

Effect of Different Amounts of Protein and Varying Proportions of Corn Silage and Alfalfa Hay on Milk Production and Nitrogen Excretion of Dairy Holstein Cows

Research Article

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ABSTRACT

Four treatments were assigned to determine the effects of dietary potential digestible fiber (pdF) and crude protein (CP) levels in mid lactating Holstein cows on milk production and nitrogen efficiency. Sixteen primiparous (n=8) and multiparous (n=8) Holstein cows (body weight (BW)=546±72 kg; days in milk (DIM)=118±50 day) were allocated to one of four diets in balanced randomized complete design in two periods (each period including 28 day). Diets consisted of 50% forage and 50% concentrate. The experimental diets were as follows: 1) 19% CP and low pdF (HPLDF); 2) 17% CP and low pdF (MPLDF); 3) 15% CP and high pdF (LPHDF) and 4) 17% CP and high pdF (MPHDF). Milk yield was similar among dietary protein levels in each treatment, when data were combined across treatments. Both milk urea nitrogen and blood urea nitrogen concentrations increased as the protein content of diet was increased. As dietary protein was increased from the lowest to the highest concentrations, mean fecal N concentration increased from 2.8 to 3.0% and urinary N from 5.8 to 7.3 g/L (P<0.05). Overall, based on N utilization as well as milk production, 17% protein in diets utilizing various proportions of alfalfa hay and corn silage as the forage source appeared sufficient for cows producing 27 kg/d of milk in this study. Reducing protein to this amount can reduce N excretion, especially environmentally labile urinary N, without affecting milk production.

KEY WORDS crude protein, dairy cow, digestive fiber, milk production, nitrogen excretion.

INTRODUCTION

The first goal in formulating diets of lactating dairy cows for protein requirements is to meet the requirements of the microbes in the rumen. The second goal is to balance the metabolizable protein (MP) requirement of the cow. An additional goal, such as not exceeding the CP requirement of the cows so that N excretion is reduced, may need to be considered. Excess CP in the diets of dairy cows contributes to growing environmental concerns related to N pollution of water resources (Tamminga, 1992). In addition, ex-

cess dietary CP can be met, resulting in unnecessary feeding expenses. Diets with lower concentrations of CP but with properly balanced ruminally degradable CP may have both an economical and environmental impact. Increased amounts of rumen undegradable protein (RUP) in the diets of cows in the mid (Arieli *et al.* 1996; Robinson *et al.* 1991) or late lactation (Robertson and Van Soest, 1981) did not result in higher milk production. This result could be explained because cows in early lactation have a greater demand for MP to meet the demands of high milk production but in later lactation, these demands decline. After peak of

milk production, the requirement for MP can be met while feeding lower CP concentration diets by maximizing DMI. Recently, there has been an effort to reduce the amount of protein fed to dairy cows to minimize N excretion and loss to the environment. Several studies (Leonardi *et al.* 2003; Wattiaux and Karg, 2004a) compared “Low” and “High” dietary protein amounts and suggested that it is possible to reduce protein from 18 (or above) to 16.5% without affecting milk production. However, other studies observed decreased milk production when dietary protein was reduced from 17.4 to 15.2% (Kalscheur *et al.* 1999) and from 18.4 to 15.1% (Broderick, 2003). Wu and Satter (2000) suggested that dietary protein may only be reduced moderately from levels producers often used (~18%) without affecting milk production and that other means including maximizing microbial protein production are needed to obtain a significant reduction in the need of dietary protein and therefore a reduction in N excretion. More research is needed to define the response curve for milk production as a function of dietary protein amount.

In addition to protein intake, the type of forage fed may be important. Corn silage and alfalfa hay are the two most common forages fed to dairy cows. Corn silage is a fermentable carbohydrate source and alfalfa is high in rumen degradable protein (RDP). The 2 feedstuffs complement each other by providing fermentable energy and available N for microbial protein synthesis in the rumen. Hence, the proportions of these forage sources in the diet could have an influence on N utilization. Dhiman and Satter (1997) showed that the efficiency of N utilization increased as the proportion of corn silage was increased to two-thirds in its combination with alfalfa. Wattiaux and Karg (2004a) showed increased milk yields when the corn silage proportion was raised from 25 to 75% in dietary forage that utilized alfalfa silage and corn silage only. The objective of this study was to determine the response in milk production and N excretion in dairy cows to dietary protein amount for diets that varied in the proportion of corn silage and alfalfa hay.

