Influence of Dietary Protein Concentration and Degradability on Performance of Lactating Cows During Hot Environmental Temperatures[1]

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ABSTRACT

To test effects of protein concentrations and degradability, 60 lactating Holstein cows in midlactation were offered the following diets during three trials between May and October in Tucson, AZ: high protein (18.4%), high degradability; high protein (18.5%), medium degradability; medium protein (16.1%), high degradability; and medium protein (16.1%), medium degradability. Diets comprised 39% alfalfa hay, 12% cottonseed hulls, 10% whole cottonseed, and 39% concentrate (DM) and concentrates contained 60, 40, 57, and 40% degradability, respectively, as determined by ficin assay. Rectal and ambient temperatures suggested that cows were under moderate to intense heat stress, as did group water intakes, which were increased about 15% by high degradability. Milk yields (3.5% FCM) and persistencies were lower for the high protein, high degradability diet than for all others. Mean DM intakes across treatments were quite high but were lower on high than medium protein; whereas ruminal ammonia and blood serum urea were higher on high protein. Milk composition, ruminal VFA, serum glucose, thyroxine, triiodothyronine, and cortisol were not affected by treatment.

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INTRODUCTION

Cattle under heat stress often have negative N balances (18) because of reduced intakes; hence, less protein is available for productive functions if dietary protein concentrations are not increased. However, too much dietary protein causes energy wastage due to excessive conversion of ammonia to urea (5, 29).

In a hot environment, Hassan and Roussel (12) observed increased feed intake and milk production in Holstein cows fed 21% CP compared with 14%. Zook (32) compared two protein solubilities (40 vs. 20%) in lactating cows subjected to heat stress or thermal neutral conditions and observed more milk on the less degradable protein during both hot and moderate conditions; but CP of rations was only 15%, somewhat lower than fed to many herds. Due to availability of high quality alfalfa hay in southwestern US, total CP in dairy cow diets is commonly as high as 19 to 20%, which often greatly exceeds NRC recommendations (25), particularly in hot weather when milk yields are low. The undegradable intake protein (UIP) in these diets could generally meet NRC recommendations of .35 to .37 because cottonseed meal, whole cottonseed, and sorghum grain are often principal ingredients.

Little information exists on the influence of excessive protein intake at varying degradabilities on milk yields and certain physiological measurements in lactating cows subjected to high environmental temperatures. The objective of this study was to compare diets containing two protein concentrations of two degradabilities for lactating cows subjected to hot summer temperatures.

MATERIALS AND METHODS

Trial 1

Twenty multiparous, midlactation Holstein cows of the University of Arizona herd at

Tucson were assigned to one of the following treatments in a 2 × 2 factorial arrangement: high protein, high degradability (HPHD); high protein, medium degradability (HPMD); medium protein, high degradability (MPHD); medium protein, medium degradability (MPMD). In high degradability diets, soybean meal and barley were major ingredients. These were replaced with corn gluten meal and milo for medium degradability diets (Table 1). Despite difference in dietary amino acid intakes from these protein supplements and grains, there are few data to suggest a difference in absorbed protein between diets and all essentially met guidelines for minimum UIP concentrations (25).

Allotment of cows to treatment was in randomized blocks, with blocks based on the 14-d pretreatment milk production. Insofar as possible, treatment groups were balanced for lactation number and days in milk (DIM). Cows received treatments for 40 d (May 8 to June 15, 1985). On average, diets (percentage of DM) consisted of 39% alfalfa hay, 10% whole cottonseed, 12% cottonseed hulls, and 39% concentrate and contained approximately 1.65 Mcal ME/kg DM (25) and 26% ADF, .76% Ca, and .36% P. Days in milk at the initiation of the study for the respective treatment groups were: 143, 155, 156, and 147.

One-half of the daily allotment of concentrate (1 kg/3 kg milk produced during pretreatment) was offered at 0600 h and the other half at 1800 h. In addition, cottonseed hulls and whole cottonseed were blended daily with concentrate. Alfalfa hay was fed ad libitum at 1800 h. Orts were determined daily at 1600 h. Concentrate was not reduced commensurate to decreases in milk yields to avoid bias favoring a treatment that might initially result in higher production. Moreover, because trial periods were only 6 to 7 wk, reducing concentrate during treatment was deemed inadvisable.

Cows were fed individually in electronic headgates (American Calan, Inc., Northwood, NH), and orts were weighed daily. Animals were kept in groups of five in open lots under metal-roofed shades. Weather data were recorded at the University of Arizona Campbell Experimental Farm Weather Station located approximately 1.0 km from the Dairy Research Center. Temperatures were measured with thermocouples placed in a shaded area and shielded from solar radiation. Rectal temperatures were determined weekly in an unshaded lane between 1300 and 1500 h for all cows using battery-operated digital read-out thermometers. Respiration rates, measured by counting breaths per 20 s, were determined while cows were resting under shades.

Cows were milked twice daily at 0500 and 1700 h, and milk yields were recorded at each milking. Cows were weighed once at 1000 h during the second and last week of the trial. Water intakes for treatment groups were measured by flow meters installed in each pen of 5 cows.

Milk samples from p.m. and a.m. milkings for each cow were collected once weekly, and the daily composite was sent to the Arizona DHIA laboratory in Phoenix for determination of fat and protein percentages by infrared analysis using a no. 13 filter. Feeds offered (concentrate and alfalfa hay) were sampled at regular intervals and composites were stored at −5°C until analyzed. Crude protein, Ca, and P were determined calorimetrically using a Technicon autoanalyzer. Protein degradability was measured according to ficin protease assay (27). Acid detergent fiber was determined according to Goering and Van Soest (9).

