EFFECT OF DIETARY PROTEIN DEGRADABILITY AND FAT ON RUMEN, BLOOD AND MILK COMPONENTS OF JERSEY AND HOLSTEIN COWS

BY

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(ABSTRACT)

Twenty-four cows in a production trial and 8 cows fitted with ruminal and duodenal cannulas were used in 4 x 4 Latin squares to investigate the effects of dietary protein degradability and supplemental fat on rumen, blood, and milk components. Diet dry matter contained 16% CP with two levels of rumen undegradable protein (RUP) obtained by substituting blood meal for soybean meal. Treatments were 29% RUP, 0% added fat; 29% RUP, 2.7% added fat (Calcium soaps of fatty acids); 41% RUP, 0% added fat; and 41% RUP, 2.7% added fat. Dry matter intake was depressed 6.2% by added fat. Plasma urea N (PUN) increased with added fat and 41% RUP, however greater changes were observed due to time of feeding. Milk production increased 7.1% in both breeds and 4% fat corrected milk increased by 8.4% in Jerseys fed added fat. Milk protein yield decreased in Holsteins fed 41% RUP. Milk protein content was reduced 7.1% by added fat and 3.9% by 41% RUP, and milk urea N (g/100g N) was increased by added fat and 41% RUP. Milk urea N followed PUN pattern throughout the day. Added fat reduced content, but not yield, of milk components. Blood meal substitution decreased content and yield of milk protein and casein N.

DEDICATION

I dedicate this dissertation to my wife, Carolina Rodriguez, my son, Diego Rodriguez and my parents, Luis and Irene Rodriguez.

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CHAPTER 1

INTRODUCTION

Many attempts have been made to increase milk production and components by feeding diets with higher crude protein (CP) or increasing amount of dietary fat. Today's high levels of milk production have forced researchers and nutritionists to find ways to improve dairy cattle diets to support greater requirements. Supplementation of bypass protein, amino acids, and fat have been tested.

The current National Research Council (N.R.C., 1985) divides dietary intake protein be divided into rumen degraded and undegraded fractions. The rumen degraded fraction is completely broken down in the rumen with partial conversion to bacterial and protozoal protein. The rumen undegraded fraction is resistant to rumen degradation and has potential to be digested and absorbed in the small intestine. In theory, ruminal bacteria cannot produce enough protein to support the requirements for maximum production and a supplemental source of rumen undegraded protein is suggested. However, results from feeding trials designed to evaluate supplemental rumen undegraded protein have been inconsistent.

Energy often is the limiting dietary factor in early lactation. A common method to increase energy supply is feeding higher concentrations of fat. Rations containing basic forages and grains usually have 3% dietary fat. In practice, the level

of fat is increased from 3 to 6% using oilseeds, animal tallow, or other ruminally unprotected fat and an extra 1 or 2% fat can be added using a protected or bypass source. Rumen protected fat is processed in a way that does not affect rumen function (Ca soaps of fatty acids) and is inert in the rumen. Acidic conditions in the abomasum cause dissociation of the calcium and fatty acids are absorbed.

Diets supplemented with fat have been associated with increased milk production and milk protein depression. Higher milk production increases demand for precursors of milk components. As proposed by N.R.C. (1985), increasing the amount of rumen undegradable protein without affecting microbial yield should increase the supply of amino acids available to the small intestine, alleviating milk protein depression caused by supplemental fat.

Most research studies evaluating milk components have been conducted with Holsteins. Therefore, the objective of this study was to evaluate the effect of protected fat and rumen undegradable protein on rumen, blood and milk components of Holstein and Jersey cows.

CHAPTER 2

REVIEW OF LITERATURE

Rumen Volatile Fatty Acids (VFA) and pH

Seymour et al. (1992) fed diets with protein degradabilities of 69.3 and 62.3% of total CP, based on corn silage, high moisture corn and either soybean meal or a 60:40 mixture of soybean meal and corn gluten meal. Total concentration of VFA's, and molar proportions of acetate, propionate and other VFA's were not different between diets. Molar percentages of butyrate (10.2 vs 9.7) and valerate (1.1 vs 1.0) were higher ($P<.01$) for the soybean meal diet. They suggested higher molar proportions may be due to more amino acid degradation from soybean meal.

A study conducted by Christensen et al. (1993) evaluated the effect of feeding 16 and 19% CP with 30 and 45% of total CP as undegradable intake protein (RUP) on rumen fermentation using a mixture of meat, corn gluten, blood, feather and fish meal. Total VFA concentration and propionate and valerate molar proportions decreased ($P<.04$) when the 45% RUP diets were fed. In contrast, acetate and butyrate molar proportions and acetate:propionate ratio were increased with higher RUP. The same reduction in total VFA, propionate, valerate and isovalerate was observed by Robinson

and McQueen (1994) when RUP was increased. Increased acetate concentration may indicate a greater fermentation of fiber due to a higher ruminal pH.

Zerbini et al. (1988) found a higher concentration of total VFA and acetate when soybean meal was fed compared to feather meal. Also, higher concentration of acetate and lower propionate and as a consequence higher acetate:propionate was found by Palmquist et al. (1993) when cows were fed a 51% RUP diet, there were no differences due to added fat.

Sklan and Tinsky (1993) used Ca soaps of fatty acids to coat protein to evaluate the effect of increasing RUP. Diets had no effect on rumen acetate, propionate or butyrate. Kim et al., (1993) reported total concentration of VFA was lower and molar concentrations of butyrate, isobutyrate, and isovalerate were higher when cows were supplemented Ca soaps of fatty acids compared to a soybean meal control diet. Lower total ruminal VFA concentration may indicate some interference with ruminal microbial activity. Palmquist and Conrad (1978) studied the effect of 5.9% ground raw soybeans and 5.7 and 10.8% hydrolyzed fat on rumen fermentation. Ground raw soybeans decreased acetate and increased propionate while hydrolyzed fat had no effect on any of the VFA's.

Drackley et al. (1994) reported added fat as tallow (5% of DM) had no effect on ruminal VFA concentration. Schauff et al. (1992) observed a lower total VFA concentration when tallow and whole soybeans were fed to supply 7% of DM as dietary fat but not at 5%. Grummer et al. (1993) reported a linear increase in total

VFA concentration when tallow increased from 0 to 3% of DM. The authors also reported a reduced molar proportion of isobutyrate and isovalerate when tallow was fed.

Ruminal pH of cows on soybean meal based diets was not different than those on corn gluten meal (Seymour et al., 1992) or a combination of blood and corn gluten meal (Robinson and McQueen, 1994). However, lower ruminal pH was observed for a soybean meal diet by Zerbini et al. (1988) compared to feather meal. Also, lower ruminal pH was reported by Broderick et al. (1993) when meat and bone meal was supplemented. The same response was observed by Christensen et al. (1993) with a mixture of byproducts. In addition, Wattiaux et al. (1994) reported increased ruminal pH in isonitrogenous diets when RUP increased using animal byproduct feeds. It seems that using protein bypass sources shift microbial fermentation towards fiber digestion because of increased pH, resulting in increased acetate concentration.

Schauff et al. (1992) reported added fat had no effect on ruminal pH at 5.2, 7.1 or 7.9 % of DM. Contrary to this, Grummer et al. (1993) found ruminal pH decreased linearly as the amount of supplemented tallow increased from 0 to 3% of DM. Surprisingly, diurnal variation of rumen pH was not significantly affected by feeding cows a TMR either 2X or 5X per day, although overall pH average for 2X fed cows was lower due to more pronounced declines after feeding (Robinson and McQueen, 1994).

Rumen Ammonia

Seymour et al. (1992) reported ruminal ammonia concentration was not different when soybean meal and a combination of soybean meal and corn gluten meal were fed, whereas Robinson and McQueen (1994) found ammonia concentration was decreased with a corn gluten and blood meal mix. Zerbini et al. (1988) observed lower concentration of ammonia in the rumen when feather meal was fed compared with a soybean meal diet. The same effect was observed by Wattiaux et al. (1994) and Broderick et al. (1993) when the amount of animal byproduct feeds was increased in isonitrogenous diets.

Christensen et al. (1993) found rumen ammonia concentration increased in diets containing 19% CP compared with 16%. Also, these authors found high RUP decreased rumen ammonia concentration regardless of the level of CP in the diet. Lower concentration of ammonia in the rumen may suggest less ammonia available for microbial protein synthesis.

Lower rumen ammonia concentration was observed by Kim et al. (1993) when Ca soaps of fatty acids were added to a soybean meal diet. Drackley et al. (1994) observed no significant difference in rumen ammonia when tallow was supplemented and Schauff et al. (1992) found no difference with tallow plus whole soybeans.

Diurnal variation of ammonia when cows were fed 2X or 5X per day was not significantly different regardless of RUP of the diet. Rumen ammonia peaks, greater

for the low RUP diets, were observed after the 2X feedings but surprisingly the same peaks were observed when cows were fed 5X (Robinson and McQueen, 1994). Gustafsson and Palmquist (1993) observed a peak in rumen ammonia one hour after feeding followed by a decline to baseline at 6 h.

Plasma Urea N

Plasma urea N can be derived at least from two sources, digestion of nitrogenous compounds within the gastrointestinal tract or amino acid catabolism in the liver (DePeters and Ferguson, 1992). Authors reported ammonia coming from rumen digestion of nitrogenous compounds can be a major contributor of plasma urea N. They suggested two routes could significantly contribute to plasma urea N concentration, flow of liquid from the rumen and absorption across the rumen wall. Once in the blood, the liver of the dairy cow has the capacity to convert ammonia to urea to prevent toxicity (Symonds et al., 1981).

Roseler et al. (1993) reported ruminal and postruminal excesses of N are eliminated from the body in the same process of hepatic urea synthesis. They concluded that rumen degradable and undegradable protein increased plasma urea N to a similar extent. However, Higginbotham et al. (1989) showed decreasing ruminal degradability from 64 to 57% reduced plasma urea N from 14 to 10.3 mg/dL in a 18.4% CP diet and from 9.8 to 7.2 mg/dL in a 16.1% CP diet. The same effect was

observed by Zerbini et al. (1988) when feather meal was fed compared to soybean meal. In addition, urea concentration in plasma was higher with urea (1.5% of DM) compared to soybean meal, meat and bone meal or the combination of the two (Broderick et al., 1993). Choung et al. (1990) reported plasma urea N increased linearly from 9.9 to 48.8 mg/dL when the amount of urea infused into the rumen increased from 0 to 480 g/d.

Harris et al. (1992) showed a linear effect when dietary protein increased from 14 to 18%, increasing plasma urea N from 10.34 to 16.72 mg/dL. Also, Tomlinson et al. (1994) reported a linear response of plasma urea N to dietary CP percentage in the diet. Plasma urea N values for the basal diet with 12% CP, soybean meal with 15%, blood meal with 15%, soybean meal with 18%, and blood meal with 18% CP were 5.21, 11.23, 10.42, 16.16 and 13.14 mg/dL, respectively. When calcium soaps of fatty acids were added to the same diets, plasma urea N values were 7.89, 10.74, 11.60, 14.93 and 14.94 mg/dL, respectively. Despite no significant differences with added fat, plasma urea N tended to be higher.

Palmquist et al. (1993) compared the effect of feeding 0 or 5% added fat (1:1 tallow and Ca soaps of fatty acids) and protein undegradability of 31 and 51% (1:1 blood and feather meal) on plasma urea N. Added fat and protein degradability increased plasma urea N concentration prefeeding and 3 h postfeeding. However, when tallow was added to the diet at 5% of DM no effect on plasma urea N was observed by Drackley et al. (1994).

No effect (Erickson et al., 1992) and a decrease in plasma urea N (West and Hill, 1990; Kim et al., 1993; Sklan and Tinsky, 1993) was reported when calcium soaps of fatty acids were added to the diet. In contrast, when sheep were fed Ca soaps of fatty acids, plasma urea N concentration increased at 4 h postfeeding (Maeng and Hwang, 1994). The same response was observed by Goodling et al. (1992) when Jerseys cows where fed a combination of vegetable:animal:Ca soaps of fatty acids. A decrease in plasma urea N concentration was observed by Palmquist and Conrad (1978) when cows were fed hydrolyzed fat. Added fat at 2.1% of DM from whole soybeans plus 2.5% or 4.0% tallow had no effect on PUN concentration (Schauff et al., 1992).

The diurnal variation of plasma urea N was evaluated by Gustafsson and Palmquist (1993). They found high producing cows had a serum urea peak 70 to 85% higher than the lowest serum urea concentration within a cow. The serum urea peak occurred 1.5 to 2.0 h after the rumen ammonia peak. Also, Miettinen and Juvonen (1990) reported serum urea levels increased after feeding in the morning but was not affected in the afternoon.

Plasma urea and milk urea N have been highly correlated ($r = .88$, Roseler et al., 1993; $r = .91$, Oltner et al., 1985; $r = .89$ for morning sampling and $r = .96$ for afternoon sampling, Miettinen and Juvonen, 1990). Also, milk urea N and dietary DIP ($r = .74$) have been found to be correlated but not dietary RUP ($r = .30$, Ropstad et al., 1989).

Milk Urea N

DePeters and Cant (1992) described the N of milk in three broad fractions: casein N, whey protein N, and non-protein N (NPN). Cerbulis and Farrell (1975) reported these three fractions constitute approximately 77.9, 17.2 and 4.9%, respectively, of the total N of milk. The distribution of the N fractions of milk can be affected by temperature, disease, parity, stage of lactation, breed and nutrition (DePeters and Cant, 1992).

The single largest contributor to milk NPN is urea (DePeters and Ferguson, 1992). Milk urea N expressed as percentage of total milk NPN ranged from 19.5 to 44.7% in diets with different dietary protein degradabilities (Roseler et al., 1993). The primary source of milk urea N is passive diffusion of urea from plasma to milk (Clark et al., 1978). DePeters and Ferguson (1992) in their review reported at least three sources of urea in milk. These were the end product of digestion, amino acid catabolism in the liver and a very small fraction can be contributed by Arginine catabolism in the mammary gland.

There are many sources of variation in milk urea N concentration, such as body weight, parity, stage of lactation, and diurnal variation (Gustafsson, 1993). Oltner et al. (1985) reported an increase of 100 kg of body weight was associated with a reduction in milk urea N concentration of 3.6 mg/dL. They also conducted a short experiment which found milk urea N concentration was 4.56 mg/dL lower in first

lactation than in older cows. These lower values for first lactation cows can be possibly explained by some of the N being partitioned towards growth.

Days in milk ranging from 100 to 200 had no effect on milk urea N (Roseler et al., 1993). Contrary to this, Gustafsson (1993) reported milk urea N was lower during the first four weeks of lactation than at other stages. He reported least squares means for weeks 1 to 4, 5 to 12 and 13 to 43 to be 25.8, 30.63 and 30.03 mg/dL.

Very few studies have been conducted to study the diurnal variation on milk urea N. Gustafsson and Palmquist (1993) found urea N in milk was equilibrated with serum urea with a lag of 1 to 2 h when the rate of change of urea N in serum was 3 to 6 mg/dL per hour. Oltner and Wiktorsson (1983) and Miettinen and Juvonen (1990) reported lower milk urea N values in morning compared to afternoon milking.

Roseler et al. (1993) compared the effects of feeding rations with different protein degradabilities on milk urea and milk NPN. The intake of undegradable and degradable protein elevated plasma and milk urea N to a similar extent. Also, fat intake increased NPN content of milk (DePeters and Cant, 1992). Wonsil (1993) reported added fat (partially hydrogenated tallow) increased the concentration of milk urea N but level of undegraded intake protein (33 and 41%) had no effect.

Casein N

Casein N concentration in bovine milk is approximately 78% of the total milk protein. The concentration of casein N decreases rapidly after parturition to a minimum at 60 days of lactation, followed by a gradual increase to the latter part of lactation (Ng-Kwai-Hang et al., 1984). Also, as the age of the cow increases, casein content decreases and whey protein content of milk increases.

A study to evaluate the effect of feeding corn or barley on milk N composition was conducted by DePeters and Taylor (1985). Casein, whey and NPN contents were not different due to type of grain. In contrast, milk casein N decreased with whole cottonseed feeding from .387 to .375%, but milk casein N as a percent of the total N was not different, although there was a tendency for reduction (DePeters et al., 1985).

DePeters et al. (1989) reported the effect on milk N composition of feeding 0 and 3.5% dietary fat (grease) and either 1.6 or 1.7 Mcal NE_l in the total ration. Adding fat depressed casein N concentration at both energy densities even though proportion of total N in the casein fraction was not affected. Drackley and Elliot (1993) found no difference in milk casein N content when partially hydrogenated tallow and Kincaid and Cronrath (1993) when Ca soaps of fatty acids were fed. Wonsil (1993) reported partially hydrogenated tallow and level of RUP (33 and 41%) had no effect on casein N concentration. The same response was found by Sandrucci et al. (1992) when blood meal was fed.

DePeters and Cant (1992) reported a summary of data from three trials in which milk N fractions were determined (Table 2.1). They found a decrease in content of total N and casein N with an increase in ether extract intake and concentration in the diet of multiparous and early lactation cows. There was no similar relationship for late lactation cows which might be attributed to positive N balance. The response in primiparous cows was similar to multiparous cows but sample size was smaller. Although, adding fat to the diet depressed casein N concentration in milk, energy intake of the cow is increased and consequently milk and casein yields were increased (Depeters et al., 1987).

TABLE 2.1. Correlations between feed variables and milk content of total N, casein and NPN.

Cows and EE[1]	Total N	Casein	NPN	
All cows (n=129)				
EE intake		-.18*	-.18*	.40**
EE, % of diet		-.22**	-.22**	.36**
Multiparous cows (n=98)				
EE intake		-.17*	-.17*	.40**
EE, % of diet		-.22**	-.22**	.35**
Primiparous cows (n=31)				
EE intake		-.21	-.23	.40**
EE, % of diet		-.21	-.23	.39**
Early lactation (n=48)				
EE intake		-.25*	-.22*	.26**
EE, % of diet		-.38*	-.32**	.22**
Late lactation (n=50)				
EE intake		-.11	-.13	.52**
EE, % of diet		-.09	-.12	.48**

[1] EE = Ether extract
* $P < .05$
** $P < .01$
Source: DePeters and Cant (1992)

In situ studies

The in situ bag technique has been used extensively to determine rates of ruminal degradation of DM and CP in different feedstuffs (Nocek et al., 1979; Nocek, 1986; Armentano et al., 1986; Murphy and Kennelly, 1987). The objective of this technique is to estimate the amount of protein and DM degraded in the rumen. The rate of DM and protein degradation varies within feedstuffs (Nocek, 1986; Erdman et al., 1987; Stallings et al., 1991), drying procedure (Armentano et al., 1986) forage type, preservation process (Nocek and Grant, 1987) and forage maturity (Stallings et al., 1991).

Nocek (1986) reported ruminal degradation rates for dry shelled and high moisture corn to be 6.1 and 5.7%/h for DM and 5.3 and 4.9%/h for CP. Lower degradation rates were reported by Nocek et al. (1979) for shelled corn (3.7 and 3.0%/h for DM and CP, respectively).

Degradation rates for soybean meal were 12.1 and 17.7%/h for DM and CP (Armentano et al., 1987) while Cozzi and Polan (1994) reported 6.47%/h for CP. The rate reported for corn gluten meal was 2.14%/h for DM (Cozzi et al., 1993), and .44%/h (Cozzi and Polan, 1994) and 1.2%/h (Cozzi et al., 1993) for CP. Nocek and Grant (1987) found the degradation rate for alfalfa silage was 11.7%/h for DM and 28.4%/h for CP. When samples were corrected for microbial contamination the rate of CP degradation was 15.2%/h.

Herrera-Saldana et al. (1990) showed that DM and CP ruminal degradabilities of corn grain were 54.4% and 69.7% and Stallings et al. (1991) reported 78.7 and 71.6%. However, Cronje (1983) found a lower CP degradability of 64.2%, Erdman et al. (1987) found 61.4%, Erasmus et al. (1994) found 56.5%, N.R.C. (1989) found 48% and Herrera-Saldana et al. (1986) 22.3%.

More consistent soybean meal DM degradabilities have been reported: 82% (Armentano et al., 1987), 78.1% (Stallings et al., 1991), 66.6% (Kirkpatrick and Kennelly, 1986). Cummins et al. (1983) reported a lower value of 56.7%. Crude protein degradabilities varied more than DM and were 93.2% (Cronje, 1986), 83% (Armentano et al., 1987), 76.9% (Stallings et al., 1991), 70% (Nocek, 1985), 67% (Erdman et al., 1987), 65% (N.R.C., 1988), 60.2% (Kirkpatrick and Kennelly, 1986), 46.2% (Erasmus et al., 1994) and 32.8% (Herrera-Saldana et al., 1986).

Corn gluten meal degradabilities are also quite variable. Murphy and Kennelly (1987) found 18.9 and 11.0%, Cozzi et al. (1993) 27.9 and 20.7% and Cummins et al. (1983) 44.0 and 50.8% for DM and CP, respectively. A CP degradability of 18.6% and 24.5% was reported by Erasmus et al. (1994) and Cronje (1983), but Herrera-Saldana et al. (1986) reported a value of 13.5%.

Erasmus et al. (1994) found a 19.2% CP disappearance from the rumen for blood meal, similar to that reported by N.R.C. (1989). Palmquist et al. (1993) and Herrera-Saldana et al. (1986) reported a lower blood meal CP degradability of 14.9% and 13.1%, respectively. Howie et al. (1994) determined ruminal degradation of

different by-pass protein feedstuffs including ring dried and batch dried blood meal. Crude protein degradation for ring dried ranged from 13.6 to 23.6% with a mean of 19% and batch dried from 5.6 to 22.4% with a mean of 15.7%. These results demonstrate large variation in ruminal degradation between two systems of processing blood meal.

Crude protein degradabilities reported for corn silage were 84.4% (Cronje, 1983), 71.7% (Erdman et al., 1987) and 69% (N.R.C., 1989). Alfalfa silage CP degradabilities were 70.9% (Erdman et al., 1987) and 77% (N.R.C., 1988). Janicki and Stallings (1988) evaluated in situ degradation of CP in various forages and preservation methods. They reported a CP degradability for corn silage of 82.2%, and 84.9% and 80.9% for alfalfa silage stored in conventional upright and oxygen limiting silos, respectively.

Nocek et al. (1979) evaluated the disappearance rates for some commonly used feedstuffs via the in situ, polyester bag technique. Nitrogen disappearance for soybean meal was 21%, cottonseed meal 41.4% and corn silage 42.7%. Corn gluten meal and ground corn had a percent residual N of 100 or above at 2 h and can be explained by microbial contamination. However, no significant differences were detected between N disappearance rate constants with or without correction for bacterial N contamination (10.6 vs 10.3%/h) when soybean meal was used (Nocek, 1985). Contrary to this, Blair and Cumming (1983) reported mean percentage microbial N contamination for soybean meal and dehydrated alfalfa after 18 h of ruminal exposure

to be 41.6 and 11.7% of total N. Mathers and Aitchison (1981) reported that nearly 20% of the residual N in alfalfa samples after 24 h of incubation was from microbial origin.

Nocek and Grant (1987) compared ruminal degradation of different forages including alfalfa stored at various DM. Dry matter degradation in hay, 60, 40 and 20% DM silages were 22.3, 11.7, 30.0 and 8.7%/h and N degradabilities were 27.1, 28.4, 29.3 and 2.1%/h. When samples were corrected for microbial N contamination rates were 18.3, 15.2, 15.3, and 7.9%/h for N degradability. Therefore, correction for bacterial N contamination changed rates of N digestion compared to noncorrected rates.

For rapidly degraded feedstuffs such as soybean meal, bacterial protein contamination does not appear to be a problem, but slowly degraded feedstuffs such as forages, contamination can result in underestimation of rates and extent of N digestion.

Total tract digestibility and microbial protein synthesis

Research has been conducted to increase nutrient availability to support milk production through increasing feed intake and rumen fermentation. Clark et al. (1992) reported microbial growth and milk production are most often limited by energy and N. They also point out the amount and source of energy and protein in the diet affect

ruminal fermentation and flow of dietary and microbial N to the small intestine. Also, N.R.C. (1985) reported bacterial crude protein (BCP) for lactating cows described as a function of the NE_l intake by the following formula:

$$BCP = 6.25\ (-30.93 + 11.45\ NE_L)$$

Passage of non-ammonia N to the small intestine increased when cows consumed greater amounts of N ($r^2 = .82$; Clark et al., 1992). Total N flow to the duodenum was increased when a combination of hydrolyzed feather meal and blood meal (Palmquist et al., 1993) or a combination of meat, blood, feather, fish and corn gluten meal was fed (Christensen et al., 1993). Contrary to this, no difference was observed when diets with feather meal (Zerbini et al., 1988), corn gluten meal (Seymour et al., 1992; Klusmeyer et al., 1990), fish meal (Klusmeyer et al., 1991a), blood meal (Waltz et al., 1989) and soybean meal were compared.

Clark et al. (1992) summarized 5 experiments and concluded the passage of microbial N to the small intestine was affected more by DMI and other dietary factors than by N intake. They also summarized 8 trials where fish, blood, corn gluten, feather meal, dried distillers grains or combinations were compared and concluded that passage of non-ammonia N to the small intestine does not increase, until around 35% of the CP fed, is supplied by supplements more resistant to rumen degradation. When resistant supplements were fed in proportions between 14 and 25% of the CP, there

was a slight decrease in passage of non-ammonia N because passage of microbial N decreased. The amount of microbial N reaching the small intestine was not different with diets containing a combination of soybean and corn gluten meal (Seymour et al., 1992), corn gluten meal alone (Klusmeyer et al., 1990) or blood meal (King et al., 1990). However, the amount of microbial N passing to the small intestine increased when soybean meal was fed compared to feather meal (Zerbini et al., 1988) and corn gluten meal was compared to cottonseed meal (King et al., 1990). A decrease in microbial N reaching the small intestine was reported when fish meal (Klusmeyer et al., 1991a) and blood meal (Waltz et al., 1989) were fed .

No significant differences were observed in the efficiency of microbial protein synthesis when corn gluten meal (Seymour et al., 1992; Klusmeyer et al., 1990), blood meal (King et al., 1990), fish meal (Klusmeyer et al., 1991a) or a combination of rumen resistant byproducts replaced part of the soybean meal (Christensen et al., 1993). However, Zerbini et al. (1988) found a greater efficiency of microbial protein synthesis when soybean meal was compared to feather meal. Also, Waltz et al. (1989) compared blood meal, feather meal and a 1:1 combination to soybean meal and found greater efficiency of microbial protein synthesis when soybean meal was used.

A reduction in rumen digestibility of DM and organic matter (OM) was observed when a high RUP diet was fed (Palmquist et al., 1993), but no difference was reported by Christensen et al. (1993) and Klusmeyer et al. (1990). Also, no difference in ruminal digestibility of DM and OM was observed when fish meal was

fed (Klusmeyer et al., 1991a). The level of undegradable protein had no effect on apparent total tract digestibility of OM (Zerbini et al., 1988; Waltz et al., 1989), OM and N (Seymour et al., 1992; King et al., 1990), or DM and OM (Christensen et al., 1993; Klusmeyer et al., 1990; Klusmeyer et al., 1991a). Christensen et al. (1993) reported a decrease in apparent total tract digestibility of N when a high RUP was fed.

Total N flow to the small intestine was not different when Ca soaps of fatty acids were fed (Klusmeyer et al., 1991a; Klusmeyer et al., 1991b). Fat supplementation has been reported to decrease microbial protein synthesis (Palmquist et al., 1993) when a mixture of 1:1 tallow and Ca soaps of fatty acids was used but no difference was observed with only Ca soaps of fatty acids (Klusmeyer et al., 1991a; Klusmeyer et al., 1991b). Efficiency of microbial protein synthesis tended to be greater with Ca soaps of fatty acids compared to the control diet (Klusmeyer et al., 1991a), but was not different in a second study (Klusmeyer et al., 1991b).

Total tract digestibility of DM was not affected by prilled fat and canola oil supplementation (Jenkins and Jenny, 1992), Ca soaps of fatty acids (West and Hill, 1990; Klusmeyer et al., 1991a; Klusmeyer et al., 1991b) or tallow, Ca soaps of fatty acids and prilled fat (Wu et al., 1993). However, Wu et al. (1993) reported tallow had a greater DM digestibility than Ca soaps of fatty acids and prilled fat. Total tract digestibility of N was increased with a combination of tallow and Ca soaps of fatty acids (Palmquist et al., 1993), hydrolyzed fat (Palmquist and Conrad, 1978), prilled fat and the combination of prilled fat and canola oil (Jenkins and Jenny, 1992), and Ca

soaps of fatty acids (Klusmeyer et al., 1991a). Contrary to this, no effect in total tract digestibility of N was found with tallow, Ca soaps of fatty acids or prilled fat (Wu et al., 1993) and with Ca soaps of fatty acids (Klusmeyer et al., 1991b).

Effect of dietary fat and RUP on milk production

It was pointed out in a review in the early 80's, the fact that fat can be added up to 3% of the total diet DM without problems and up to 5% in high producing herds (Palmquist and Jenkins, 1980). More recently, there has been a lot of interest in feeding an extra 2 to 3% fat in a form that resists degradation in the rumen. Recent studies have been conducted to increase milk production and persistency by feeding different fat sources, whole soybeans and tallow (Schauff et al., 1992), extruded soybeans (Kim et al., 1993), cottonseeds (Sklan et al., 1992) and Ca soaps of fatty acids (Schneider et al., 1988; Sklan et al., 1989; Erickson et al., 1992; Tomlinson et al., 1994).

In some studies DMI was not different compared to the control diet when fat was added (Schneider et al., 1988; Schauff et al., 1992; Klusmeyer et al., 1991a; Erickson et al., 1992; Wu et al., 1993; Tomlinson et al., 1994) while others observed a decrease (Klusmeyer et al., 1991b; Kim et al., 1993). Milk production was not improved when cows were fed whole soybeans and tallow (Schauff et al., 1992), Ca soaps of fatty acids plus whole cottonseeds (Kincaid and Cronrath, 1993) or Ca soaps

of fatty acids alone (Klusmeyer et al., 1991a; West and Hill, 1990). Lack of response when fat is fed may be due to supplementation in mid or late lactation when cows are in a positive energy balance or due to genetic potential of the herd.

Contrary to this, milk production increased when Ca soaps of fatty acids were supplemented (Schneider et al., 1988; Klusmeyer et al., 1991b; Erickson et al., 1992; Tomlinson et al., 1994). Wu et al. (1993) reported increased milk production with tallow, Ca soaps of fatty acids and prilled fat. Also, Goodling et al. (1992) observed an increase in milk yield and 4% FCM when Jersey cows where fed an animal vegetable mix and Ca soaps of fatty acids.

Schneider et al. (1990) evaluated the effect of feeding Ca soaps of fatty acids and injecting bovine somatotropin (BST) in lactating dairy cows. Feed intake and milk production were not affected by added fat or BST but interestingly, apparent feed efficiency (kg of 3.5% FCM/DMI) was increased by Ca soaps of fatty acids and a larger increase was observed when fat and BST were together.

The N.R.C. (1989) reported "as milk production increases, a substantial amount of additional dietary protein from a protein supplement must escape ruminal fermentation to meet the animal's requirement for protein". Responses to feeding RUP have been contradictory. Zimmerman et al. (1992), Klusmeyer et al. (1991a), Christensen et al. (1993) and Tomlinson et al. (1994) reported no change in milk production when high RUP was fed. In contrast, Pires et al. (1994) found a decrease in milk production when feeding a high level of bypass protein.

Rakes et al. (1992) reported the level of bypass protein did not affect milk production in Jersey cows when the data was analyzed for primiparous and multiparous cows together. However, when primiparous cows were analyzed separately, more milk and FCM was produced when treated bypass soybean meal was fed. In addition, Sandrucci et al. (1992) reported no difference in milk production when blood meal was fed at 2.5% of ration DM.

An increase in DMI was observed by Zimmerman et al. (1992) with a high RUP diet, whereas Klusmeyer et al. (1991a), Tomlinson et al. (1994) and Christensen et al. (1993) found no difference between high RUP and soybean meal. The same response was observed by Sandrucci et al. (1992) with blood meal. A decrease in DMI was reported when a high RUP diet (Wattiaux et al., 1994) and blood meal were fed (Pires et al., 1994).

Effect of dietary fat and RUP on milk fat production

Milk fat content was increased when fat was added to the diet using whole soybeans and tallow (Schauff et al., 1992), Ca soaps of fatty acids (Schneider et al., 1988; Klusmeyer et al., 1991a; Klusmeyer et al., 1991b) and a mix of animal:vegetable:Ca soaps of fatty acids (Goodling et al., 1992). Also, West and Hill (1990) reported that milk fat content increased when Ca soaps of fatty acids were fed to Holstein and Jersey cows. Conversely, no effect was observed with Ca soaps of

fatty acids (Schneider et al., 1990; Erickson et al., 1992; Tomlinson et al., 1994) and Ca soaps of fatty acids and whole cottonseeds (Kincaid and Cronrath, 1993).

Schauff et al. (1992) found an increase in milk fat yield with whole soybeans and tallow. The same response was observed by Klusmeyer et al. (1991b) and Schneider et al. (1988) with Ca soaps of fatty acids, and by Kincaid and Cronrath (1993) with Ca soaps of fatty acids and whole cottonseeds. Also, Wu et al. (1993) using tallow, Ca soaps of fatty acids and prilled fat found an increase in milk fat yield. Increases in milk fat yield are due mainly to increases in milk production.

Zimmerman et al. (1992) and Klusmeyer et al. (1991a) reported no change in milk fat yield and percentage with a high RUP diet. However, Tomlinson et al. (1994) reported a decrease in fat content with feather meal and Sandrucci et al. (1992) with blood meal. In contrast, Christensen et al. (1993) and Wattiaux et al. (1994) found high RUP diets increased milk fat content. Wonsil (1990) reported that energy status of the animal, DMI and stage of lactation may influence milk fat production. Variable responses in milk fat content with high RUP diets may be due to one of these factors.

Effect of dietary fat and RUP on milk fatty acids

Palmquist and Jenkins (1980) reported under normal conditions approximately one half of the fatty acids in milk fat are derived from de novo synthesis, 40 to 45%

from the diet, and less than 10% from adipose tissue. The ruminal biohydrogenation of unsaturated fatty acids accounts for the high degree of saturation of ruminant milk and adipose tissue. However, if the fat is protected from ruminal activity, not only the biohydrogenation process can be overcome but also, can prevent interference with fiber digestion. The digestion and absorption of protected fat in the small intestine is unimpaired and unsaturated fatty acids can be incorporated into milk fat and adipose tissue (Annison, 1983).

Higher weight percentages ranging from 8 to 42% of C_6 to C_{14} were found in Jerseys compared to Holsteins regardless of the diet (Palmquist and Beaulieu, 1992). Authors also reported that $C_{18:0}$ was 13% higher and $C_{18:1}$ 15% lower in Jersey cows. The ratio of $C_{18:0}/C_{18:1}$ was .54 for Jersey cows regardless of fat supplementation but in Holsteins this ratio decreased from .45 to .39 when Ca soaps of fatty acids were fed. In a second study, they compared Jerseys and Holsteins fed roasted soybeans. Jerseys had more C_6 to C_{14} by 15% more than Holsteins but the ratio of $C_{18:0}/C_{18:1}$ was the same.

Added fat as whole soybeans and tallow decreased concentration of short and medium chain and $C_{16:0}$ milk fatty acids, while increased $C_{18:0}$ and $C_{18:1}$ (Schauff et al., 1992). A similar response was observed by Kim et al. (1993) when extruded soybeans were fed. A decrease in C_{10} , C_{12} and $C_{18:0}$ and an increase in $C_{16:0}$ and $C_{16:1}$ was observed with Ca soaps of fatty acids (Tomlinson et al., 1994). Schneider et al. (1990) found a decrease in C_{12}, C_{14} and $C_{18:0}$ and a higher proportion of C_{16} and

$C_{18:1}$. However, no significant differences were observed in the fatty acid composition of milk from C_8 to $C_{18:3}$ when cows were supplemented with Ca soaps of fatty acids (Schneider et al., 1988).

Wu et al. (1993) compared diets containing tallow, Ca soaps of fatty acids, or prilled fat on milk fatty acid composition. Added fat decreased percentages of C_6 to $C_{14:0}$ and increased C_{16} (14%) and C_{18} (22%). Ca soaps of fatty acids and prilled fat increased $C_{16:0}$ by 8.5 and 3.5% more than tallow respectively. Tallow increased $C_{18:0}$ by 15.6% more than the other two sources of fat.

No differences were observed in milk fatty acid composition when cows were fed a high RUP diet (Zimmerman et al., 1992). However, lower short chain fatty acids from C_6 to C_{12} were reported by Tomlinson et al. (1994) with feather meal.

Effect of dietary fat and RUP on milk protein production

Wu and Huber (1994) compiled data of trials from 1970 to 1992 that evaluated fat source and milk protein concentration. Fat supplements were categorized into animal-vegetable, Ca salts of fatty acids, prilled fat, protected tallow, tallow, whole cottonseeds, whole soybeans, and yellow grease. They reported occurrence and magnitude of milk protein depression were similar regardless of fat source, and concluded the major reason for depression may be due to insufficient amino acids available to the mammary gland when fat supplementation increases milk yield.

Different hypotheses have been proposed to explain milk protein depression when fat is added: glucose deficiency (Smith et al., 1978), insulin resistance (Palmquist and Moser, 1981), decreased mammary blood flow (Cant et al., 1993), somatotropin deficiency (Casper and Schingoethe, 1989) and amino acid supply to the mammary gland for milk protein synthesis (Wu and Huber, 1994). Also, DePeters and Cant (1992) suggested a dilution effect for milk protein depression when milk yield increases. This effect can be calculated as:

$$\left(1 - \frac{\textit{percentage increase protein yield}}{\textit{percentage increase milk yield}}\right) X\ 100$$

In some studies, milk protein percentage was not affected by added fat as whole soybeans and tallow (Schauff et al., 1992) or Ca soaps of fatty acids (Schneider et al., 1988; Schneider et al., 1990; Tomlinson et al., 1994). In contrast, Ca soaps of fatty acids decreased milk protein content although yield was not affected (Klusmeyer et al., 1991a; Klusmeyer et al., 1991b; Erickson et al., 1992; Goodling et al., 1992; Wu et al., 1993). A similar response was observed by Wu et al. (1993) with tallow or prilled fat and by Drackley and Elliot (1993) with partially hydrogenated tallow. Also, West and Hill (1990) reported milk protein content but not yield decreased when Ca soaps of fatty acids were fed to Holstein and Jersey cows.

Fat supplementation decreased solids non-fat content but not yield when Ca soaps of fatty acids were fed (Klusmeyer et al., 1991b; Erickson et al., 1992). Also, partially hydrogenated tallow reduced solids non-fat but not yield (Drackley and Elliot, 1993). Contrary to this, Klusmeyer et al. (1991a) found no difference in solids non-fat and yield. Kim et al. (1993) reported added fat as extruded soybeans or Ca soaps of fatty acids had no effect on lactose content, but yield was increased. Sandrucci et al. (1992) found no change in lactose content with blood meal while Klusmeyer et al. (1990) reported solids non-fat content and yield were decreased when corn gluten meal was fed.

Zimmerman et al. (1992) reported a decrease in milk protein content with soybean meal plus undegradable protein, Klusmeyer et al. (1990) with corn gluten meal, and Tomlinson et al. (1994) with blood meal and feather meal. When whole or ground roasted soybeans were fed the same response was observed (Pires et al., 1994). Sandrucci et al. (1992) and Pires et al. (1994) observed no change in milk protein content with blood meal and Christensen et al. (1993) with a mix of rumen resistant byproducts. Contrary to this, Komaragiri and Erdman (1992) found an increase in milk protein percentage with a high RUP diet.

CHAPTER 3

EFFECT OF DIETARY PROTEIN DEGRADABILITY AND FAT ON RUMEN, BLOOD AND MILK COMPONENTS OF JERSEY AND HOLSTEIN COWS (ABSTRACT)

Twelve Holstein and 12 Jersey cows were used in six 4 x 4 Latin squares to investigate the effects of dietary protein degradability and supplemental fat on rumen, blood and milk components. Diet dry matter contained 16% CP with two levels of rumen undegradable protein (RUP) obtained by substituting blood meal for soybean meal. Treatments were 29% RUP, 0% added fat; 29% RUP, 2.7% added fat (Calcium soaps of fatty acids); 41% RUP, 0% added fat; and 41% RUP, 2.7% added fat. Total mixed ration DM consisted of 30% corn silage, 29% alfalfa haylage and 41% concentrate. Dry matter intake (DMI) was depressed 6.2% by added fat. Plasma urea N (PUN) at 0700 h increased in Jerseys fed added fat and in Holsteins fed 41% RUP, but at 1600 h increased only in Jerseys fed added fat. Milk production increased 7.1% in both breeds and 4% fat corrected milk 8.4% in Jerseys fed added fat. Milk protein (kg/d) was reduced by 41% RUP in Holsteins. Milk protein content was reduced 7.1 and 3.9%, and milk urea N (g/100g total N) was increased 4.9 and 8.5% by added fat and 41% RUP, respectively. Added fat reduced concentration, but not yield, of milk components. Blood meal substitution decreased concentration and yield of milk protein and casein N.

Materials and Methods

Experimental Design and Diets

Twelve Holstein and 12 Jersey cows (35 to 73 days postpartum) were used in six 4 x 4 Latin squares balanced for carryover effects. Experimental periods were 21 d. Cows were randomized and blocked within breed into three squares according to pretreatment milk production. Cows were housed in the Calan Door area at the Virginia Tech Dairy Center and breeds were separated to avoid competition. Ingredients and chemical composition of the diets are shown in Table 3.1. Diets were formulated to be isonitrogenous (17% CP) and to have 29 and 41% rumen undegradable protein **(RUP).** Blood meal was substituted for soybean meal on a protein equivalent basis to increase the RUP level. Each level of RUP was formulated without and with 2.7% added fat **(F)**. A portion of shelled corn was substituted with Ca soaps of fatty acids (MEGALAC, Church & Dwight Co., Inc.). Corn gluten meal was added to supply an equivalent amount of the corn protein that was removed. Diets containing added fat had a greater Net Energy of lactation and were calculated to have 5.8 to 5.9% total fat. All diets were formulated to have 31% neutral detergent fiber and 19% acid detergent fiber.

Concentrate ingredients were premixed and stored until added to corn and alfalfa silages in a Uebler mixer (Model 780, Uebler Manufacturing Co., Vernon, NY)

prior to feeding. The total mixed diets were fed as total mixed diets at 1000 and 1400 h.

Measurements and Sampling

Cows were fed to have between 5 and 10% daily orts, which were checked each morning and feeding rates adjusted. Dry matter intake was measured on days 7 through 10 and 14 through 17 of each period. Feed refusals were weighed at 0630 on an electronic scale. Forages were sampled weekly and concentrates in the third week of each period. Each sample was taken in duplicate, and one was sent to the Virginia Tech Forage Testing Laboratory and the other frozen for further analysis. Body weights were recorded at d 1, 20 and 21. Milk production was recorded daily by a Surge computerized system. Samples for milk fatty acids were collected at 1300 h on d 18 of each period. Samples were centrifuged in a Beckman J2-21 centrifuge (Beckman Instruments, Columbia, MD) with a JA-20 rotor at 5930 x *g* for 30 min and placed at -20°C until the fat layer was firm (approximately 10 min). The fat layer was removed and stored at -20°C. Samples for milk composition were taken in duplicate at 0100 and 1300 h on d 19 and 20 of each period. Samples were collected in 50 mL DHIA milk containers with preservative (Bronopol) and stored at 2°C at the Dairy Nutrition Laboratory. One sample was submitted to the DHIA Laboratory on d 21 of

each period for milk fat, solids non-fat and lactose analysis. The duplicate sample was analyzed for total N, casein N and milk urea N.

Blood samples (10 mL) were collected from the jugular vein at 0700 and 1600 h on d 20 of each period for plasma preparation. Blood samples were combined with 100 μL of heparinized saline with concentration of 2.86 units/μL (Sodium salt heparin, Sigma Chemical Co., St. Louis, MO) and immediately placed on ice. Samples were taken to the Dairy Nutrition Laboratory and centrifuged in a Beckman J2-21 centrifuge (Beckman Instruments, Columbia, MD) with a JA-20.1 rotor at 3220 x *g* for 15 min. Plasma was divided into duplicate samples prior to storage at -20°C.

Ruminal fluid was taken at 1600 h on d 20 of each period by applying vacuum to an esophageal tube fitted with a suction strainer. A total of 50 mL was collected into 50 mL plastic tubes, immediately placed on ice and taken to the Dairy Nutrition Laboratory. Duplicates 5 mL aliquots were preserved with 1 mL of 25% H_3PO_4 for ammonia analysis. Duplicates 5 mL aliquots were preserved with 1 mL of 25% H_3PO_4 and 1 mL of 30 mM isocaproic acid (internal standard) for VFA analysis, prior to storage at -20°C. Ruminal pH was measured on the remaining portion of each sample.

Analyses

Concentrate and forage DM were determined by drying overnight at 60°C in a forced air oven. Dried samples were ground through a 1 mm screen in a Cyclotec mill (Tecator 1093, Hoganas, Sweden) and analyzed for total N (AOAC, 1990), ether extract (AOAC, 1990), acid detergent fiber (Goering and Van Soest, 1970) and neutral detergent fiber (Van Soest et al., 1991). Milk samples were analyzed for fat and lactose content by infrared spectroscopy (Fossomatic 605, B filter), and total milk N by Kjeldahl analysis (AOAC, 1990).

Milk urea N was determined in triplicate by urease method (Weatherburn, 1967). Ten μL of milk sample were placed into a 10 mL glass tube and incubated for 20 min with 200 μL of urease solution (Sigma Chemical Co., St. Louis, MO) with concentration of .104 units urease/μl sample. One mL of reagent one (50 g phenol and 250 mg sodium nitroferricyanide/L) and reagent two (25 g sodium hydroxide and 42 mL 8% sodium hypochlorite/L) were added. Samples were vortexed and incubated at room temperature overnight. Absorbance was read on a spectrophotometer (Spectronic 1001, Bausch & Lomb, Rochester, NY) at a wavelength of 625 nm. Milk urea N was also measured using Azotest strips (Compagnie Chimique D'Aquitaine, Lalande de Pomerol, France). Samples were placed into a 10 mL beaker, a strip was incubated in the milk for 5 min and rinsed with cold tap water. A subjective

evaluation of strip colors was made by two observers and compared to standard colors on the bottle.

Milk casein N was determined using the procedure of Aschaffenburg and Drewry (1959). Samples were warmed to room temperature and mixed thoroughly. A 2.5 mL sample, 17.5 mL of distilled water and .25 mL of 10% acetic acid were placed in a 250 mL Erlenmeyer flask and mixed. Flasks were placed in a water bath at 40°C for 10 min. Precipitated samples were filtered through Whatman #2 (Whatman, Clifton, NJ) filter paper and rinsed three times with distilled water. Once drained, filter papers were folded and put into a Kjeldahl tube for N analysis (AOAC, 1990).

Milk fatty acids were determined by extracting and methylating by direct transesterification (Outen et al., 1976) using undecenoic acids (NU-Check-Prep Inc., Elysian, MN) as internal standard. A Hewlett Packard 5890A gas chromatograph equipped with a flame ionization detector (Hewlett Packard Co., Sunvale, CA) and an auto-sampler was used for analysis. Samples were split 100:1 onto a 30 m DB-225 glass capillary column with .25 mm i.d. and .15 μm film thickness (J & W Scientific, Folsom, CA). Temperature for the injector and detector was at 225°C. Runs were initiated by a temperature program at 60°C, warmed to 205°C at a rate of 5°C/min, held for 12 min and warmed to 220°C at a rate of 5°C/min and held for 7 min.

Rumen samples were thawed and filtered through a GN-6 filter (Geldman Sciences, Ann Arbor, MI) using syringe and 25 mm Millipore swinnex filter holder (Millipore Corporation, Bedford, MA). Plasma and filtered rumen samples were

analyzed for urea N and ammonia, respectively (Weatherburn, 1967). Filtered rumen samples were analyzed for VFA by gas chromatography by injecting .5 μL of sample into a Varian Vista 6000 chromatograph (Varian Instruments, Palo Alto, CA) equipped with a flame ionization detector, a Varian Vista 4270 integrator (Varian Instruments, Palo Alto, CA), and a 6' x ¼" o.d. and 2 mm i.d. glass column (Supelco Inc., Bellfonte, PA) packed with GP 10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb WAW. The analysis was isothermal with temperatures of 115°C for the column, 170°C for the injector and 180°C for the detector. Nitrogen gas was used as a carrier with a flow of 80 mL/min and hydrogen and air as detector gases with flows of 40 and 60 mL/min, respectively. Rumen pH was measured on unfiltered rumen samples within 60 min after collection using an Accumet pH Meter (Fisher Scientific, Raleigh, NC).

Statistical Analyses

Data were analyzed by ANOVA using the general linear models procedure of PC-SAS (1990). Data are presented as least squares means and standard errors of the mean. Variation in the dependent variable, Y, was described as follows:

$$Y_{ijklmn} = \mu + B_i + PR_j + (BxPR)_{ij} + C_{(ij)k} + PE_l + PT_m + F_n + (PTxF)_{mn} + (BxPT)_{im} + (PRxPT)_{jm} + (BxPRxPT)_{ijm} + (BxF)_{in} + (PRxF)_{jn} + (BxPRxF)_{ijn} + (BxPTxF)_{imn} + (PRxPTxF)_{jmn} + (BxPRxPTxF)_{ijmn} + E_{ijklmn}$$

where:

Y_{ijklmn} = dependent variable

μ = overall mean of Y

B_i = effect of breed (i = 1..2)

PR_j = effect of previous production (j = 1..3)

$(BxPR)_{ij}$ = interaction of breed and previous production

$C_{(ij)k}$ = effect of cow k within breed and production (k = 1..4)

PE_l = effect of period (l = 1..4)

PT_m = effect of level of dietary RUP (m = 1..2)

F_n = effect of dietary fat (n = 1..2)

$(PTxF)_{mn}$ = interaction of RUP and fat

$(BxPT)_{im}$ = interaction of breed and RUP

$(PRxPT)_{jm}$ = interaction of previous production and RUP

$(BxPRxPT)_{ijm}$ = interaction of breed, previous production and RUP

$(BxF)_{in}$ = interaction of breed and fat

$(PRxF)_{jn}$ = interaction of previous production and fat

$(BxPRxF)_{ijn}$ = interaction of breed, previous production and fat

$(BxPTxF)_{imn}$ = interaction of breed, RUP and fat

$(PRxPTxF)_{jmn}$ = interaction of previous production, RUP and fat

$(BxPRxPTxF)_{ijmn}$ = interaction of breed, previous production, RUP and fat

E_{ijklmn} = random residual error

Breed differences were tested by dividing the breed mean square by the mean square of cow within breed and production. Contrasts for fat and RUP were calculated within each breed. Contrast for fat represents the comparison of diets without added fat to those with added fat, all at average RUP. Contrast for RUP represents the comparison of 29% RUP diets to 41% RUP, all at average fat. Breed contrast represents the comparison of the least square means for Holstein versus Jersey averaged across all treatments.

RESULTS AND DISCUSSION

Diets were formulated to be 17% CP but the quality of the alfalfa silage decreased throughout the experiment and the mean was 16%. Values from Dair4 ration evaluation software (Stallings et al., 1993) were used to formulate RUP levels 29 and 41% of total CP. Diets containing added fat had greater NE_L and less non-fiber carbohydrates due to Megalac replacing corn. Diets were formulated to have 3.2 (no added fat) and 5.9% (added fat) total fat. However, lower concentrations of total fat, 2.4 and 5.1% respectively, were obtained due to lower ether extract than reported by N.R.C. (1989) for corn silage, corn grain and blood meal. Neutral and acid detergent fibers were similar but tended to be slightly greater in the 29% RUP diets because more soybean meal was used.

Dry matter intake decreased 6.0% for Holsteins and 6.4% for Jerseys when fat was added to the diet (Table 3.2). Dry matter intake of Holsteins was 5.1 kg/d greater than Jersey cows. Expressed as percent of body weight, DMI decreased 5.3% for Holsteins and 6.0% for Jerseys when fat was supplemented. Jerseys had greater intake, 4.0% compared to 3.6% for Holsteins, when expressed as percent of body weight. Similar reductions in DMI were reported by Klusmeyer et al. (1991b) and by Kim et al. (1993) when Ca soaps of fatty acids were fed. However, Drackley et al. (1994) reported liquid fat decreased DMI in Jerseys but not in Holsteins. On the other hand, Schneider et al. (1988) and Erickson et al. (1992) found no difference in DMI

when Ca soaps of fatty acids were fed. Reduction of DMI may be a result of metabolic control (N.R.C., 1988) because energy intake in diets with added fat was similar to diets with no fat.

Holsteins increased milk yield 6.9% and Jerseys 7.4% when cows were fed added fat. Holsteins produced 10.5 kg/d of milk more than Jerseys. Higher milk production has been reported when Ca soaps of fatty acids (Schneider et al., 1988; Klusmeyer et al., 1991b; Erickson et al., 1992; Tomlinson et al., 1994) and when tallow, Ca soaps of fatty acids or prilled fat were supplemented (Wu et al., 1993). Contrary to this, Klusmeyer et al. (1991a) and West and Hill (1990) found no difference when cows were fed Ca soaps of fatty acids. Milk yield expressed as 4% FCM increased 8.4% for Jerseys with added fat. Holsteins produced 5.2 kg/d more of 4% FCM than Jerseys. Differences between breeds in 4% FCM were smaller than milk yield due to higher fat content in Jersey milk.

Holsteins and Jerseys increased production of milk per Mcal of NE_L by 6.7 and 8.6%, respectively. Holsteins produced .94 kg and Jerseys .85 kg of milk/Mcal NE_L. However, only Jerseys increased 9.7% production of 4% FCM/Mcal NE_L in response to added fat. Also, Jerseys had a greater efficiency of production, 0.99 kg/Mcal NE_L compared to 0.90 kg/Mcal NE_L for Holsteins. Greater efficiency of FCM/Mcal NE_L in Jerseys compared to Holsteins is due to a greater milk fat content in Jersey milk. Schneider et al. (1990) and Wu et al (1993) found Ca soaps of fatty acids increased feed efficiency (3.5% FCM/DMI). Increased milk yield and efficiency of production

in Jerseys may be due to increased uptake of dietary fatty acids by the mammary gland compared to Holsteins when Ca soaps of fatty acids were fed.

Body weight change per period when fat was fed was 10.2 kg lower for Holsteins and 8.4 kg lower for Jerseys. Holsteins gained more weight (10.2 kg/period) than Jerseys (3.5 kg/period). Loss of weight in Jerseys and reduced gain in Holsteins may be due to increased milk production and not to decreased DMI when fat was supplemented, because total energy intake between diets was similar (Holsteins 35.91 and 35.93 Mcal NE_l, Jerseys 27.83 and 27.73 Mcal NE_l, for no fat and added fat respectively). Drackley et al. (1994) reported Jerseys lost weight and Holsteins did not when liquid fat was added to the diet. However, DMI was reduced in Jerseys but did not change in Holsteins. Drackley et al. reported interpretation of differences in body weight change is difficult in a Latin square design with 28-d periods. In other studies, no changes in body weight were observed when fat was added to the diet (West and Hill, 1990; Palmquist et al., 1993), but milk yield was not increased and DMI was not reduced by addition of fat. Our data showed that level of RUP in the diet had no significant effect on DMI, milk production, efficiency of production or body weight gain.

Table 3.3 contains yields of milk components. Milk fat yield increased 8.9% for Jerseys with added fat. Fat yield was not different between breeds. Fat yield in Jerseys increased when fat was supplemented, mainly due to an increase in milk production because milk fat concentration was not changed. However, in Holsteins

regardless of increased milk yield, there was no response in fat yield due to a decrease in milk fat content. Different results were reported by West and Hill (1990) where Holsteins had a greater milk fat yield compared to Jerseys when Ca soaps of fatty acids were fed. Erickson et al. (1992) found no difference in milk fat yield while Kim et al. (1993) reported an increase when cows were fed Ca soaps of fatty acids.

Protein yield was not affected by added fat. However, 41% RUP decreased protein yield 4.4% for Holsteins. Holsteins produced 0.17 kg/d more milk protein than Jerseys. Despite no differences in milk yield when cows were fed 41% RUP diets, a reduction in protein yield was observed in Holsteins mainly due to a reduction in protein percentage of milk. A similar response was observed by Klusmeyer et al. (1990) when cows were fed corn gluten meal.

Lactose yield increased 5.5% for Holsteins and 10.2% for Jerseys when cows were supplemented with added fat. Holsteins produced 0.51 kg/d more milk lactose than Jerseys. Solids non-fat (SNF) yield increased 3.2% for Holsteins and 6.0% for Jerseys with added fat. Holsteins produced 0.73 kg/d more milk SNF than Jerseys. Increased lactose and SNF yield was mainly due to increased milk production in Holsteins when cows were fed added fat. A similar response was observed by Kim et al. (1993) by feeding Ca soaps of fatty acids. However, in Jerseys, increased lactose yield was due to a combination of increased milk yield and lactose content, but SNF yield was only due to milk yield when fat was supplemented.

Holsteins decreased milk fat content 5.8% when fat was added (Table 3.4). Jerseys had a greater milk fat content of 5.1% compared to 3.7% for Holsteins. A protein by fat interaction occurred when Jerseys fed added fat decreased milk fat content with 29% RUP and increased with 41% RUP. West and Hill (1990) reported Ca soaps of fatty acids increased milk fat content in Jerseys and Holsteins, whereas Drackley et al. (1994) found decreased milk fat content when Jerseys and Holsteins were fed liquid fat. Contrary to these, milk fat content was not affected when Ca soaps of fatty acids were fed (Schneider et al., 1990). As reported by Wonsil (1990), milk fat production can be affected by DMI, stage of lactation and energy status of the animal.

Protein content decreased 6.3 and 7.8% when fat was fed, and 4.2 and 3.6% with 41% RUP for Holsteins and Jerseys, respectively. Jerseys had a greater milk protein content of 3.7%, compared to 3.1% for Holsteins. Decreased milk protein content, but not yield, was reported when Ca soaps of fatty acids were fed (West and Hill, 1990; Erickson et al., 1992; Kim et al., 1993; Wu et al., 1993). However, Palmquist and Conrad (1978) and Schneider et al. (1990) reported no difference in milk protein content when fat was added to the diet. Wu and Huber (1994) suggested milk protein depression when fat is fed may be due to insufficient supply of amino acids because of increased milk production. Also, DePeters and Cant (1992) proposed depression may be due to a dilution effect. Using their formula:

our data suggest that 99.98% of the depression is due to dilution because milk yield

$$Dilution = (1 - \frac{percentage\ increase\ protein\ yield}{percentage\ increase\ milk\ yield}) \times 100$$

increased but milk protein yield did not when fat was added to the diet.

Insufficient supply of amino acids (AA) or an imbalance could be the reason milk protein content was decreased when 41% RUP diets were fed. Similar results were observed by Klusmeyer et al. (1990) with corn gluten meal, and Tomlinson et al. (1994) with blood and feather meal. King et al. (1990) compared diets high in corn grain and corn silage supplemented with blood meal, corn gluten meal or cottonseed meal. When cottonseed meal and corn gluten meal were supplemented at 45% of the ration CP, lysine was one of the less abundant AA in plasma. However, when blood meal was supplemented, lysine concentration increased. Chandler (1989) reported an AA index for protein sources based on chemical score of AA. He concluded bacterial crude protein had greater biological value than any rumen resistant protein, and branched-chain AA were the most limiting. With his index, soybean meal had the greatest biological value. Polan (1992) reported "soybean meal is as balanced as any" compared to AA in milk protein. Leucine, isoleucine and valine are in greater concentration in milk protein than in bacterial protein and most feedstuffs, and also they appeared to be the most limiting in corn-based diets. Milk protein depression in

our diets may indicate branched-chain AA were limiting due to the substitution of blood meal for soybean meal in the 41% RUP diets.

Jerseys increased lactose content 1.4% with added fat and 1.6% with 41% RUP. Breeds were not different in milk lactose content. No difference in lactose content was reported by Kim et al. (1993) and Wu et al. (1993) when Ca soaps of fatty acids were fed. Also, no change in lactose content was observed with blood meal (Sandrucci et al., 1992). Increased lactose content in Jerseys may be due to increased use of amino acid as substrate for gluconeogenesis because supplemented fat diets increased plasma urea N at 0700 h and 1600 h.

Solids non-fat decreased 2.6% in Holsteins and 2.5% in Jerseys when fat was added. Jerseys had a greater concentration of SNF, 9.48% compared to 8.66% for Holsteins. Decreased SNF with added fat was mainly due to decreased milk protein content. Contrary to this, no difference in SNF was observed regardless of decreased protein content when Ca soaps of fatty acids were fed (Kim et al., 1993).

Total milk N content decreased 6.3 and 7.7% when fat was added, and 4.3 and 3.7% with 41% RUP for Holsteins and Jerseys, respectively (Table 3.5). Jerseys had a greater concentration of total milk N than Holsteins (.583 versus .481 g/100 g of milk). As explained previously, reasons for decreased N content may be explained by dilution due to increased milk production because yield of total milk N was not decreased by added fat. However, yield of total milk N was decreased when 41% RUP diets were fed to Holsteins and may be due to an insufficient or imbalanced

supply of AA to the mammary gland. Similar reductions in total N content of milk have been observed (DePeters et al., 1989; Drackley and Elliot, 1993; Kincaid and Cronrath, 1993).

