (Bull et al., 1976). Lipid addition caused increases in energy digestibility when protected tallow (Kronfeld and Donoghue, 1980) or whole cottonseed (Smith et al., 1981) was fed, but prilled fat (Schauff and Clark, 1989) and calcium salts of fatty acids (Jenkins and Palmquist, 1984) did not influence energy digestibility.

Nitrogen digestibility also may be influenced by fat supplementation. Digestibility of nitrogen was increased when whole cottonseed was added to a basal diet (Smith et al., 1981) and addition of hydrolyzed fat increased nitrogen digestibility (Palmquist and Conrad, 1978). Other studies reported no change in nitrogen digestibility due to addition of tallow (Jenkins and Palmquist, 1984; and Palmquist and Conrad, 1980).

Free fatty acids combine with metal ions, especially calcium, in the rumen and distal portions of the digestive tract to form insoluble salts. Digestibility of calcium and magnesium were reduced by the addition of tallow (Jenkins and Palmquist, 1984) and protected tallow in the diet (Kronfeld and Donoghue, 1980). Other studies indicated that digestibility of calcium was not affected by prilled fat (Schauff and Clark, 1989), calcium salts of palm oil (Filley et al., 1987) or hydrolyzed fat

(Palmquist and Conrad, 1978). Addition of protected tallow did not influence plasma concentrations of calcium and magnesium (Kronfeld et al., 1980). It is apparent that interactions between dietary calcium and supplemental lipid are not consistent.

The objective of this study was to determine the effects of breed and breed and dietary treatment on energy, nitrogen, calcium, and magnesium balance in Holstein and Jersey cows fed a control or tallow supplemented diet.

MATERIAL AND METHODS

Eight mature Holsteins and 8 mature Jerseys were used to determine nutrient balances at 8 and 21 wk of lactation. All cows were fed a control diet (Table 8) from calving to 8 wk. Four cows of each breed were fed a tallow supplemented diet (Table 8) from 9 through 21 wk. The remaining 4 cows in each breed were fed the control diet through 21 wk.

Total collections of feces, urine, and milk were conducted in a small 6-stanchion tie stall barn. Four to 6 cows were grouped by stage of lactation (8 wk + 6 days) to determine nutrient balances in 3 one-week periods. The same grouping procedure was used to determine nutrient balances again at 21 wk. Cows were moved into the barn

the day before starting each trial (day 1) and remained in individual tie stalls until the end of the 5th day. The beginning of day 1 started at 0800 h. Body weights were determined before cows entered and immediately after cows were removed from the barn.

Diets were fed as two equal feedings at 0800 and 2000 h each day. Each diet was sampled prior to feeding. At 0700 h feed refusals (orts) were weighed and sampled. Cows were milked at 0400 h and 1600 h.

All feces and urine produced in a 24 h period was collected. As feces was excreted, it was removed from the plywood floor with a trowel and placed in individual covered containers. Someone was "on duty" at all times throughout each day to insure that feces were collected and properly stored at all times. Urine was collected via soft, molded urine cups designed in the laboratory of Dr. R. L. Belyea, in the Department of Dairy Science, University of Missouri, Columbia, MO. The installation procedure for the external urine cups involved several steps. Two days before the cows were moved into the barn, the area behind the hip bone was clipped and cleaned. Velcro anchor patches were glued on the rump and thigh on each side of the cow. When the cows were moved into the trial room, straps connected the urine cup to the velcro patches. The urine cup was positioned correctly when the

top of the cup was approximately 5 cm below the anus. A drainage tube connected the urine cup to the top of a storage container a few inches below floor level. Storage containers were 1 gallon polypropylene jugs, which were emptied into a 5-gallon polyethylene container throughout the day as each of the small jugs was filled. Sulfuric acid (50% w/v) is added (50 ml in 1 gallon) to the urine containers.

Total weight of feces and urine was recorded daily at 0800 h. Feces in the storage container was mixed throughly before sampling. Two pre-weighed aluminum pans were filled with approximately 300 g wet feces. Urine in the large storage container was mixed throughly before removing two 100 ml samples. After the 1600 h milking, 2 ml of milk for every .45 kg produced were kept refrigerated and composited with a sample from the 0400 h milking. Composited daily milk samples and the urine samples were frozen for later analyses. Feed, orts, and fecal samples were weighed, then dried at 60 C until weight was constant. Dried samples were ground through a 2 mm screen in a Wiley mill and composited for a weekly sample. Frozen daily samples of milk and urine were thawed, mixed throughly and composited, then refrozen in 5 ml aliquots for later analyses. Milk and urine dry matter content were determined by freeze-drying.

All the balance trial samples were analyzed for fat (except urine), energy, nitrogen, calcium, and magnesium content. Percent fat was determined by ether extraction using a Tecator Soxtec System HT 1043 Extraction Unit (Tecator AB in Hoganas, Sweden). Nitrogen content was determined by Kjeldahl procedure using a Tecator Kjeltec System 1002 Distilling Unit. Energy content was measured by bomb calorimetry using a Parr Adiabatic Calorimeter (Parr, Moline, Illinois). Samples for mineral analysis were digested to remove organic components by the wet digestion procedure (Emerson, 1975). Calcium and magnesium concentrations were determined by atomic absorption spectrophotometry (Ramberg et al., 1975) using digested solution diluted in 0.1% w/v lanthanum chloride.

In order to evaluate intake and milk production by breed, several ratios were calculated. Milk (kg) per kg of DMI (M/DMI), DMI (kg) per kg metabolic body weight (MBW) (DMI/MBW), milk (kg) per kg MBW (M/MBW), and milk energy (LE) output per unit of NE_L intake (LE/NE_L) were analyzed for the feeding period between the 2 balance trials. Milk energy (Mcal) was calculated as suggested by Tyrrell and Reid (1965) and NE_L was determined using NRC (1989) feed values. The above ratios were calculated for each breed at 12, 14, 16, 18, and 20 wk, with the respective ratios at 8 wk used as a covariate.

Statistical analysis. The effect of breed at 8 wk and the effects of breed and dietary treatment at 21 wk were evaluated using General Linear Models (SAS, 1985). The model used in the analysis of dependent variables at 21 wk is given below:

$$Y_{ijk} = u + B_i + D_j + (BD)_{ij} + e_{ijk}$$

where:

Y_{ijk} is observed dependent variable

u is mean of Y

B_i is fixed effect of i^{th} breed; i=1,2

D_j is fixed effect of j^{th} diet; j=1,2

$(BD)_{ij}$ is the interaction between breed and diet

e_{ijk} is random residual

Diet and breed by diet interaction were removed from the model to evaluate breed differences at 8 wk. For analyses concerning nitrogen, calcium, and magnesium status, the dependent variables included amounts for intake, fecal, urinary, milk, and digested. Apparent digestibility and overall balance for each of these dietary measurements were also evaluated. For energy status, the above variables plus metabolizable energy (ME), tissue energy (TE), calculated heat production, and partial efficiency of ME utilization for LE (LE/ME) were investigated. Metabolizable energy was calculated using the equation suggested by Moe and Tyrrell (1976). The ratios M/DMI,

DMI/MBW, M/MBW, and LE/NE_L were analyzed using an analysis of covariance as listed on p. 90.

RESULTS AND DISCUSSION

Least-squares means for DMI, initial BW and milk production and composition during the balance trial at 8 wk are given in Table 20. Holsteins had higher DMI, BW, and milk yield than Jerseys, but body weight loss as a percent of original weight was not significantly different between breeds. Jerseys had higher percent fat, protein, and magnesium than Holsteins in milk.

Energy Balance

Least-squares means for energy measurements at 8 wk are presented in Table 21. Energy intake was greater for Holsteins. However, fecal energy was not significantly different. Therefore, apparent digestibility of the control diet was higher for the Holsteins. Metabolizable energy, which was calculated from DE, was also higher for the Holsteins. Milk (LE) and maintenance energy (MaE) was higher for Holsteins than Jerseys. However, mobilized tissue energy was higher for Jerseys, due to greater body weight loss during the balance trial. Calculated total heat production was higher for the Holsteins. The partial efficiency of ME utilization for lactation (LE/ME) was higher for Jerseys than Holsteins.

Table 20. Least-squares means for dry matter intake (DMI), initial body weight (BW), body weight change, milk yield, and percent fat, protein, solids-not-fat (SNF), calcium (Ca), and magnesium (Mg) at wk 8.

	Holstein	Jersey	SE
DMI, kg	22.8*	16.0	.4
initial BW, kg	616*	432	12
BW change, %	-2.0	-3.0	.6
milk, kg	38.8*	25.3	1.0
fat, %	3.42	4.68*	.25
protein, %	2.98	3.45*	.10
SNF, %	8.77	9.09	1.08
Ca, %	.221	.212	.010
Mg, %	.025	.032*	.003

* Significant difference (P<.05) due to breed.

Table 21. Least-squares means for intake (IE), fecal (FE), digested (DE), metabolizable (ME), urinary (UE), milk (LE), tissue (TE), and maintenance energy (MaE), with calculated total heat production (CalcHE), digestibility, and partial efficiency of ME utilization for milk production (LE/ME) at 8 wk.

Factor	Holstein	Jersey	SE
IE, Mcal/d	77.8*	53.2	1.5
FE, Mcal/d	24.9	27.5	1.4
DE, Mcal/d	52.9*	25.7	1.8
Digestibility, %	67.9*	48.3	2.4
UE, Mcal/d	2.9*	2.2	.1
ME, Mcal/d[a]	43.2*	18.8	1.7
LE, Mcal/d	28.7*	21.4	1.2
TE, Mcal/d	10.0	14.0	3.0
MaE, Mcal/d	9.8*	7.4	.1
CalcHE, Mcal/d[b]	31.3*	18.0	3.2
LE/ME, %	67.0	119.0*	10

[a] calculated using ME = -0.45 + 1.01DE (Moe and Tyrrell, 1976)

[b] calculated using HE = IE - (FE + UE + LE + TE) (NRC, 1981)

* Significant difference (P<.01) due to breed.

Breed was a significant source of variation for partitioning of intake energy. Since there are obvious differences in body size, differences in intake and output of energy were expected. The significantly lower digestibility of energy in Jerseys at 8 wk, due to high output of fecal energy, could have been due to the extreme stress of confinement that the Jerseys experienced. There were obvious differences in behavior between Jerseys and Holsteins to indicate that Jerseys were under more stress at 8 wk. One Jersey remained standing for 36 h before lying down and other Jerseys lost more body weight than expected.

Because ME is calculated from DE, the differences in DE would also be reflected in ME. Metabolizable energy as a percentage of DE varies linearly between 80, at 50% digestibility, and 88, at 80% digestibility (Moe and Tyrrell, 1976). The ME as a percent of DE in Holsteins averaged 81.5% and in Jerseys averaged 72.3% at 8 wk. The energy digestibility by Jerseys at 8 wk (48.3%) was lower than the lower limit used for the equation of Moe and Tyrrell (1976). This could explain why ME as a percent of DE was 72% in Jerseys, lower than the 80% that is normally expected.

The partial efficiency of ME utilization for milk production was also influenced by breed. Partial

efficiencies estimates were described by Moe et al. (1971). In lactating animals, efficiency of ME utilization for milk production was 64% compared to 75% for body weight gain. Partial efficiencies of ME utilization for milk at 8 wk was 67% for Holsteins and 120% for Jerseys. The estimate of 120% for Jerseys is unrealistic. Since ME is calculated from DE, the partial efficiency is subject to the errors noted previously. Calculated ME intake was lower than LE output. The deficit between milk energy output and ME intake was met by mobilized body tissue.

Because of the unrealistic results for Jerseys at 8 wk, the effects of breed on nitrogen, calcium, and magnesium parameters at 8 wk will be included in the remaining chapter, but not emphasized.

Least-squares means for intake, body weight, and production measures at 21 wk are given in Table 22. Holsteins had higher DMI, body weights and milk production than Jerseys. Milk from Jerseys had higher percent fat, protein, SNF, calcium, and magnesium. Diet did not influence any of these measurements during this wk.

Energy parameters for the second balance trial at 21 wk are listed in Table 23. Significant breed effects were similar to those found at 8 wk except that apparent digestibility of the control diet energy was similar for

Table 22. Least-squares means for dry matter intake (DMI), initial body weight (BW), body weight change, milk yield, and percent fat, protein, solids-not-fat (SNF), calcium (Ca), and magnesium (Mg) at wk 21.

	Holstein		Jersey		
	Control	Tallow	Control	Tallow	SE
DMI, kg[a]	25.1	24.0	16.9	17.2	1.1
initial BW, kg[a]	658	618	417	455	18
BW change, %	-1.5	-0.1	-0.8	-1.4	1.0
milk, kg[a]	34.2	36.9	20.9	20.5	1.6
fat, %[a]	3.45	3.31	4.98	5.18	.23
protein, %[a]	3.17	2.99	3.75	3.98	.18
SNF, %[a]	9.84	9.36	10.35	10.39	1.6
Ca, %[a]	.208	.227	.285	.288	.023
Mg, %[a]	.025	.026	.033	.037	.004

[a] Significant difference ($P<.01$) due to breed.

Table 23. Least-squares means for intake (IE), fecal (FE), digested (DE), metabolizable (ME), urinary (UE), milk (LE), tissue (TE), and maintenance energy (MaE), with calculated total heat production (CalcHE), digestibility, and partial efficiency of ME utilization for milk production (LE/ME) at 21 wk.

	Holstein		Jersey		
Factor	Control	Tallow	Control	Tallow	SE
IE, Mcal/d[ab]	91.4	101.2*	61.3	69.5*	4.6
FE, Mcal/d[a]	36.2	34.5	24.6	25.7	2.0
DE, Mcal/d[ab]	55.2	66.7*	36.7	43.7*	3.1
Digestibility, %[b]	60.5	65.9*	59.7	62.7*	1.3
UE, Mcal/d	2.9	3.0	2.7	2.5	.3
ME, Mcal/d[abc]	44.5	56.5*	28.9	36.4*	2.2
LE,Mcal/d[a]	24.6	24.9	18.2	18.3	1.3
TE, Mcal/d	7.4	.3	3.9	6.9	4.8
MaE, Mcal/d[a]	10.3	9.9	7.4	7.8	.2
CalcHE, Mcal/d[ad]	35.1	39.0	19.8	29.8	4.3
LE/ME, %[ab]	55.6*	44.2	63.1*	50.7	2.60

[a] Significant difference (P<.01) due to breed.

[b] Significant difference (P<.05) due to diet.

[c] calculated using ME = -0.45 + 1.01DE (Moe and Tyrrell, 1976).

[d] calculated using HE = IE - (FE + UE + LE + TE) (NRC, 1981)

* Significant difference (P<.05) due to diet within breed.

both breeds. At 21 wk, ME as a percent of DE averaged 82.6% in Holsteins and 81.5% in Jerseys, because apparent digestibility of energy was between 50 and 80% for both breeds. The apparent digestibility was similar between breeds because Jerseys did not exhibit stress at 21 wk as they did at 8 wk. Dietary treatment had a significant influence on IE, DE, ME, digestibility, and LE/ME. Intake energy was greater for all cows recieving the tallow diet, but fecal energy output was similar. Therefore amount of digested energy was greater for tallow fed cows in both breeds. The digestibility was also greater for cows fed tallow diet. As a result, metabolizable energy was higher for cows fed dietary tallow than cows fed the control diet. The partial efficiency of ME utilization for lactation was lower for both breeds fed tallow. Other studies have indicated no change in energy intake when diets are supplemented with various lipids (Horner et al., 1986; and Steele et al., 1984). Our results indicated higher ME intake due to tallow addition for both breeds. In contrast, Steele (1984) reported no differences in ME intake between cows fed tallow and those fed a basal diet. However, addition of soybean oil decreased ME intake per day (Steele, 1985). Output of energy in milk was not influenced by dietary treatment in this study. Similar results were found by Steele (1984) when cows were fed

supplemental tallow. Addition of protected tallow to a diet has been shown to increase overall digestibility of dietary energy (Kronfeld and Donoghue, 1980). Increasing amounts of whole cottonseed were also found to increase in energy digestibility (Smith et al., 1981). In contrast, Palmquist and Conrad (1980) found no change in digestibility when 2 levels of tallow were added to a basal diet. Other studies comparing basal and fat supplemented diets also indicated no change in energy digestibility (Jenkins and Palmquist, 1984; and Schauff and Clark, 1989).

Our results indicated a decrease in the partial efficiency of ME utilization for milk production in Holsteins and Jerseys fed supplemental tallow. In contrast, utilization of ME did not differ between basal, tallow, or soybean oil diets fed to dairy cows (van der Honing, 1980). An increase in efficiency of utilization of ME for lactation by cows fed protected tallow has been reported by Kronfeld et al. (1980). The range of LE/ME values reported by the above authors were similar to the values provided by this study.

Nitrogen

Least-squares means for nitrogen measurements at 8 wk are given in Table 24. Breed significantly influenced daily intake, fecal, urinary, and milk nitrogen. Apparent

Table 24. Least-squares means for the nitrogen in intake, feces, urine, and milk, with digestibility and overall nitrogen balance at 8 wk.

Factor	Holstein	Jersey	SE
Intake, g/d	635*	448	10
Feces, g/d	231*	188	10
Urine, g/d	171*	115	9
Milk, g/d	180*	134	6
Digestibility, %	63.6*	58.1	1.7
Balance, g/d	53*	8	11

* Significant difference (P<.01) due to breed.

digestibility and overall balance of nitrogen were higher for Holstein cows.

Table 25 contains the least-squares means for nitrogen measurements at 21 wk. Apparent digestibility of nitrogen was similar for both breeds. Nitrogen balance was greater for Holsteins, despite considerable variation among cows. There were no significant differences due to dietary treatment.

In agreement with our results, both Jenkins and Palmquist (1984) and Palmquist and Conrad (1984) did not find any alteration in nitrogen digestibility between basal and tallow supplemented diets. Nitrogen digestibilities of 65.8 % for diets containing tallow fatty acids and 61.6 % for diets containing calcium soaps of tallow fatty acids (Jenkins and Palmquist, 1984) were similar to the nitrogen digestibilities found in this study. Digestibilities of nitrogen (range of 61.5-69.5%) found by Palmquist and Conrad (1980) also were similar. Other studies, in which rapeseed (Murphy et al., 1987), ground soybeans (Palmquist and Conrad, 1978), or blended animal-vegetable fat (Palmquist and Conrad, 1980) were fed, indicated no change in nitrogen digestibility due to added fat.

Table 25. Least-squares means for the nitrogen in intake, feces, urine, and milk, with digestibility and overall nitrogen balance at 21 wk.

	Holstein		Jersey		
Factor	Control	Tallow	Control	Tallow	SE
Intake, g/d[a]	681	654	454	455	32
Feces, g/d[a]	256	230	166	163	14
Urine, g/d[a]	219	208	159	164	9
Milk, g/d[a]	169	169	123	127	7
Digestibility, %	62.4	65.0	63.4	64.0	1.5
Balance, g/d[a]	38	47	6	1	16

[a] Significant difference ($P<.05$) due to breed.

Calcium

Least-squares means for calcium measurements at 8 wk are presented in Table 26. There were significant breed differences for intake, and milk calcium. Apparent digestibility of calcium was higher for Holsteins. Both Holsteins (-9.7g) and Jerseys (-21.5g) were in negative calcium balance.

Breed differences at 21 wk included intake, fecal, urinary, and milk calcium (Table 27). Holsteins had higher fecal and urinary excretion of calcium than Jerseys. Apparent digestibility of calcium, however, was similar between breeds.

Intake of calcium was higher for cows fed tallow diet versus control diet. The amount of calcium absorbed (data not shown) was significantly higher for cows fed tallow, because apparent digestibility of calcium was similar for both dietary treatments. Calcium excretion in urine, however, was lower for Holstein cows fed tallow and tended to be lower for Jerseys fed tallow. As a result, calcium retention was significantly higher for all cows fed tallow. Analysis of the tallow diet at 21 wk indicated higher concentration of calcium than in the control diet, despite the fact that both diets were formulated to have the same calcium content. The difference in concentration of calcium may be due to diet sampling method. During the

Table 26. Least-squares means for the calcium in intake, feces, urine, and milk, with digestibility and overall calcium balance at 8 wk.

Factor	Holstein	Jersey	SE
Intake, g/d	147*	89	2.4
Fecal, g/d	56.8	46.9	4.7
Urine, g/d	13.4	9.4	1.7
Milk, g/d	86.4*	53.9	4.7
Digestibility, %	61.2*	47.2	4.3
Balance, g/d	-9.7	-21.5	6.0

* Significant difference (P<.05) due to breed.

Table 27. Least-squares means for the calcium in intake, feces, urine, and milk, with digestibility and overall calcium balance at 21 wk.

	Holstein		Jersey		
Factor	Control	Tallow	Control	Tallow	SE
Intake, g/d[ab]	156	185*	105	143*	9
Fecal, g/d[a]	70.6	73.1	44.6	61.9	10
Urine, g/d[ab]	29.6*	7.6	11.7	8.7	5
Milk, g/d[a]	71.0	82.8	57.3	59.1	5
Digestibility, %	51.6	60.9	57.0	55.7	6.6
Balance, g/d[b]	-15.8	21.8*	.3	13.1*	8

[a] Significant difference (P<.05) due to breed.

[b] Significant difference (P<.01) due to diet.

* Significant difference (P<.05) due to diet within breed.

balance trials the complete diets were sampled for analysis. During the rest of the experimental period, the forages and concentrate components of each diet were sampled separately, then analyzed.

Several reports indicated that dietary fat supplementation may alter calcium digestibility. Digestibility of calcium was decreased 76% when a protected tallow diet was added to a basal diet (Kronfeld and Donoghue, 1980). The authors suggested the response was due to formation of insoluble soaps in the intestine. Jenkins and Palmquist (1984) also reported that dietary tallow decreased calcium digestibility. Digestibility was reduced the least when tallow was fed as fatty acids and was reduced the most when tallow was fed as soaps. Another study concerning corn oil supplementation found decreases in apparent and true digestibility of calcium (Tillman and Brethour, 1958). However, several reports are in agreement with results of this study (ie. no change in calcium digestibility due to fat addition) (Filley et al., 1987; Palmquist and Conrad, 1978; and Schauff and Clark, 1989).

Magnesium

Magnesium data for the balance trial at 8 wk are listed in Table 28. Breed differences were found for magnesium intake, fecal output, apparent digestibility and

overall magnesium balance. Holsteins were higher for all measurements. Both breeds were in positive magnesium balance at this point of lactation, but Holsteins were in greater positive magnesium balance than Jerseys.

Similar breed effects (Table 29) were found at 21 wk, that apparent digestibility was similar between breeds. Again, all cows were in positive magnesium balance, with Holsteins in greater positive balance than Jerseys. Diet significantly influenced overall magnesium balance due to differences in intake and urinary magnesium (P=.10) and apparent digestibility (P=.11) that approached significance. The amount of magnesium absorbed was higher for all cows fed tallow. Also, there was a trend (P=.11) toward greater apparent digestibility of magnesium for all cows fed dietary tallow. Within each breed, magnesium retention was significantly higher for cows fed the tallow diet.

Jenkins and Palmquist (1984) reported decreases in apparent digestibility (determined by total collection) when tallow was added as free acids or calcium soaps. Apparent digestibility of magnesium in their control diet was 27.7% versus 18.7% when tallow fatty acids were added and 15.6% when calcium soaps of tallow fatty acids were added. The lower magnesium digestibility in their study compared to this study may be due to amount of

Table 28. Least-squares means for the magnesium in intake, feces, urine, and milk, with digestibility and overall magnesium balance at 8 wk.

Factor	Holstein	Jersey	SE
Intake, g/d	80.5*	37.0	6.2
Fecal, g/d	20.7*	14.4	2.3
Urine, g/d	5.7	5.1	.6
Milk, g/d	9.6	8.2	.9
Digestibility, %	72.5*	58.3	5.2
Balance, g/d	44.0*	9.7	6.6

* Significant difference ($P<.05$) due to breed.

Table 29. Least-squares means for the magnesium in intake, feces, urine, and milk, with digestibility and overall magnesium balance at 21.

	Holstein		Jersey		
Factor	Control	Tallow	Control	Tallow	SE
Intake, g/d[a]	82.5	93.8	49.5	69.2	8.7
Feces, g/d[a]	21.5	17.1	12.5	12.8	3.0
Urine, g/d	8.5	6.3	7.5	6.9	.8
Milk, g/d	8.7	9.5	7.0	7.5	1.1
Digestibility, %	72.8	81.7	56.6	81.2	9.9
Balance, g/d[ab]	43.7	60.9*	22.6	42.0*	8.0

[a] Significant difference (P<.05) due to breed.

[b] Significant difference (P<.05) due to diet.

* Significant difference (P<.05) due to diet within breed.

supplemental fat. Level of supplemental fat was 4.5% compared to 2% in this study. Kronfeld and Donoghue (1980) also found lowered magnesium digestibility when protected tallow was fed. In contrast, magnesium digestibility was higher when a diet containing hydrolyzed fat was fed (38.7% versus a control diet of 25.4%) (Palmquist and Conrad, 1978).

Intake and production ratios

The ratios of M/DMI, DMI/MBW, M/MBW, and LE/NE_L were evaluated to determine their influence during the dietary treatment period. Holsteins had higher ratios than Jerseys for M/DMI, DMI/MBW, and M/MBW. Thus, Holsteins seem to have a greater capacity for feed intake and seem to be more efficient in their conversion of consumed feed to milk. However, Jerseys had higher percent fat, protein, and SNF in their milk. When the breeds were compared on the basis of milk energy output per unit of NE_L intake, Holsteins and Jerseys had a similar ratios.

Table 30 contains least-squares means for the ratio of M/DMI by dietary treatment for the experimental period. Holsteins fed tallow had higher M/DMI ratio at 18 and 20 wk than Holsteins fed the control diet. Control and tallow fed Jersey cows had similar ratios at all lactation points. Holsteins fed the tallow diet had greater milk yield, but similar DMI, compared to Holsteins fed the

Table 30. Least-squares means for milk yield (kg) per kg of dry matter intake.

	Holstein[a]			Jersey		
Week	Control	Tallow	SE	Control	Tallow	SE
12	1.52	1.64	.07	1.27	1.31	.15
14	1.52	1.73	.09	1.28	1.26	.08
16	1.43	1.60	.08	1.28	1.20	.11
18	1.38	1.55*	.05	1.29	1.20	.10
20	1.41	1.69*	.06	1.12	1.21	.09

[a] Significant difference ($P<.05$) due to breed.

* Significant difference ($P<.05$) due to diet within breed.

control diet all through the dietary treatment period (Tables 17 and 19). This trend was not seen in the Jerseys because milk yield was similar for both diets.

The least-squares means for DMI/MBW by dietary treatment in Holstein and Jersey cows are given in Table 31. There were no significant differences due to diet within either breed.

Least-squares means in Table 32 are for the ratio of M/MBW by dietary treatment within breed. In Holsteins, tallow fed cows had a higher ratio at all observations except 20 wk. The difference was due to higher milk yield by tallow-fed cows. Tallow fed Jersey cows had a higher ratio at 20 wk. At wk 14, effects of dietary tallow on this ratio approached significance (P=.10). These results are different than results reported by Palmquist and Conrad (1978). The authors reported that milk production by Jersey cows was increased with 6 and 20% fat addition, but Holsteins increased milk production with the 6% fat addition only. Jerseys responded with more milk per unit metabolic body size than did Holsteins. The authors suggested that the response was due to greater output of milk in relation a capacity of the rumen. Our results showed that Jerseys did not respond to a 2% tallow addition with increased milk yield as compared to results from Palmquist and Conrad (1978).

Table 31. Least-squares means for dry matter intake (kg) per kg metabolic body weight.

	Holstein[a]			Jersey		
Week	Control	Tallow	SE	Control	Tallow	SE
12	.194	.209	.013	.173	.182	.013
14	.193	.197	.007	.171	.191	.010
16	.204	.205	.010	.180	.190	.016
18	.206	.202	.010	.173	.193	.013
20	.201	.174	.011	.181	.186	.007

[a] Significant difference ($P<.05$) due to breed.

Table 32. Least-squares means for milk yield (kg) per kg metabolic body weight.

	Holstein[a]			Jersey		
Week	Control	Tallow	SE	Control	Tallow	SE
12	.293	.343*	.007	.217	.235	.016
14	.289	.343*	.006	.210	.247	.012
16	.288	.330*	.007	.222	.232	.016
18	.282	.317*	.009	.213	.237	.008
20	.279	.296	.011	.197	.230*	.006

[a] Significant difference ($P<.05$) due to breed.

* Significant difference ($P<.05$) due to diet within breed.

Table 33 contains the least-square means for LE/NE_L by diet within breed. The ratio was not influenced by dietary treatment at any point in either breed. There were large variations, as shown by large standard errors, for both breeds. Holstein cows fed tallow had higher energy consumption, but produced more milk. The net effect was no change in the ratio. Jersey cows fed tallow consumed more energy, but did not produce more milk. There was a tendency for a lower ratio, but the difference was not significant.

In relating the various ratios (Tables 31 to 33) to energy balance at 21 wk, several observations are reinforced. Dry matter intake (kg) per kg of MBW was not different due to diet. This indicates that the higher energy intake by tallow-fed cows is due to the increased energy density of the tallow diet. In addition, the apparent digestibility of energy was higher for cows fed the tallow diet. Not only did the tallow fed cows have higher energy intakes, they also digested this energy more efficiently. The increased energy of the tallow diet was used to produce a higher volume of milk in Holsteins, but not Jerseys. This is shown in the ratio of milk yield (kg) per kg MBW at 4 out of 5 lactation points, where tallow fed Holsteins had a higher ratio than controls. However, during the balance trial at 21 wk, milk energy

Table 33. Least-squares means for milk energy output (Mcal) per unit (Mcal) of NE_L intake.

	Holstein			Jersey		
Week	Control	Tallow	SE	Control	Tallow	SE
12	.695	.655	.026	.708	.633	.065
14	.642	.740	.039	.665	.590	.053
16	.675	.664	.040	.728	.637	.071
18	.628	.626	.015	.689	.600	.064
20	.648	.675	.023	.602	.590	.053

output was similar for control and tallow fed cows in both breeds. In Holsteins fed tallow, the milk volume was greater, but the solids content of the milk was not. Tallow-fed cows were consuming more energy, but were not increasing the energy output into milk. Thus, partial efficiency of ME utilization for lactation was lower than the efficiency in control cows. This observation is reinforced by the ratio of milk energy output (Mcal) per unit of NE_l intake. No differences were present between cows on control or tallow diet.

Holstein cows fed tallow were gaining more weight (Table 17) than controls during the fat feeding period. During the balance at 21 wk, tallow fed cows lost less weight (as reflected by a smaller tissue energy value) than control cows. Holstein cows were using the increased energy from the tallow diet, although maybe not as efficiently, to increase volume of milk and also to gain body tissue.

Summary

There were obvious differences between Holstein and Jersey cows during the balance trials and the fat feeding period. At 8 wk, Jerseys performed poorly. This is reflected in greater body weight loss, and significantly lower apparent digestibilities of energy, nitrogen, calcium, and magnesium. The only reasonable explanation

for this is that Jerseys responded more than Holsteins to the stress of confinement. At 21 wk, apparent digestibilities for all variables were similar. During the fat feeding period, Jerseys fed tallow did not increase milk yield or body weight gain. Metabolic efficiency was apparently decreased by dietary tallow in Jerseys. Additional energy intake was partitioned into the calculated total heat production.

Breed and diet at 21 wk had significant influences on energy, calcium, and magnesium balance. Dietary tallow increased the amount of energy intake, absorbed energy, and metabolizable energy, but did not increase milk energy output in either breed. Therefore, partial efficiency of ME utilization for lactation was lower for cows fed tallow. Nitrogen status was not influenced by dietary treatment. Cows fed tallow excreted less calcium in the urine and were in greater positive calcium balance than control cows. Also, tallow-fed cows were in greater positive magnesium balance. Unlike energy digestibility, dietary tallow did not significantly influence nitrogen, calcium, or magnesium digestibility.

CHAPTER 6

CHANGES IN BONE STRENGTH, MINERAL CONTENT AND HISTOLOGICAL MEASUREMENTS IN LACTATING HOLSTEIN AND JERSEY COWS

INTRODUCTION

Bone is a dynamic living tissue that is continuously undergoing remodeling throughout life. Very few investigations, however, have been conducted on factors that affect bone in the adult animal. The physiological demand for calcium during lactation in dairy cattle is similar to that of the antler cycle in deer. During antler growth in male deer, large amounts of calcium and phosphorus are mobilized from the skeleton to support growth of antlers (Banks et al., 1968b; and Choa et al., 1984). Skeletal resorption of minerals was highest when calcification of antlers was highest (Stephenson and Brown, 1984). Chemical composition per unit volume (mM/ml) of calcium, phosphorus, and magnesium was lower in bone during the rapid antler growth period (Banks et al., 1968b). Histological evaluation of rib samples from deer (Banks et al., 1968a) indicated bone resorption and porosity was greatest during peak antler growth.

During lactation, substantial amounts of calcium are mobilized from bone to support milk synthesis. Little and McMeniman (1973) reported that concentration of calcium,

phosphorus, and ash in rib biopsies were significantly lower in lactating sheep. Calcium contribution from bone during lactation in rats has been calculated. Brommage (1989) determined that 19% of calcium transferred to milk was derived from the maternal skeleton with the maternal diet providing the remainder. This amount (24 mg/d) might be a serious drain on the maternal system.

The density or specific gravity of bone may be changed due to excessive mobilization of minerals. Little (1972) observed that specific gravity of rib tissue was lower in cattle fed a phosphorus deficient ration than in cattle fed adequate phosphorus. Specific gravity of rib samples from lactating sheep were lower than those of non-lactating sheep (Little and McMeniman, 1973). In deer, rib density was the lowest during peak antler growth (Banks et al., 1968b). From these studies, changes in rib density or specific gravity were attributed to substantial loss of bone mineral due to the demand for calcium to support either lactation or antler growth.

The objective of this study was to evaluate the effects of breed and stage of lactation on bone shear, density, mineral concentrations, and histological measurements in Holstein and Jersey cows.

MATERIAL AND METHODS

Rib biopsies were performed on 8 Holstein and Jersey cows at 7, 60 and 150 d of lactation. Eight bone samples, 4 from each breed, were taken at each sampling point. Biopsied samples were removed from the middle of the 11^{th} or 12^{th} rib on either the left or right side. The rib was chosen as an indicator of mineralization status because ribs and axial skeleton are more sensitive to resorption and remodeling than the long bones (Hill, 1962). Rib biopsy procedure was similar to that described in Little, (1972). Cow were tranquilized with Rompun (xylazine) and maintained under anesthesia with guaifenesin IV drip during the surgery. At the midpoint of the rib, a 5-9 cm section of bone was extracted using a wire bone saw. The section of bone was immediately washed in saline. The bone was then cut into 4 segments using a band saw. A large portion (3 cm) was saved for specific gravity and shear stress test determinations and the three smaller pieces were used for mineral content, and histological evaluation. The portion reserved for the organic histologic evaluation was placed in Bouins fixative for 2 wk. The other bone samples were kept frozen until used.

Specific gravity and shear stress measurements were determined using the same sample. Specific gravity was measured by the water displacement technique described by

Mohsenin (1978). The equation used to calculate specific gravity (SP) as follows:

$$SP = \frac{\text{weight in air x specific gravity of water}}{\text{weight of displaced water}}$$

Shear test was conducted using a Model TMS Instron Testing Machine. The testing procedure is similar to that of Wilson et al. (1984). The specimen was placed on the test apparatus (see Appendix, Figure 1) with the flatest side down. The crosshead speed was 10 mm/min and chart speed was 100 mm/min. The load on the machine was full scale (25 kN). Once the machine was started, the force-deformation curve through the point of rupture was recorded. The calculation of shear strength (stress) was determined by:

$$S = F / 2 * A$$

where:

S = shear stress (kg/cm^2)

F = applied force (kg)

A = cross sectional area (cm^2)

Mineral content was determined as follows. First, the bone samples were initially digested in order to remove all organic material (Emerson, 1975). The final digestion solution contained only inorganic components. Calcium and magnesium were determined by atomic absorption spectrophotometry (Perkin-Elmer Model 403) using solution

diluted 1:10,000 in 0.1% lanthanum chloride. Concentration of standards were 1 to 5 ug calcium/ml and 0.1 to 0.5 ug magnesium/ml. Percent phosphorus was measured by colorimetric determination (Hill, 1972).

The inorganic section of bone was used to distinguish the mineralized from non-mineralized areas. For routine histological study, the evaluation of fresh undecalified bone (a condition where the tissue deviates minimally from the living bone) is the optimal choice. The section of bone for inorganic histological evaluation was cut using a Buehler Isomet low speed saw equipped with a diamond wheel blade. Sections were cut at 50 to 100 um. This method gave high quality sections that were suitable for quantitative light microscopic examination (Gilbertson, 1977; Wallin et al., 1985; and Weaker and Richardson, 1978). These cut sections were then stained in Villanueva bone stain and processed according to the procedure of Villanueva (1974). This stain selectively colors osteoid seams and preserves tissue details found in fresh, mineralized sections of bone.

A quantitative evaluation of compact bone was performed using mineralized sections. Since the Haversian system or osteon is the basic functional unit of compact bone, an evaluation of number, size, and distribution of osteons was conducted. The light microscope was equipped with a 1.11 mm square grid in the eyepiece. The area within the grid was

assessed for osteon characteristics. Grid evaluations have been routinely employed by others (Frost et al., 1962; and Kelin and Frost, 1964). The cross-section of the bone was divided into 4 regions. Within each region (see Appendix, Figure 2), cortical bone was again divided into 3 circular zones (outer, middle, and inner) with the inner zone located adjacent to the marrow cavity and the outer zone located beneath the periosteum. In these zones, repeated grid observations were made. Classification of osteons were: (1) small (25 to 125 um), (2) medium (125.1 to 250 um), and (3) large (250.1 to 375 um).

Decalcified samples were used to evaluate organic matrix and cell types. Since minerals are removed by decalcification, the cell types associated with bone (osteoblasts, osteocytes, and osteoclasts) were easily identified. After fixation in Bouins, the samples were washed in 70 % ethyl alcohol over a period of 5 to 7 days with complete changes each day. The commercial decalcifying agent (Surgipath Decalcifier II) employed was a combination of hydrochloric acid and ethylenediamine tetraacetic acid (EDTA). The hydrochloric acid causes rapid decalcification while EDTA binds the calcium ions and prevents serious swelling of the tissue (Urban, 1981). After decalcification, the sections were embedded in glycol methacrylate (Hott and Marie, 1987). Bone samples were then

sectioned (3 to 6 um) with a glass knife, stained with Azure II, and evaluated using the light microscope.

Statistical Analysis. Stage of lactation, breed of cow, and rib location effects on bone density, shear stress test, calcium, phosphorus, and magnesium percent in bone were evaluated by the General Linear Models (SAS, 1985) using the model below:

$$Y_{ijkl} = u + B_i + D_j + (BD)_{ij} + R_k + (BR)_{ik} + (DR)_{jk} + e_{ijkl}$$

where:

Y_{ijkl} is observed dependent variable

u is mean of Y

B_i is fixed effect of i^{th} breed; i=1,2

D_j is fixed effect of j^{th} day; j=1,3

$(BD)_{ij}$ is the interaction between breed and day

R_k is fixed effect of k^{th} rib location; k=1,2

$(BR)_{ik}$ is the interaction between breed and rib location

$(DR)_{jk}$ is the interaction between day and rib location

e_{ijkl} is random residual.

The histological evaluation of inorganic bone included the effects of breed, stage of lactation, rib location, and circular zone on the count, classification, and distribution of osteons. The analysis used the General Linear Models

(SAS, 1985). The model is given below:

$$Y_{ijklmn} = u + B_i + C_{(i)j} + D_k + DC_{(i)jk} + (BD)_{ik} + L_l + LC_{(i)jl} + (BL)_{il} + (DL)_{kl} + (DL)C_{(i)jkl} + Z_m + ZC_{(i)jm} + (BZ)_{im} + (DZ)_{km} + (DZ)C_{(i)jkm} + (LZ)_{lm} + (LZ)C_{(i)jlm} + e_{ijklmn}$$

where:

Y_{ijklmn} is observed dependent variable

u is mean of Y

B_i is fixed effect of i^{th} breed; i=1,2

$C_{(i)j}$ is random effect of j^{th} cow within i^{th} breed

D_k is fixed effect of k^{th} day; k=1,2

$DC_{(i)jk}$ is interaction between day and cow within breed

$(BD)_{ik}$ is the interaction between breed and day

L_l is fixed effect of l^{th} rib location; l=1,2

$LC_{(i)jl}$ is interaction between rib location and cow within breed

$(BL)_{il}$ is the interaction between breed and rib location

$(DL)_{kl}$ is the interaction between day and rib location

$(DL)C_{(i)jkl}$ is interaction among day and rib location and cow within breed

Z_m is fixed effect of m^{th} zone; m=1,3

$ZC_{(i)jm}$ is interaction between zone and cow within breed

$(BZ)_{im}$ is the interaction between breed and zone

$(DZ)_{km}$ is the interaction between day and zone

$(DZ)C_{(i)jkm}$ is interaction among day and zone and cow within breed

$(LZ)_{lm}$ is the interaction between rib location and zone

$(LZ)C_{(i)jlm}$ is interaction among rib location and zone and cow within breed

e_{ijklmn} is random residual

Each main effect or 2-way interaction between main effects were tested for significance ($P<.05$) using the interaction of each with cow within breed.

RESULTS AND DISCUSSION

Least-squares means for specific gravity and shear stress by breed are given in Table 34. Specific gravity measurement was significantly influenced by breed. Jerseys had a higher specific gravity (1.73 g/ml) of bone than Holsteins (1.67 g/ml). As for shear stress, Jersey and Holstein cows showed no statistical differences. Stage of lactation did not affect either specific gravity or shear stress (Table 35). Shear stress was not affected by rib location (11th or 12th), but rib location effects on specific gravity approached significance ($p=.11$) (Table 36). The specific gravity values in this study were similar to those of 1.68 and 1.58 g/ml reported for beef cattle by Little (1972). The lower specific gravity, 1.58 g/ml, occurred after the cattle were on a phosphorus deficient diet for 6 wk. Regarding lactation effects on bone,

Table 34. Least-squares means for specific gravity (SP), (g/ml), shear (kg/cm^2), and percent calcium (Ca), phosphorus (P), and magnesium (Mg) in bone by breed.

Breed[a]	SP	Shear	Ca	P	Mg
H	1.67	358	30.4	11.9	.919
J	1.73*	373	28.9	11.4	.725
SE	.01	21	2.2	.3	.10

[a] Breed: H = Holstein and J = Jersey.

* Significant difference (P<.01) due to breed.

Table 35. Least-squares means for specific gravity (SP), (g/ml), shear (kg/cm^2), and percent calcium (Ca), phosphorus (P), and magnesium (Mg) in bone by day of lactation.

Day	SP	Shear	Ca	P	Mg
7	1.67	354	25.9	11.8	.754
60	1.71	394	28.7	11.5	.791
150	1.71	348	34.4	11.7	.920
SE	.01	26	2.7	.4	.012

Table 36. Least-squares means for specific gravity (SP), (g/ml), shear (kg/cm^2), and percent calcium (Ca), phosphorus (P), and magnesium (Mg) in bone by rib location (Rib).

Rib	SP	Shear	Ca	P	Mg
11	1.71	366	31.6	11.7	.850
12	1.68	365	27.7	11.6	.794
SE	.01	21	2.2	.3	.102

lactating sheep were reported to have lower specific gravity (1.47 g/ml) than non-lactating sheep (1.63 g/ml) (Little and McMeniman, 1973). In agreement, Little et al. (1978) found that lactating sheep were much more sensitive to a phosphorus deficient diet than were non-lactating sheep because specific gravity of bone was greatly decreased in the lactating group. Interpretation of these results indicate that lactation necessitates a high demand for minerals and bone must supply these minerals.

Lower specific gravity was expected at the 60 d observation, which was peak lactation and peak demand for calcium, but this was not the case. Results also indicated significant breed differences for specific gravity. No other study has investigated this possibility or has studied these bone parameters in dairy cattle. Results also indicated a trend (P=.11) in specific gravity difference between the 11th and 12th rib. Other studies using rib biopsies did not state which rib or ribs they used for the specific gravity determination (Banks et al., 1968b; Little, 1972; amd Little and McMeniman, 1973). There was large sample variation for shear stress with no differences due to breed, day, or bone location. No reports of ruminant bone stress studies were found in the literature. However, Crenshaw et al. (1981b) reported significant dietary influences on shear stress in ribs of growing swine. Pigs

fed .8% calcium, and .8% phosphorus in the diet had higher shear stress than pigs fed .4% calcium and .4% phosphorus.

There were no significant differences in percent calcium, phosphorus, or magnesium in bone (Table 34) due to breed or day of lactation (Table 35). Effect of stage of lactation on percent calcium in bone approached significance (P=.11) with percent calcium increasing as lactation advanced. The location of rib did not influence any of these mineral parameters (Table 36). Rib calcium content was higher that of beef cattle (18.3%) (Little, 1972). Calcium content of rib in this study was similar to the 24.5% percent calcium determined in dry, fat-free bone by Little (1972). Bones in this study were frozen fresh and remained frozen for a period of time (1 to 8 mo) before analysis, therefore, some dessication may have taken place. This would elevate the percent minerals in bone. Phosphorus content was also higher than that of 8.6% in fresh bone reported by Little (1972), but similar to the 11.5% phosphorus in dry, fat-free bone. A study conducted with sheep (Little and McMeniman, 1973) did not find any calcium percentage difference between fresh bone of lactating sheep versus non-lactating sheep, but percent bone phosphorus differed. Non-lactating sheep had significantly higher percent phosphorus in bone than lactating sheep.

All two-way interactions between main effects were also studied. The interaction between breed and rib location was significant only for percent calcium in bone (See Appendix, Table 4). Jerseys had higher percent calcium in the 11th rib than the 12th rib, but this trend was not apparent in Holsteins. The interaction between day of lactation and rib location had a significant influence on percent calcium and magnesium (See Appendix, Table 5). At 7 and 60 d of lactation, calcium and magnesium percent in bone were higher in the 11th than the 12th rib. At 150 d, the trends were reversed, with higher percents of both minerals in the 12th rib. All other 2-way interactions were non-significant.

In the histological evaluation of the bone samples, the osteons demonstrated a mature configuration. The homogenous staining of osteons and interstitial bone was indicative of this maturity. If osteoclasts were actively resorbing bone mineral then resorption surfaces would be identified by scalloped surfaces. Very few resorption areas were present, however, indicating bone stability at all observations. Some resorption spaces and filling osteons (indicating bone formation) were present, but the majority of osteons were in a stable and mature configuration. Similar observations were found in deer during initial stages of antler growth (Banks et al., 1968a). The staining procedure provided visual details of the Haversian systems that were

consistent with normal mature bone structure. These details included the radiating canaliculi, lacunar spaces, Haversian canals, Volkman's canals, and osteoid material. Since visual differences in remodeling state of these bones were not found, a quantitative histological evaluation was employed.

Neither breed (Table 37), stage of lactation (Table 38), rib location (Table 39), or zone (Table 40) had any influence on osteon classification and distribution. There was extreme variation in osteon classification and distribution within each cow and between cows.

Sections of organic bone were evaluated for cell types. Abundant osteocytes were apparent in every section. A limited number of osteoblasts and osteoclasts were found. During active resorption, one would expect the occurence of osteoclasts in sufficient numbers for histological determination. Increased bone remodeling would presumably show the presence of both osteoclasts and osteoblasts. The large number of osteocytes and very small numbers of osteoblasts and osteoclasts indicates that these samples came from anatomically stable bone.

Although cows were in negative calcium balance at 60 d (see Table 26, Chapter 5), it was not apparent in specific gravity, shear stress, organic and inorganic histological evaluation, and percent phosphorus and magnesium. However,

Table 37. Least-squares means for distribution of osteons by breed.

Breed[a]	Small[b]	Medium[c]	Large[d]	Total
H	5.31	6.79	7.74	19.8
J	4.85	6.94	7.47	19.4
SE	1.30	.99	.61	1.7

[a] Breed designation: H = Holstein and J = Jersey.

[b] Small: 25 to 125 um.

[c] Medium: 125.1 to 250 um.

[d] Large: 250.1 to 375 um.

Table 38. Least-squares means for distribution of osteons by day of lactation.

Day	Small[a]	Medium[b]	Large[c]	Total
7	6.80	8.81	5.46	21.1
60	4.94	5.17	8.19	18.4
150	3.49	6.61	9.14	19.2
SE	1.70	1.28	.79	2.3

[a] Small: 25 to 125 um.

[b] Medium: 125.1 to 250 um.

[c] Large: 250.1 to 375 um.

Table 39. Least-squares means for distribution of osteons by location of rib.

Rib	Small[a]	Medium[b]	Large[c]	Total
11	3.19	7.07	8.57	18.7
12	6.96	6.65	6.62	20.4
SE	1.58	1.07	.60	1.7

[a] Small: 25 to 125 um.

[b] Medium: 125.1 to 250 um.