In the last week of the experiment, rumen contents were sampled by stomach tube 2 to 3 h after the a.m. feeding from three cows randomly selected from each treatment. Rumen fluid was strained through four layers of cheesecloth and a 10-ml aliquot was acidified with .5 ml of .1 N HCl and centrifuged at 2000 × *g* for 20 min. Supernatant was frozen for later analysis of ammonia nitrogen according to Chaney and Marbach (3) and for VFA by GLC with a 80/120 Carbopack B-DA/4 Carbowax 20 m column. Samples of coccygeal vein blood were obtained at biweekly intervals. Blood was centrifuged at 2000 × *g* and serum frozen at −5°C until analyzed for urea N using the phenol-hypochlorite procedure (1), glucose by the hexokinase method (Sigma Chemical Co., St. Louis, MO), and thyroxine (T_4), triiodothyronine (T_3), and cortisol by double-antibody radioimmunoassay (Diagnostic Product Corp., Los Angeles, CA).

Trial 2

Based on 14-d pretreatment milk yields, 24 multiparous Holstein cows in midlactation were

TABLE 1. Ingredient and nutrient composition of feeds used in the study (%).[1]

Item	Trial 1 Concentrate				Trial 2 Concentrate			
	HPHD	HPMD	MPHD	MPMD	HPHD	HPMD	MPHD	MPMD
Ingredients, % of DM								
Ground corn	56.4	57.0	57.7	58.0	54.9	54.6	58.9	64.0
Barley[2]	12.9	. . .	28.7	. . .	24.2	. . .	31.6	. . .
Sorghum grain[2]	. . .	12.3	. . .	26.0	. . .	27.5	. . .	26.2
Soybean meal	24.9	. . .	7.6	. . .	15.6	. . .	4.0	. . .
Corn gluten meal	. . .	24.9	. . .	9.1	. . .	12.6	. . .	4.3
Animal fat	3.8	3.3	4.0	4.0	3.3	3.3	3.5	3.5
Mineral-vitamin mix[2]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Nutrients, % of DM								
Crude protein	22.0	24.1	15.0	15.4	16.4	16.4	11.5	11.7
ADF	4.4	4.0	3.9	4.5	5.4	4.7	5.0	4.5
Ca	.19	.23	.21	.20	.19	.15	.14	.17
P	.50	.46	.47	.45	.45	.43	.40	.42
NE_l, Mcal/kg	2.06	2.05	2.07	2.04	2.05	2.03	2.06	2.02

Item	Trial 3 Concentrate				For all trials		
					Alfalfa	Cottonseed	
	HPHD	HPMD	HPHD	MPMD	Hay	Whole	Hulls
Ingredients, % of DM							
Ground corn	41.4	53.6	50.0	75.8	. . .	. . .	. . .
Barley[2]	20.4	. . .	32.3	. . .	. . .	. . .	. . .
Soybean meal	27.9	. . .	7.6	. . .	. . .	. . .	. . .
Cottonseed meal	. . .	14.7	. . .	3.5	. . .	. . .	. . .
Brewers dried grains	. . .	16.4	. . .	8.3	. . .	. . .	. . .
Meat and bone meal	. . .	7.3	. . .	2.8	. . .	. . .	. . .
Animal fat	3.5	3.5	3.3	3.3	. . .	. . .	. . .
Mineral-vitamin mix[4]	6.8	4.5	6.8	6.3	. . .	. . .	. . .
Nutrients, % of DM							
Crude protein	21.3	20.4	12.9	13.1	20.4	23.5	5.9
ADF	5.8	8.4	5.2	5.0	32.0	44.5	66.0
Ca	. . .	. . .	. . .	. . .	1.69	.23	.18
P	.67	.77	.65	.37	.23	.53	.12
NE_l, Mcal/kg	1.96	1.88	1.96	1.91	1.42	2.23	.98

[1]HP = High protein; MP = medium protein; HD = high degradability; MD = medium degradability. Concentrates were fed at 39% of DM, with alfalfa hay, cottonseed hulls and whole cottonseed at 39, 12 and 10%, respectively.

[2]Barley and sorghum grain were dry rolled.

[3]Comprised 1% trace-mineral (TM) salt, .2% $CaCO_3$, .6% dicalcium phosphate, and 11,500, 6600, and 15 IU of vitamins A, D, and E/kg.

[4]Comprised 1.3% TM salt, .4% MgO, .8% $NaHCO_3$, 1.7% Bentonite, .8% limestone (except MD diets), 1.5% dicalcium phosphate (except HPMD), and vitamins similar to Trials 1 and 2.

assigned to the four treatment groups similar to Trial 1. Treatments were for 50 d from August 22 to October 8, 1985. Average DIM on August 22 for the respective groups were: 104, 87, 121, 115. Feeding methods, diets, weighing, feed sampling and analysis, and other management practices were similar to Trial 1, except percentage protein was reduced about 1.2% for all rations (Table 2) because of lower initial milk production of cows (28 vs. 34 kg/d). Rumen and blood were not sampled during Trial 2 due to time and labor constraints.

Trial 3

Sixteen multiparous Holstein cows in midlactation were assigned to the same treatments in a manner similar to Trial 1. Treatment diets were fed for 47 d from July 25 to September 10, 1986. Average DIM on July 25 for the respective treatments were: 147, 159, 164, and 132. Feeding strategy, weighing, and feed analyses were similar to Trials 1 and 2. The medium degradability concentrates in Trial 3 contained dried brewers grains and cottonseed meal for less degradable protein sources (Table 1) because of a temporary unavailability of corn gluten meal. Blood and rumen sampling and analyses were similar to Trial 1.

Statistical Analysis

Least square means for milk production and composition, DM intake, and feed efficiency (FCM yield/DM intake) data were analyzed by covariate ANOVA as described by Gill (8). