

## Effect of Feeding High Forage Diets with Supplemental Fat on Blood Metabolites, Rumen Fermentation and Dry Matter Digestibility in Dairy Cows

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**ABSTRACT** : Fifty mid-lactation Holstein cows were used in a six-week feeding trial to study effects of high-forage, and high-fat diets on blood constituents, rumen fermentation and dry matter digestibility. Cows were divided into 10 replicates, each consisting of five cows. Each cow was assigned to a control (diet 1) or one of the four experimental diets (high-forage (75%), high-fat (7.5%) (diet 2); high-forage, medium-fat (5.0%) (diet 3); medium forage (65%), high-fat (diet 4); medium-forage, medium-fat (diet 5)), or a control diet containing about 50% forage and 2% fat. All diets were isonitrogenous (17.7% crude protein). The forage mixture consisted of 20% alfalfa hay, 40% alfalfa haylage, and 40% corn silage. Supplemental fat included 80% rumen-protected fat and 20% yellow grease. A non-significant difference was observed in concentrations of blood glucose for cows on different experimental and control diets. Plasma nonesterified fatty acids (NEFA) were higher in cows consuming experimental diets than those consuming the control diet. However, differences in NEFA concentrations in the plasma of cows consuming diets with different forage and fat levels were not significant. Rumen pH, concentration of volatile fatty acids (VFA) in rumen contents, and dry matter digestibility of control and experimental diets, and diets with different levels of forage and supplemental fat did not differ significantly. (*Asian-Aus. J. Anim. Sci.* 2000. Vol. 13, No. 4 : 451-456)

**Key Words** : High-Forage Diets, Supplemental Fat, Blood Metabolites, Rumen Fermentation

### INTRODUCTION

High producing dairy cows require high amounts of energy which cannot be met through conventional diets during peak periods of milk production. Therefore, cows are in negative energy balance during this period. Inadequate energy intake causes decreased milk production and increased susceptibility of cows to metabolic disorders such as ketosis, fatty liver, and downer cow syndrome. (Curtis et al., 1985). High concentrations of free fatty acids in the diet tend to suppress rumen fiber digestion. Fatty liver and ketosis are interrelated metabolic disorders that are manifested during periods of negative energy balance of dairy cows, especially during early lactation.

Supplemental fat in dairy rations provides fatty acids as an energy source for tissues and precursors for mammary triglyceride synthesis. It is well established that supplying long-chain fatty acids from blood to the mammary gland decreases *de novo* synthesis of fatty acids (Grummer, 1992). Higher concentrations of free fatty acids in the diet tend to suppress rumen fiber digestion. Protected fat in the form of prills are not digested in the rumen, however, but pass to the small intestine where greater than 90% digestibility is achieved. Development of ruminally inert fats, such as calcium salts of fatty acids (CSFA)

and prilled fatty acids, has partially overcome some negative effects of normal fats, such as free oils and oils with fatty acids of medium-chain length or unsaturated chains, on ruminal fermentation (Schauff and Clark, 1989; Jerred et al., 1990).

Fiber content of the diet is one key nutritional factor that affects the performance response to any supplemental fat source (Canale et al., 1990). High forage diets promote increased use of fat by maintaining normal rumen characteristics (mainly pH) and by providing surfaces to adsorb fat. Thus, fat and forages complement each other by optimizing energy intake and rumen function (Palmquist, 1987). The optimum effective fiber content of diets containing supplemental fat needs to be determined.

Little research work has been done to explore the possibility of combining high proportions of forage with high fat to attain an acceptable energy intake for high producing cows. The objective of the current study was to develop schemes of feeding to utilize higher amounts of forage and fat for dairy cows and their effects on blood metabolites, rumen fermentation and dry matter digestibility in these animals.

### MATERIALS AND METHODS

Fifty multiparous mid-lactation Holstein cows were used to evaluate the effect of feeding high forage diets with supplemental fat on blood metabolites, rumen fermentation and dry matter digestibility. These cows were approximately of the same age, same body

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Received June 9, 1998; Accepted May 10, 1999

size and almost of the same body weight. The five experimental diets to be evaluated in the present study were as follows:

- Diet 1 : 50% Forage and 2% added Fat (Control)
- Diet 2 : 75% Forage and 7.5% added Fat
- Diet 3 : 75% Forage and 5% added Fat
- Diet 4 : 65% Forage and 7.5% added Fat
- Diet 5 : 65% Forage and 5% added Fat

The four experimental diets B through E contained forage i.e. 20% alfalfa hay, 40% alfalfa hataige, and 40% corn silage on a DM basis. the added fat contained 80% protected fat (Energy Booster EB 100, Milk Specialties Company, Dundee, IL) and 20% yellow grease. Diets were formulated to meet the requirements of a mature Holstein cow producing 90 lb of milk with 3.4% fat. All diets were iso-nitrogenous and ranged from 1.74 to 1.89 Mcal of NE<sub>i</sub> per kg of dry matter. The cows were randomly allotted to the five experimental diets such that each diet received ten cows. The cows were fed the experimental diets for a period of six weeks. Within the 6 wk experimental period cows were maintained on the normal herd diet during wk 1 to establish baseline values. Durins wk 2, cows were shifted step wise to the experimental diets.

Blood samples were taken from a jugular vein on the last day of wk 1, 3, 4, 5 and 6. Blood was collected in three 10 ml culture tubes containing 50 USP units of heparin and one tube containing 75 ml of 4% NaF. Tubes were kept in ice until blood samples were centrifuged for 5 minutes at 10,000×g. Plasma was harvested and stored at -20°C.

Samples of rumen contents were collected via stomach tube on the last day of wk 1 and 6. Sample pH was determined immediately. Samples were then acidified by adding 1% of 50% sulfuric acid (H<sub>2</sub>SO<sub>4</sub>), centrifuged at 30,000×g for 10 min, and the supernatant was collected and stored at -20°C for VFA analysis.

Cows from replications four through seven were fed chromic oxide (Cr<sub>2</sub>O<sub>3</sub>) mixed in the TMR at 0.15% of diet DM to determine digestibility of diets

during wk 6. Fecal "grab" samples were taken during the last 3 d at 6 h intervals. Sample collection times were staggered for 2 h daily to have a representative sample for every 2 h interval during a 24 h period. For each collection, a 50 g sample was weighed and composited to form one sample per cow. Samples were stored at -20°C until analyzed for chromium content. Fecal samples were dried in a hot air oven at 55°C for 48 hours and ground through a 1 mm screen in a Wiley mill. The ground samples then were analyzed for chromium (Williams et al., 1962).

Plasma glucose concentrations were determined spectrophotometrically by using a commercial kit (Sigma glucose kit #315-500, Sigma Chemical Co., St. Louis, MO). Nonesterified fatty acid concentrations were determined by using a modification of commercial kit (NEFA-C kit, Wako Chemical Co. USA, Dallas, TX) as described by Drackley (1989). Concentrations of βHBA were determined from plasma samples. One ml of plasma was deproteinized by using 2 ml of 0.3 N Ba(OH)<sub>2</sub> and 2 ml of 5% ZnSO<sub>4</sub> (Somogyi, 1945) The protein free filtrates were then assayed enzymatically for β-hydroxybutyrate (Williamson and Mellanby, 1974).

Concentrations of VFA in rumen fluid were determined by gas chromatography (Erwin et al., 1961) by utilizing an automated gas chromatograph (model 4600; Varian, Palo Alto, CA) using a packed column (Supelco, 1990).

The data were subjected to analysis of variance by using general linear model procedures of SAS (SAS Institute, 1990). Three orthogonal linear contrasts were constructed to test differences between control and the treatments, forage levels, and fat levels.

## RESULTS AND DISCUSSION

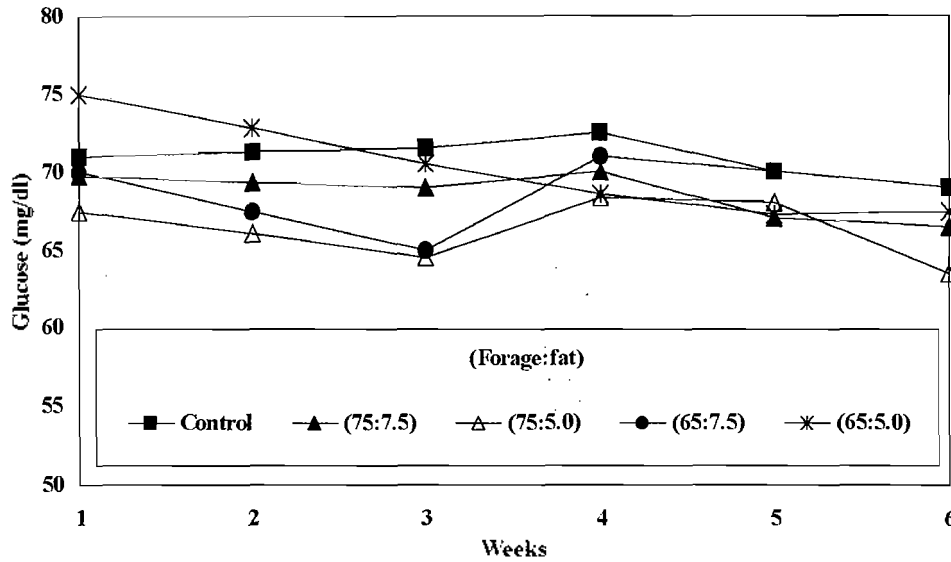
### Blood metabolites

Average glucose concentrations (table 1) in the blood of cows fed the control or one of the four treatment diets were 70.72 and 67.35 mg/dl respectively (p<0.05). No differences were observed in plasma concentrations of glucose in the blood of cows fed diets with different levels of forage or fat.

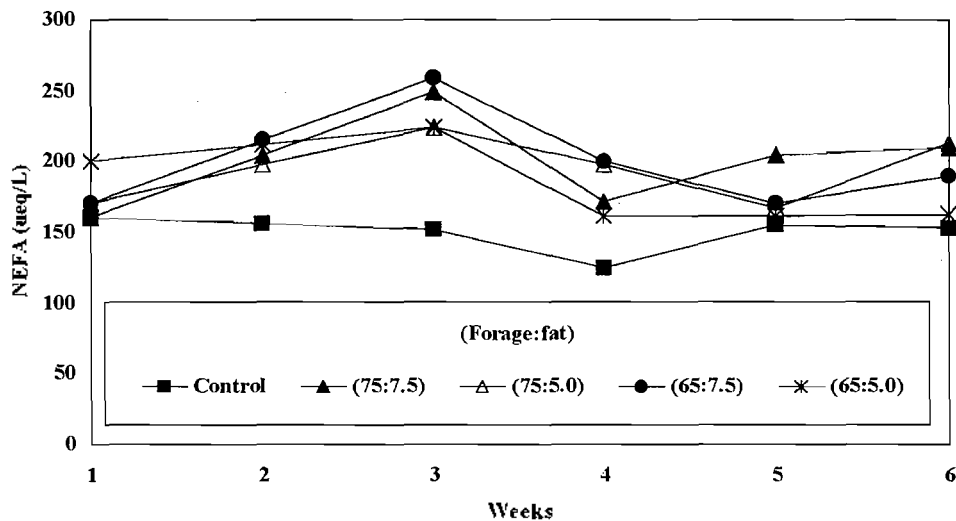
**Table 1.** Average plasma glucose, nonesterified fatty acids (NEFA), and β-hydroxybutyrate (βHBA) concentrations of cows during wk 3, 4, 5, and 6 of the experimental period

Item levels	Diets (forage:fat)					Contrasts		
	1 (Control)	2 (75:7.5)	3 (75:5.0)	4 (65:7.5)	5 (65:5.0)	Control vs. treatments	Forage levels	Fat levels
Glucose (mg/dl)	70.72	64.57	65.66	67.94	68.23	**	NS	NS
NEFA (meq/L)	148.53	212.42	205.54	209.54	187.60	**	NS	NS
βHBA (mg/dl)	3.87	4.75	5.69	5.32	4.99	**	NS	NS

\*\* p<0.01, NS=non-significant.



**Figure 1.** Plasma glucose concentrations of cows fed the control or the treatment diets during the experimental period (wk 3-6) (Pooled SEM=2.17). The numbers in parenthesis in the legend box are the percent forage (75 or 65) followed by the percent supplemental fat (7.5 or 5.0) in the diets.

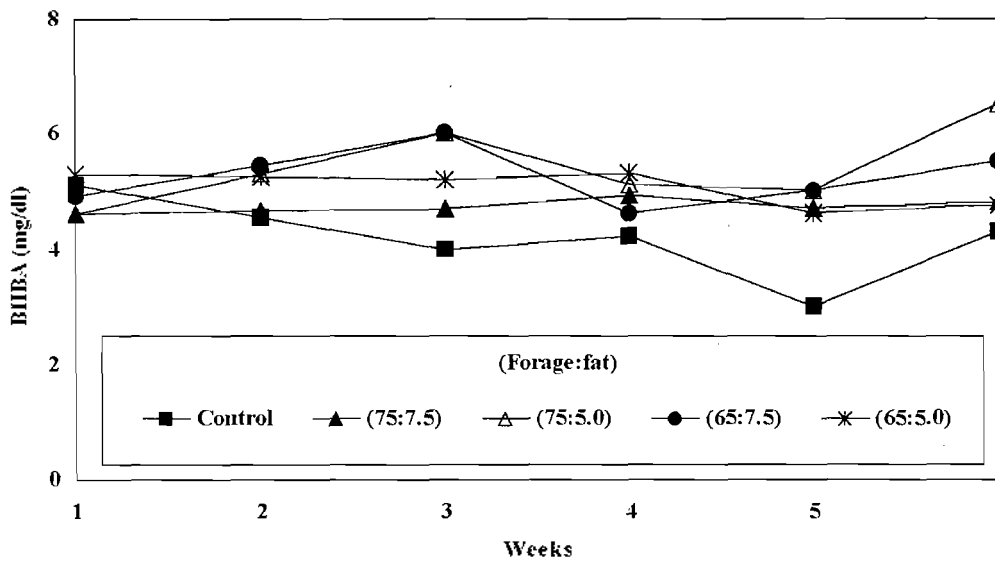


**Figure 2.** Plasma nonesterified fatty acids (NEFA) concentrations of cows fed the control or the treatment diets during the experimental period (wk 3-6) (Pooled SEM=20.02). The numbers in parenthesis in the legend box are the percent forage (75 or 65) followed by the percent supplemental fat (7.5 or 5.0) in the diets.

Comparing the treatment with the pretreatment period revealed that overall glucose concentration in the blood of all cows was lower during the treatment period. Decreases among all the diets over the experimental period were consistent and no diet by week interactions were noted (figure 1).

Plasma NEFA concentrations (table 1, figure 2)

were higher in the blood of cows fed one of the four treatment diets than in blood of cows fed the control diet, whereas in NEFA concentrations were greatest during the first week of treatment for cows on all four treatment diets, whereas NEFA concentrations for the control diet decreased during the experimental period when compared with the pretreatment period.



**Figure 3.** Plasma  $\beta$ -hydroxybutyrate ( $\beta$ HBA) concentrations of cows fed the control or the treatment diets during the experimental period (wk 3-6) (Pooled SEM=0.683). The numbers in parenthesis in the legend box are the percent forage (75 or 65) followed by the percent supplemental fat (7.5 or 5.0) in the diets.

NEFA concentrations for cows fed the 75% forage diet with high levels of supplemental fat never decreased to the level of the pretreatment period; however, they did decrease during wk 4 to wk 6 compared with wk 3 concentrations. Blood NEFA concentrations for all treatment diets were highest during wk 3 suggesting that cows needed a long adjustment period before they were able to utilize larger amounts of dietary fatty acids. Cows fed the diet containing 65% forage and 5% fat had lower plasma NEFA concentrations during wk 4-6 than during the pretreatment period (174.9 vs. 201.20 meq/L) indicating that increased forage may help keep NEFA concentrations at low levels.

Concentrations of  $\beta$ HBA (table 1) were lower for cows fed the control diet compared with the four groups fed treatment diets (3.87 vs. 5.19 mg/dl). No differences were noted in  $\beta$ HBA concentrations for cows fed diets having different levels of forage and fat (table 1).  $\beta$ HBA concentrations during the experimental period remained almost constant (figure 3).

Other studies on cows fed supplemental fat reported either no effect (Palmquist and Conrad, 1978) or increased plasma glucose concentrations with fat supplementation (Cervantes, 1992; Jenkins and Jenny, 1989). Results of our study are in agreement with those conducted by Palmquist and Moser (1981) and Erickson et al. (1992) where decreases in plasma glucose concentration were reported. Changes in plasma concentrations of NEFA are observed commonly in cows supplemented with fat, probably

due to hydrolysis of increased blood triglycerides (DePeters et al., 1989; Schauff et al., 1992). Grummer and Carrol (1991) concluded that plasma NEFA concentrations almost always increase when supplemental fat is fed. The results of the current study are similar to those of other workers (DePeters et al., 1989; Canale et al., 1990; Cervantes, 1992) who found that plasma NEFA concentrations in lactating cows supplemented with dietary fat are consistently increased.

### Rumen fermentation

#### 1) pH

The rumen pH for cows fed the control diet or one of the four treatment diets during the treatment period was 6.62 and 6.67 respectively (table 2). There were no differences in pH of the rumen between different dietary groups during the treatment period. These results are in agreement with those obtained by Schauf and Clark (1989) indicating that feeding rumen protected fat at up to 7.2% of the ration DM did not adversely affect rumen pH. Other workers (Grummer, 1988; Schauff and Clark, 1990) also have reported that rumen protected fat fed up to 5% of ration DM does not change rumen pH.

#### 2) Volatile fatty acids

The average concentrations of VFA and acetate:propionate ratios during experimental period are given in table 2. Average concentrations of acetate, propionate,

**Table 2.** Average rumen pH, volatile fatty acid (VFA) concentrations, and acetate:propionate ratios in rumen content of cows during wk 6 of the experimental period

Item	Diets (forage:fat)					Contrasts		
	1 (Control)	2 (75:7.5)	3 (75:5.0)	4 (65:7.5)	5 (65:5.0)	Control vs. treatments	Forage levels	Fat levels
pH	6.62	6.63	6.70	6.68	6.67	NS	NS	NS
	mM					Significance		
Acetate	68.02	55.91	61.55	63.77	67.36	NS	NS	NS
Propionate	18.42	15.42	13.99	17.12	16.43	NS	NS	NS
Isobutyrate	0.99	0.63	0.73	0.62	0.81	*	NS	NS
Butyrate	11.62	9.48	9.72	10.81	12.07	NS	NS	NS
Isovalerate	1.79	1.78	1.72	1.44	1.83	NS	NS	NS
Valerate	1.44	1.33	1.25	1.37	1.36	NS	NS	NS
Acetate:propionate	4.28	4.70	3.95	4.29	4.08	NS	NS	NS

\*  $p < 0.05$ , NS=non-significant.

isobutyrate, butyrate, isovalerate and valerate were 63.3, 16.28, 0.76, 10.74, 1.71 and 1.35 mM, respectively. The acetate:propionate ratio for the experimental period averaged 4.26. No differences were noted in rumen VFA and acetate:propionate ratios between cows fed the control diet or one of the four treatment diets and between cows fed diets with different levels of forage or fat. Acetate concentrations for cows fed high forage, high fat diets were lower during the treatment period when compared with pretreatment period. For cows fed diets with 75% forage and 5% fat, acetate concentrations tended to remain constant, whereas acetate concentrations increased for cows fed diets with 65% forage at both 5.0 and 7.5% fat and for those fed control diets. Propionate concentrations were lower for cows fed the control diet or one of the treatment diets during treatment compared with the pretreatment period. No differences in acetate:propionate ratios were found between cows fed the control and treatment diets during the treatment period. These results are in agreement with those of other workers (Grummer et al., 1993; Kim et al., 1990; Weakly et al., 1990) who reported little or no effect of supplemental fat on rumen VFA concentrations or acetate:propionate ratios.

### 3) Dry matter digestibility

No differences were observed in the DM digestibility of control or treatment diets (table 3). The expected low DM digestibility of diets containing high levels of forage was apparently prevented by supplementing with higher proportions of highly digestible fat. The digestibility of control and treatment diets ranged from 73.90 to 76.10%. These results are in agreement with those reported by Elliot et al. (1994), Palmquist and Conrad (1980), Schauff and Clark (1989), and West and Hill (1990).

**Table 3.** Average dry matter digestibility in cows fed different experimental diets

Diet (forage:fat) <sup>1</sup>	Digestibility (%)	Contrasts	Significance
1 (Control)	75.36	Control vs. treatments	NS
2 (75:7.5)	75.23	Forage levels	NS
3 (75:5.0)	74.00	Fat levels	NS
4 (65:7.5)	76.10		
5 (65:5.0)	73.90		

<sup>1</sup> Percentage of dietary dry matter. NS=non-significant.

## CONCLUSIONS

Supplemental fat had no effect on concentrations of blood glucose but plasma nonesterified fatty acids and  $\beta$ HBA were higher. No differences in rumen pH, concentration of volatile fatty acids (VFA) in the rumen content and DM digestibility of control and experimental diets were observed. The expected decrease of DM digestibility of diets containing 75% forage was apparently prevented by supplementation with higher proportions of highly digestible fat.

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