Casein N content decreased 6.9 and 8.3% when fat was fed, and 4.6 and 4.8% with 41% RUP for Holsteins and Jerseys, respectively. Jerseys had a greater concentration of casein N (.452 versus .360 g/100 g of milk) than Holsteins. Casein N concentration was reduced when cows were fed whole cottonseeds (DePeters et al., 1985) or grease (DePeters et al., 1989). However, no difference was reported when cows were fed partially hydrogenated tallow (Drackley and Elliot, 1993) or Ca soaps of fatty acids (Kincaid and Cronrath, 1992). Depressed casein N content may be due to insufficient supply of amino acids when cows were fed 41% RUP diets, and to dilution when fed added fat because casein N yield decreased with 41% RUP but not with added fat. Milk urea N concentration as g/100 g of milk (average of 0100 h and 1300 h sampling) in Holsteins decreased 4.2% with added fat and increased 4.0% with 41% RUP. Holsteins had a greater concentration (.024 versus .021 g/100 g of milk) compared to Jerseys.

Casein N concentration expressed as percent of total N decreased 1.0% in Holsteins with added fat, and 1.1% in Jerseys with 41% RUP. Jerseys had a greater concentration of casein N, 77.5% compared to 74.9% for Holsteins. Contrary to this, DePeters et al. (1985) and DePeters et al. (1989) reported no difference in casein N as a percent of total N when cows were fed fat.

Milk urea N concentration expressed as percent of total N increased 8.1% with added fat in Jerseys, and 10.8 and 5.8% with 41% RUP for Holsteins and Jerseys, respectively. Holsteins had a greater concentration of milk urea N (5.07 versus 3.73 g/100 g of total N) compared to Jerseys. Similar results were reported by DePeters et al. (1985 and 1989), where milk NPN increased when cows were fed added fat. Although in these studies milk NPN was measured instead of milk urea N, we can assume milk urea N was increased because urea is the largest contributor of milk NPN (DePeters and Ferguson, 1992). In summary, our data supported DePeters and Cant (1992) who found total milk N and milk casein N content were negatively correlated while milk NPN content was positively correlated to ether extract intake and concentration in the diet. Also total milk N and casein N decreased and NPN increased as a percent of the total milk N when diets were supplemented with added fat.

The concentration of milk fatty acids are in Tables 3.6 and 3.7, and yields in Tables 3.8 and 3.9. The concentration of $C_{4:0}$ increased 3.1% in Holsteins by added fat. A similar response was observed in Holsteins but not in Jerseys when supplemented with .75 kg/d of Ca soaps of fatty acids (Palmquist et al., 1993). Added fat decreased short and medium chain fatty acids ($C_{6:0}$ to $C_{12:0}$), and the $C_{6:0}$ decreased 11.1% and $C_{12:0}$ 30.6%. Palmquist et al. (1993) reported added fat depressed de novo synthesis of $C_{6:0}$ to $C_{12:0}$ and greatest depression in greater chain length. He concluded the inhibition when chain length increases is consistent with condensation

of acetyl units with a preformed 4 carbon primer. Smaller reductions were observed in $C_{14:0}$ and $C_{14:1}$ of 19.8% and 23.0%, respectively, when supplemental fat was added. Palmquist et al. (1993) reported these smaller reductions in $C_{14:0}$ are due to the partial origin of $C_{14:0}$ from intestinal fatty acids.

Concentration of $C_{15:0}$ was reduced 33.6% in Holsteins and 32.9% in Jerseys with added fat. However, concentrations of $C_{18:0}$ and $C_{18:1}$ increased 7.6 and 11.7%, and 30.3 and 30.7% for Holsteins and Jerseys, respectively, when fat was supplemented. Added fat as whole soybeans and tallow decreased concentrations of short and medium chain and $C_{16:0}$ milk fatty acids, while increasing $C_{18:0}$ and $C_{18:1}$ (Schauff et al., 1992). A decrease in C_{10} , C_{12} and $C_{18:0}$ and an increased in $C_{16:0}$ and $C_{16:1}$ was observed when Ca soaps of fatty acids were fed (Tomlinson et al., 1994). Schneider et al. (1990) found a decrease in C_{12}, C_{14} and $C_{18:0}$ and a greater proportion of C_{16} and $C_{18:1}$. However, no significant differences were observed in the fatty acid composition of milk from C_8 to $C_{18:3}$ when cows were supplemented with Ca soaps of fatty acids (Schneider et al., 1988). Wu et al. (1993) compared diets containing tallow, Ca soaps of fatty acids, or prilled fat on milk fatty acid composition. Added fat decreased percentages of C_6 to $C_{14:0}$ and increased C_{16} (14%) and C_{18} (22%). Ca soaps of fatty acids and prilled fat increased $C_{16:0}$ by 8.5 and 3.5% more than tallow, also tallow increased $C_{18:0}$ by 15.6%, more than the other two sources of fat.

The 41% RUP diets decreased milk fat $C_{15:0}$ (9.5% for Holsteins and 6.4% for Jerseys) and $C_{18:2}$ (8.5% for Holsteins and 7.5% for Jerseys) and increased butyric acid

by 4.3% in Jerseys. No differences were observed in milk fatty acid composition when cows were fed a high RUP diet (Zimmerman et al., 1992), but fewer short chain fatty acids from C_6 to C_{12} were reported by Tomlinson et al. (1994) when feather meal was fed.

Compared to Holsteins, higher concentrations of C_6 to C_{14} ranging from 12.5 to 28.4% were observed in Jerseys regardless of the diet. A similar increase ranging from 8 to 42% for C_6 to C_{14} was found in Jerseys compared to Holsteins regardless of the diet (Palmquist and Beaulieu, 1992). Authors also reported that $C_{18:0}$ was 13% higher and $C_{18:1}$ was 15% lower in Jersey cows. In our experiment, $C_{18:0}$ was 9.5% higher and $C_{18:1}$ was 20.6% less in Jersey cows. The ratio of $C_{18:0}/C_{18:1}$ was decreased by added fat from .60 to .51 in Jerseys and from .44 to .36 in Holsteins. Also 41% RUP diets decreased the ratio by 5.1% in Jerseys. However, Jerseys had a greater ratio compared to Holsteins (.55 and .40, respectively) regardless of the diet. Palmquist and Beaulieu (1992) reported the ratio of $C_{18:0}/C_{18:1}$ was .54 for Jerseys regardless of fat supplementation but in Holsteins this ratio decreased from .45 to .39 when Ca soaps of fatty acids were fed. In a second study, Palmquist and Beaulieu compared Jerseys and Holsteins fed roasted soybeans. Jerseys increased the concentration of C_6 to C_{14} by 15% above Holsteins but the ratio of $C_{18:0}/C_{18:1}$ was the same. They concluded Holsteins had a greater compensation for decreased short chain fatty acid synthesis by increasing desaturase activity. Figures 3.1 and 3.2 show the change in concentration and yield of fatty acids compared to the control diet. Overall,

short and medium chain fatty acid concentrations were less affected and long chain yield was higher in Jerseys when fat was supplemented, suggesting a greater efficiency of de novo synthesis and incorporation of dietary fatty acids into milk fat.

Table 3.10 contains plasma urea N at 0700 and 1600 h and milk urea N at 0100 and 1300 h. Plasma urea N at 0700 h in Jerseys increased 8.0% with added fat, and with 41% RUP Holsteins increased 9.7%. Overall concentration of plasma urea N at 0700 h was not different by breed. Increased plasma urea N at 0700 h with added fat and 41% RUP may be due to increased hepatic deamination of amino acids (DePeters and Ferguson, 1992). Plasma urea N at 1600 h increased in Jerseys 8.7% when fat was added. There was no difference with 41% RUP, however, there was a protein by fat interaction in Holsteins because plasma urea N concentration increased when fat was fed with 41% RUP but decreased when fed with 29% RUP. Overall concentration at 1600 h was not different by breed. Palmquist et al. (1993) found diets with added fat and high RUP increased plasma urea N prefeeding and 3 h postfeeding. However, no change (Erickson et al., 1992) and a decrease in plasma urea N (West and Hill, 1990 and Kim et al., 1993) was reported when Ca soaps of fatty acids were fed. In our study there is no explanation why the protein by fat interaction occurred at 1600 h, because plasma urea N concentration at 0700 in both breeds and at 1600 h in Jerseys increased when fat was added to the diet. Concentrations at 1600 h were higher than at 0700 h due to time of sampling relative

to feeding. Sampling at 1600 h was 2 h after feeding and an increased rumen ammonia concentration was observed at this time as shown in Figure 4.8.

Milk urea N at 0100 h was not affected by added fat or 41% RUP, however, Holsteins had a greater concentration (22.9 mg/dL) than Jerseys (20.2 mg/dL). At 1300 h, added fat decreased milk urea N by 4.7% for Holsteins, however, with 41% RUP milk urea N increased by 5.7%. Holsteins had a greater concentration of milk urea N (25.2 mg/dL) compared to Jerseys (22.7 mg/dL). Why concentration of milk urea N at 1300 h decreased in Holsteins when fat was fed can not be explained. Increased concentration of milk urea N at 1300 h in Holsteins fed 41% RUP may be due to increased concentration of plasma urea N at 0700 h. Roseler et al. (1993) reported that intake of undegradable and degradable protein elevated plasma and milk urea N to a similar extent. However, Wonsil (1993) reported added fat increased milk urea N but level of undegradable protein had no effect.

Rumen pH increased 6.1% in Holsteins fed 41% RUP diets (Table 3.11). However, overall rumen pH was not different by breed. Increased rumen pH when cows are fed rumen undegradable feeds have been reported with different protein sources (Zerbini et al., 1988; Broderick et al., 1993; Christensen et al., 1993; Wattiaux et al., 1994). Apparently feeding these sources shifts microbial fermentation towards fiber digestion, due to increased pH resulting in increased acetate concentrations. However, Seymour et al. (1992) and Robinson and McQueen (1994) found no differences.

Jerseys had a greater rumen ammonia concentration (13.4 mg/dL) than Holsteins (7.9 mg/dL). When cows were fed 41% RUP diets rumen ammonia decreased 34.3% for Holsteins and 30.2% for Jerseys. Similar results were observed with a corn gluten and blood meal mix (Robinson and McQueen, 1994) and feather meal (Zerbini et al., 1988). However, Seymour et al. (1992) found no difference when corn gluten meal was fed. Decreased rumen ammonia concentration is due to increased resistance to microbial degradation in the rumen of the protein source fed.

Jerseys had a greater concentration of total VFA (83.8 mmol/L) compared to Holsteins (76.3 mmol/L). Total VFA concentration decreased 14.6% for Holsteins and 5.6% for Jerseys when 41% RUP was fed. Similar reductions in rumen ammonia concentration have been observed when high RUP diets were fed (Zerbini et al., 1988; Christensen et al., 1993; Robinson and McQueen, 1994). However, Seymour et al. (1992) found no difference with corn gluten meal. Lower total ruminal VFA concentration may indicate some interference with ruminal microbial activity.

Table 3.12 contains rumen VFA concentrations. Acetate concentration increased 3.1% in Holsteins and 1.9% in Jerseys when fed 41% RUP diets. However, when the same diets were fed to Holsteins propionate concentration decreased by 8.0%. Similar increased acetate and decreased propionate concentration was found by Christensen et al. (1993), Palmquist et al. (1993) and Robinson and McQueen (1994) with high RUP diets. However, Seymour et al. (1992) found no difference in acetate and propionate concentration with corn gluten meal. Increased acetate and decreased

propionate may indicate greater fermentation of fiber with 41% RUP perhaps due to higher rumen pH. Acetate:propionate ratio increased with 41% RUP diets by 11.5 and 6.9% for Holsteins and Jerseys, respectively. Ratios between breeds were not different. Christensen et al. (1993) and Palmquist et al. (1993) reported an increase in the ratio when high RUP diets were fed.

Valerate concentration decreased with 41% RUP by 12.1 and 9.1% for Holsteins and Jerseys, respectively. Reduced concentrations of valerate was also observed by Seymour et al. (1992) and may indicate decreased ruminal fermentation of AA when high RUP diets are fed. Butyrate, isobutyrate and isovalerate were not affected by diet or breed.

Correlation coefficients of rumen ammonia, plasma and milk urea N are shown in Table 3.13. Rumen ammonia at 1600 h was not correlated with plasma urea N at 0700 h, or milk urea N at 0100 and 1300 h. Rumen ammonia was correlated with plasma urea N at 1600 h (r = .64 overall, .41 for Holsteins and .74 for Jerseys). No significant correlations of rumen ammonia and plasma urea N at 0700 h and milk urea N are mainly due to time of sampling. Rumen fluid was sampled at 1600 h and this may be the reason high concentration of rumen ammonia (2 h after feeding) had a high correlation with plasma urea N at 1600 h.

Plasma urea N at 0700 h was correlated with plasma urea N at 1600 h (r = .70 overall, .85 for Holsteins and .59 for Jerseys). Correlations (r) between plasma urea N at 0700 h and milk urea N at 0100 and 1300 h were .65 and .65 overall, .77 and .77

for Holsteins, and .60 and .64 for Jerseys, respectively. Plasma urea N at 1600 h was correlated with milk urea N at 0100 h (r = .42 overall, .69 for Holsteins and .40 for Jerseys) and with milk urea N at 1300 h (r = .39 overall, .72 for Holsteins and .32 for Jerseys). Milk urea N at 0100 h was correlated with milk urea N at 1300 h (r = .82 overall, .84 for Holsteins and .72 for Jerseys). Correlations between plasma urea N at 0700 h and milk urea N at 0100 and 1300 h are very similar and may be a result of sampling before feeding. In contrast, lower correlations were observed between the 1600 h plasma urea N and milk urea N and may be due to increased concentrations of plasma urea N at 1600 h.

Azotest strips were correlated ($P < .01$) to milk urea N (r = .46 overall, .38 for Holsteins and .62 for Jerseys). Prediction of milk urea N by Azotest strips was very poor as observed in Figure 3.3 (r = .46). In our study Azotest strips predicted 85% of the samples equal to or above of 25 mg/dL, however, the urease procedure verified only 23%. Roseler and Chase (Personal communication) compared the ability to measure urea N of Sigma 535-A test kit (Diacetylmonoxime reaction) and Azotest strips. Twelve different concentrations were prepared with distilled water and analyzed with both procedures. A low relationship between the two methods was found ($r^2 = .38$). They reported timing is critical in the interpretation of Azotest strips, intense rinsing can result in reduction of color intensity on the strips and color bars on the Azotest strip bottle are difficult to interpret. However, in a second study (Roseler and Chase, personal communication) where 20 cows in four different diets were used

to compared the same procedures they reported a high correlation (r^2 = .82). Reasons of disagreements between experiments were not discussed. It is difficult to compare these data to ours because we used a urease reaction to measure urea N while they used diacetylmonoxime reaction. The use of a cow-side test for milk urea N is important because it can give a quick measurement on the farm. Roseler et al. (1990) reported gross deficiencies or excesses of total protein intake may be monitored by plasma urea N and milk urea N.

Summary and Conclusions

Total VFA concentration can be used as an indicator of extent of ruminal fermentation while ruminal ammonia can be used as an indicator of available N for microbial protein production. When 41% RUP diets were fed, rumen ammonia and total VFA were decreased, though to a greater extent in Holsteins compared to Jerseys. Also, when Holsteins were fed 41% RUP diets, an increase in ruminal pH and acetate concentration and a decrease in propionate concentration was observed. However, when Jerseys were fed 41% RUP diets, only acetate concentration increased, suggesting a lesser influence of 41% RUP diets on ruminal fermentation in Jerseys compared to Holsteins. Supplemental dietary fat did not affect any of the ruminal parameters measured.

Greater concentrations of plasma urea N were observed when Jersey cows were fed added fat. Also, greater concentrations of plasma urea N were observed in Holsteins fed 41% RUP diets despite reduced ruminal ammonia. This might indicate increased use in Holsteins of dietary or body reserve amino acids as substrate for gluconeogenesis with subsequent loss of N as urea.

Dry matter intake decreased when cows were fed added fat, but total daily energy intake did not change. Increased milk production when cows were fed added fat, and decreased dry matter intake, suggests a greater efficiency of conversion of feed to milk. However, when milk was corrected for fat content, Jerseys had a greater

efficiency of production than Holsteins because milk fat content decreased in Holsteins but not in Jerseys. Greater efficiency of production in Jerseys in response to supplemental fat may be due to greater efficiency of de novo fatty acid synthesis and enhanced incorporation of dietary fatty acids into milk fat. Although interpretation of body weight changes in experiments with short periods is difficult, Jerseys gained less weight per period, suggesting a greater partitioning of nutrient in body reserves towards milk production.

Fatty acid synthesis requires energy (ATP) and reducing equivalents (NADPH). The main source of reducing equivalents for lipogenesis is the pentose phosphate pathway. Enhanced incorporation of dietary fatty acids into milk fat triglyceride may spare glucose used to produce reducing equivalents or ATP, thus providing more glucose for lactose production. This suggestion is supported by the fact that Jersey cows had a greater lactose content in milk when their diets were supplemented with fat compared to Holsteins.

Decreased milk protein and casein N content when cows were fed added fat may be due to dilution from increased milk production because milk protein and casein N yields were not decreased. However, decreased content and yields of milk protein and casein N in Holsteins, but not Jerseys, fed 41% RUP diets may indicate limited microbial protein yield during ruminal fermentation, resulting in an imbalanced supply of amino acids for absorption in the small intestine. In contrast, increased milk urea N as a percent of the total milk N, when Jerseys were fed supplemental fat and

Holsteins were fed 41% RUP, may be due to transfer of plasma urea N to milk when plasma urea was elevated.

Table 3.1. Dietary ingredients and chemical composition of diets.

Item	Treatments			
	29% RUP	29% RUP+F	41% RUP	41% RUP+F
Ingredients, % DM				
Corn Silage	30.0	30.0	30.0	30.0
Alfalfa Silage	29.0	29.0	29.0	29.0
Corn grain	25.7	21.7	29.9	25.9
Soybean Meal	13.1	13.1	5.5	5.5
Blood Meal			3.4	3.4
Corn Gluten Meal		0.6		0.6
Fat		3.4		3.4
Mineral/vitamin[1]	2.2	2.2	2.2	2.2
Chemical Composition				
CP, % of DM	15.9	15.9	16.0	16.0
NE_L, Mcal/kg	1.54	1.65	1.56	1.65
ADF, % of DM	20.6	20.5	20.0	19.9
NDF, % of DM	30.3	30.0	29.6	29.3
NFC, % of DM	44.0	41.3	45.0	42.4
RUP, % of CP	29.0	29.1	40.7	40.6
EE, % of DM	2.4	5.1	2.4	5.1
Ca, % of DM	.98	1.28	.97	1.27
P, % of DM	.56	.55	.53	.52

RUP = Rumen undegraded protein
F = Fat as Ca soaps of fatty acids
NFC = Non-fiber carbohydrates
EE = ether extract
[1] Contained: 18% $NaHCO_3$; 16% Ca; 6.5% P; 3.5% K; 2.2% Mg; 3.2% S; 5.8% Cl; .027% Fe; .013% Cu; .0003% Co; .11% Mn; .13% Zn; .002% I; .0005% Se; and 110,000 IU of vitamin A/kg; 44,000 IU of vitamin D/kg, and 550 IU of vitamin E/kg.

Table 3.2. Daily dry matter intake, milk production and efficiency of milk production in response to dietary fat (F) and rumen undegradable protein (RUP).

		Treatments					Contrasts (P <.05)			
	B[1]	29% RUP	29% RUP+F	41% RUP	41% RUP+F	SEM	FAT	RUP	INT	BC[2]
DMI, kg/d	H	23.2	22.0	23.1	21.6	.4	.01	NS	NS	.01
	J	18.0	17.1	17.9	16.6		.01	NS	NS	
DMI, % BW[3]	H	3.75	3.59	3.76	3.51	.08	.01	NS	NS	.05
	J	4.17	3.95	4.14	3.86		.01	NS	NS	
Milk Yield,	H	32.3	34.8	33.2	35.2	.5	.01	NS	NS	.01
kg/d	J	22.5	23.0	22.7	24.5		.01	NS	NS	
4% FCM[4],	H	31.2	32.6	32.4	32.9	.6	NS	NS	NS	.01
kg/d	J	26.3	27.6	25.8	28.8		.01	NS	NS	
Milk/Mcal NE_L,	H	.91	.96	.92	.99	.05	.02	NS	NS	.05
kg	J	.82	.88	.82	.90		.01	NS	NS	
FCM/Mcal NE_L,	H	.87	.89	.89	.92	.03	NS	NS	NS	.05
kg	J	.95	1.01	.93	1.05		.01	NS	NS	
BW change,	H	13.8	3.8	16.9	6.6	2.8	.01	NS	NS	.01
kg/period	J	4.3	-1.2	11.2	-.1		.01	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

[3] Dry matter intake as a percent of body weight

[4] 4% fat corrected milk

Table 3.3. Milk component yields in response to dietary fat (F) and rumen undegradable protein (RUP).

		Treatments					Contrasts (P <.05)			
	B[1]	29% RUP	29% RUP+F	41% RUP	41% RUP+F	SEM	FAT	RUP	INT	BC[2]
		---------- kg/d ----------								
Fat	H	1.22	1.24	1.28	1.26	.03	NS	NS	NS	NS
	J	1.15	1.20	1.11	1.26		.01	NS	NS	
Protein	H	1.06	1.05	1.01	1.01	.02	NS	.04	NS	.01
	J	.87	.88	.85	.84		NS	NS	NS	
Lactose	H	1.62	1.72	1.64	1.72	.03	.01	NS	NS	.01
	J	1.10	1.20	1.11	1.24		.01	NS	NS	
SNF	H	2.91	3.00	2.87	2.97	.05	.06	NS	NS	.01
	J	2.15	2.26	2.13	2.28		.01	NS	NS	

INT = Protein x fat interaction
SNF = Solids non-fat
[1] B = Breeds, H = Holstein, J = Jersey
[2] BC = Breed contrast

Table 3.4. Milk component content in response to dietary fat (F) and rumen undegradable protein (RUP).

		Treatments					Contrasts (P <.05)			
	B[1]	29% RUP	29% RUP+F	41% RUP	41% RUP+F	SEM	FAT	RUP	INT	BC[2]
		------- g/100 g milk ------								
Fat	H	3.72	3.55	3.87	3.60	.09	.02	NS	NS	.01
	J	5.19	4.98	5.00	5.19		NS	NS	.04	
Protein	H	3.27	3.00	3.06	2.94	.04	.01	.01	NS	.01
	J	3.95	3.64	3.80	3.51		.01	.01	NS	
Lactose	H	4.88	4.89	4.95	4.91	.03	NS	NS	NS	NS
	J	4.91	4.98	4.97	5.06		.01	.03	NS	
SNF	H	8.83	8.57	8.72	8.52	.05	.01	NS	NS	.01
	J	9.65	9.40	9.55	9.34		.01	NS	NS	

INT = Protein x fat interaction
SNF = Solids non-fat
[1] B = Breeds, H = Holstein, J = Jersey
[2] BC = Breed contrast

Table 3.5. Nitrogen fractions of milk in response to dietary fat (F) and rumen undegradable protein (RUP).

		Treatments					Contrasts (P <.05)			
	B[1]	29% RUP	29% RUP+F	41% RUP	41% RUP+F	SEM	FAT	RUP	INT	BC[2]
		----- g/100 g milk -------								
Total	H	.513	.470	.480	.461	.006	.01	.01	NS	.01
	J	.619	.570	.596	.550		.01	.01	NS	
Casein	H	.386	.352	.360	.343	.005	.01	.01	NS	.01
	J	.484	.443	.460	.423		.01	.01	NS	
Urea[3]	H	.0244	.0227	.0247	.0243	.0005	.05	.06	NS	.01
	J	.0215	.0212	.0214	.0215		NS	NS	NS	
		----- g/100 g total N ---								
Casein	H	75.4	74.7	75.1	74.4	.4	.06	NS	NS	.01
	J	78.2	77.7	77.2	77.0		NS	.03	NS	
Urea	H	4.77	4.86	5.24	5.43	.10	NS	.01	NS	.01
	J	3.51	3.74	3.66	4.00		.01	.05	NS	

INT = Protein x fat interaction
[1] B = Breeds, H = Holstein, J = Jersey
[2] BC = Breed contrast
[3] Average of 0100 and 1300 h

Table 3.6. Concentration of milk fatty acids in response to dietary fat (F) and rumen undegradable protein (RUP).

		Treatments					Contrasts (P <.05)			
	B[1]	29% RUP	29% RUP+F	41% RUP	41% RUP+F	SEM	FAT	RUP	INT	BC[2]
		- g/100 g of fatty acids --								
4:0	H	2.49	2.53	2.51	2.68	.05	.07	NS	NS	NS
	J	2.50	2.58	2.62	2.68		NS	.05	NS	
6:0	H	1.38	1.15	1.37	1.19	.02	.01	NS	NS	.01
	J	1.46	1.35	1.52	1.39		.01	NS	NS	
8:0	H	.93	.68	.91	.73	.02	.01	NS	NS	.01
	J	1.01	.86	1.05	.89		.01	NS	NS	
10:0	H	2.31	1.53	2.23	1.54	.05	.01	NS	NS	.01
	J	2.74	2.09	2.79	2.15		.01	NS	NS	
12:0	H	2.98	1.89	2.88	1.92	.08	.01	NS	NS	.01
	J	3.55	2.56	3.61	2.66		.01	NS	NS	
14:0	H	12.27	9.40	12.01	9.46	.18	.01	NS	NS	.01
	J	12.76	10.63	13.11	10.72		.01	NS	NS	
14:1	H	1.25	.95	1.19	.92	.03	.01	NS	NS	.01
	J	.95	.77	1.02	.75		.01	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

Table 3.7. Concentration of milk fatty acids in response to dietary fat (F) and rumen undegradable protein (RUP).

		Treatments					Contrasts (P <.05)			
	B[1]	29% RUP	29% RUP+F	41% RUP	41% RUP+F	SEM	FAT	RUP	INT	BC[2]
		- g/100 g of fatty acids --								
15:0	H	1.48	.93	1.28	.90	.03	.01	.01	NS	NS
	J	1.39	.94	1.32	.87		.01	.07	NS	
16:0	H	42.84	42.44	43.77	42.79	.53	NS	NS	NS	.05
	J	45.24	44.67	45.61	44.44		NS	NS	NS	
16:1	H	2.12	2.02	2.10	1.95	.07	NS	NS	NS	.01
	J	1.73	1.59	1.72	1.62		NS	NS	NS	
18:0	H	8.40	9.18	8.42	8.92	.25	.02	NS	NS	.05
	J	9.33	10.20	8.73	9.98		.01	NS	NS	
18:1	H	18.94	24.79	19.00	24.66	.44	.01	NS	NS	.01
	J	15.16	19.56	14.96	19.82		.01	NS	NS	
18:2	H	2.55	2.45	2.27	2.30	.04	NS	.01	NS	.01
	J	2.12	2.14	1.95	1.99		NS	.01	NS	
18:0/18:1	H	.44	.37	.44	.36	.01	.01	NS	NS	.01
	J	.62	.52	.58	.50		.01	.03	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

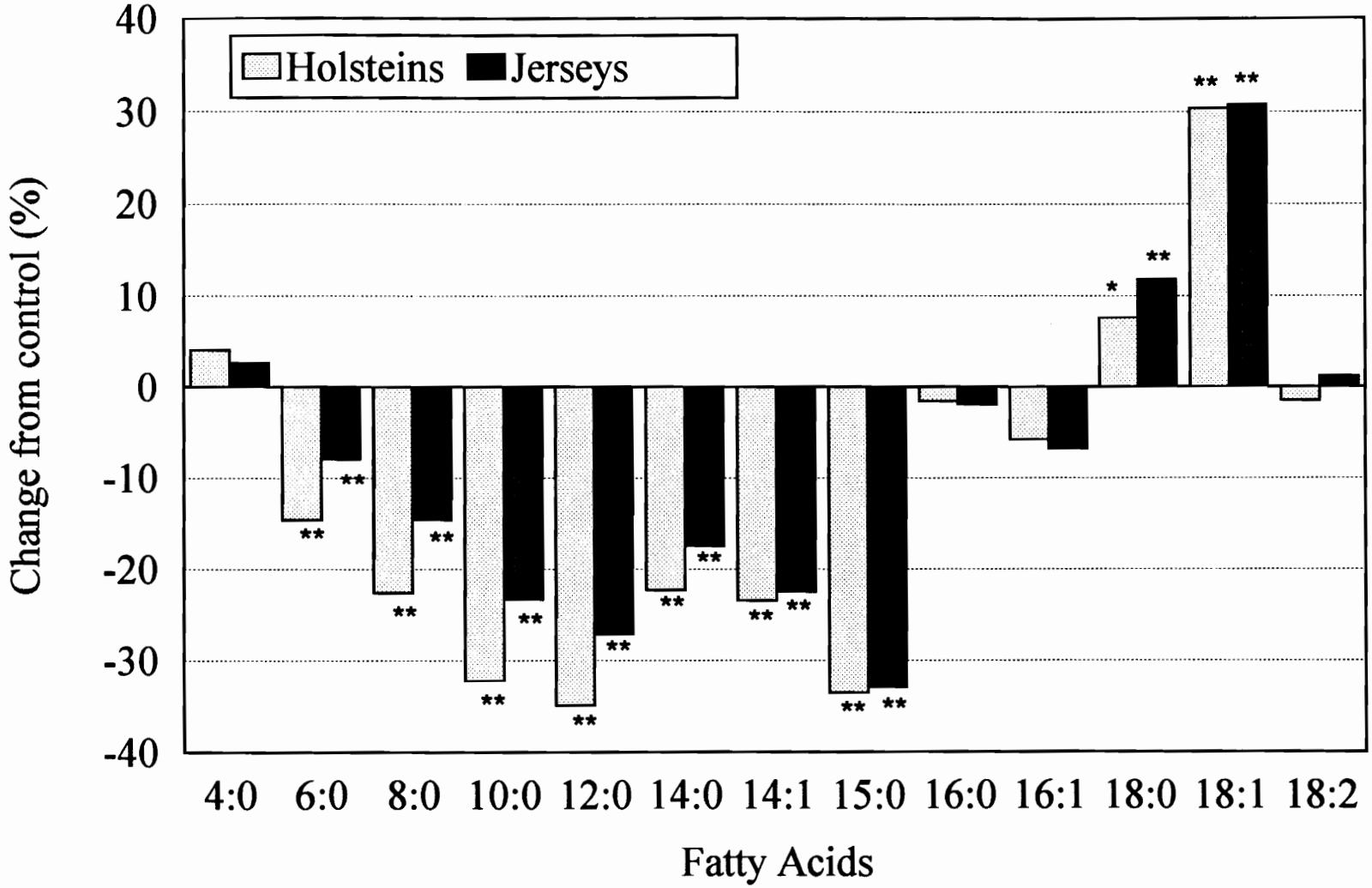

Figure 3.1. Concentration of milk fatty acids in response to dietary fat, added fat differs from no fat ** ($P < .01$), * ($P < .05$).

Table 3.8. Yield of milk fatty acids in response to dietary fat (F) and rumen undegradable protein (RUP).

		Treatments					Contrasts (P <.05)			
	B[1]	29% RUP	29% RUP+F	41% RUP	41% RUP+F	SEM	FAT	RUP	INT	BC[2]
		---------- g/d ----------								
4:0	H	30.5	31.7	32.5	34.1	1.1	NS	.05	NS	NS
	J	29.0	31.0	29.4	33.7		.01	NS	NS	
6:0	H	16.9	14.4	17.4	15.2	.6	.01	NS	NS	NS
	J	16.9	16.3	17.1	17.6		NS	NS	NS	
8:0	H	11.2	8.6	11.7	9.3	.4	.01	NS	NS	NS
	J	11.7	10.3	11.7	11.4		.04	NS	NS	
10:0	H	27.8	18.7	28.2	19.3	.9	.01	NS	NS	.01
	J	31.5	25.3	31.0	27.4		.01	NS	NS	
12:0	H	35.7	23.3	36.6	23.8	1.2	.01	NS	NS	.01
	J	40.8	31.1	40.1	33.8		.01	NS	NS	
14:0	H	148.9	116.9	153.3	118.2	3.8	.01	NS	NS	NS
	J	146.6	128.0	146.0	135.4		.01	NS	NS	
14:1	H	15.4	11.8	15.4	11.6	.5	.01	NS	NS	.01
	J	10.6	9.2	11.1	9.5		.01	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

Table 3.9. Yield of milk fatty acids in response to dietary fat (F) and rumen undegradable protein (RUP).

		Treatments					Contrasts (P <.05)			
	B[1]	29% RUP	29% RUP+F	41% RUP	41% RUP+F	SEM	FAT	RUP	INT	BC[2]
		---------- g/d ----------								
15:0	H	18.0	11.6	16.5	11.1	.7	.01	NS	NS	NS
	J	15.8	11.4	14.7	10.9		.01	NS	NS	
16:0	H	527.9	528.8	563.8	539.9	15.6	NS	NS	NS	NS
	J	521.1	537.6	509.3	561.3		.04	NS	NS	
16:1	H	26.1	25.1	27.5	24.1	1.1	.05	NS	NS	.01
	J	19.8	19.1	19.3	20.6		NS	NS	NS	
18:0	H	101.4	114.3	104.6	113.0	4.0	.01	NS	NS	NS
	J	108.0	123.6	97.1	126.8		.01	NS	NS	
18:1	H	227.6	307.4	240.0	308.7	8.6	.01	NS	NS	.01
	J	174.8	234.8	165.6	251.3		.01	NS	NS	
18:2	H	30.7	30.4	28.8	28.7	.9	NS	.05	NS	.01
	J	24.2	25.8	21.6	25.3		.01	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

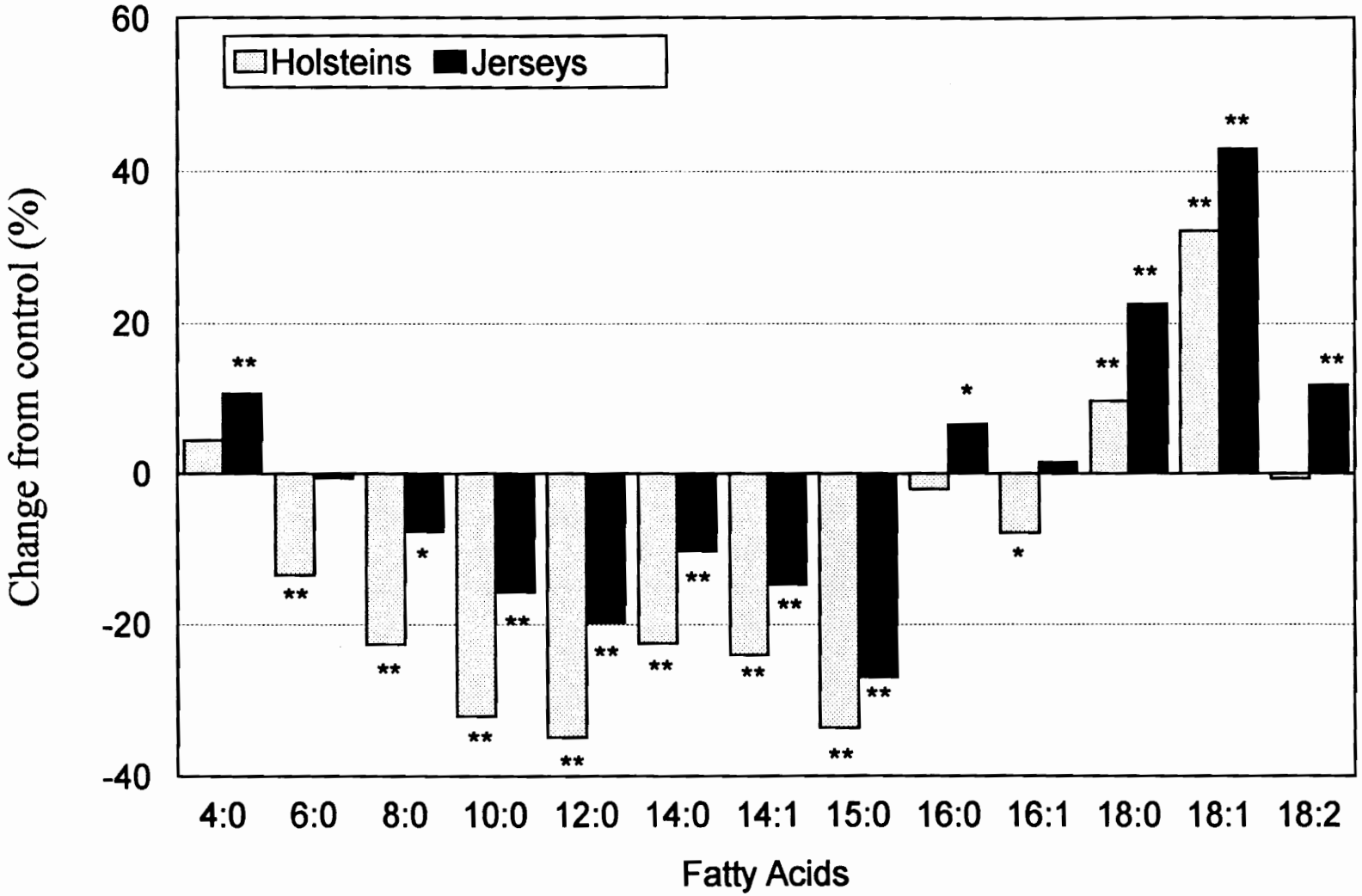

Figure 3.2. Yield of milk fatty acids in response to dietary fat, added fat differs from no fat ** ($P < .01$), * ($P < .05$).

Table 3.10. Plasma (PUN) and milk (MUN) urea N in response to dietary fat (F) and rumen undegradable protein (RUP).

		Treatments					Contrasts (P <.05)			
	B[1]	29% RUP	29% RUP+F	41% RUP	41% RUP+F	SEM	FAT	RUP	INT	BC[2]
		---------- mg/dL ----------								
PUN 0700	H	14.6	15.0	16.1	16.4	.4	NS	.01	NS	NS
	J	15.3	16.1	14.9	16.5		.01	NS	NS	
PUN 1600	H	16.8	16.2	16.2	17.9	.5	NS	NS	.05	NS
	J	18.1	19.0	16.8	18.8		.01	NS	NS	
MUN 0100	H	23.4	22.0	23.3	23.1	.7	NS	NS	NS	.01
	J	20.1	20.2	19.9	20.5		NS	NS	NS	
MUN 1300	H	25.5	23.5	26.1	25.7	.5	.02	.01	NS	.01
	J	23.0	22.3	22.9	22.5		NS	NS	NS	

INT = Protein x fat interaction
[1] B = Breeds, H = Holstein, J = Jersey
[2] BC = Breed contrast

Table 3.11. Rumen pH, ammonia and total VFA concentration in response to dietary fat (F) and rumen undegradable protein (RUP).

		Treatments					Contrasts (P <.05)			
	B[1]	29% RUP	29% RUP+F	41% RUP	41% RUP+F	SEM	FAT	RUP	INT	BC[2]
pH	H	6.67	6.36	6.91	6.92	.13	NS	.01	NS	NS
	J	6.64	6.69	6.64	6.74		NS	NS	NS	
Ammonia,	H	10.1	9.0	5.9	6.7	1.4	NS	.03	NS	.01
mg/dL	J	14.7	17.0	9.9	12.2		NS	.01	NS	
Total VFA	H	81.5	83.2	70.2	70.5	1.9	NS	.01	NS	.05
mmol/L	J	85.6	87.0	80.7	82.1		NS	.02	NS	

INT = Protein x fat interaction
[1] B = Breeds, H = Holstein, J = Jersey
[2] BC = Breed contrast

Table 3.12. Concentration of rumen VFA in response to dietary fat (F) and rumen undegradable protein (RUP).

		Treatments					Contrasts (P <.05)			
	B[1]	29% RUP	29% RUP+F	41% RUP	41% RUP+F	SEM	FAT	RUP	INT	BC[2]
		----- moles/100 moles -----								
Acetate	H	66.3	66.6	68.5	68.4	.4	NS	.01	NS	NS
	J	66.6	66.7	67.6	68.4		NS	.01	NS	
Propionate	H	18.8	18.1	16.7	17.1	.4	NS	.01	NS	NS
	J	17.6	18.0	17.4	16.7		NS	NS	NS	
Butyrate	H	10.9	11.2	10.9	10.6	.2	NS	NS	NS	NS
	J	11.5	11.1	11.2	10.9		NS	NS	NS	
Valerate	H	1.58	1.56	1.40	1.37	.06	NS	.01	NS	NS
	J	1.66	1.64	1.47	1.54		NS	.02	NS	
Isobutyrate	H	.91	.90	.89	.93	.03	NS	NS	NS	NS
	J	.93	.92	.88	.95		NS	NS	NS	
Isovalerate	H	1.54	1.63	1.53	1.53	.05	NS	NS	NS	NS
	J	1.60	1.59	1.49	1.57		NS	NS	NS	
Acet:Prop	H	3.60	3.69	4.12	4.02	.10	NS	.01	NS	NS
	J	3.80	3.76	3.90	4.15		NS	.02	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

Table 3.13. Correlation coefficients for rumen ammonia, plasma and milk urea N.

	B[1]	RAN	PUN 0700	PUN 1600	MUN 0100	MUN 1300
RAN[2] 1600	O	-	.14	.64**	.00	-.10
	H	-	.16	.41**	.23	.16
	J	-	.14	.74**	.13	-.01
PUN[3] 0700	O		-	.70**	.65**	.65**
	H		-	.85**	.77**	.77**
	J		-	.59**	.60**	.64**
PUN[3] 1600	O			-	.42**	.39**
	H			-	.69**	.72**
	J			-	.40**	.32*
MUN[4] 0100	O				-	.82**
	H				-	.84**
	J				-	.72**

[1] B = Breeds, O = overall, H = Holstein, J = Jersey
[2] Rumen ammonia N
[3] Plasma urea N
[4] Milk urea N
* P < .05
** P <.01

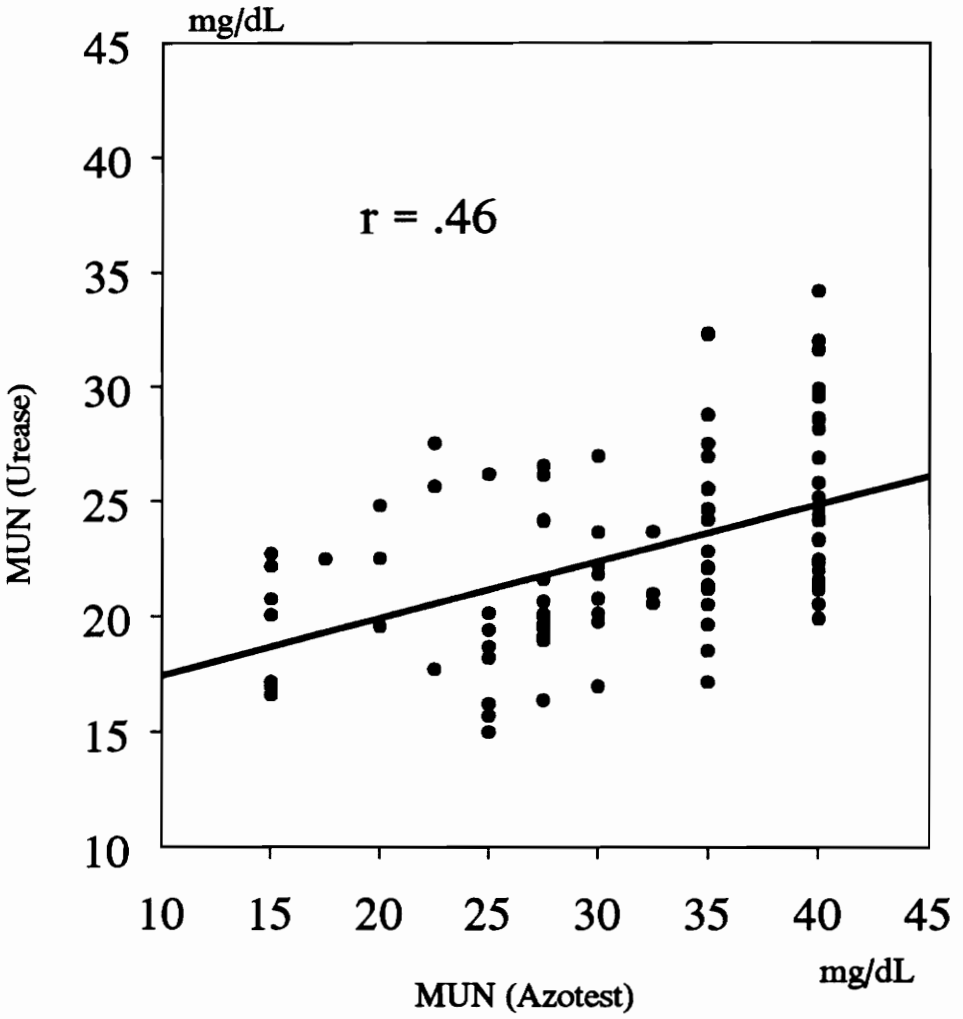

Figure 3.3. Relationship between concentration of urea in milk determined by Azotest and Urease analysis

CHAPTER 4

DIURNAL VARIATION OF MILK AND PLASMA UREA NITROGEN OF HOLSTEIN AND JERSEY COWS IN RESPONSE TO DIETARY PROTEIN DEGRADABILITY AND ADDED FAT

(ABSTRACT)

Four Holstein and 4 Jersey cows fitted with ruminal and duodenal cannulas, were used in two 4 x 4 Latin squares to investigate the effects of dietary protein degradability and supplemental fat on rumen, blood and milk components. Diet dry matter (DM) contained 16.2% CP with two levels of rumen undegradable protein (RUP) obtained by substituting blood meal for soybean meal. Treatments were 29% RUP, 0% added fat; 29% RUP, 2.7% added fat (Ca soaps of fatty acids); 41% RUP, 0% added fat; and 41% RUP, 2.7% added fat. Total mixed ration DM consisted of 30% corn silage, 29% alfalfa haylage and 41% concentrate. Feeding times were 1000 and 1400 h. Milk urea N (MUN), plasma urea N (PUN) and rumen ammonia were measured every 4 h over a 24 h period. Dry matter intake decreased 6.7% by added fat. Rumen ammonia concentration was between 25 and 45% lower when 41% RUP diets were fed. Overall, concentration of PUN (mg/dL) and milk components were influenced by diet. However, MUN (5.3 versus 3.8 mg/dL) was higher in Holsteins compared with Jerseys. Both PUN and MUN increased within 2 h after the 1000 h feeding followed by a decline 6 h after the 1400 feeding. In this short term study fat supplementation had no affect on milk production and yield of milk components. Higher RUP due to blood meal substitution increased yield of milk components. Plasma urea N and MUN did not differ due to dietary treatments, but varied throughout the day in relation to feeding.

Materials and Methods

Experimental Design and Diets

Four Holstein and 4 Jersey cows (31 to 61 days postpartum) fitted with ruminal and duodenal cannulas, were used in two 4 x 4 Latin squares, one for each breed, balanced for carryover effects in 15-d periods. Cows were housed in tie stalls in the Dairy Nutrition Research Barn at the Virginia Tech Dairy Center. Ingredients and chemical composition of the diets are shown in Table 4.1. Diets were formulated to be isonitrogenous (17% CP) and to have 29 and 41%, rumen undegradable protein **(RUP)** and blood meal was substituted for soybean meal on a protein equivalent basis to increase the RUP level. Each level of RUP was formulated without and with 2.7% added fat **(F).** A portion of shelled corn was substituted with Ca soaps of fatty acids (MEGALAC, Church & Dwight Co., Inc.). Corn gluten meal was added to supply an equivalent amount of the corn protein that was removed. Diets containing added fat had a greater NE_L and were calculated to have 5.8 to 5.9% total fat. All diets were formulated to have 31% neutral detergent fiber and 19% acid detergent fiber.

Concentrate ingredients were premixed and stored until added to corn and alfalfa silages in a Uebler mixer (Model 780, Uebler Manufacturing Co., Vernon, NY) prior to feeding. The total mixed diets were fed as total mixed diets at 1000 and 1400 h. Immediately after the last period was completed an in situ study was performed in

four of the eight fistulated cows (two Holsteins and two Jerseys). For this study, cows were fed a diet containing 35% RUP and 4.1% total fat with the same feed ingredients listed in Table 4.1, starting 8 days before the 72 h incubation.

Measurements and Sampling

Cows were fed to have between 5 and 10% daily orts, which were checked each morning and feeding rates adjusted. Dry matter intake was measured on days 11 through 15 of each period. Feed refusals were weighed at 0700 h on an electronic scale. Body weights were recorded on d 1 in the first three periods and on days 1 and 16 in the last period. Forages were sampled weekly and concentrates once each period. Each sample was taken in duplicate, one was sent to the Virginia Tech Forage Testing Laboratory and the other frozen for the in situ work and for further analysis. Chromic oxide (Fisher Scientific, Fair Lawn, NJ) and cobalt ethylenediaminetetraacetate (Co-EDTA) were used as digestibility and flow markers as described by Uden et al. (1980). From d 9 through d 15 of each period, 15 g of chromic oxide and 120 mL of Co-EDTA solution (7.5 g of Co-EDTA) were administered at 12 h intervals (0700 and 1900 h) through the rumen cannula. Chromic oxide and Co-EDTA were sprinkled on the top of the rumen contents in a preformed pocket leaving the paper bag inside the rumen. On d 15 only the 0700 h administration was performed.

A jugular vein catheter was installed on d 14 at 1400 h and removed on d 15 at 2200 h. Catheters were prepared at the Dairy Nutrition Laboratory and submerged in alcohol until implantation. Three to five minutes before catheterization cows were given .75 mL of Butorphanol i.v. (10 mg/mL) as an analgesic. The jugular vein catheter was Tygon® tubing (.02 mm i.d., .06 mm o.d., Fisher Scientific Co., Pittsburgh, PA) inserted through a 14 G x 1½" hypodermic needle (Monoject, Sherwood Medical, St. Louis, MO) placed temporarily in the jugular vein. After the catheter was inserted the hypodermic needle was removed and a one-way stopcock (Baxter K70, Valencia, CA) fitted with a Luer stub adapter (Intramedic®, Becton Dickinson and Co., Franklin Lakes, NJ) and PRN adapter (Luer Lok® adapter, Becton Dickinson, Sandy, UT) was connected to the tubing. Patches for catheters protection made at the Dairy Nutrition Laboratory were placed on the right side of cow's necks. Catheters were flushed with 10 mL of .9% saline followed by 5 mL of heparinized saline (200 u/mL, sodium salt heparin, Sigma Chemical Co.,St. Louis, MO).

Cows were milked in tie stalls with a bucket milking system. The initial milking prior to sampling was at 1600 h on d 14 in order to establish a 4 h interval for milkings. Milk, blood, rumen, duodenal and fecal samples were collected every four hours. Sampling times started at 2000 h on d 14 and ended at 2000 h on d 15. Daily milk production was recorded with a Surge computerized system throughout each period, except during the collection period on d 14 to 15 when milk production a 4 h intervals was weighed on an electronic scale.

Samples for milk composition were taken in duplicate every 4 h. Samples were collected into 50 mL DHIA milk containers with preservative (Bronopol), placed on ice and stored at 2°C at the Dairy Nutrition Laboratory. One sample was submitted to the DHIA Laboratory on d 16 of each period for milk fat, solids non-fat and lactose analysis. The duplicate sample was analyzed for total N, casein N and milk urea N.

Blood samples were collected with a 10 cc syringe (Becton Dickinson & Co, Franklin Lakes, NJ) connected to the one-way stopcock to draw blood (10 mL) from the jugular vein for plasma preparation. After blood was drawn, catheters were flushed with 5 mL of .9% saline and 2 mL of heparinized saline. Blood samples were combined with 100 μL of heparinized saline with concentration of 2.86 units/μL (Sodium salt heparin, Sigma Chemical Co., St. Louis, MO) and immediately placed on ice. Samples were taken to the Dairy Nutrition Laboratory and centrifuged in a Beckman J2-21 centrifuge (Beckman Instruments, Columbia, MD) with a JA-20.1 rotor at 3220 x *g* for 15 min. Plasma was divided into duplicate samples prior to storage at -20°C.

Ruminal fluid was taken by siphoning through the rumen cannula with an esophageal tube fitted with a suction strainer, placed into a PVC pipe with holes drilled to allow the passage of liquid. Approximately 100 mL of rumen fluid were discarded and then a total of 50 mL collected into 50 mL plastic tubes, immediately placed on ice and taken to the Dairy Barn Nutrition Laboratory. Duplicates of 5 mL aliquots were preserved with 1 mL of 25% H_3PO_4 for ammonia analysis. Duplicates

of 5 mL aliquots were preserved with 1 mL of 25% H_3PO_4 and 1 mL of 30 mM isocaproic acid (internal standard) for VFA analysis, prior to storage at -20°C. After samples for rumen ammonia and VFA were prepared, ruminal pH was measured on the remaining portion of sample.

Duodenal and fecal samples were taken every 4 h from 2000 h on d 14 to 1600 h on d 15. Duodenal samples were taken from the cannula into a large plastic beaker. The first surge was discarded and then a 1.5 L sample was taken. Two 250 mL subsamples were taken using a 140 mL cup after agitation to resuspend the solid particles. A handful of feces was taken with a plastic insemination sleeve, and placed into a ziplock bag. Samples were stored at 2°C at the Dairy Nutrition Laboratory until the end of the sampling when duodenal and fecal samples were composited.

One duodenal subsample from each collection time was frozen as a reserve. The remaining subsamples were combined into a 2 L glass beaker and stirred to resuspend solid particles. Two composited subsamples (250 mL) were then taken upon agitation and frozen until analyzed. Each fecal sample from each cow was mixed within the plastic glove and transferred into a 250 mL sampling cup. At the time of transfer, a portion of the sample at each collection time was placed into another 250 mL sampling cup as duplicate. All samples were frozen until analyzed.

One L of ruminal fluid was taken at the end of each sampling period to isolate ruminal bacteria in the same way as described for ammonia and VFA. Samples were stored overnight at 2°C and processed the next morning. An aliquot of 250 mL was

centrifuged in a IECB-22 centrifuge (International Equipment Co., Needham Heights, MA) at 200 x *g* for 10 min. The supernatant was kept and centrifuged at 30,000 x *g* for 20 min., the pellet was then rinsed and resuspended with distilled water. This procedure was repeated twice and then frozen until analyzed for total N and cytosine.

The in situ technique was used to determine CP and DM degradability of feeds. Individual forage samples were ground with dry ice through a 5 mm screen in a Thomas-Wiley laboratory mill (Arthur Thomas Company, Philadelphia, PA). Corn, soybean meal, corn gluten meal and blood meal were composited and incubated. Bags were made of Pecap polyester (Tetko Inc., Lancaster, NY) with a pore size 58 microns and with a size of 10.16 x 17.78 cm. Between 5 and 14 g of feed sample was placed in each bag for a feed exposure of 15 mg/cm^2 of DM (Nocek, 1985). Bags were closed with 4" Bar-Lok Cable ties (Dennison Manufacturing Co., Framingham, MA), tied in duplicate and attached to a chain with 7.5" Bar-Lok Cable ties (Dennison Manufacturing Co., Framingham, MA) and grouped according to incubation times.

The in situ study started 8 days (at 1800 h) after the last period was completed. Prior to incubation, bags were soaked in warm water for 15 min and then introduced in the rumen through the rumen cannula at 72, 48, 24, 12, 6 and 2 h before all bags were removed. The 0 h bags were soaked in warm water for 15 min. All bags were removed from the rumen at the same time, rinsed in cold tap water and duplicates were separated. Three successive cold water machine rinses (3-5 min) with agitation were conducted until rinse water was clear. Bags were dried at 60°C in a forced air

oven until weight was constant. Bags and residues were weighed to determine DM disappearance, then duplicate samples were removed from the bags and composited for each incubation time. Composite samples were ground through a 1 mm screen in a Cyclotec mill (Tecator 1093, Hoganas, Sweden) and stored for later analysis.

Analyses

Concentrate and forage DM were determined by drying overnight at 60°C in a forced air oven. Dried samples were ground through a 1 mm screen in a Cyclotec mill (Tecator 1093, Hoganas, Sweden) and analyzed for total N (AOAC, 1990), ether extract (AOAC, 1990), acid detergent fiber (Goering and Van Soest, 1970), neutral detergent fiber (Van Soest et al., 1991) and organic matter (600°C for 4 h). Milk samples were analyzed for fat and lactose content by infrared spectroscopy (Fossomatic 605, B filter) and total milk N by Kjeldahl analysis (AOAC, 1990).

Milk urea N was determined in triplicate by urease method (Weatherburn, 1967). Ten μL of milk sample were placed into a 10 mL glass tube and incubated for 20 min with 200 μL of urease solution (Sigma Chemical Co., St. Louis, MO) with concentration of .104 units urease/μL sample. One mL of reagent one (50 g phenol and 250 mg sodium nitroferricyanide/L) and reagent two (25 g sodium hydroxide and 42 mL 8% sodium hypochlorite/L) were added. Samples were vortexed and incubated

at room temperature overnight. Absorbance was read on a spectrophotometer (Spectronic 1001, Bausch & Lomb, Rochester, NY) at a wavelength of 625 nm.

Milk casein N was determined using the procedure of Aschaffenburg and Drewry (1959). Samples were warmed to room temperature and mixed thoroughly. A 2.5 mL sample, 17.5 mL of distilled water and .25 mL of 10% acetic acid were placed in a 250 mL Erlenmeyer flask and mixed. Flasks were placed in a water bath at 40°C for 10 min. Precipitated samples were filtered through Whatman #2 (Whatman, Clifton, NJ) filter paper, rinsed 3 times with distilled water, drained, and placed into a Kjeldahl tube for N analysis (AOAC, 1990).

Rumen samples were thawed and filtered through a GN-6 filter (Geldman Sciences, Ann Arbor, MI) using syringe and 25 mm Millipore swinnex filter holder (Millipore Corporation, Bedford, MA). Plasma and filtered rumen samples were analyzed for urea N and ammonia, respectively (Weatherburn, 1967). Filtered rumen samples were analyzed for VFA by gas chromatography by injecting .5 µL of sample into a Varian Vista 6000 chromatograph (Varian Instruments, Palo Alto, CA) equipped with a flame ionization detector, a Varian Vista 4270 integrator (Varian Instruments, Palo Alto, CA), and a 6' x ¼" o.d. and 2 mm i.d. glass column (Supelco Inc., Bellfonte, PA) packed with GP 10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb WAW. The analysis was isothermal with temperatures of 115°C for the column, 170°C for the injector and 180°C for the detector. Nitrogen gas was used as a carrier with a flow of 80 mL/min and hydrogen and air as detector gases with flows of 40

and 60 mL/min, respectively. Rumen pH was measured on unfiltered rumen samples within 60 min after collection using an Accumet pH Meter (Fisher Scientific, Raleigh, NC).

Frozen duodenal and fecal samples were dried in a Dura-Top freeze dryer (FTS® Systems Inc., Stone Ridge, NY). Samples were analyzed for DM, total N (AOAC, 1990) and organic matter (600°C for 4 h). Approximately .5 g of each sample was placed into digestion tubes and digested with nitric, perchloric and sulfuric acid and hydrogen peroxide (SAC, 1973). Two mL of nitric and perchloric acid were added to each sample and samples were placed in a hood overnight. The next day, tubes were placed in a heated aluminum block after two drops of hydrogen peroxide were added to each sample. Block temperature was set between 100 and 120°C and kept constant until white fumes evolved. Then 1 mL of sulfuric acid was added and block temperature raised to 150°C. Digestion was considered complete when samples had turned a reddish color and were non-reactive. Tubes were cooled in a rack and next morning diluted up to 50 mL with distilled deionized water. Samples were filtered through Whatman # 1 filter paper (Whatman, Clifton, NJ) and stored at 2°C until analyzed. Cobalt was determined with a Varian Vista AA-475 atomic absorption spectrophotometer (Varian Instruments, Palo Alto, CA) with an air-acetylene flame at 240.7 nm wavelength (slit width .2 mm) and chromium was determined with an acetylene-nitrous oxide flame at 357.9 nm wavelength (slit width .5 mm).

Apparent digestibility coefficients (ADC) of DM, organic matter and N were calculated by the indirect method as described by Schneider and Flatt (1975):

$$ADC = 100 - \left(100 \; X \; \frac{\%\ indicator\ feed \; X \; \%\ nutrient\ feces}{\%\ indicator\ feces \; X \; \%nutrient\ feed} \right)$$

A cytosine assay (Zerbini, 1984) was performed on corn and alfalfa silages, whole duodenal contents, and bacteria samples to calculate the amount of microbial contamination in forages, percent of microbial N reaching the abomasum, and cytosine:N ratio in bacteria. Samples (250 mg) were placed into a 15 mL screw top tube and 2.5 mL of perchloric acid added. Tubes were vortexed and allowed to stay in the hood at room temperature overnight. Hydrolysis was conducted using a block heater at 90°C and vortexing every 15 min. Tubes were cooled to room temperature and diluted with approximately 5 mL of .2 M NH_3HPO_4. Diluted samples were transferred to a 100 mL volumetric flask, 3.5 mL of NH_4OH were added to adjust pH, and taken to volume. An aliquot of sample was filtered through a .45 µm Nylaflo filter (Geldman Sciences, Ann Arbor, MI) using syringe and filter holder. Cytosine was analyzed by a high pressure liquid chromatography HPLC 2510 (Varian Instruments, Palo Alto, CA) with a 25 cm Partisil 10 SCX column (Whatman, Clifton,

NJ). The mobile phase was .15 M ammonium phosphate adjusted at pH 3.53 at flow rate of 0.6 mL/min. Injection volume for all samples was 10 µL. A LINEAR UVIS 200 detector (Linear Instruments Corporation, Edison Way, NV) was set at 254 nm with an absorbance range of 0.2 AUFS and connected to a HP 3393A integrator (Hewlett Packard Company, Avondale, PA).

Microbial N as a percent of the total N reaching the duodenum (%MND) was calculated as Zerbini (1984):

$$\%MND = 100\ X\ \frac{\left(\frac{\textit{microbial N mg/g DM}}{\textit{microbial cytosine µM/g DM}}\ x\ \textit{duodenal cytosine µM/g DM}\right)}{\textit{duodenal N mg/g DM}}$$

In situ samples were analyzed for total N (AOAC, 1990). Protein degradability was calculated with the indirect method described by Janicki (1988) and with the direct method. The indirect method describes fractions A, B and C as follows:

A = NPN or true protein that is degraded very rapidly with a fractional disappearance rate equal to infinity,

B = Protein that is degraded at a measurable rate similar to the rate of passage (k_d = .02 to .07/h), and

C = protein that is not degraded.

Rate of passage and degradation at any time usually affects only fraction B. A monoexponential rate of decay was used to describe degradation rate of fraction B and to estimate its value at 0 time. The residual material after 72 h was considered to be the undegraded fraction and was designated as fraction C. Fraction C was subtracted from the percent remaining at times 2 to 24 h and the natural log was calculated. Degradation constants (k_d) were calculated by regressing the natural log of each of these fractions on time. The degradation constant is represented by the slope of this line and fraction B is represented by the antilog of the intercept. The rapidly degraded fraction A was calculated by the following equation:

$$A = 100 - (B + C)$$

A linear regression of log data on time for the indirect method adapted from Janicki (1986) is presented in Figure 4.1. The direct method describes fractions A, B and C as follows:

A = NPN or true protein that is solubilized rapidly in warm water and is calculated subtracting from 100 the percent remaining at 0 time after soaking in warm water.

B = Protein that is degraded at a measurable rate similar to the rate of passage and is calculated subtracting from 100 the sum of fractions A and C.

C = Protein that is undegraded in the rumen and is the percent remaining at 72 h.

The rate of degradation of fraction B was calculated using the same procedure described in the indirect method. Percent of protein degradability (D) for indirect and direct methods were calculated after calculating the % of protein in fractions A, B and C using the equation of Ørskov and McDonald (1979):

$$D = A + \frac{(B \; X \; k_d)}{(k_d + k_r)}$$

where:

D = Protein degradability (%),

A = Fraction readily degraded (%),

B = Fraction degraded at a measured rate (%),

k_d = Protein degradation constant of fraction B,

k_r = Rumen turnover rate assumed to be .05/h.

In Situ Bag Technique

TOTAL N & DM = A + B + C

	READILY DEGRADED	DEGRADED AT MEASURABLE	NOT DEGRADED
	E = INFINITE	E = .1/h	K = 0
	A = TOTAL -B -C	B = $e^{intercept}$	C = 72 h res/orig.

Fraction of feed remaining in the rumen at any given point of time = $B \cdot e^{-kt} + C$ (for $t > 0$)

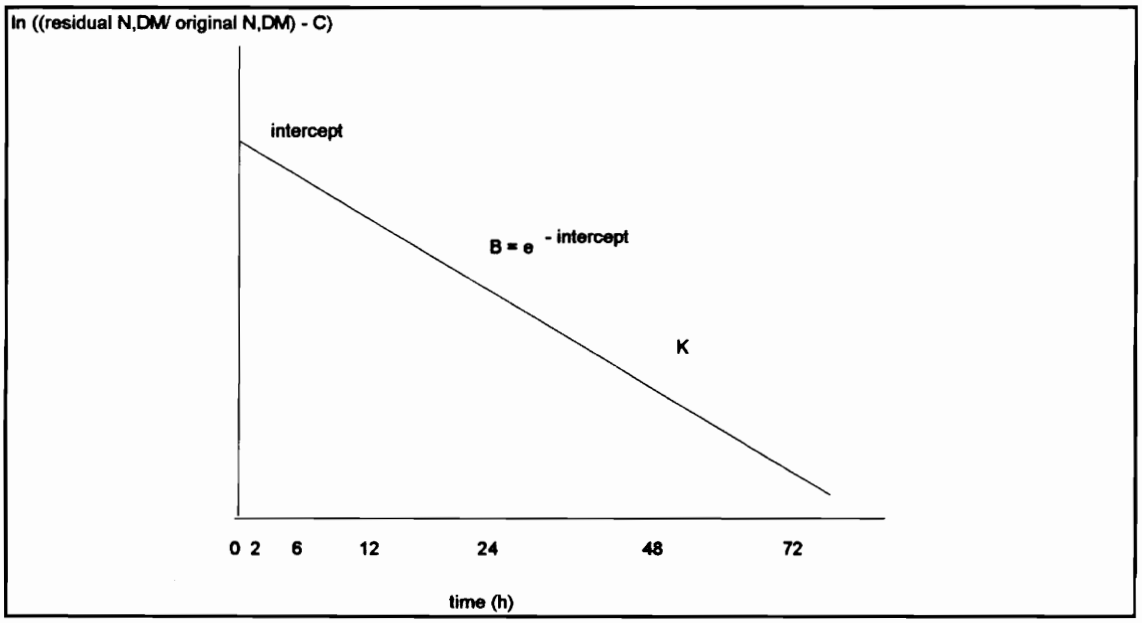

Figure 4.1. Regression of the log on time used to describe rumen degradation of N and DM

Statistical Analyses

Data were analyzed by ANOVA and repeated measures using the general linear models procedure of PC-SAS (1990). Data are presented as least squares means and standard errors of the mean. Variation in the dependent variable, Y, was described as follows:

$$Y_{ijklm} = \mu + B_i + C_{(i)j} + PE_k + PT_l + F_m + (PTxF)_{lm} + (BxPT)_{il} + (BxF)_{im} + (BxPTxF)_{ilm} + Error\text{-}A_{ijklm} + T_n + (T \times all\ effects) + Error\text{-}B_{ijklmn}$$

where:

Y_{ijklm} = dependent variable

μ = overall mean of Y

B_i = effect of breed (i = 1..2)

$C_{(i)j}$ = effect of cow j within breed (j = 1..4)

PE_k = effect of period (k = 1..4)

PT_l = effect of level of dietary RUP (l = 1..2)

F_m = effect of fat (m = 1..2)

$(PTxF)_{lm}$ = interaction of RUP and fat

$(BxPT)_{il}$ = interaction of breed and RUP

$(BxF)_{im}$ =	interaction of breed and fat
$(BxPTxF)_{ilm}$ =	interaction of breed, RUP and fat
Error-A_{ijklm} =	random error (among cows)
T_n =	effect of time (n = 1..7)
(T x all effects) =	interaction of time and all other effects
Error-B_{ijklmn} =	random residual error (within cows)

Breed differences were tested by dividing the breed mean square by the mean square of cow within breed. Contrasts for fat and RUP were calculated within each breed. Contrast for fat represents the comparison of diets without added fat to those with added fat, all at average RUP. Contrast for RUP represents the comparison of 29% RUP diets to 41% RUP, all at average fat. Breed contrast represents the comparison of the least square means for Holstein versus Jersey averaged across all treatments.

Dry matter intake, milk production, milk component content and yield were tested for all effects, except time. Diurnal variation of milk component, plasma, and ruminal parameters were tested for all effects including time.

RESULTS AND DISCUSSION

Diets were formulated to be 17% CP but because the quality of the alfalfa silage decreased throughout the experiment the mean was 16.2%. Values from Dair4 ration evaluation software (Stallings et al., 1993) were used to calculate RUP levels of 29 and 41% of total CP. Diets containing added fat had greater NE_L and less non-fiber carbohydrates due to Megalac replacing corn. Diets were formulated to have 3.2% (no added fat) and 5.9% (added fat) total fat. However, lower concentrations of total fat, 2.5 and 5.2% respectively, were obtained due to lower ether extract than reported by N.R.C. (1989) for corn silage, ground corn and blood meal. Neutral and acid detergent fibers were similar but tended to be slightly greater in the 29% RUP diets because more soybean meal was used.

Dry matter intake decreased 7.7 and 5.4% when fat was added to the diet in Holsteins and Jerseys, respectively (Table 4.2). Dry matter intake between breeds was not different. However, a protein by fat interaction occurred when Holsteins fed added fat and 41% RUP consumed less DMI compared to 29% RUP diets. Expressed as percent of body weight, DMI decreased 7.6% in Holsteins and 4.5% in Jerseys when fat was supplemented. Dry matter intake expressed as percent of body weight was not different between breeds. Similar reductions in DMI were observed in cows used in the experiment reported in Chapter 3. However, total energy intake was similar between diets, 31.25 and 30.64 Mcal/d for Holsteins and 25.83 and 25.97 Mcal/d for

Jerseys, without and with added fat, respectively. Klusmeyer et al. (1991b) and Kim et al. (1993) also reported a reduction in DMI when Ca soaps of fatty acids were fed. On the other hand, Schneider et al. (1988) and Erickson et al. (1992) found no difference in DMI when Ca soaps of fatty acids were supplemented. Reduction of DMI may be a result of metabolic control (N.R.C., 1988) due to similar energy intake when cows were supplemented with fat.

Although the objective of this experiment was not to measure production, milk yield was increased 8.0% in Holsteins fed 41% RUP diets. Holsteins produced 8.8 kg/d more milk than Jerseys. Cows did not respond to added fat, contrary to the results reported in Chapter 3. However, variation between cows in both experiments was similar (error mean square = 2.82 and 2.16, for Chapter 3 and 4, respectively), but response to added fat in Holsteins and Jerseys was 2.26 and 1.67 kg/d for Chapter 3, and 2.16 and 0 kg/d for Chapter 4. Lack of significant response to added fat in Jerseys used in this experiment, may be due to the length of the periods used (15 d) but in Holsteins appears to be due to fewer cows. Klusmeyer et al (1991a) found no difference when cows were fed Ca soaps of fatty acids and high forage diets (66% of DM) with experimental periods of 14 d. Tomlinson et al. (1994) concluded effects of Ca soaps of fatty acids on milk yield were not expressed until wk 4 but protein effects were expressed by wk 3.

Milk yield expressed as 4% FCM in Holsteins increased 7.6% when RUP diets were fed, mainly due to increased milk production because milk fat content was not

affected. Production of 4% FCM per Mcal of NE_L consumed increased 6.1% in Holsteins fed added fat. A similar response to fat was observed in Chapter 3 cows fed added fat. Lack of response in efficiency of production in Jerseys compared to cows used in Chapter 3 may be due to using shorter experimental periods.

Table 4.3 contains milk component yields. Fat yield was not affected by diets or breed. However, Jerseys used in the experiment reported in Chapter 3 increased milk fat yield with added fat due to increased milk yield. Protein yield was not affected by added fat. However, 41% RUP increased protein yield 11.1% in Holsteins. Contrary to this, Holsteins used in the experiment reported in Chapter 3 reduced protein yield when 41% RUP diets were fed. Increased protein yield was due to increased milk production when Holsteins were fed 41% RUP because milk protein content was not affected.

Lactose yield increased 7.7% in Holsteins fed 41% RUP, and Holsteins produced 0.49 kg/d more milk lactose than Jerseys. Contrary to this, Holsteins and Jerseys used in the experiment reported in Chapter 3 increased lactose yield with added fat. Solids non-fat yield increased 6.8% in Holsteins fed 41% RUP diets. Contrary to this, in cows used in the experiment reported in Chapter 3, SNF yield increased with added fat. Increased lactose and SNF yields are due to increased milk production when Holsteins were fed 41% RUP diets because milk component contents were not affected.

Milk fat content was not different by diet (Table 4.4). However, Jerseys had a greater milk fat content 5.4% compared to 3.8% in Holsteins. Similar values were observed in cows used in the experiment reported in Chapter 3, but Holsteins decreased fat content with added fat. In Chapter 3, milk protein content decreased in both breeds with added fat and 41% RUP, and lactose content increased in Jerseys fed added fat. However, in this study protein and lactose content were not affected by diet. Solids non-fat content decreased 2.1% in Holsteins fed added fat and 1.1% in Jerseys fed 41% RUP. Decreased SNF content may be due to a non-significant decrease in milk protein content when Holsteins and Jerseys were fed these diets.

Nitrogen fractions of milk are shown in Table 4.5. Total N and casein N content were not affected by diet. However, Jerseys had a greater concentration of total N (.562 vs .457 g/100 g of milk) and casein N (.441 vs .346 g/100 g of milk) than Holsteins. Contrary to this, when cows used in Chapter 3 were fed added fat and 41% RUP diets a decrease in total milk N and casein N was observed. Milk urea N concentration decreased 6.5% in Holsteins fed added fat. A similar response was observed when Chapter 3 Holsteins were fed added fat.

Casein N concentration expressed as percent of total N was not different by diet or breed. Similar results were reported by DePeters et al. (1985 and 1989) where no difference in casein N concentration was observed when cows were fed added fat. Contrary to this in Chapter 3, Holsteins fed added fat and Jerseys fed 41% RUP had reduction in casein N concentration. Milk urea N concentration expressed as percent

of total N was not affected by diet. Holsteins had a greater concentration of milk urea N (5.28 vs 3.74 g/100 g of total N) compared to Jerseys. Contrary to this, cows in Chapter 3 increased milk urea N when fed added fat and 41% RUP. In this study milk components were sampled seven times during the day and the average of the last six used to compare the effect of the diets. Lack of significant differences in milk component contents in this experiment compared to Chapter 3 may be due to the diurnal variation of the components or less degrees of freedom used in the statistical analysis.

Milk yield (kg/milking) increased in Holsteins fed added fat at 0400 and 2000 h. When 41% RUP diets were fed, Jerseys increased milk yield at 1600 h and Holsteins at 2000 h (Table 4.6). Milk yield changed across time (Figure 4.2), however, response to dietary treatments were not different. Variation in milk yield throughout the day may be explained by responses of cows to different milking personnel between milking intervals.

Table 4.7 contains milk fat content over a 24 h period. Fat content varied across time, however, content was not affected by diets. Jerseys had a greater milk fat concentration compared to Holsteins throughout the 24 h period. Fat content decreased to levels similar to Chapter 3 by 0400 h in Jerseys and by 0000 h in Holsteins (Figure 4.3). A similar trend was observed by Bartsch et al. (1981) when cows were milked every three hours and by Jurco (1967) when cows were milked every hour. The relative high fat concentration at the beginning of the sampling

period could have been due to residual milk from a previous milking (Bartsch et al., 1981). Also, milk fat content decreased when cows were milked twice compared to thrice a day (Allen et al., 1986; Barnes et al., 1990) although another study found no difference when cows were milked twice compare to four times a day (Hillerton et al., 1990).

Milk protein content over a 24 h period is shown in Table 4.8. Protein content and response of protein content to added fat and 41% RUP varied across time. Overall milk protein content decreased before feeding. Fat supplementation reduced milk protein concentration in Holsteins after feeding (Figure 4.4). Also, 41% RUP reduced protein concentration in Jerseys at 0400 and 1200 h (Figure 4.5). Jerseys had a greater milk protein concentration throughout the 24 h period. Bartsch et al. (1981) reported milk protein content did not change when cows were milked and fed every 3 h in a 24 h period. However, when cows were milked every 3 h and fasted 24 h, milk protein content decreased from 3.65 to 3.15%. Reduced protein content before feeding may be due to insufficient supply of amino acids to the mammary gland (Wu and Huber, 1994).

Table 4.9 contains casein N content over a 24 h period. Casein N concentration decreased in Holsteins fed added fat at 0400 h and fed 41% RUP at 1200 h. Casein N content and response of casein N to 41% RUP varied across time (Figure 4.6). Decreased casein N concentration before feeding may be due to limited supply of amino acids to the mammary gland (Wu and Huber, 1994), and decrease up

to 2 h after feeding may be due to lag time needed for rumen fermentation, flow and absorption of digesta. Iotsov (1956) reported rate of milk, milk fat and milk protein secretion varied during 24 h periods. The highest rate of secretion was during the day, with a decrease towards evening and being lowest at night. Although feeding times were not reported these variations could be related to feeding management.

Milk lactose content over a 24 h period was not affected by diet or breed (Table 4.10.) , except at 2000 h when Jerseys fed 41% RUP increased lactose concentration, however, content varied across time. Lactose concentration peaked between feedings (Figure 4.7) followed by a decrease two hours after the second feeding. Increased lactose after feeding may be partially be due to increase propionate after feeding.

Plasma urea N in a 24 h period was not affected by diet or breed (Table 4.11). In Chapter 3, Jerseys fed added fat and Holsteins fed 41% RUP increased plasma urea N. Although no dietary effects were observed, plasma urea N concentration varied across time (Figure 4.8), increasing from 0000 to 0400 h and at 0800 to 1200 h and decreasing from 0400 to 0800 h and at 1600 to 2000 h. Increased plasma urea N after feeding may be partially due to increased rumen ammonia concentration. Gustafsson and Palmquist (1993) reported plasma urea N increased 2 hours after feeding.

Milk urea N in a 24 h period is shown in Table 4.12. Milk urea N decreased in Holsteins fed 41% RUP at 1200 and 2000 h. Diet had no effect on milk urea N at any other time. However, across time, milk urea N concentration varied (Figure 4.8),

decreasing from 2000 to 0000 h and from 1600 to 2000 h and increasing from 0800 to 1600 h. There was a breed difference at 1200 and 1600 h when Holsteins had a greater milk urea N concentration before and after feeding. Similar results were observed in Chapter 3 cows where Holsteins had a greater concentration of milk urea N at 0100 and 1300 h. In this study, concentration of milk urea N followed very closely the pattern of plasma urea N. However, in the study of Gustafsson and Palmquist (1993), where samples were collected every hour, there was a lag for equilibration between milk urea and plasma urea N of 1 h.

Table 4.13 contains rumen ammonia concentration over a 24 h period. Rumen ammonia concentration in Holsteins decreased at 0000 h and increased at 1200 h when fat was fed. However, a protein by fat interaction occurred at 0000 h when Holsteins fed added fat with 29% RUP had decreased ammonia concentration, but was not different with 41% RUP. Increased rumen ammonia after the first feeding in Holsteins fed added fat, may be due to lower concentration of NSC in these diets. However, in Chapter 3 added fat did not affect in any rumen parameter. When 41% RUP diets were fed, rumen ammonia concentration was lower overall, and in Holsteins, at all times except at 1600 h (Figure 4.9). However, when the same diets were fed to Jerseys, a lower concentration was observed only at 2000 and 0400 h. Rumen ammonia concentration, response of ammonia to added fat and response to 41% RUP varied across time. Rumen ammonia peak was observed 2 h after first feeding only, when the concentration doubled from the lowest (0800 h) to the highest point (1200

h). Peak on low RUP diets was 12.6% greater than high RUP diets. A similar increase in rumen ammonia concentration through a 24 h period and peaks after feeding were observed when soybean meal was compared to blood and corn gluten meal (Robinson and McQueen, 1994). Also, Gustafsson and Palmquist (1993) observed a clear rumen ammonia peak one hour after feeding. Decreased rumen ammonia concentration is due to increased resistance of protein feeds to microbial degradation in the rumen.

Rumen pH over a 24 h period is shown in Table 4.14. Rumen pH was not different by diet except at 1600 h when Jerseys fed 41% RUP diets had a greater pH and at 2000 h when Holsteins fed added fat had a lower pH. Contrary to this, only Holsteins fed 41% RUP diets in Chapter 3 had a greater rumen pH. Jerseys had a greater rumen pH at 0800 h compared to Holsteins. Rumen pH (Figure 4.10) and response of pH to 41% RUP diets changed across time, increasing 6 h prior to 1000 h feeding followed by a decrease 6 h after the 1000 h feeding. A similar pH reduction after feeding was observed by Robinson and McQueen (1994). This reduction may be due to increased availability of fermentable substrate after feeding. The subsequent increase in pH may be due to a reduction in substrates and a shift in fermentation to fiber digestion before next feeding.

Total VFA were not affected by diet or breed except at 0800 h when Jerseys had a lower total VFA concentration compared to Holsteins (Table 4.15). Contrary to this, Total VFA concentration decreased in cows used in Chapter 3 when

supplemented with 41% RUP. However, total VFA concentration changed across time decreasing before feeding (least at 0800 h) and increasing to nadir at 0000 h (Figure 4.11). The VFA response to feeding indicates decreased fermentable substrate before feeding followed by an increased availability after feeding.

Acetate concentration (Table 4.16) increased at 0800 and 1600 h in Holsteins fed added fat. However, concentration did not differ by breed. Concentration of acetate increased across time when 41% RUP was fed. Similar to this, Holsteins and Jerseys used in Chapter 3 increased acetate concentration when fed 41% RUP diets. However, propionate concentration (Table 4.17) was not affected by diet or breed. Contrary to this, Holsteins used in Chapter 3 had a smaller propionate concentration when fed 41% RUP diets. Butyrate concentration (Table 4.18) was not affected by diet or breed except at 1200 h when Holsteins fed added fat had a decreased concentration. However, acetate, propionate and butyrate concentrations varied across time (Figure 4.12). Acetate concentration increased from 0400 to 0800 h before feeding and decreased from 0800 to 1200 h after feeding. Propionate concentration decreased from 0400 to 0800 h and increased from 0800 to 1200 h after feeding. Butyrate concentration decreased from 0400 to 1200 h and increased from 1200 to 1600 h. Increased acetate and decreased propionate may indicate lower availability of readily fermentable carbohydrates and greater number of fiber digesting bacteria before feeding.

Across breeds, valerate concentration was less when 41% RUP diets were fed (Table 4.19). Smaller concentrations due to high RUP diet occurred in Holsteins at 0000 h and in Jerseys at 2000, 0000, 0400 and 0800 h. Also, lower valerate concentrations were observed in Chapter 3 with 41% RUP diets. Concentrations and response to 41% RUP changed across time (Figure 4.13), reaching a low at 0800, followed by a peak at 1200 h.

When Holsteins were fed 41% RUP diets a decrease in isobutyrate concentration was observed at 0000, 0400 and 0800 h (Table 4.20). There was a breed difference at 0400 and 0800 h when Jerseys had a greater isobutyrate concentration than Holsteins. When analyzed across time (Figure 4.14) isobutyrate concentrations changed, increasing from 2000 to 0800 h and decreasing at 2000 h. Reduced concentrations of butyrate and valerate with high RUP diets were also observed by Seymour et al. (1992) and may indicate decreased ruminal fermentation of AA.

Isovalerate (Table 4.21 and Figure 4.15) and acetate:propionate ratio (Table 4.22) were not affected by diet or breed. However, concentration of isovalerate and ratio of acetate and propionate changed across time. Isovalerate concentrations increased from 0800 to 1200 h and decreased from 1200 to 2000 h, and acetate:propionate increased from 0400 to 0800 h and decreased from 0800 to 1200 h (Figure 4.11). Increased ratios resulted from both increased acetate and decreased propionate concentrations before feeding.

Apparent total tract DM, organic matter and N digestibilities are shown in Table 4.23. Digestibilities for DM, OM and N were not different by diet or breed. Also, no difference was observed in total tract digestibility of OM (Zerbini et al., 1988; Waltz et al., 1989) and OM and N (Seymour et al., 1992; King et al., 1990) or DM and OM (Christensen et al., 1993; Klusmeyer et al., 1990; Klusmeyer et al., 1991a) when high RUP diets were fed. However, Christensen et al. (1993) reported a decrease in apparent total tract digestibility of N when a high RUP diet was fed. Supplemented fat has been shown to have no effect on total tract digestibility of DM (West and Hill, 1990; Jenkins and Klusmeyer et al., 1991a; Klusmeyer et al., 1991b; Jenny, 1992; Wu et al., 1993). However, some studies have reported increased total tract digestibility of N when fat is supplemented (Palmquist and Conrad, 1978; Jenkins and Jenny, 1992; Palmquist et al., 1993).

Microbial N (% of total N) reaching the duodenum was not different by diet or breed (Table 4.23). Contrary to this, Zerbini (1984) reported reduced microbial N reaching the abomasum of calves fed corn gluten meal or feather meal compared to a soybean meal diet. The N.R.C. (1985) reported microbial growth is not limited until rumen ammonia concentrations fall below 3 to 5 mg/dL. Lack of change in microbial N reaching the duodenum when 41% RUP diets were fed may be due to adequate ammonia for microbial synthesis, because throughout the 24 h period rumen ammonia was above 5 mg/dL.

Correlation coefficients (between cows) of rumen ammonia, plasma and milk urea N are shown in Table 4.24. Rumen ammonia was correlated equal or greater to ($r = .4$) with plasma urea N. Plasma and milk urea N were highly correlated at all points except 0800 and 1200 h.

Table 4.25 contains DM fractions, degradation rates of DM, and rumen degradabilities of DM of feeds using the direct method. There were no differences between breeds in degradation rates of DM, except for alfalfa silage, where fraction A in Holsteins was greater than Jerseys and fraction C in Jerseys was greater than Holsteins. Soybean meal DM was the most degradable (82.3%). Similar values were reported by Armentano et al. (1987) (82%) and Stallings et al. (1991) (78.1%), although Cummins et al. (1983) reported a value of 56.7%. Corn silage DM degradability was 64.4%. Ground corn DM degradability was 61.1%, greater than the degradability reported by Herrera-Saldana et al. (1986) of 54.4% but lower than degradability reported by Stallings et al. (1991) of 78.8%. Alfalfa silage DM degradability was 59.1%. Corn gluten meal DM degradability was 20.7%, similar to that reported by Murphy and Kennelly (1986) of 18.9%, although Cozzi et al. (1993) and Cummins et al. (1983) reported 27.9 and 44.0%, respectively. Blood meal DM degradability was the lowest at 3.6%.

Crude protein fractions, degradation rates of CP, and rumen degradabilities of CP of feeds using the direct method are shown in Table 4.26. Corn and alfalfa silage were analyzed for cytosine to correct for microbial contamination. However, no

cytosine was found at any time in any of the forage samples analyzed. There were no differences between breeds in degradation rate of CP, CP degradability, or CP fractions except for alfalfa silage, where fraction A in Holsteins was greater than Jerseys. Soybean meal CP was degraded at 84.2%, and similar results were reported by Armentano et al. (1987), however, lower values were reported by Kirkpatrick and Kennelly (1986). Alfalfa silage CP degradability was 79.0%, similar to degradability reported by Stallings et al. (1988), but higher than that reported by Erdman et al. (1987). Corn silage CP degradability was 70.3%, similar to degradability reported by Erdman et al. (1987), but lower than degradabilities reported by Stallings et al. (1988) (82.2%) and Cronje (1983) (84.4%). Ground corn CP degradability was 58.1%, similar to Erasmus et al. (1994), however, greater values were reported by Erdman et al. (1987), Herrera-Saldana et al. (1990) and Stallings et al. (1991). Corn gluten meal CP degradability was 13.6%, similar to that reported by Murphy and Kennelly (1987) while Cozzi et al. (1993) and Cummins et al. (1983) reported greater degradabilities of 20.7 and 50.8%, respectively. Blood meal CP degradability was 6.8%, although greater values were observed by Herrera-Saldana (1990), Palmquist et al. (1993) and Erasmus et al. (1994) of 13.1, 14.9, and 19.2%, respectively.

Table 4.27 contains DM fractions, degradation rates of DM, and rumen degradabilities of DM of feeds using the indirect method. There were no differences between breeds in DM degradation rates or degradability. Alfalfa silage fraction C was greatest in Jerseys, whereas, corn silage fraction A was greatest in Holsteins.

Also, ground corn fraction A was greatest in Holsteins. Blood meal fraction A was negative because this method overestimates fraction B in blood meal due to the lag from time 0 to 2 h of incubation. Soybean meal DM was degraded at 76.6%, followed by corn silage at 64.2%, ground corn at 60.6%, alfalfa silage at 58.2%, corn gluten meal at 22.4% and blood meal at 1.9%.

Crude protein fractions, degradation rates of CP, and rumen degradabilities of CP of feeds using the indirect method are shown in Table 4.28. There were no differences between breeds in CP degradation rates or degradability, and fractions B and C. Fraction A in alfalfa silage and ground corn was greatest in Holsteins. Fraction A in soybean and blood meal were negative because fraction B was overestimated by rapid CP degradation and is demonstrated by the steep slope in soybean meal and a delay in lag time from 0 to 2 h of incubation in blood meal. Alfalfa silage CP was the most degradable at 77.2%, followed by soybean meal (74.0%), corn silage (71.3%), ground corn (60.4%), corn gluten meal (13.8%) and blood meal (4.9%).

Dry matter and CP degradabilities were very similar between methods except for soybean and blood meals. The indirect method estimated fraction A by subtracting 100 from the intercept. Very steep slopes (fast rate of degradation) or lag times (no degradation between times) for some feedstuffs like soybean and blood meal, respectively, would give erroneous results. Diets were originally formulated to have 29% and 41% RUP. However, when diets were recalculated with in situ results from

the direct method, the RUP level was 23.3% and 40.8%. The lower value for the 29% RUP was mainly due to greater CP degradability for soybean meal (84.2% versus 70%) and ground corn (58.1% versus 35%) with in situ compared to Dair4, respectively. The 41% RUP calculations were not much different because CP degradability for blood meal was lower (6.8% versus 18%) using in situ method compared to Dair4.

Summary and Conclusions

Many factors can change ruminal fermentation. However, one important factor determining microbial protein yield is availability of N (peptides, amino acids and ammonia) in the rumen. Although in this study microbial N (% of total N) in the abomasum was not affected by diet, lower ruminal ammonia, valerate and isobutyrate concentrations may indicate increased resistance of protein to ruminal degradation when 41% RUP diets were fed. Regardless of the diet fed, some diurnal variation in ruminal fermentation can be expected due to the way in which rumen microbe populations reproduce, grow and pass to the small intestine. A major factor influencing bacteria growth in the rumen besides the diet, is availability of fermentable substrate (amount and time of feeding). This suggestion is supported by the fact that before feeding, ruminal ammonia, total VFA, propionate and valerate concentrations were lowest, and ruminal pH and acetate concentration were highest. However after feeding, concentrations of ruminal ammonia, total VFA, propionate, valerate, and isovalerate increased and acetate and pH decreased.

Differences in ruminal fermentation, especially ammonia concentration, due to level of RUP did not affect plasma urea N or milk urea N. The major factor affecting rumen ammonia, plasma urea N, and milk urea N was time relative to feeding. Increased concentrations of plasma and milk urea N after feeding may be partially due

to increased ruminal ammonia. However, plasma and milk urea N concentrations were most likely affected by other factors such as use of amino acids for gluconeogenesis.

Substrates such as acetate, glucose, β-hydroxy-butyrate and preformed fatty acids are used for milk fat synthesis, and glucose is used for lactose synthesis. Though, milk protein arises from preformed plasma proteins, the major source is synthesis from free amino acids in the secretory cells of the mammary gland. Milk components, especially protein, casein and lactose, changed throughout the day. Diurnal variation of milk N components may indicate variable supply of amino acids related to feeding times.

Table 4.1. Dietary ingredients and chemical composition of diets.

Item	Treatments			
	29% RUP	29% RUP+F	41% RUP	41% RUP+F
Ingredients, % DM				
Corn Silage	30.0	30.0	30.0	30.0
Alfalfa Silage	29.0	29.0	29.0	29.0
Corn grain	25.7	21.7	29.9	25.9
Soybean Meal	13.1	13.1	5.5	5.5
Blood Meal			3.4	3.4
Corn Gluten Meal		0.6		0.6
Fat		3.4		3.4
Mineral/vitamin	2.2	2.2	2.2	2.2
Chemical Composition				
CP, % of DM	16.1	16.2	16.2	16.3
NE_L, Mcal/kg	1.57	1.68	1.59	1.68
ADF, % of DM	19.3	19.3	18.7	18.7
NDF, % of DM	29.6	29.3	29.0	28.6
NFC, % of DM	44.3	41.6	45.4	42.8
RUP, % of CP	29.0	29.1	40.7	40.6
EE, % of DM	2.5	5.2	2.5	5.2
Ca, % of DM	.98	1.28	.97	1.27
P, % of DM	.56	.55	.53	.52

RUP = Rumen undegraded protein
F = Fat as Ca soaps of fatty acids
NFC = Non fiber carbohydrates
EE = ether extract
[1] Contained: 18% $NaHCO_3$; 16% Ca; 6.5% P; 3.5% K; 2.2% Mg; 3.2% S; 5.8% Cl; .027% Fe; .013% Cu; .0003% Co; .11% Mn; .13% Zn; .002% I; .0005% Se; and 110,000 IU of vitamin A/kg; 44,000 IU of vitamin D/kg, and 550 IU of vitamin E/kg.

Table 4.2. Daily dry matter intake, milk production and efficiency of milk production in response to dietary fat (F) and rumen undegradable protein (RUP).

		Treatments					Contrasts (P <.05)			
	B[1]	29% RUP	29% RUP+F	41% RUP	41% RUP+F	SEM	FAT	RUP	INT	BC[2]
DMI, kg/d	H	19.1	18.4	20.5	18.1	.3	.01	NS	.02	NS
	J	16.4	15.2	16.3	15.7		.02	NS	NS	
DMI, % BW[3]	H	3.62	3.45	3.80	3.41	.08	.01	NS	NS	NS
	J	3.99	3.77	4.03	3.88		.03	NS	NS	
Milk Yield,	H	29.2	31.4	32.7	32.8	.7	NS	.01	NS	.05
kg/d	J	22.6	21.7	22.8	23.8		NS	NS	NS	
4% FCM[4],	H	27.8	31.2	32.0	31.5	1.0	NS	.01	NS	NS
kg/d	J	27.0	26.4	27.2	28.6		NS	NS	NS	
FCM/Mcal NE_L,	H	.93	1.01	.99	1.03	.06	.06	NS	NS	NS
kg	J	1.06	1.04	1.05	1.09		NS	NS	NS	

INT = Protein x fat interaction
[1] B = Breeds, H = Holstein, J = Jersey
[2] BC = Breed contrast
[3] Dry matter intake as a percent of body weight
[4] 4% fat corrected milk

Table 4.3. Milk component yields in response to dietary fat (F) and rumen undegradable protein (RUP).

		Treatments					Contrasts (P <.05)			
	B[1]	29% RUP	29% RUP+F	41% RUP	41% RUP+F	SEM	FAT	RUP	INT	BC[2]
		---------- kg/d ----------								
Fat	H	1.07	1.24	1.26	1.22	.05	NS	NS	NS	NS
	J	1.19	1.18	1.20	1.27		NS	NS	NS	
Protein	H	.86	.87	.99	.94	.03	NS	.01	NS	NS
	J	.84	.78	.80	.81		NS	NS	NS	
Lactose	H	1.51	1.63	1.69	1.69	.04	NS	.01	NS	.05
	J	1.13	1.09	1.15	1.20		NS	NS	NS	
SNF	H	2.64	2.78	2.91	2.87	.07	NS	.02	NS	NS
	J	2.14	2.04	2.13	2.21		NS	NS	NS	

INT = Protein x fat interaction
SNF = Solids non-fat
[1] B = Breeds, H = Holstein, J = Jersey;
[2] BC = Breed contrast

Table 4.4. Milk component content in response to dietary fat (F) and rumen undegradable protein (RUP).

		Treatments					Contrasts (P <.05)			
	B[1]	29% RUP	29% RUP+F	41% RUP	41% RUP+F	SEM	FAT	RUP	INT	BC[2]
		------- g/100 g milk ------								
Fat	H	3.69	3.96	3.89	3.69	.16	NS	NS	NS	.01
	J	5.30	5.51	5.32	5.38		NS	NS	NS	
Protein	H	2.98	2.75	3.05	2.90	.10	NS	NS	NS	.05
	J	3.72	3.65	3.56	3.45		NS	NS	NS	
Lactose	H	5.15	5.16	5.16	5.11	.04	NS	NS	NS	NS
	J	4.97	4.99	5.04	5.05		NS	NS	NS	
SNF	H	9.01	8.80	8.91	8.75	.05	.01	NS	NS	.05
	J	9.44	9.44	9.35	9.34		NS	.06	NS	

INT = Protein x fat interaction
SNF = Solids non-fat
[1] B = Breeds, H = Holstein, J = Jersey;
[2] BC = Breed contrast

Table 4.5. Nitrogen fractions of milk in response to dietary fat (F) and rumen undegradable protein (RUP).

		Treatments					Contrasts (P <.05)			
	B[1]	29% RUP	29% RUP+F	41% RUP	41% RUP+F	SEM	FAT	RUP	INT	BC[2]
		----- g/100 g milk -------								
Total	H	.464	.429	.478	.458	.016	NS	NS	NS	.05
	J	.582	.571	.556	.542		NS	NS	NS	
Casein	H	.347	.337	.358	.343	.011	NS	NS	NS	.01
	J	.457	.442	.438	.428		NS	NS	NS	
Urea[3]	H	.0248	.0226	.0240	.0230	.0006	.03	NS	NS	NS
	J	.0216	.0208	.0210	.0214		NS	NS	NS	
		----- g/100 g total N ---								
Casein	H	74.9	78.3	75.1	75.5	1.6	NS	NS	NS	NS
	J	78.5	77.8	78.9	78.9		NS	NS	NS	
Urea	H	5.42	5.60	5.25	4.86	.34	NS	NS	NS	.05
	J	3.57	3.66	3.78	3.97		NS	NS	NS	

INT = Protein x fat interaction
[1] B = Breeds, H = Holstein, J = Jersey;
[2] BC = Breed contrast
[3] Average of 24 h

Table 4.6. Milk yield in response to dietary fat (F) and rumen undegradable protein (RUP) over a 24 h period in cows fed at 1000 and 1400 h.

Time	B[1]	Treatments				SEM	Contrasts (P <.05)			
		29% RUP	29% RUP+F	41% RUP	41% RUP+F		FAT	RUP	INT	BC[2]
		------- kg/milking --------								
Yield 2000	H	4.41	5.61	5.50	4.94	.53	NS	NS	NS	NS
	J	5.08	4.68	4.67	5.00		NS	NS	NS	
Yield 0000	H	5.17	4.19	5.23	5.27	.40	NS	NS	NS	NS
	J	3.33	3.75	4.26	4.09		NS	NS	NS	
Yield 0400	H	5.20	6.58	5.78	5.91	.24	.01	NS	NS	NS
	J	4.58	3.87	3.64	4.06		NS	NS	NS	
Yield 0800	H	5.08	4.42	5.89	4.88	.40	.06	NS	NS	NS
	J	3.05	3.04	3.54	3.91		NS	NS	NS	
Yield 1200	H	5.08	6.09	5.64	6.01	.35	.07	NS	NS	NS
	J	4.42	4.56	4.33	4.18		NS	NS	NS	
Yield 1600	H	4.57	5.30	5.18	5.31	.24	NS	NS	NS	NS
	J	3.75	2.86	3.89	4.13		NS	.02	NS	
Yield 2000	H	4.12	4.87	4.96	5.46	.21	.01	.01	.04	NS
	J	3.50	3.61	3.12	3.35		NS	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

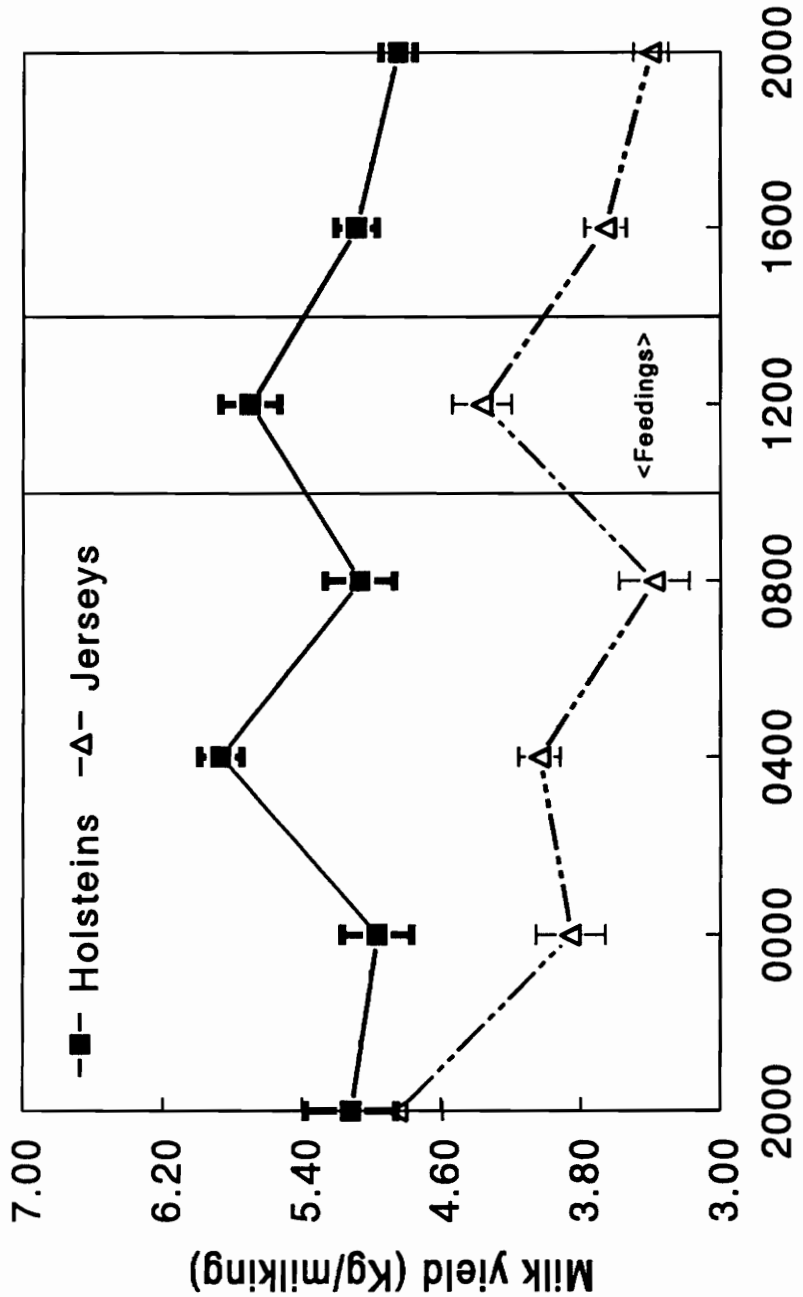

Figure 4.2. Effect of time on milk yield, breeds did not differ

Table 4.7. Milk fat content in response to dietary fat (F) and rumen undegradable protein (RUP) over a 24 h period in cows fed at 1000 and 1400 h.

Time	B[1]	Treatments				SEM	Contrasts (P <.05)			
		29% RUP	29% RUP+F	41% RUP	41% RUP+F		FAT	RUP	INT	BC[2]
		---------- % ----------								
FAT 2000	H	4.11	4.71	4.90	3.91	.51	NS	NS	NS	.01
	J	6.44	6.93	6.00	6.45		NS	NS	NS	
FAT 0000	H	3.75	3.70	3.63	3.72	.42	NS	NS	NS	.01
	J	5.67	5.57	6.15	5.54		NS	NS	NS	
FAT 0400	H	3.78	3.82	3.83	3.73	.20	NS	NS	NS	.01
	J	5.11	5.40	5.02	5.02		NS	NS	NS	
FAT 0800	H	3.72	3.68	3.91	3.39	.25	NS	NS	NS	.01
	J	4.69	4.93	5.02	5.04		NS	NS	NS	
FAT 1200	H	3.43	4.09	3.66	3.60	.23	NS	NS	NS	.05
	J	4.36	5.19	4.82	4.73		NS	NS	NS	
FAT 1600	H	3.43	3.73	3.61	3.41	.20	NS	NS	NS	.01
	J	5.15	4.68	5.10	5.26		NS	NS	NS	
FAT 2000	H	3.35	3.53	3.55	3.86	.23	NS	NS	NS	.05
	J	4.98	4.95	4.58	5.16		NS	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

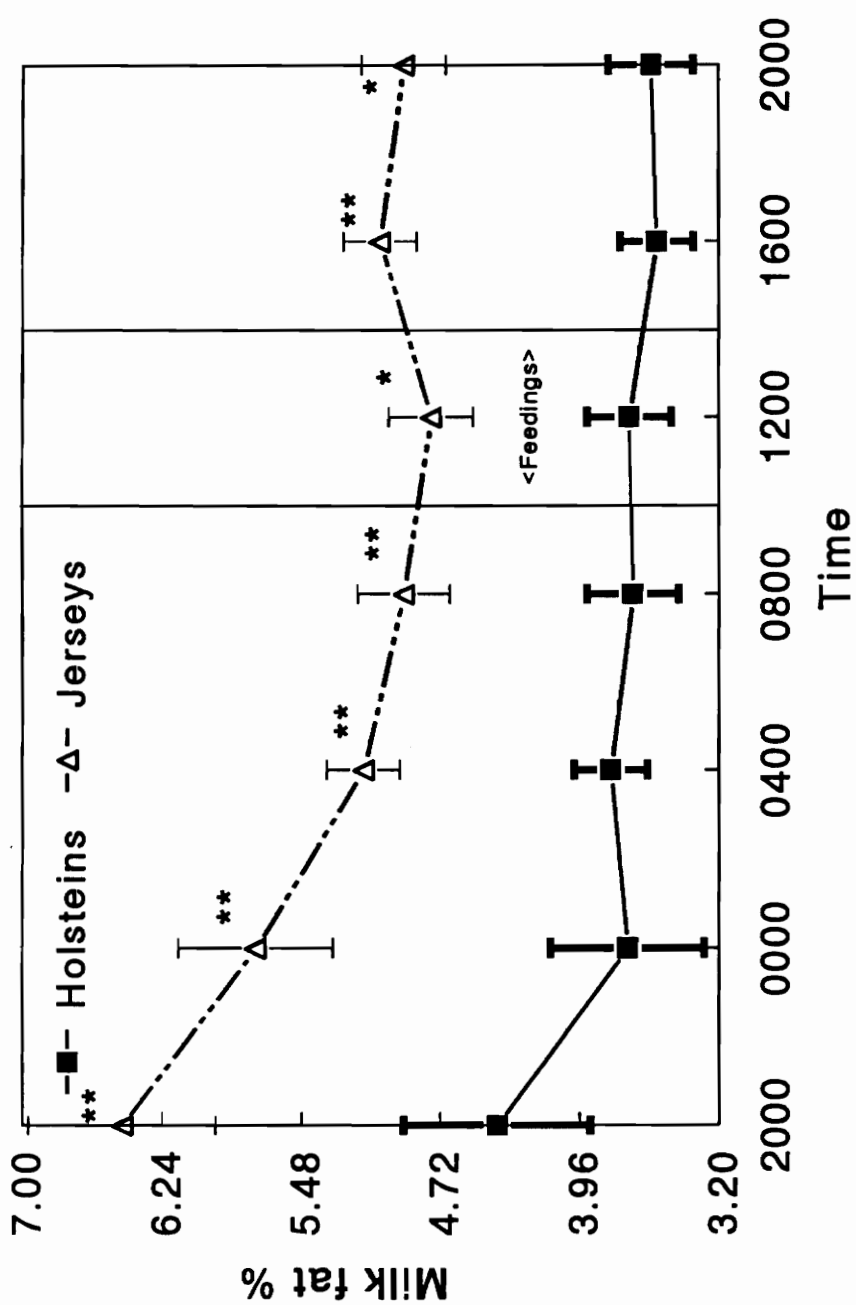

Figure 4.3. Effect of time on milk fat content, breeds differ **(P<.01), *(P<.05)

Table 4.8. Milk protein content in response to dietary fat (F) and rumen undegradable protein (RUP) over a 24 h period in cows fed at 1000 and 1400 h.

Time	B[1]	Treatments				SEM	Contrasts (P <.07)			
		29% RUP	29% RUP+F	41% RUP	41% RUP+F		FAT	RUP	INT	BC[2]
		---------- % ----------								
PROT 2000	H	3.27	3.05	2.93	2.97	.08	NS	.03	NS	.05
	J	3.84	3.73	3.72	3.55		NS	NS	NS	
PROT 0000	H	3.15	2.89	2.99	2.83	.07	.02	NS	NS	.05
	J	3.80	3.75	3.68	3.61		NS	NS	NS	
PROT 0400	H	3.07	2.82	2.96	2.86	.08	NS	NS	NS	.05
	J	3.83	3.71	3.62	3.54		NS	.05	NS	
PROT 0800	H	3.05	2.89	2.98	2.90	.08	NS	NS	NS	.05
	J	3.68	3.63	3.54	3.54		NS	NS	NS	
PROT 1200	H	2.99	2.86	2.94	2.88	.08	NS	NS	NS	.05
	J	3.68	3.60	3.50	3.40		NS	.05	NS	
PROT 1600	H	3.16	2.89	3.18	2.98	.09	.03	NS	NS	.05
	J	3.72	3.70	3.63	3.58		NS	NS	NS	
PROT 2000	H	3.14	2.83	3.07	2.84	.10	.02	NS	NS	.05
	J	3.88	3.73	3.69	3.55		NS	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

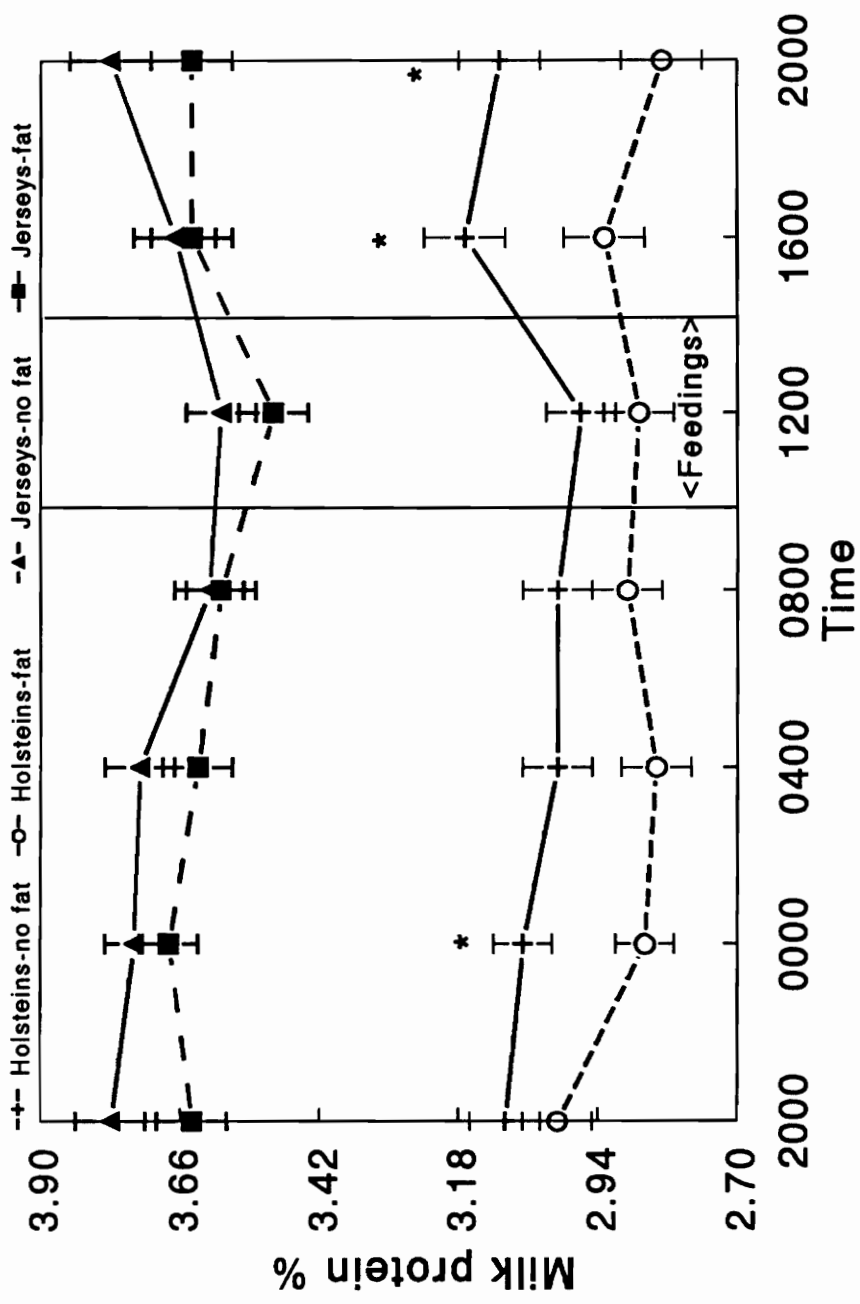

Figure 4.4. Effect of added fat on milk protein content, fat differs *(P<.05)

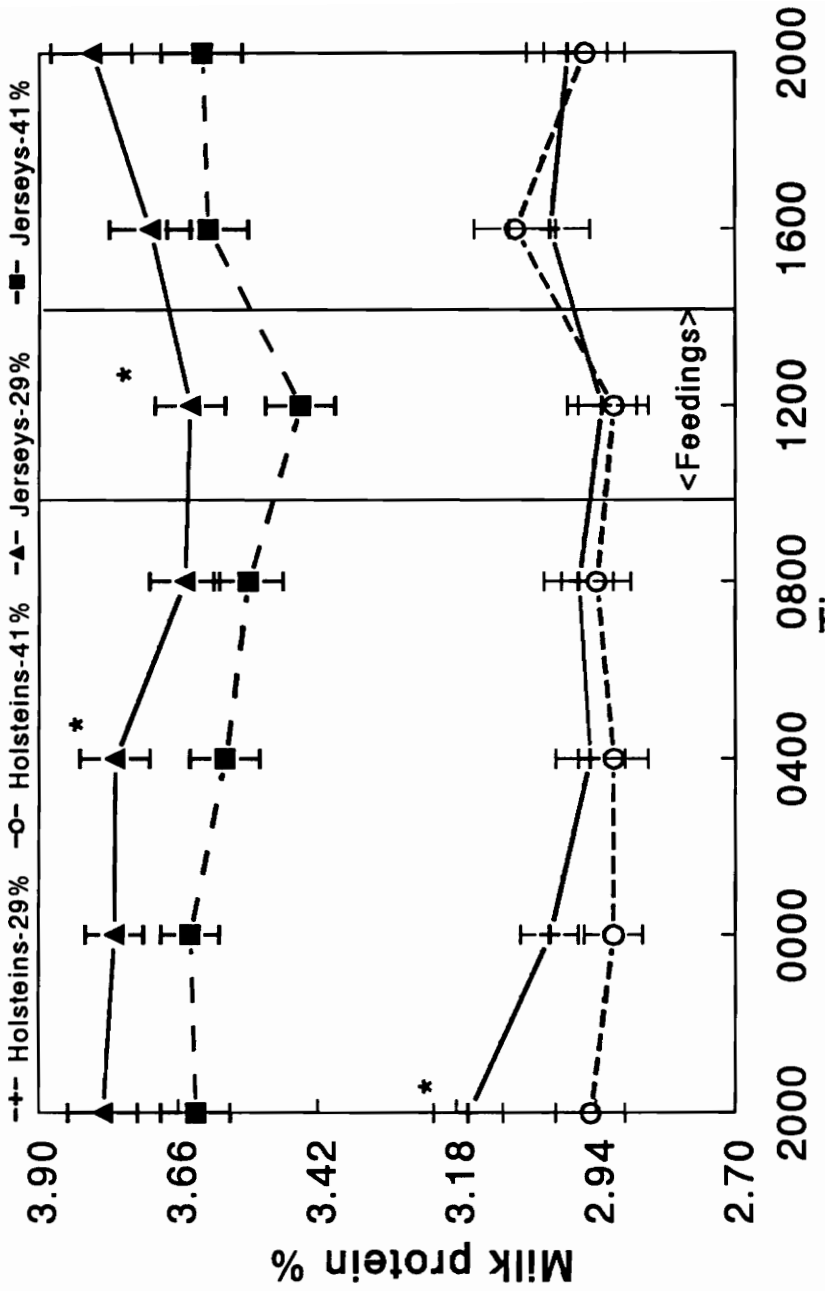

Figure 4.5. Effect of RUP on milk protein content, RUP differs *(P<.05).

Table 4.9. Milk casein N content in response to dietary fat (F) and rumen undegradable protein (RUP) over a 24 h period in cows fed at 1000 and 1400 h.

Time	B[1]	Treatments				SEM	Contrasts (P <.07)			
		29% RUP	29% RUP+F	41% RUP	41% RUP+F		FAT	RUP	INT	BC[2]
		---------- % ----------								
CASEIN 2000	H	.380	.347	.348	.350	.010	NS	NS	.06	.05
	J	.462	.453	.452	.423		NS	NS	NS	
CASEIN 0000	H	.375	.338	.362	.333	.011	NS	NS	NS	.01
	J	.464	.454	.445	.440		NS	NS	NS	
CASEIN 0400	H	.366	.336	.358	.343	.009	.05	NS	NS	.01
	J	.470	.452	.444	.433		NS	NS	NS	
CASEIN 0800	H	.369	.341	.354	.342	.010	NS	NS	NS	.01
	J	.457	.450	.432	.426		NS	NS	NS	
CASEIN 1200	H	.359	.340	.350	.345	.010	NS	.05	NS	.01
	J	.454	.438	.426	.418		NS	NS	NS	
CASEIN 1600	H	.275	.330	.365	.343	.039	NS	NS	NS	.05
	J	.433	.410	.437	.423		NS	NS	NS	
CASEIN 2000	H	.337	.330	.357	.356	.018	NS	NS	NS	.01
	J	.463	.444	.445	.424		NS	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

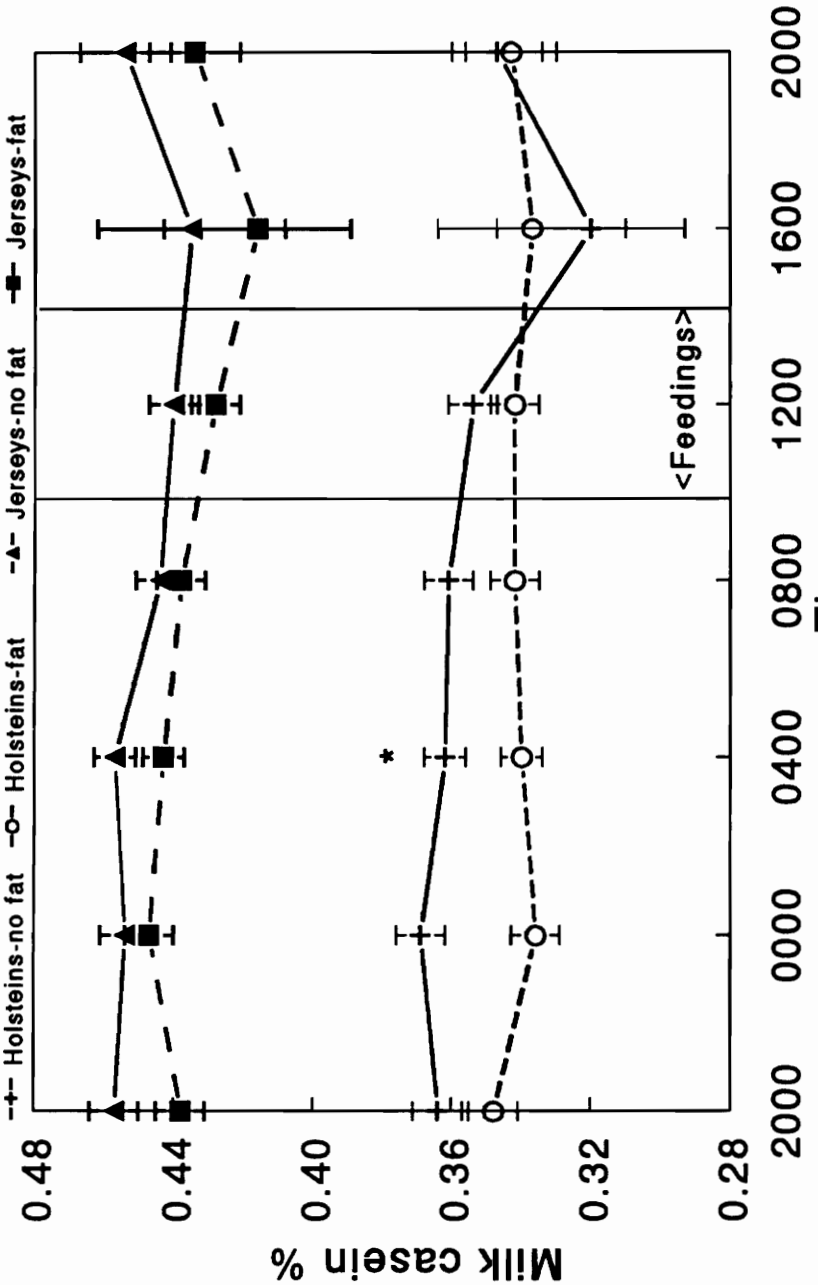

Figure 4.6. Effect of added fat on casein N content, fat differs *(P<.05).

Table 4.10. Milk lactose content in response to dietary fat (F) and rumen undegradable protein (RUP) over a 24 h period in cows fed at 1000 and 1400 h.

Time	B[1]	Treatments				SEM	Contrasts (P <.05)			
		29% RUP	29% RUP+F	41% RUP	41% RUP+F		FAT	RUP	INT	BC[2]
		---------- % ----------								
LACT 2000	H	5.11	5.08	5.11	5.10	.05	NS	.04	NS	NS
	J	4.91	4.89	5.02	5.00		NS	NS	NS	
LACT 0000	H	5.09	5.07	5.13	5.06	.06	NS	NS	NS	NS
	J	4.82	4.84	4.92	4.97		NS	NS	NS	
LACT 0400	H	5.11	5.12	5.10	5.04	.06	NS	NS	NS	NS
	J	4.98	4.94	5.05	5.07		NS	NS	NS	
LACT 0800	H	5.18	5.14	5.16	5.14	.05	NS	NS	NS	NS
	J	4.99	5.08	5.05	5.11		NS	NS	NS	
LACT 1200	H	5.24	5.25	5.22	5.19	.04	NS	NS	NS	NS
	J	5.08	5.09	5.10	5.12		NS	NS	NS	
LACT 1600	H	5.15	5.20	5.12	5.12	.05	NS	NS	NS	NS
	J	5.02	5.05	5.06	5.06		NS	NS	NS	
LACT 2000	H	5.10	5.20	5.16	5.11	.05	NS	NS	NS	NS
	J	4.96	5.01	5.06	5.03		NS	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

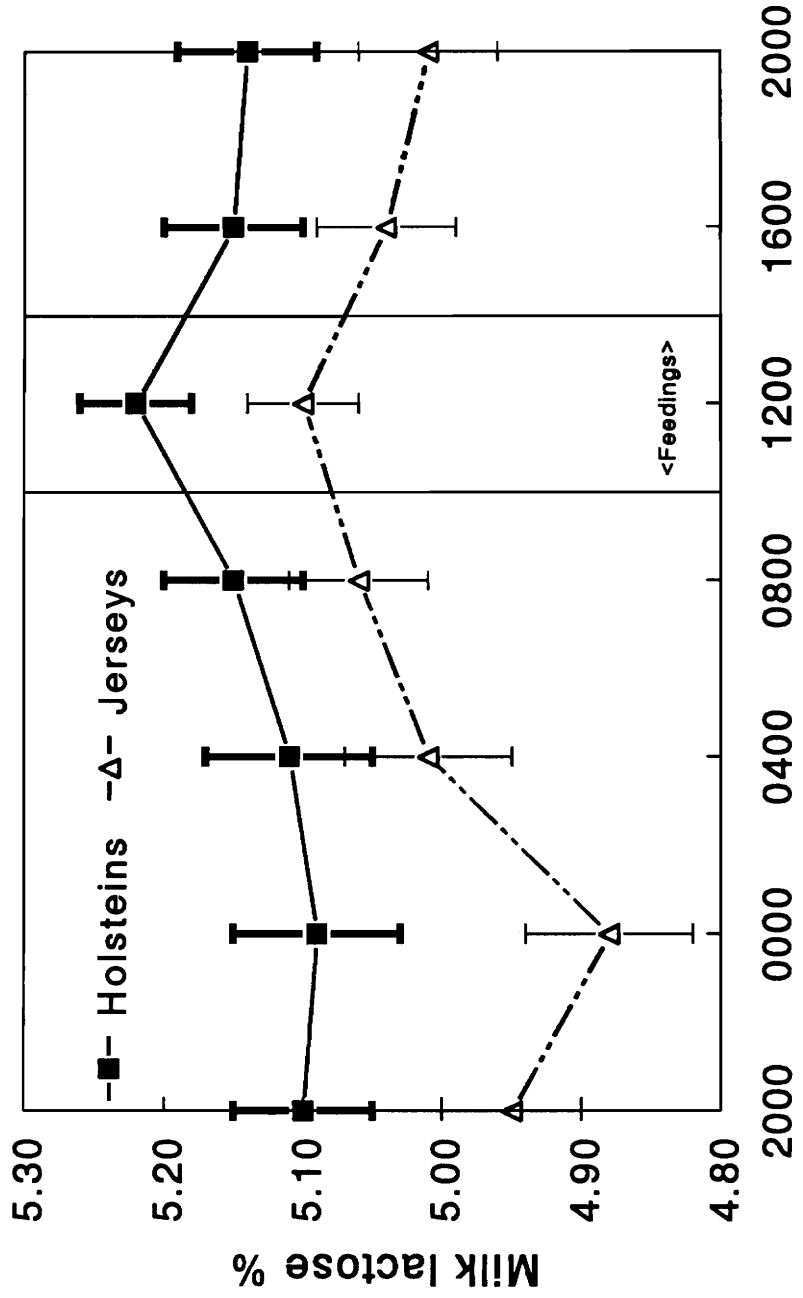

Figure 4.7. Effect of time on milk lactose content, breeds did not differ.

Table 4.11. Plasma urea N (PUN) in response to dietary fat (F) and rumen undegradable protein (RUP) over a 24 h period in cows fed at 1000 and 1400 h.

Time	B[1]	Treatments				SEM	Contrasts (P <.05)			
		29% RUP	29% RUP+F	41% RUP	41% RUP+F		FAT	RUP	INT	BC[2]
		--------- mg/dL ----------								
PUN 2000	H	15.8	14.5	14.8	13.7	1.2	NS	NS	NS	NS
	J	16.0	15.3	12.3	14.8		NS	NS	NS	
PUN 0000	H	15.3	14.1	16.0	13.0	1.1	NS	NS	NS	NS
	J	13.9	14.8	12.7	15.0		NS	NS	NS	
PUN 0400	H	16.4	14.7	15.4	14.4	.9	NS	NS	NS	NS
	J	15.8	16.2	15.0	16.5		NS	NS	NS	
PUN 0800	H	15.6	15.3	14.0	14.3	.7	NS	NS	NS	NS
	J	14.7	15.2	14.5	16.1		NS	NS	NS	
PUN 1200	H	16.7	17.5	16.3	15.7	.9	NS	NS	NS	NS
	J	18.1	18.7	17.7	19.1		NS	NS	NS	
PUN 1600	H	17.0	18.5	16.7	16.3	1.0	NS	NS	NS	NS
	J	18.0	19.0	18.2	17.8		NS	NS	NS	
PUN 2000	H	16.7	15.7	16.0	15.1	.9	NS	NS	NS	NS
	J	15.2	16.0	14.5	15.5		NS	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] B = Breed contrast

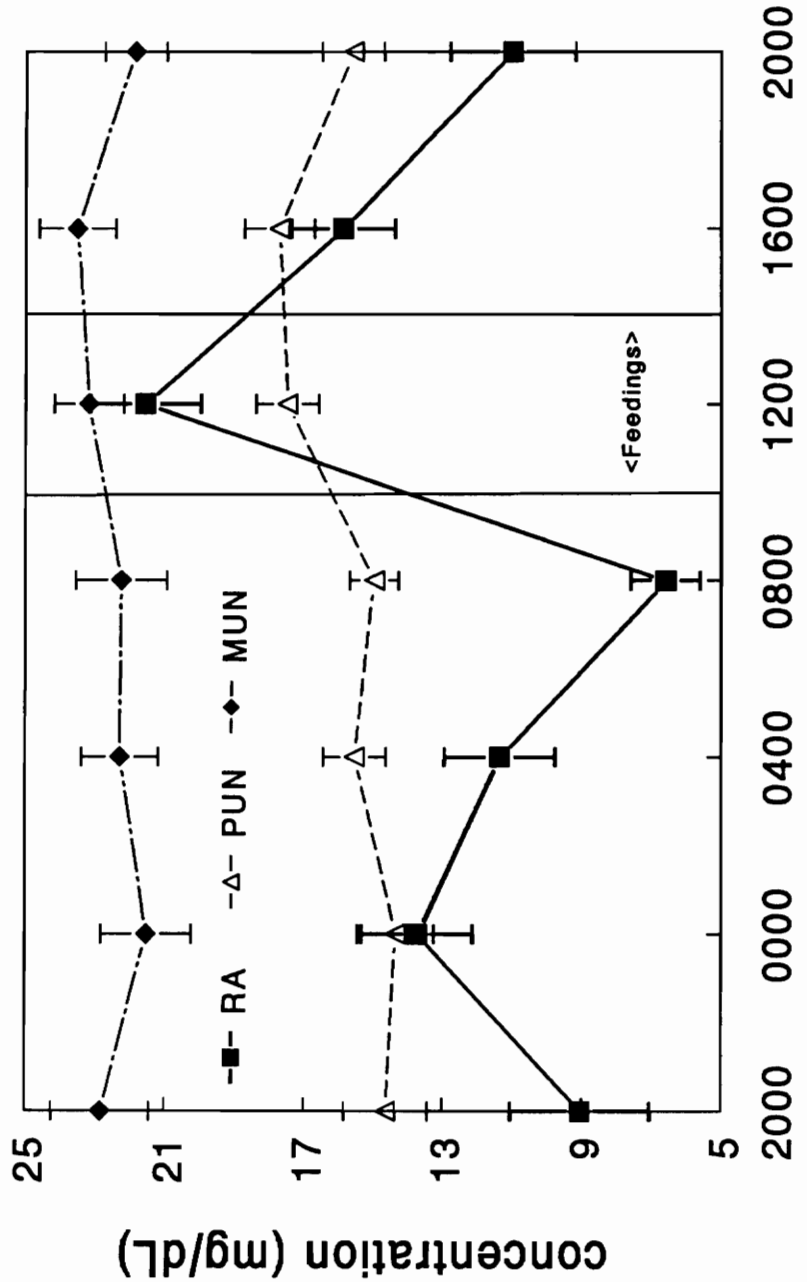

Figure 4.8. Effect of time on rumen ammonia, PUN and MUN

Table 4.12. Milk urea N (MUN) in response to dietary fat (F) and rumen undegradable protein (RUP) over a 24 h period in cows fed at 1000 and 1400 h.

Time	B[1]	Treatments				SEM	Contrasts (P <.05)			
		29% RUP	29% RUP+F	41% RUP	41% RUP+F		FAT	RUP	INT	BC[2]
		---------- mg/dL ---------								
MUN 2000	H	24.2	25.3	22.6	21.8	1.4	NS	NS	NS	NS
	J	24.2	22.7	20.4	21.4		NS	NS	NS	
MUN 0000	H	23.8	21.8	22.5	21.6	1.3	NS	NS	NS	NS
	J	21.0	20.1	19.8	21.8		NS	NS	NS	
MUN 0400	H	24.2	22.3	23.3	24.4	1.1	NS	NS	NS	NS
	J	21.7	20.7	20.7	21.0		NS	NS	NS	
MUN 0800	H	24.8	21.1	24.6	25.1	1.3	NS	NS	NS	.05
	J	21.3	19.2	21.4	20.4		NS	NS	NS	
MUN 1200	H	26.1	23.9	24.7	22.4	1.0	.05	NS	NS	.05
	J	21.8	21.2	22.2	23.2		NS	NS	NS	
MUN 1600	H	26.3	24.0	25.1	23.2	1.1	NS	NS	NS	NS
	J	22.6	22.9	21.5	22.6		NS	NS	NS	
MUN 2000	H	23.7	22.5	24.2	21.8	.9	.06	NS	NS	NS
	J	21.3	20.9	20.6	19.9		NS	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

Table 4.13. Rumen ammonia (RAN) in response to dietary fat (F) and rumen undegradable protein (RUP) over a 24 h period in cows fed at 1000 and 1400 h.

Time	B[1]	Treatments				SEM	Contrasts (P <.05)			
		29% RUP	29% RUP+F	41% RUP	41% RUP+F		FAT	RUP	INT	BC[2]
		---------- mg/dL ---------								
RAN 2000	H	15.5	12.1	10.4	7.6	2.0	NS	.03	NS	.01
	J	12.1	7.1	3.5	4.0		NS	.01	NS	
RAN 0000	H	20.9	12.5	11.2	10.9	1.6	.01	.01	.03	NS
	J	13.3	14.2	10.7	12.0		NS	NS	NS	
RAN 0400	H	16.3	14.8	7.9	9.2	1.6	NS	.01	NS	NS
	J	12.3	13.1	8.4	8.5		NS	.02	NS	
RAN 0800	H	9.4	9.8	5.6	4.7	1.0	NS	.01	NS	NS
	J	5.8	6.9	4.9	5.3		NS	NS	NS	
RAN 1200	H	20.1	27.0	18.2	22.4	1.6	.01	.07	NS	NS
	J	19.8	24.9	20.0	19.7		NS	NS	NS	
RAN 1600	H	17.7	12.9	14.4	13.5	1.5	NS	NS	NS	NS
	J	14.3	18.9	16.7	18.3		NS	NS	NS	
RAN 2000	H	13.4	14.5	10.0	10.4	1.8	NS	.06	NS	NS
	J	11.5	11.3	6.5	9.9		NS	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

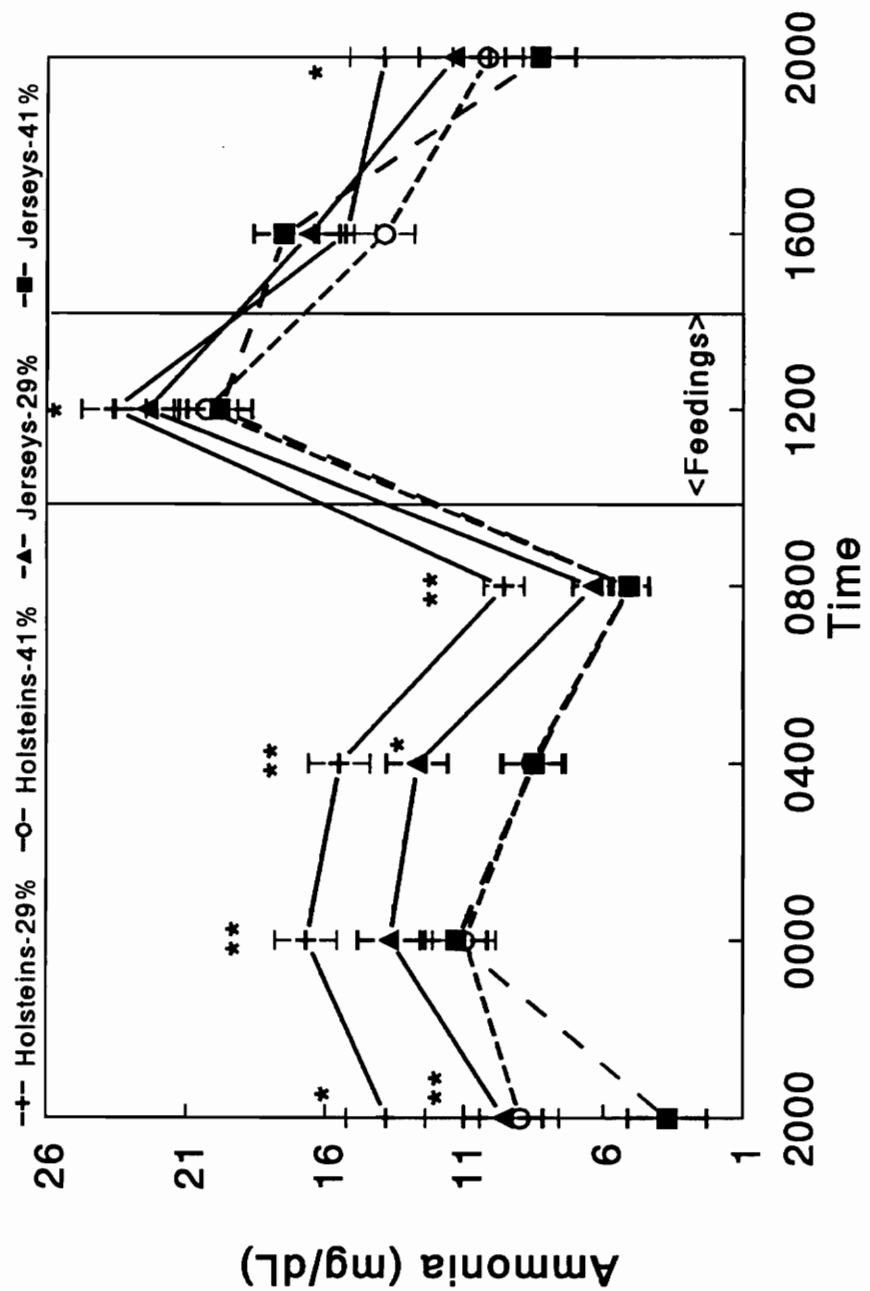

Figure 4.9. Effect of RUP on ruminal ammonia, RUP differs **(P<.01),*(P<.07)

Table 4.14. Rumen pH in response to dietary fat (F) and rumen undegradable protein (RUP) over a 24 h period in cows fed at 1000 and 1400 h.

Time	B[1]	Treatments				SEM	Contrasts (P <.05)			
		29% RUP	29% RUP+F	41% RUP	41% RUP+F		FAT	RUP	INT	BC[2]
pH 2000	H	5.54	6.13	6.16	6.13	.18	NS	NS	NS	NS
	J	6.03	6.22	6.23	6.28		NS	NS	NS	
pH 0000	H	5.97	6.23	6.09	6.13	.09	NS	NS	NS	NS
	J	6.22	6.28	6.23	6.28		NS	NS	NS	
pH 0400	H	6.26	6.41	6.21	6.28	.11	NS	NS	NS	NS
	J	6.49	6.42	6.41	6.36		NS	NS	NS	
pH 0800	H	6.50	6.67	6.51	6.49	.07	NS	NS	NS	.05
	J	6.90	6.80	6.90	6.79		NS	NS	NS	
pH 1200	H	6.32	6.41	6.39	6.33	.07	NS	NS	NS	NS
	J	6.56	6.56	6.55	6.57		NS	NS	NS	
pH 1600	H	6.07	6.18	6.18	6.14	.07	NS	NS	NS	NS
	J	6.26	6.12	6.31	6.36		NS	.05	NS	
pH 2000	H	6.15	6.26	6.05	6.25	.07	.05	NS	NS	NS
	J	6.17	6.18	6.19	6.22		NS	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

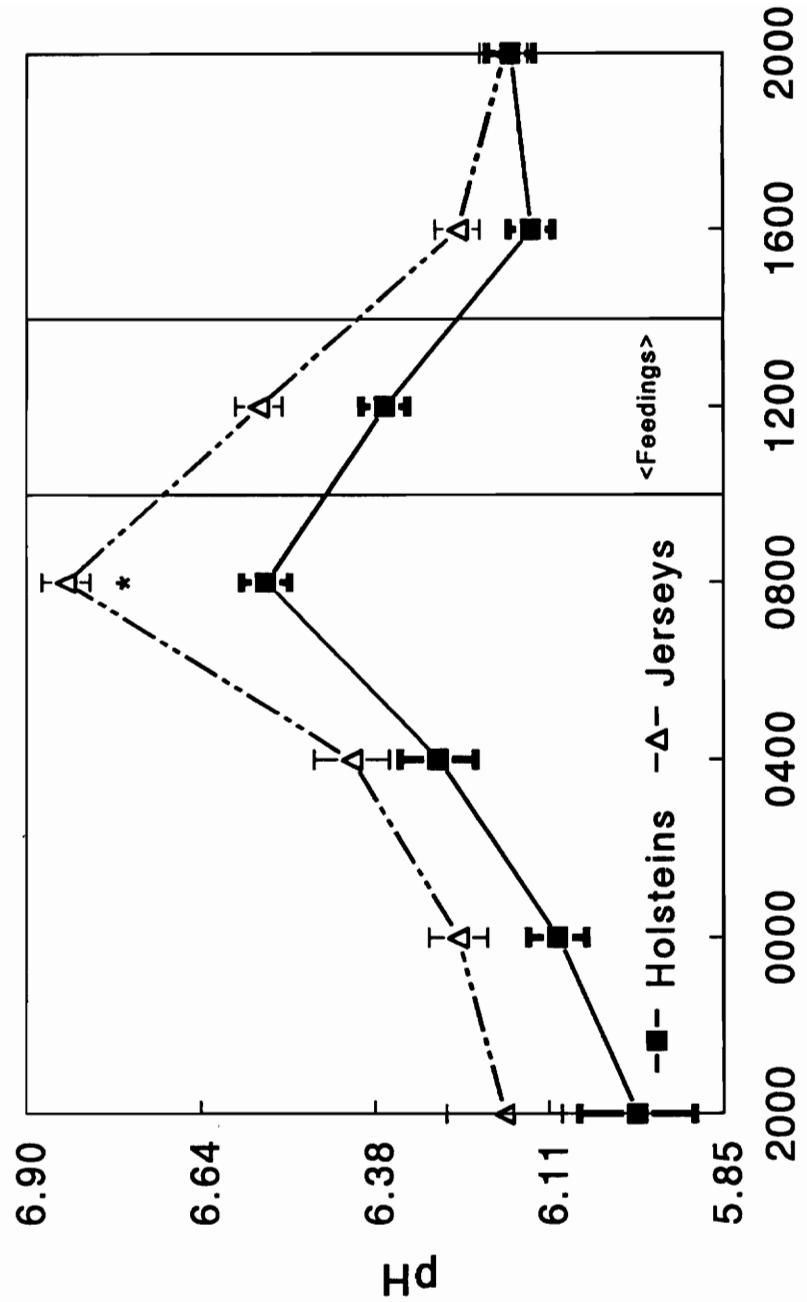

Figure 4.10. Effect of time on ruminal pH, breeds differ *(P<.05).

Table 4.15. Total VFA in response to dietary fat (F) and rumen undegradable protein (RUP) over a 24 h period in cows fed at 1000 and 1400 h.

Time	B[1]	Treatments				SEM	Contrasts (P <.05)			
		29% RUP	29% RUP+F	41% RUP	41% RUP+F		FAT	RUP	INT	BC[2]
		---------- mmol/L -------								
VFA 2000	H	110.8	92.1	96.6	87.0	7.5	NS	NS	NS	NS
	J	101.8	95.3	94.0	94.6		NS	NS	NS	
VFA 0000	H	103.7	96.6	99.6	95.0	4.2	NS	NS	NS	NS
	J	100.9	102.8	104.7	100.4		NS	NS	NS	
VFA 0400	H	104.3	92.2	93.0	98.4	4.1	NS	NS	.05	NS
	J	93.6	98.0	94.3	103.4		NS	NS	NS	
VFA 0800	H	84.4	87.2	84.7	83.0	4.7	NS	NS	NS	.01
	J	68.1	72.9	65.8	78.3		NS	NS	NS	
VFA 1200	H	96.5	89.2	86.2	88.6	6.1	NS	NS	NS	NS
	J	76.1	88.4	86.7	84.8		NS	NS	NS	
VFA 1600	H	111.5	94.4	98.7	90.4	6.5	NS	NS	NS	NS
	J	103.3	102.6	98.6	101.5		NS	NS	NS	
VFA 2000	H	100.9	99.5	93.6	96.1	5.9	NS	NS	NS	NS
	J	103.4	100.9	97.7	94.4		NS	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

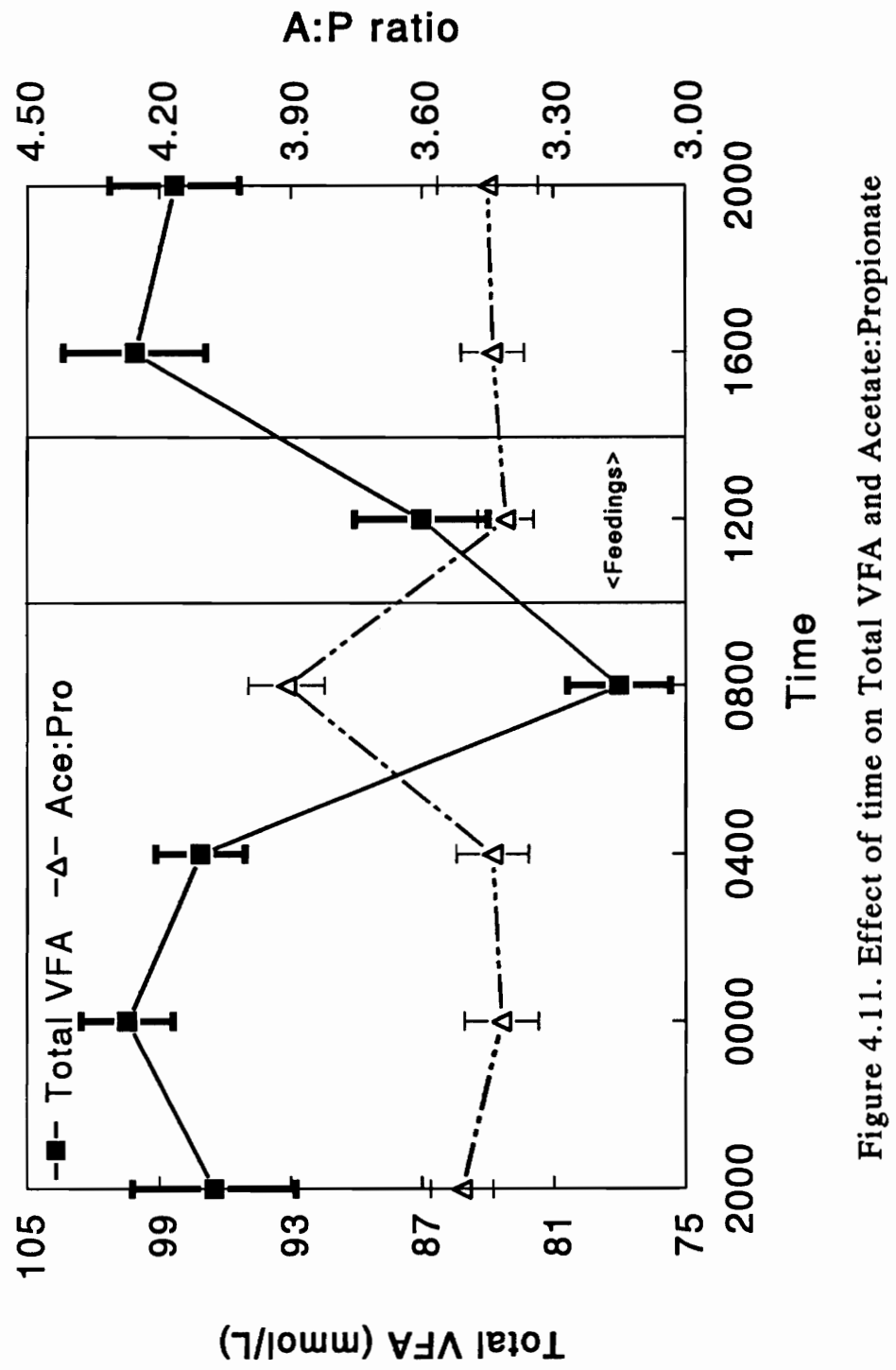

Figure 4.11. Effect of time on Total VFA and Acetate:Propionate

Table 4.16. Acetate concentration in response to dietary fat (F) and rumen undegradable protein (RUP) over a 24 h period in cows fed at 1000 and 1400 h.

Time	B[1]	Treatments				SEM	Contrasts (P <.05)			
		29% RUP	29% RUP+F	41% RUP	41% RUP+F		FAT	RUP	INT	BC[2]
		----- moles/100 moles -----								
ACE 2000	H	62.8	64.6	63.5	63.4	.6	NS	NS	NS	NS
	J	65.4	66.0	67.0	66.4		NS	NS	NS	
ACE 0000	H	62.1	63.9	63.5	63.2	.6	NS	NS	NS	NS
	J	65.3	64.8	66.2	65.7		NS	NS	NS	
ACE 0400	H	63.1	64.1	63.6	62.9	.6	NS	NS	NS	NS
	J	65.3	64.9	66.4	66.1		NS	NS	NS	
ACE 0800	H	64.3	67.2	65.4	65.7	.6	.02	NS	.05	NS
	J	68.2	67.4	69.1	67.8		NS	NS	NS	
ACE 1200	H	63.1	63.3	63.7	63.6	.5	NS	NS	NS	NS
	J	65.5	64.9	66.3	65.4		NS	NS	NS	
ACE 1600	H	62.6	64.9	63.9	64.2	.6	.05	NS	NS	NS
	J	65.8	65.3	66.0	64.6		NS	NS	NS	
ACE 2000	H	62.8	63.7	65.6	63.7	1.3	NS	NS	NS	NS
	J	63.1	65.7	67.1	66.2		NS	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

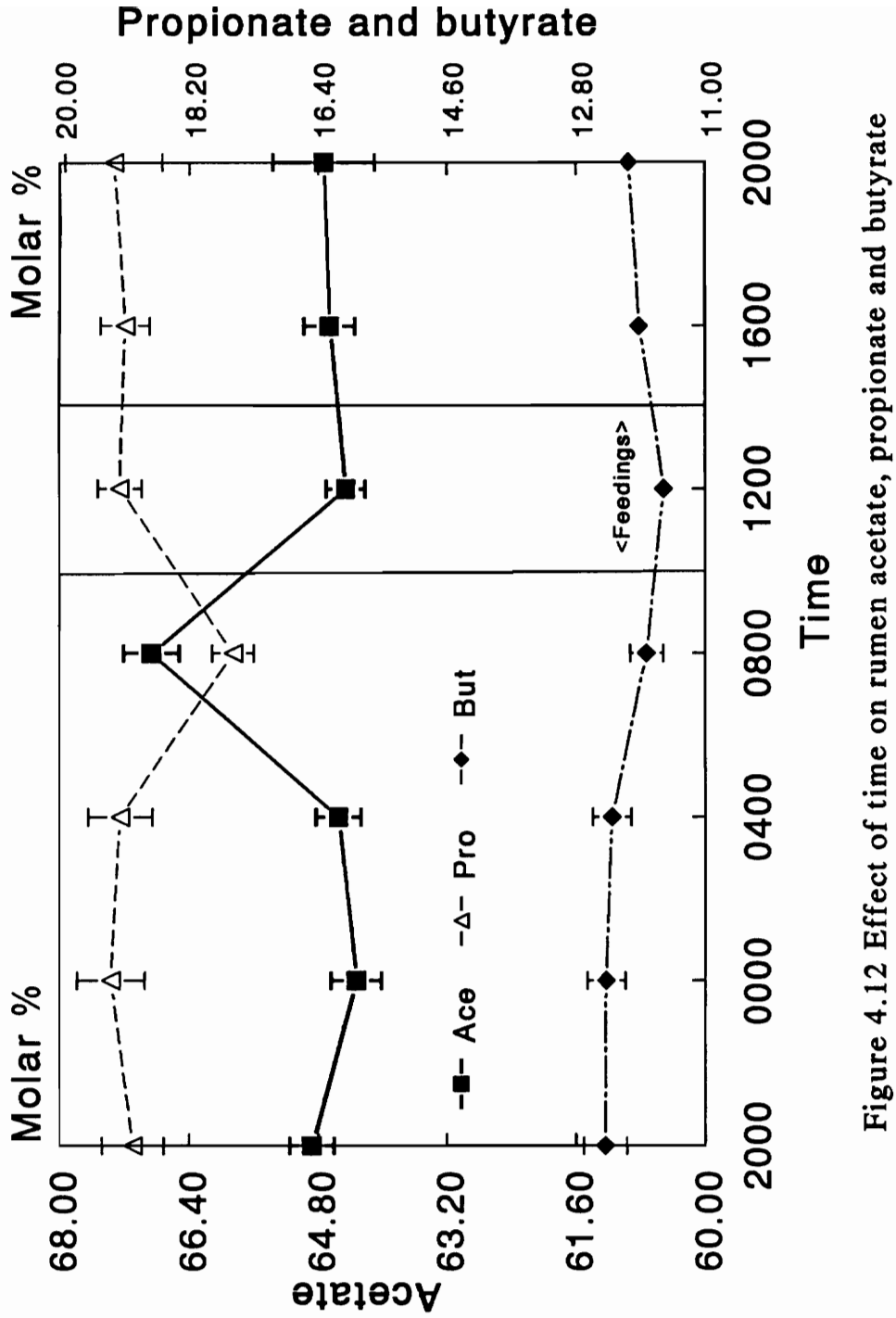

Figure 4.12 Effect of time on rumen acetate, propionate and butyrate

Table 4.17. Propionate concentration in response to dietary fat (F) and rumen undegradable protein (RUP) over a 24 h period in cows fed at 1000 and 1400 h.