MATERIALS AND METHODS

Sixteen Holstein cows (n=8 multiparous and n=8 primiparous) were used to determine the effect of varying the dietary protein content and different source of forage on milk production and N excretion. At the start of the study, cows were 118 ± 50 DIM (day in milk) (Mean \pm SE), weighed 545 ± 93 kg and had been consuming treatment diets since d 21 postpartum. The experiment was designed as a randomized complete design. Cows were assigned randomly within 1 of 4 dietary treatments (for each treatment two primiparous and two multiparous). The experiment consisted of 2 peri-

ods lasting 28 d each (21 d of adaptation and 7 d of sampling). The diets were as follows: 1) 19% CP and low potential digestible fiber (pdF) (HPLDF); 2) 17% CP and low pdF (MPLDF); 3) 15% CP and high pdF (LPHDF) and 4) 17% CP and high pdF (MPHDF). The diets were balanced for pdF by using different levels of dried alfalfa hay and corn silage in forage proportion of diets (Table 1). Diets used in all trials consisted of forage and concentrate at a ratio of 50:50 (DM basis) (Table 2). Alfalfa hay and corn silage were the forage sources used in the treatments. Treatments 1, 2 and 3, 4 used a different alfalfa hay: corn silage ratio and these ratios were 20:80 and 80:20 for treatments 1, 2 and 3, 4, respectively. The targeted protein content of the diet was 19, 17, 15 or 17% for the 4 treatments.

Diets were offered *ad libitum* andorts were kept at 10% of the amount offered. The actual amounts of feed offered and refused were recorded daily to obtain net intake for individual animals. The body weight (BW) of cows was measured on two consecutive days at the beginning of the trial and at the end of each period. Three milk samples were taken from consecutive morning, noon and evening milking during the last day in each period. Fecal and urine samples were taken during the last 4 d of the collection week of each period. Feces were sampled from the rectum and urine during urination with stimulation. Fecal samples were pooled to obtain a composite for individual cows in each period. Samples of urine were acidified to pH < 4 using 4 M HCl and frozen. These samples were later thawed and a composite was generated for each cow during each period.

Blood samples (10 mL) were collected once during the collection week from the coccygeal vein into evacuated tubes containing EDTA.

Milk samples were analyzed for fat, protein, lactose, urea N using infrared spectroscopy (Foss Electric). Feed and fecal samples were dried at 55 °C in a forced air oven for 48 h. Ground samples were analyzed for DM (102 °C), CP based on Kjeldahl N (AOAC, 1990). Urine samples were also analyzed for CP using the AOAC method (AOAC, 1990). Blood plasma was obtained by centrifugation at 4 °C and $3000 \times g$ for 15 min and analyzed for urea N concentration (Urea Nitrogen kit 580). Urine samples were analyzed for creatinine concentration (Sigma Creatinine Kit 555) to estimate urine volume (Valadares *et al.* 1999).

Rumen fluid pH was determined within 3 min of collection using a handheld pH meter (Twin pH meter model B-213; Spectrum Technologies Inc., Plainfield, IL). Also, 1 mL of rumen fluid was acidified with 20 μ L of 50% trichloroacetic acid and frozen until analysis for NH₃ N concentration. Body weight was recorded using weighing scales. The body condition score (BCS), recorded simultaneously using scale of 1 (thin) to 5 (fat) with increments of 0.25.

Table 1 Food rations based on the percentage of dry matter

Feeds	Different levels of CP (%)			
	19	17	15	17
Ingredient, % of DM				
Dried alfalfa	40.42	40.42	9.62	9.60
Corn silage	9.62	9.62	40.42	40.33
Barley grain ground	3.85	12.3	13.47	7.2
Corn grain ground	16.84	12.99	10.11	13.44
Wheat bran	2.41	4.33	1.92	1.44
Fish meal	2.41	1.92	2.41	3.12
Sugar beet pulp	4.33	4.81	7.22	4.32
Soybean meal	13.47	7.22	8.18	12.48
Cottonseed meal	4.81	4.81	4.81	6.24
Calcium carbonate	0.48	0.48	0.48	0.48
NaCl	0.38	0.38	0.38	0.38
Sodium bicarbonate	0.48	0.48	0.48	0.48
Vitamin mineral	0.48	0.48	0.48	0.48

Table 2 Nutrient composition of diets

Feeds	F:C ratio			
	50:50	50:50	50:50	50:50
Crude protein, %	19.08	17.01	15.04	17.04
RUP, (% of CP)	37.65	37.83	37.58	37.74
RDP, (% of CP)	62.32	62.17	62.42	62.26
NEL, (Mcal per kg)	1.60	1.57	1.70	1.73
EE, %	3.20	3.13	3.25	3.43
NDF, %	33.00	33.00	34.00	33.50
PeNDF, %	23.51	23.61	21.84	21.55
NFC, %	39.30	40.17	42.91	41.84
Starch, %	19.63	21.8	28.44	27.64
Ca, %	0.99	0.95	0.96	0.98
P, %	0.48	0.47	0.45	0.50

RUP: rumen undegradable protein, RDP: rumen degradable protein, NEL: net energy lactation, EE: ether extract, NDF: natural detergent fiber, PeNDF: physical effective NDF and NFC: non fiber carbohydrate.

Data were analyzed separately for each trial with a balanced randomized complete block design. The MIXED procedure of SAS (SAS, 1999) was used for the analysis using the following model:

$$Y_{ijkn} = \mu + T_i + P_j + \text{Time}_k + (T \times P)_{ij} + (T \times \text{Time})_{ik} + \text{COW}_n(t)_i + e_{ijhn}$$

Where:

Y: observation.

μ : overall mean.

T: treatments effect.

P: period.

e: residual error.

For all analyses, differences were considered significant at ($P < 0.05$), unless specified.

RESULTS AND DISCUSSION

Dry matter intake was not affected by dietary protein concentration in any of the treatments but cows fed the diets

with high pdF had greater ($P < 0.05$) DMI (Table 3). Broderick (2003) showed small increases in DMI as dietary protein was increased from 15.1 to 18.4%, whereas no change was reported by Davidson *et al.* (2003) with dietary protein ranging from 16.5 to 19.4% or by Wattiaux and Karg (2004a) using protein of 16.5 to 17.9%. The BW of cows was similar among treatments in all treatments.

There was no effect of dietary protein concentration on milk yield in any of the treatments (Table 4). The lack of response in milk production is consistent with others' observations that milk yield did not change when dietary protein was varied from 17.2 to 19.0% (Sannes *et al.* 2002), from 16.8 to 19.4% (Davidson *et al.* 2003) and from 16.7 to 18.4% (Broderick, 2003). In the study of Broderick (2003), milk yield decreased when dietary protein was reduced to 15.1%. Milk protein content, milk fat yield, lactose and 3.5% fat corrected milk (FCM) did not change in any of the treatments (Leonardi *et al.* 2003). Kung and Huber (1983) reported no change in milk fat or protein concentration with diets varying in protein from 11 to 17%, whereas Cunningham *et al.* (1996) showed increased milk fat and protein percentages and yields when dietary protein was

increased from 14.5 to 16.5%, but no changes when protein was further increased to 18.5%. Milk urea N concentration increased ($P<0.05$) with dietary protein in all 4 treatments (Table 5). Diets used in diet 3 contained less protein than those used in the other trials, resulting in the lowest milk urea nitrogen (MUN) and blood urea N concentrations. Analysis of combined data from all treatments showed that MUN linearly increased from 10.1 to 15.2 mg/dL (SEM 0.5) and blood urea N from 11.1 to 15.8 mg/dL (SEM 0.4)

as dietary protein was increased from the lowest to the highest levels. The observation on the relationship between dietary protein level and MUN and blood urea N concentrations is also consistent with those made in many other studies (Broderick and Clayton, 1997; Chapa *et al.* 2001; Sannes *et al.* 2002).

Apparent digestibility of DM increased ($P<0.05$) as the digestibility of fiber and protein content of the diet was increased (Table 6).

Table 3 Dry matter intake and BW of cows fed diets containing different amounts of protein in treatments utilizing different ratios of alfalfa hay to corn silage

Variable	Diets				SEM	P-value
	1	2	3	4		
DMI, kg/d	17.87 ^{bc}	17.46 ^c	18.44 ^{ab}	18.6 ^a	0.37	0.01
BCS	3.62	3.53	3.68	3.63	0.05	0.07
BCS change	0.2	0.22	0.33	0.23	0.04	0.20
BW, kg	570.8	570	571.3	571.3	2.50	0.27
BW gain, kg/d	16.68	15.89	17.15	17.14	2.56	0.27
Ruminal pH	6.71	6.61	6.63	6.65	0.14	0.30

The means within the same row with at least one common letter, do not have significant difference ($P>0.05$).

1. high protein, low potential digestible fiber, 2. mid protein, low potential digestible fiber, 3. low protein, high potential digestible fiber and 4. mid Protein, high potential digestible fiber.

DIM: days in milk, BCS: body condition score; BW: body weight.

SEM: standard error of the means.

Table 4 Milk yield and components of cows fed diets containing different amounts of protein in treatments utilizing different ratios of alfalfa hay to corn silage

Variable	Diets				SEM	P-value
	1	2	3	4		
Milk, kg/d	27.13	25.61	25.71	27.56	2.21	0.89
Milk corrected with 3.2	24.84	23.23	22.17	26.02	1.87	0.49
Fat, %	2.91	2.94	2.77	3.09	0.24	0.84
Fat yield, kg/d	0.78	0.74	0.68	0.86	0.06	0.36
protein, %	3.12	3.13	3.21	3.18	0.06	0.67
protein, kg/d	0.97	0.8	0.81	0.86	0.05	0.75
Lactose, %	4.83	4.89	4.84	4.91	0.07	0.86
Lactose, kg/d	1.33	1.32	1.22	1.34	0.01	0.83

The means within the same row with at least one common letter, do not have significant difference ($P>0.05$).

1. high protein, low potential digestible fiber, 2. mid protein, low potential digestible fiber, 3. low protein, high potential digestible fiber and 4. mid Protein, high potential digestible fiber.

SEM: standard error of the means.

Table 5 Milk urea N (MUN) and blood urea N (BUN) concentrations of cows fed diets containing different amounts of protein in treatments utilizing different ratios of alfalfa hay to corn silage

Item	Diets				SEM	P-value
	1	2	3	4		
MUN, mg/dL	15.2 ^a	14.3 ^{ab}	10.1 ^c	13.6 ^b	0.5	0.03
BUN, mg/dL	15.8 ^a	14.2 ^{ab}	11.1 ^c	13.3 ^b	0.4	0.02

The means within the same row with at least one common letter, do not have significant difference ($P>0.05$).

1. high protein, low potential digestible fiber, 2. mid protein, low potential digestible fiber, 3. low protein, high potential digestible fiber and 4. mid Protein, high potential digestible fiber.

SEM: standard error of the means.

The highest digestibility occurred with the 17% CP in treatment 4.

The digestibility was low for treatment 2. Wattiaux and Karg (2004b) showed no changes in DM digestibility when dietary protein was changed from 16.5 to 17.7%. Changes in the apparent digestibility of protein were not consistent either.

Decreasing dietary protein has been reported to result in decreases (Wright *et al.* 1998; Kauffman and St-Pierre, 2001; Broderick, 2003; Wattiaux and Karg, 2004b) or no changes (Noftsker and St-Pierre, 2003) in protein apparent digestibility. Fecal and urinary N concentrations increased linearly as dietary protein was increased for all treatments (Table 7).

Table 6 Apparent digestibility of nutrients in cows fed diets containing different amounts of protein in treatments utilizing different ratios of alfalfa hay to corn silage

Items	Diets				SEM	P-value
	1	2	3	4		
Dry matter (DM), %	67.67 ^{bc}	66.28 ^c	68.12 ^{ab}	69.54 ^a	1.48	0.04
Crude protein (CP), %	71.58	69.41	68.19	69.73	1.62	0.23

The means within the same row with at least one common letter, do not have significant difference ($P>0.05$).

1. high protein, low potential digestible fiber, 2. mid protein, low potential digestible fiber, 3. low protein, high potential digestible fiber and 4. mid Protein, high potential digestible fiber.

SEM: standard error of the means.

Table 7 Fecal and urinary N concentrations in cows fed diets containing different amounts of protein in treatments utilizing different ratios of alfalfa hay to corn silage

Items	Diets				SEM	P-value
	1	2	3	4		
Fecal N, %	2.87 ^a	2.74 ^{ab}	2.47 ^c	2.69 ^b	0.04	0.04
Urinary N, g/L	6.59 ^a	6.29 ^{ab}	5.17 ^{bc}	5.81 ^c	0.23	0.02

The means within the same row with at least one common letter, do not have significant difference ($P>0.05$).

1. high protein, low potential digestible fiber, 2. mid protein, low potential digestible fiber, 3. low protein, high potential digestible fiber and 4. mid Protein, high potential digestible fiber.

SEM: standard error of the means.

The result on urinary N concentration is consistent with several other studies that reported increased urinary N excretion with increased dietary protein (Tomlinson *et al.* 1996; Castillo *et al.* 2001).

CONCLUSION

Using 4 treatments that varied in the proportion of alfalfa hay and corn silage varying dietary protein from 15 to 19% did not affect milk yield but increased N excretion. Based on the data from all 4 treatments and the evaluation using the NRC (2001) ration formulation program, dietary protein at 17% was sufficient for cows producing 27 kg/d of milk and fed diets containing various proportions of alfalfa hay and corn silage. Reducing protein to this amount can reduce N excretion, especially environmentally labile urinary N, without affecting milk production.

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