[c] Large: 250.1 to 375 um.

Table 40. Least-squares means for distribution of osteons by circular zone in bone.

Zone	Small[a]	Medium[b]	Large[c]	Total
Inner	1.64	5.67	9.18	16.5
Middle	5.33	6.18	6.97	18.5
Outer	8.26	8.73	6.64	23.7
SE	1.48	1.11	.69	2.0

[a] Small: 25 to 125 um.

[b] Medium: 125.1 to 250 um.

[c] Large: 250.1 to 375 um.

stage of lactation effects on percent calcium in bone did approach significance (P=.11). Further studies are certainly needed in dairy cattle in order to understand the mineral contribution from bone to the calcium homeostasis during lactation.

In conclusion, the rib biopsy samples taken from Holstein and Jersey cows exhibited similar results for specific gravity, shear stress, percent minerals in bone, and histological evaluations. Stage of lactation, breed, and rib location had little influences on these bone parameters. However, breed significantly influenced specific gravity of bone with bone from Jerseys exhibiting a higher specific gravity than bone from Holsteins. Our results indicate that these cows had stable bone configurations throughout the experimental period.

SUMMARY AND CONCLUSIONS

Stage of lactation and breed effects on plasma hormones, glucose, and calcium

Stage of lactation significantly influenced plasma hormones, glucose, and calcium, but most of the variation was found at calving. Plasma somatotropin concentration was higher in both breeds at 2 wk before calving than the day of calving. Jerseys had higher somatotropin on day after calving than Holsteins. In contrast, insulin declined from prepartum to calving. Plasma insulin was not different between breeds at either day after calving. At calving, the ratio of somatotropin to insulin was higher in both breeds, as compared to prepartum observations. On the day after calving, however, Jerseys had a higher ratio than Holsteins. Plasma glucose was lower on day of calving in both breeds, as compared to prepartum concentrations. Holsteins, however, at calving had higher plasma glucose than Jerseys.

Plasma parathyroid hormone was elevated 4-fold from prepartum to calving, but breed differences were not detected. Plasma 1,25-dihydroxyvitamin D_3 increased 2-fold from prepartum to calving in both breeds. However, Jerseys had higher 1,25-dihydroxyvitamin D_3 than Holsteins on day of and day after calving.

Plasma calcium concentrations declined from 2 wk before calving to calving. Jerseys had lower total calcium than Holsteins at both days after calving. Ionized calcium and normalized ionized calcium followed the same trend as total calcium. The greater degree of hypocalcemia in Jerseys was accompanied by higher concentration of 1,25-dihydroxyvitamin D_3, as noted above.

Concentrations of somatotropin, insulin, parathyroid and 1,25-dihydroxyvitamin D_3 at 4 and 8 wk were indicative of metabolic status during early lactation. Plasma somatotropin and ratio of somatotropin to insulin declined from parturition to 4 wk, due primarily to an increase in insulin concentration as lactation advanced. Plasma glucose was similar between calving and 4 wk. Plasma parathyroid hormone and 1,25-dihydroxyvitamin D_3 were lower at 4 wk than at calving. Breed differences were not detected at 4 or 8 wk for any of the above parameters.

Total calcium in plasma increased and was normal by 2 wk postpartum. Plasma calcium concentrations were similar between breeds at 2, 4, 6, and 8 wk. Ionized calcium as a percent of total calcium was higher at calving. After calving, percent ionized calcium gradually decreased through 8 wk.

Several ratios were calculated to evaluate intake and production due to large breed differences in body size,

feed intake, and milk production. Milk produced (kg) per kg DMI, DMI (kg) per kg MBW, and milk (kg) per kg MBW were higher for
Holsteins than Jerseys. When breeds were compared on the basis of milk energy output per unit of NE_L intake, however, Holsteins and Jerseys were similar.

In conclusion, stage of lactation had a significant influence on all variables, but most of the variation occurred at calving. Breeds responded differently to the stress of calving as indicated by different concentrations of plasma hormones, calcium, and glucose. After calving, breeds were similar. The higher ionized calcium as a percent of total calcium at calving may have been due to stimulation of calcium mobilization from bone by elevated concentrations of parathyroid hormone and 1,25-dihydroxyvitamin D_3.

Dietary treatment effects on plasma hormones, glucose, and calcium

During the fat-feeding period, which was past peak lactation, Holsteins had higher plasma somatotropin than Jerseys. Holsteins fed tallow tended to have a lower somatotropin concentration than Holsteins fed the control diet. Holsteins and Jerseys fed tallow had significantly higher concentrations of total and ionized calcium at 20

wk. Despite the difference, ionized calcium as a percent of total calcium was not changed.

Holsteins fed tallow produced more milk and gained more body weight than those fed the control diet. These trends were not present in Jerseys. Holsteins fed tallow diet had lower protein % at 18 and 20 wk and lower SNF % at 20 wk than Holsteins fed the control diet. Jerseys had similar milk composition whether fed control or tallow diet.

Dietary treatment and breed effects on nutrient balances

The balance trial conducted at 8 wk of lactation showed that apparent digestibility of energy in the control diet was higher for Holsteins (67.9%) than Jerseys (48.3%). Jerseys apparently experienced more stress due to the collection procedures than Holsteins. Comparisons at 8 wk, therefore, contributed little to the understanding of breed differences. Data from Jerseys at 21 wk, however, were within normal ranges and comparisons based on breed and diet were reliable.

Nitrogen digestibility and balance at 21 wk were not influenced by dietary treatment or breed. Energy intake and apparent digestibility were greater for Holstein and Jersey cows fed tallow diet. Absorbed energy and metabolizable energy were also greater. However, Holstein

and Jersey cows fed tallow had lower partial efficiency of ME utilization for lactation than cows fed the control diet.

Intake and amount of absorbed calcium was greater for cows fed tallow, due to a slightly higher calcium concentration in the tallow diet. However, calcium retention was greater for Holsteins and Jerseys fed tallow, due to lower excretion of calcium in urine. Absorbed magnesium was greater for tallow-fed cows in both breeds, due to slightly higher intake of magnesium (P=.10) and apparent digestibility of magnesium (P=.11). As a result, cows fed the tallow diet retained more magnesium.

Holsteins fed tallow had a higher ratio of milk (kg) per kg DMI at 18 and 20 wk. Dietary treatment did not affect this ratio in Jerseys. Holsteins fed tallow also had higher milk (kg) per kg MBW at 12, 14, 16, and 18 wk than Holsteins fed the control diet. However, the ratio of milk energy output (Mcal) per Mcal NE_L intake was not influenced by diet in either breed.

In conclusion, dietary tallow addition increased energy intake, absorbed energy, and metabolizable energy in both breeds. The additional energy was used less efficiently, because the partial efficiency of ME utilization for lactation was lower in tallow-fed cows. Dietary tallow addition did not influence dietary nitrogen

utilization. Calcium and magnesium retention, however, were increased in cows fed tallow.

Stage of lactation and breed effects on bone characteristics

Stage of lactation had no effect on rib specific gravity, shear stress, or percent minerals in bone. Specific gravity and shear stress were similar for cows at 7, 60, and 150 d of lactation. Day of lactation did not influence phosphorus or magnesium content in bone. Effects of day of lactation on percent calcium in bone approached significance (P=.11), with percent calcium became progessively higher as lactation advanced. Histological evaluation of rib indicated that stage of lactation was not a significant influence on histological structure of bone taken from rib.

Breed did not significantly alter any bone characteristics, except specific gravity. Jerseys had higher specific gravity (1.73 g/ml) than Holsteins (1.67 g/ml). The difference might indicate a greater propensity for calcium mobilization from bone of Holsteins to meet lactation requirements. If so, it supports data indicating that Jerseys are more susceptible to parturient paresis.

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APPENDIX

Table 41. Least-squares means for milk fat percent by breed.

Breed[a]	Diet[b]	Week of lactation					
		8	12	14	16	18	20
H	C	3.45	3.81	3.29	3.45	3.67	3.71
H	T	3.36	3.34	3.60	3.37	3.37	3.45
SE			.38	.12	.22	.16	.13
J	C	4.55	4.75	4.21	4.61	4.45	4.48
J	T	4.94	4.83	4.27	4.61	4.99	4.76
SE			.43	.30	.30	.40	.42

[a] Breed: H = Holstein and J = Jersey.

[b] Diet: C = control and T = tallow.

Table 42. Least-squares means for milk protein percent.