Time	B[1]	Treatments				SEM	Contrasts (P <.05)			
		29% RUP	29% RUP+F	41% RUP	41% RUP+F		FAT	RUP	INT	BC[2]
		----- moles/100 moles -----								
PRO 2000	H	20.8	19.0	21.4	20.9	.9	NS	NS	NS	NS
	J	18.1	17.7	17.2	16.9		NS	NS	NS	
PRO 0000	H	21.3	19.4	21.0	21.2	.9	NS	NS	NS	NS
	J	17.9	18.6	17.7	17.4		NS	NS	NS	
PRO 0400	H	20.8	19.0	20.9	21.6	.9	NS	NS	NS	NS
	J	18.1	18.2	17.4	17.3		NS	NS	NS	
PRO 0800	H	20.4	17.3	19.1	19.5	.7	NS	NS	.03	NS
	J	16.3	16.6	15.4	16.2		NS	NS	NS	
PRO 1200	H	19.9	19.8	19.8	20.4	.6	NS	NS	NS	NS
	J	18.5	18.7	18.0	18.3		NS	NS	NS	
PRO 1600	H	20.3	18.8	19.8	19.8	.7	NS	NS	NS	NS
	J	18.1	18.3	18.6	19.0		NS	NS	NS	
PRO 2000	H	21.1	20.1	17.7	20.4	1.3	NS	NS	NS	NS
	J	21.1	18.0	17.7	18.0		NS	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

Table 4.18. Butyrate concentration in response to dietary fat (F) and rumen undegradable protein (RUP) over a 24 h period in cows fed at 1000 and 1400 h.

Time	B[1]	Treatments				SEM	Contrasts (P <.05)			
		29% RUP	29% RUP+F	41% RUP	41% RUP+F		FAT	RUP	INT	BC[2]
		----- moles/100 moles -----								
BUT 2000	H	12.6	12.4	11.3	12.0	.6	NS	NS	NS	NS
	J	15.6	15.6	12.3	13.3		NS	NS	NS	
BUT 0000	H	12.3	12.5	11.5	11.8	.5	NS	NS	NS	NS
	J	12.6	12.6	12.5	13.1		NS	NS	NS	
BUT 0400	H	12.2	12.7	11.7	11.7	.5	NS	NS	NS	NS
	J	12.5	12.6	12.4	12.8		NS	NS	NS	
BUT 0800	H	11.5	11.6	11.8	11.3	.5	NS	NS	NS	NS
	J	11.7	12.1	12.1	12.5		NS	NS	NS	
BUT 1200	H	12.5	11.9	11.6	11.3	.3	.05	NS	NS	NS
	J	11.3	11.5	11.1	11.5		NS	NS	NS	
BUT 1600	H	12.7	12.0	11.8	11.6	.4	NS	NS	NS	NS
	J	11.8	12.0	11.4	12.1		NS	NS	NS	
BUT 2000	H	12.2	12.4	12.3	12.0	.3	NS	NS	NS	NS
	J	12.0	12.4	11.5	11.9		NS	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

Table 4.19. Valerate concentration in response to dietary fat (F) and rumen undegradable protein (RUP) over a 24 h period in cows fed at 1000 and 1400 h.

Time	B[1]	Treatments				SEM	Contrasts (P <.05)			
		29% RUP	29% RUP+F	41% RUP	41% RUP+F		FAT	RUP	INT	BC[2]
		----- moles/100 moles -----								
VAL 2000	H	1.32	1.55	1.30	1.33	.07	NS	NS	NS	NS
	J	1.58	1.37	1.27	1.22		NS	.01	NS	
VAL 0000	H	1.61	1.49	1.41	1.40	.05	NS	.02	NS	NS
	J	1.56	1.47	1.35	1.39		NS	.02	NS	
VAL 0400	H	1.35	1.36	1.34	1.32	.04	NS	NS	NS	NS
	J	1.47	1.52	1.36	1.31		NS	.01	NS	
VAL 0800	H	1.15	1.06	1.07	1.06	.04	NS	NS	NS	NS
	J	1.05	1.13	.96	.94		NS	.01	NS	
VAL 1200	H	1.58	1.74	1.63	1.66	.06	NS	NS	NS	NS
	J	1.84	1.91	1.83	1.81		NS	NS	NS	
VAL 1600	H	1.54	1.58	1.56	1.60	.05	NS	NS	NS	NS
	J	1.76	1.75	1.58	1.78		NS	NS	NS	
VAL 2000	H	1.38	1.38	1.52	1.45	.08	NS	NS	NS	NS
	J	1.47	1.42	1.41	1.45		NS	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

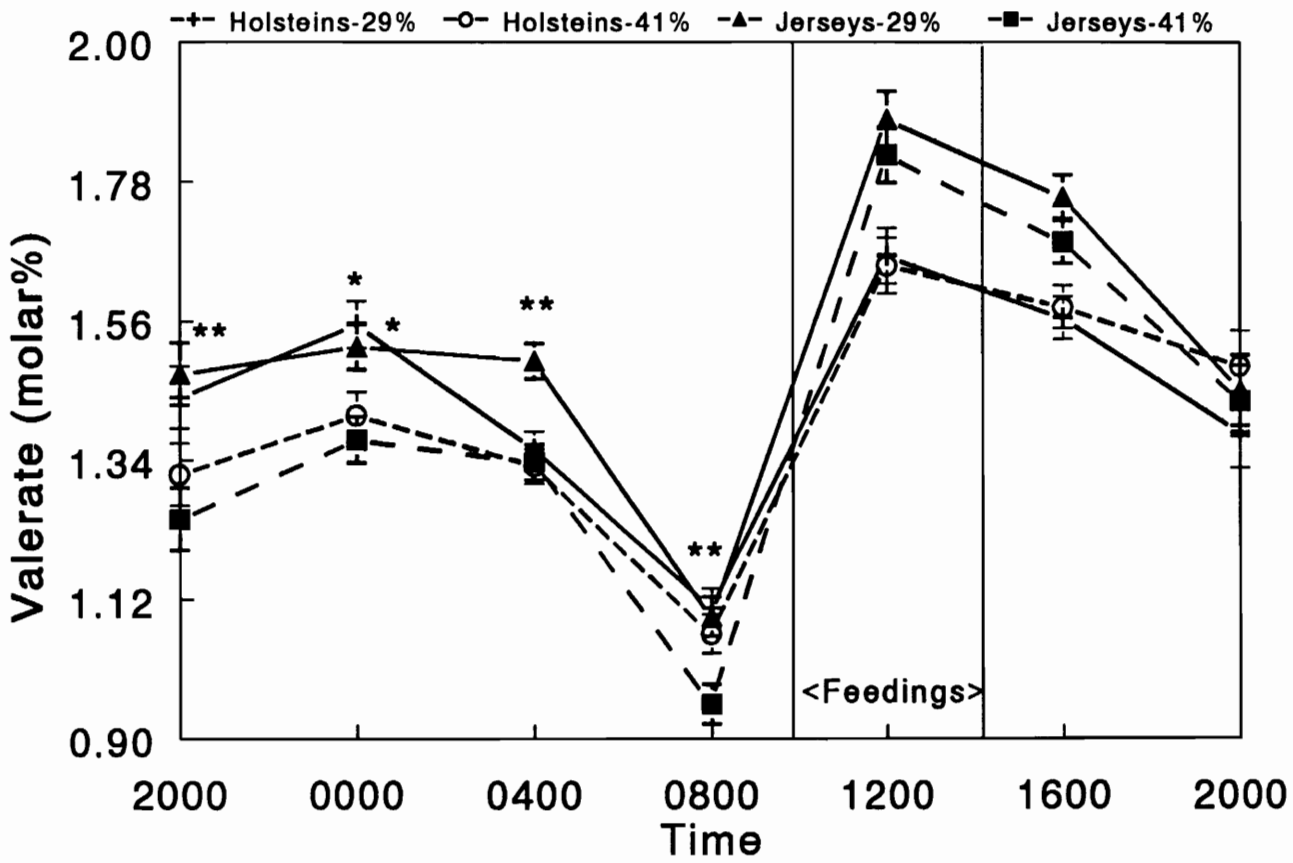

Figure 4.13. Effect of RUP on ruminal valerate, RUP differs **(P<.01),*(P<.05)

Table 4.20. Isobutyrate concentration in response to dietary fat (F) and rumen undegradable protein (RUP) over a 24 h period in cows fed at 1000 and 1400 h.

Time	B[1]	Treatments				SEM	Contrasts (P <.05)			
		29% RUP	29% RUP+F	41% RUP	41% RUP+F		FAT	RUP	INT	BC[2]
		----- moles/100 moles -----								
ISB 2000	H	.77	.77	.79	.72	.05	NS	NS	NS	NS
	J	.85	.80	.79	.79		NS	NS	NS	
ISB 0000	H	.86	.93	.81	.76	.05	NS	.07	NS	NS
	J	.94	.95	.81	.91		NS	NS	NS	
ISB 0400	H	.83	.98	.80	.75	.05	NS	.03	NS	.01
	J	1.01	1.07	.97	.95		NS	NS	NS	
ISB 0800	H	.88	1.1	.81	.83	.08	NS	.04	NS	.01
	J	1.14	1.20	1.03	1.05		NS	NS	NS	
ISB 1200	H	.92	1.11	1.03	.99	.06	NS	NS	NS	NS
	J	1.01	1.12	1.08	1.11		NS	NS	NS	
ISB 1600	H	.92	.90	.92	.90	.04	NS	NS	NS	NS
	J	.91	.92	.90	.93		NS	NS	NS	
ISB 2000	H	.81	.77	.90	.82	.06	NS	NS	NS	NS
	J	.85	.88	.84	.83		NS	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

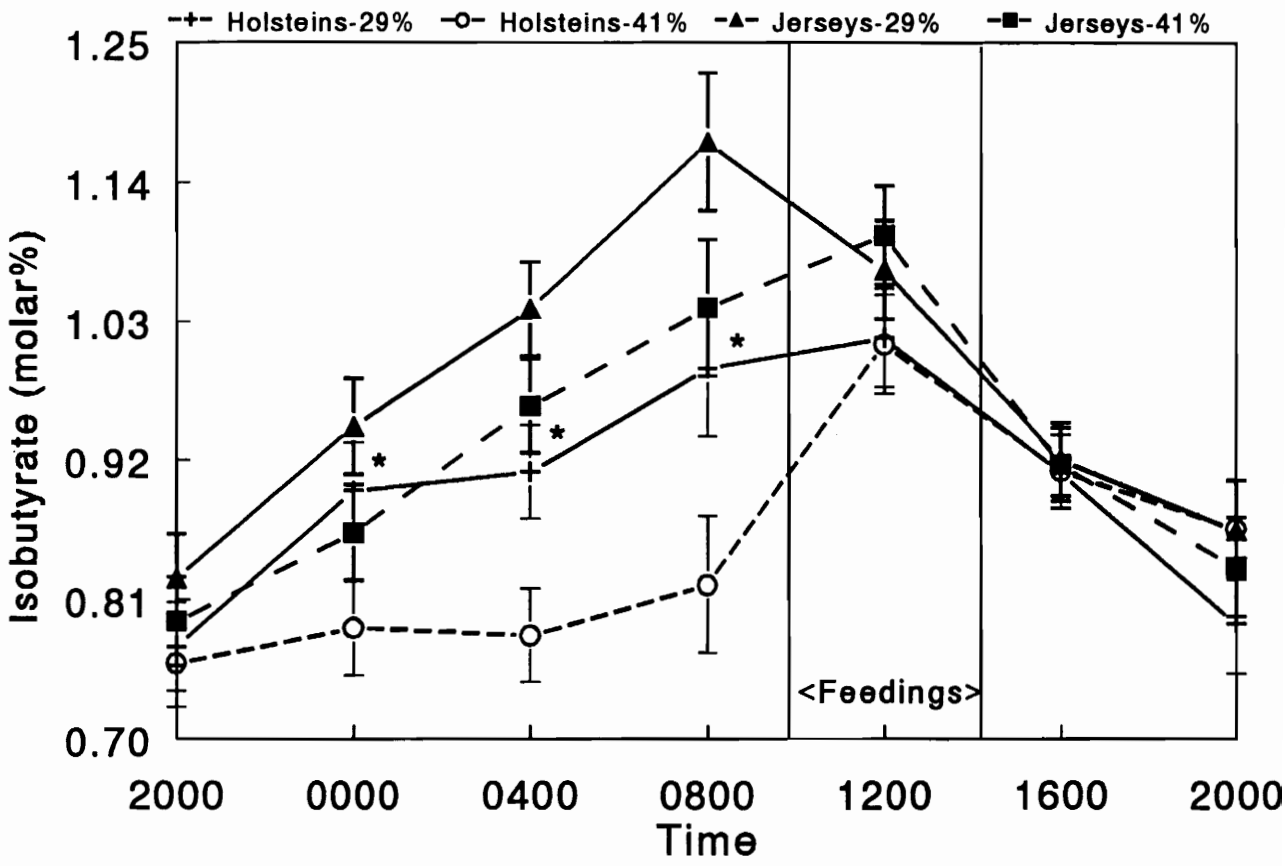

Figure 4.14. Effect of RUP on ruminal isobutyrate, RUP differs *(P<.07).

Table 4.21. Isovalerate concentration in response to dietary fat (F) and rumen undegradable protein (RUP) over a 24 h period in cows fed at 1000 and 1400 h.

Time	B[1]	Treatments				SEM	Contrasts (P <.05)			
		29% RUP	29% RUP+F	41% RUP	41% RUP+F		FAT	RUP	INT	BC[2]
		----- moles/100 moles -----								
ISV 2000	H	1.73	1.74	1.72	1.57	.14	NS	NS	NS	NS
	J	1.56	1.48	1.42	1.39		NS	NS	NS	
ISV 0000	H	1.81	1.76	1.79	1.59	.11	NS	NS	NS	NS
	J	1.68	1.64	1.41	1.47		NS	NS	NS	
ISV 0400	H	1.77	1.82	1.72	1.60	.13	NS	NS	NS	NS
	J	1.65	1.75	1.48	1.51		NS	NS	NS	
ISV 0800	H	1.69	1.83	1.79	1.56	.12	NS	NS	NS	NS
	J	1.68	1.64	1.41	1.56		NS	NS	NS	
ISV 1200	H	2.07	2.19	2.11	2.03	.10	NS	NS	NS	NS
	J	1.86	1.87	1.75	1.89		NS	NS	NS	
ISV 1600	H	1.98	1.76	2.00	1.85	.14	NS	NS	NS	NS
	J	1.62	1.72	1.52	1.60		NS	NS	NS	
ISV 2000	H	1.64	1.68	1.99	1.64	.16	NS	NS	NS	NS
	J	1.57	1.60	1.50	1.62		NS	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

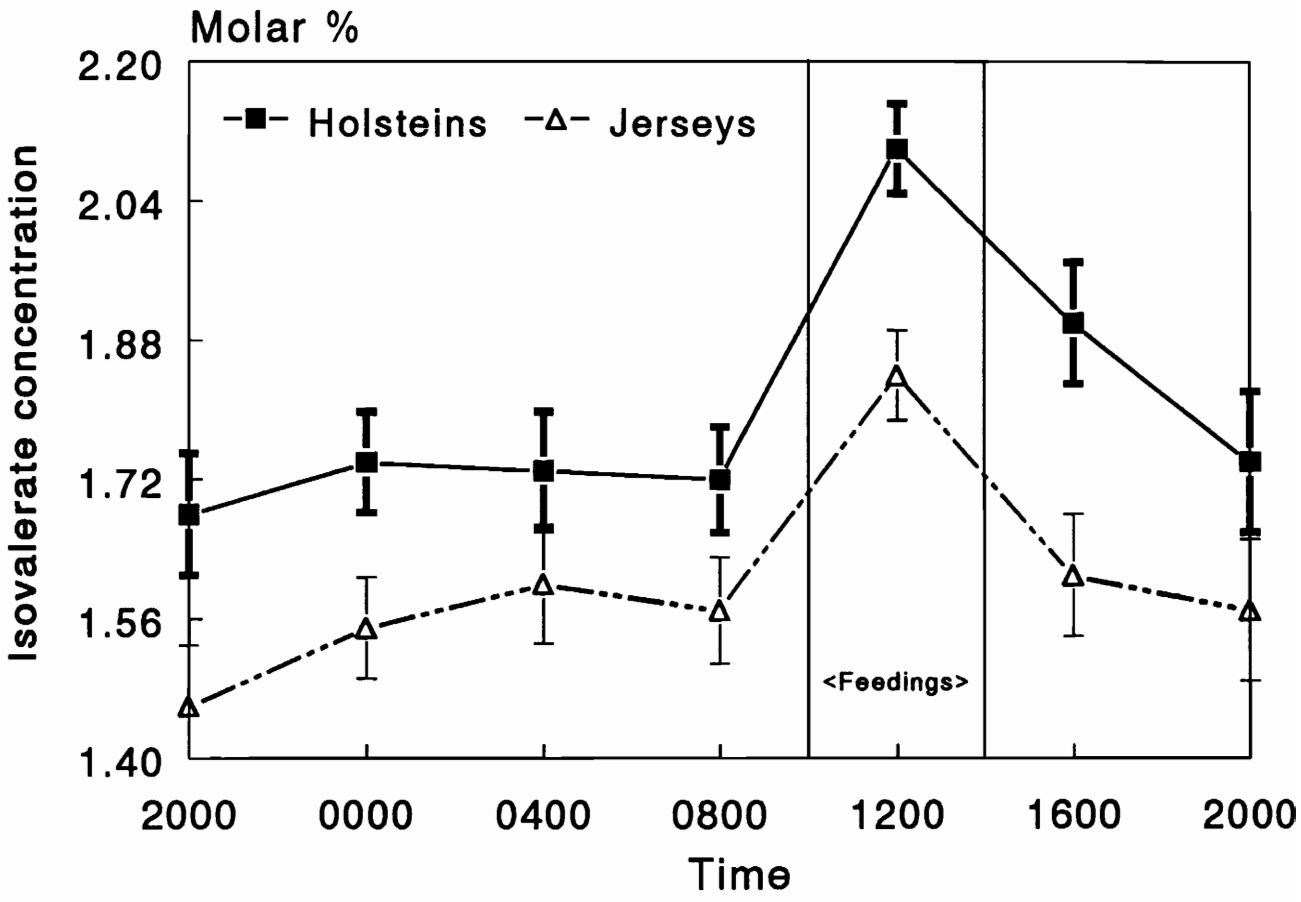

Figure 4.15. Effect of time on ruminal isovalerate, breeds did not differ.

Table 4.22. Acetate:Propionate ratio in response to dietary fat (F) and rumen undegradable protein (RUP) over a 24 h period in cows fed at 1000 and 1400 h.

Time	B[1]	Treatments				SEM	Contrasts (P <.05)			
		29% RUP	29% RUP+F	41% RUP	41% RUP+F		FAT	RUP	INT	BC[2]
A:P 2000	H	3.14	3.43	3.18	3.08	.14	NS	NS	NS	NS
	J	3.63	3.74	3.90	3.96		NS	NS	NS	
A:P 0000	H	3.05	3.31	3.19	3.05	.17	NS	NS	NS	NS
	J	3.66	3.50	3.77	3.81		NS	NS	NS	
A:P 0400	H	3.18	3.39	3.19	2.91	.16	NS	NS	NS	NS
	J	3.64	3.58	3.84	3.83		NS	NS	NS	
A:P 0800	H	3.29	3.98	3.55	3.42	.17	NS	NS	.04	NS
	J	4.19	4.09	4.52	4.22		NS	NS	NS	
A:P 1200	H	3.27	3.21	3.28	3.14	.12	NS	NS	NS	NS
	J	3.55	3.49	3.72	3.61		NS	NS	NS	
A:P 1600	H	3.19	3.47	3.34	3.27	.14	NS	NS	NS	NS
	J	3.64	3.61	3.56	3.42		NS	NS	NS	
A:P 2000	H	3.16	3.22	3.73	3.20	.22	NS	NS	NS	NS
	J	3.13	3.68	3.79	3.69		NS	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

Table 4.23. Apparent total tract dry matter, organic matter and N digestibilities, and microbial N (% of total N) in the abomasum.

		Treatments					Contrasts (P <.05)			
	B[1]	29% RUP	29% RUP+F	41% RUP	41% RUP+F	SEM	FAT	RUP	INT	BC[2]
Dry matter,%	H	67.8	65.3	68.6	63.8	2.1	NS	NS	NS	NS
	J	64.0	65.3	62.9	62.8		NS	NS	NS	
Organic	H	68.8	66.6	69.6	64.8	2.1	NS	NS	NS	NS
matter,%	J	64.8	66.5	64.1	63.9		NS	NS	NS	
Nitrogen,%	H	65.4	64.9	65.9	62.6	2.1	NS	NS	NS	NS
	J	62.1	64.9	60.8	63.4		NS	NS	NS	
Microbial N,	H	44.2	47.6	43.3	43.4	3.8	NS	NS	NS	NS
% total N	J	44.5	41.7	44.8	41.7		NS	NS	NS	

INT = Protein x fat interaction

[1] B = Breeds, H = Holstein, J = Jersey

[2] BC = Breed contrast

Table 4.24. Correlation coefficients (between cows) for rumen ammonia, plasma and milk urea N in a 24 h period.

	Time[1]	PUN[3]	MUN[4]
RAN[2]	2000	.40*	.36*
PUN		-	.70**
RAN	0000	.52**	.54**
PUN		-	.62**
RAN	0400	.41*	.43*
PUN		-	.57**
RAN	0800	.37*	.14
PUN		-	.30
RAN	1200	.37*	.43*
PUN		-	.28
RAN	1600	.40*	.37*
PUN		-	.46**
RAN	2000	.26	.31
PUN		-	.84**

[1] Sampling times
[2] Rumen ammonia N
[3] Plasma urea N
[4] Milk urea N
* $P < .05$
** $P < .01$

Table 4.25. Dry matter fractions, degradation rate of fraction B (K_d) and rumen degradability (D) of DM of forages and byproducts using the direct method.

		FRACTIONS				
	B[1]	A	B	C	K_d	D
AS[2]	O	34.3**	40.6	25.1*	8.0	59.1
	H	35.0	40.3	24.7	7.7	59.2
	J	33.6	40.8	25.6	8.2	59.0
CS[3]	O	49.1	33.9	17.0	4.2	64.4
	H	49.1	34.3	16.6	3.6	63.5
	J	49.0	33.6	17.4	4.7	65.2
C[4]	O	9.5	88.1	2.4	7.1	61.1
	H	8.7	89.8	1.5	6.5	59.3
	J	10.3	86.4	3.3	7.8	62.9
SBM[5]	O	30.0	68.9	1.1	16.1	82.3
	H	30.1	68.9	1.0	15.4	81.6
	J	29.9	68.9	1.2	16.9	83.0
BM[6]	O	-	23.0	77.0	.9	3.6
	H	-	24.1	75.9	1.2	4.5
	J	-	22.0	78.0	.6	2.6
CGM[7]	O	4.0	62.1	33.9	1.8	20.7
	H	4.0	65.0	31.0	1.7	20.5
	J	4.0	59.2	36.8	2.0	20.9

[1] Breeds, O = overall, H = Holstein, J = Jersey
[2] Alfalfa silage
[3] Corn silage
[4] Corn grain
[5] Soybean meal
[6] Blood meal
[7] Corn gluten meal
* $P < .05$ Holsteins differ from Jerseys
** $P < .01$ Holsteins differ from Jerseys

Table 4.26. Crude protein fractions, degradation rate of fraction B (K_d) and rumen degradability (D) of CP of forages and byproducts using the direct method.

		FRACTIONS				
	B[1]	A	B	C	K_d	D
AS[2]	O	56.4*	31.9	11.7	12.6	79.0
	H	56.8	32.1	11.1	10.3	78.4
	J	55.9	31.8	12.3	15.0	79.6
CS[3]	O	65.1	14.4	20.5	3.3	70.3
	H	65.1	14.8	20.1	2.4	69.2
	J	65.0	14.0	21.0	4.3	71.5
C[4]	O	4.6	91.5	3.9	7.1	58.1
	H	3.7	93.0	3.3	6.4	56.0
	J	5.4	90.1	4.5	7.8	60.2
SBM[5]	O	20.4	79.0	.6	21.3	84.2
	H	20.6	78.9	.5	19.6	83.3
	J	20.3	79.1	.6	22.9	85.0
BM[6]	O	-	28.0	72.0	1.4	6.8
	H	-	27.8	72.2	1.8	7.4
	J	-	28.2	71.8	1.0	6.3
CGM[7]	O	6.2	53.9	39.9	.8	13.6
	H	6.1	57.5	36.4	.7	13.3
	J	6.2	50.4	43.4	.9	13.9

[1] Breeds, O = overall, H = Holstein, J = Jersey
[2] Alfalfa silage
[3] Corn silage
[4] Corn grain
[5] Soybean meal
[6] Blood meal
[7] Corn gluten meal
* $P < .05$ Holsteins differ from Jerseys
** $P < .01$ Holsteins differ from Jerseys

Table 4.27. Dry matter fractions, degradation rate of fraction B (K_d) and rumen degradability (D) of DM of forages and byproducts using the indirect method.

		FRACTIONS				
	B[1]	A	B	C	K_d	D
AS[2]	O	31.8	43.1	25.1*	8.0	58.2
	H	31.3	44.0	24.7	7.7	57.9
	J	32.1	42.3	25.6	8.2	58.4
CS[3]	O	48.5**	34.5	17.0	4.2	64.2
	H	50.5	32.9	16.6	3.6	64.3
	J	46.6	36.0	17.4	4.7	64.0
C[4]	O	7.8**	89.8	2.4	7.1	60.6
	H	13.8	84.6	1.6	6.5	61.5
	J	1.8	94.9	3.3	7.8	59.6
SBM[5]	O	4.8	94.1	1.1	16.1	76.6
	H	6.5	92.5	1.0	15.4	76.1
	J	3.0	95.7	1.3	16.9	77.1
BM[6]	O	-2.0	25.0	77.0	.9	1.9
	H	-1.9	25.9	75.9	1.2	3.0
	J	-2.1	24.1	78.0	.6	.8
CGM[7]	O	6.3	59.8	33.9	1.8	22.4
	H	6.7	62.3	31.0	1.7	22.5
	J	5.9	57.3	36.8	2.0	22.2

[1] Breeds, O = overall, H = Holstein, J = Jersey
[2] Alfalfa silage
[3] Corn silage
[4] Corn grain
[5] Soybean meal
[6] Blood meal
[7] Corn gluten meal
* $P < .05$ Holsteins differ from Jerseys
** $P < .01$ Holsteins differ from Jerseys

Table 4.28. Crude protein fractions, degradation rate of fraction B (K_d) and rumen degradability (D) of CP of forages and byproducts using the indirect method.

		FRACTIONS				
	B[1]	A	B	C	K_d	D
AS[2]	O	49.9*	38.4	11.7	12.6	77.2
	H	54.0	34.9	11.1	10.3	77.4
	J	45.8	41.8	12.4	15.0	77.0
CS[3]	O	65.8	13.7	20.5	3.3	71.3
	H	67.6	12.3	20.1	2.4	71.7
	J	64.0	15.0	21.0	4.3	70.9
C[4]	O	9.8*	86.3	3.9	7.1	60.4
	H	15.9	80.9	3.2	6.4	61.3
	J	3.7	91.8	4.5	7.8	59.5
SBM[5]	O	-35.2	134.6	.6	21.3	74.0
	H	-24.6	124.1	.5	19.6	74.2
	J	-45.9	145.3	.6	22.9	73.8
BM[6]	O	-2.6	30.5	72.0	1.4	4.9
	H	-2.8	30.6	72.2	1.8	5.4
	J	-2.4	30.5	71.8	1.0	4.5
CGM[7]	O	6.4	53.7	39.9	.8	13.8
	H	6.8	56.8	36.4	.7	14.0
	J	5.9	50.6	43.5	.9	13.6

[1] Breeds, O = overall, H = Holstein, J = Jersey
[2] Alfalfa silage
[3] Corn silage
[4] Corn grain
[5] Soybean meal
[6] Blood meal
[7] Corn gluten meal
* $P < .05$ Holsteins differ from Jerseys
** $P < .01$ Holsteins differ from Jerseys

CONCLUSIONS

Supplemental fat increased milk yield and efficiency of production and may be due to increased uptake of dietary fatty acids by the mammary gland. However, when fat was supplemented, milk protein content decreased due to a dilution effect, because milk volume increased while milk protein yield did not change.

Lower milk protein concentration and yield in Holsteins due to feeding 41% RUP may be due to an imbalanced supply of amino acids for absorption in the small intestine. Also, microbial protein yield could be affected because 41% RUP diets had lower ruminal VFA's and ammonia concentration.

Regardless of differences in rumen ammonia concentration due to level of RUP, no differences in plasma urea N and milk urea N were detected. Plasma and milk urea N concentrations were most likely affected by other factors such as use of amino acids for gluconeogenesis. The major factor influencing diurnal changes in rumen ammonia, plasma urea N and milk urea N is feeding time and frequency. Milk urea N followed plasma urea N in diurnal pattern.

Variation in milk components, especially protein, casein N, milk urea N, and lactose, and digesta fermentation patterns throughout the day may indicate cows should be fed more often during the day to minimize variation.

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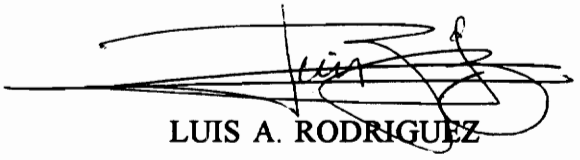

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