Breed[a]	Diet[b]	Week of lactation					
		8	12	14	16	18	20
H	C	2.98	3.13	3.24	3.31	3.35*	3.36*
H	T	2.92	3.09	3.02	3.12	3.12	3.00
SE			.05	.10	.09	.08	.10
J	C	3.39	3.73	3.96	3.92	3.91	3.93
J	T	3.42	3.73	3.92	3.82	4.14	3.91
SE			.17	.16	.15	.15	.17

[a] Breed: H = Holstein and J = Jersey.

[b] Diet: C = control and T = tallow.

* Significant difference (P<.10) due to diet within breed.

Table 43. Least-squares means for milk solids-not-fat percent.

Breed[a]	Diet[b]	Week of lactation					
		8	12	14	16	18	20
H	C	8.64	8.61	8.85	8.81	8.89	8.95*
H	T	8.33	8.73	8.59	8.58	8.61	8.39
SE			.06	.11	.10	.10	.14
J	C	9.00	9.36	9.51	9.47	9.41	9.38
J	T	8.96	9.30	9.54	9.41	9.44	9.46
SE			.13	.14	.11	.14	.17

[a] Breed: H = Holstein and J = Jersey.

[b] Diet: C = control and T = tallow.

* Significant difference (P<.05) due to diet within breed.

Table 44. Least-squares means for bone specific gravity (SP) (g/ml), shear (kg/cm^2), and percent calcium (Ca), phosphorus (P), and magnesium (Mg) by breed and rib location.

Breed[b]	Rib	SP	Shear	Ca	P	Mg
H	11	1.69	361	29.5	11.6	.912
H	12	1.64	355	31.4	12.3	.927
J	11	1.73	371	33.7*	11.8	.789
J	12	1.74	374	24.2	11.0	.660
SE		.02	30	3.1	.5	.145

[a] Breed: H = Holstein and J = Jersey.

* Significant difference (P<.10) due to rib location within breed.

Table 45. Least-squares means for bone specific gravity (SP) (g/ml), shear (kg/cm^2), and percent calcium (Ca), phosphorus (P), and magnesium (Mg) by day and rib location.

Day	Rib	SP	Shear	Ca	P	Mg
7	11	1.67	371	29.6*	12.1	.85*
7	12	1.68	337	22.2	11.5	.65
60	11	1.74	356	36.7*	11.5	1.10*
60	12	1.66	432	20.7	11.4	.53
150	11	1.71	370	28.5	11.5	.65
150	12	1.69	326	40.3*	12.0	1.19*
SE		.02	37	3.7	.6	.18

* Significant difference ($P<.01$) due to rib location within breed.

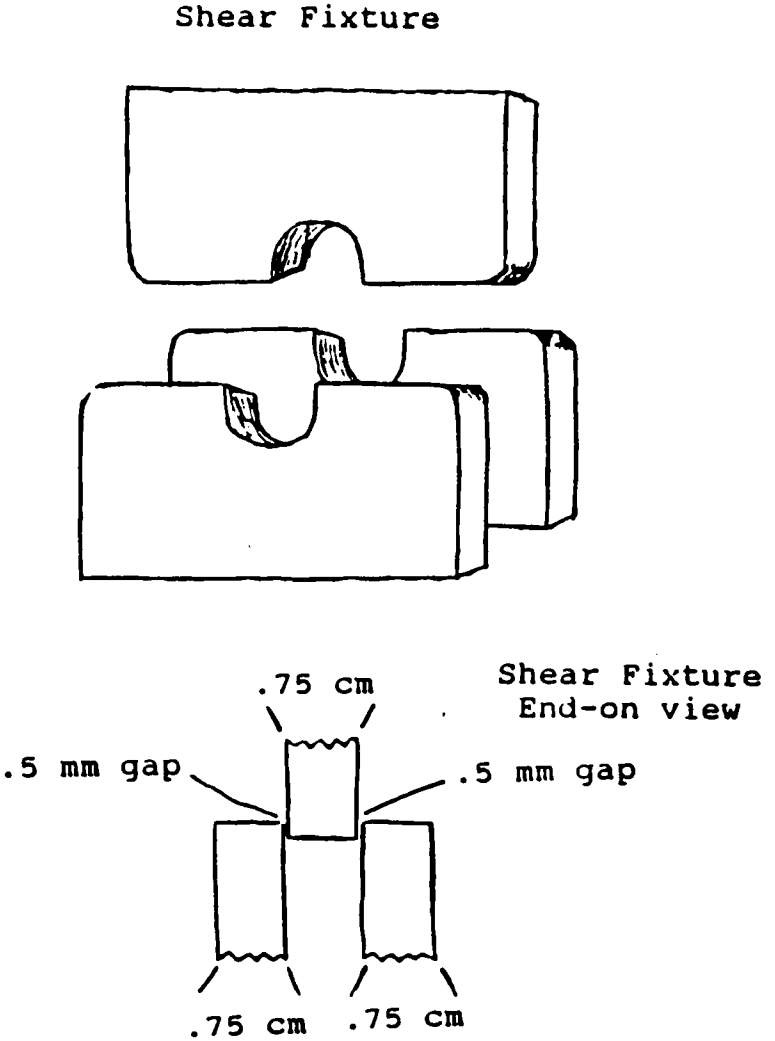

Figure 1. Diagram of shear fixtures used on Instrom Model 1123 Testing Machine for bone strength determination.

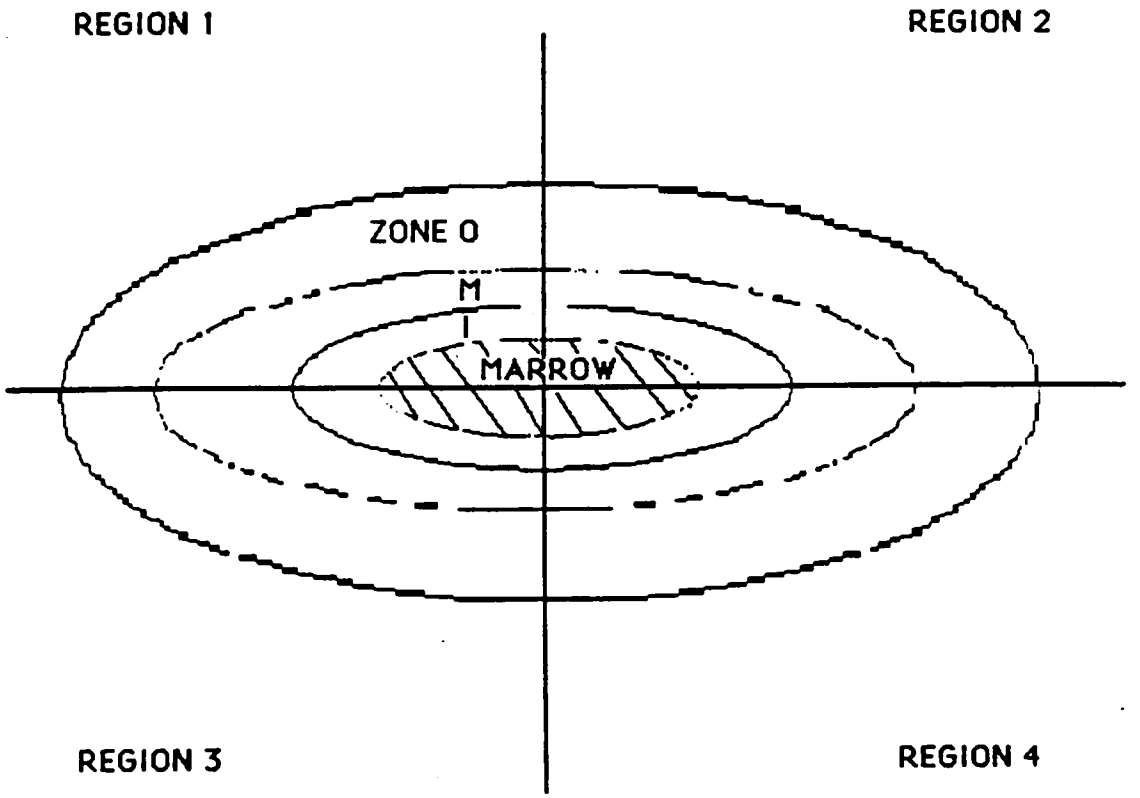

Figure 2. Diagram of cross-sectional area of bone divided into 4 regions and 3 circular zones.

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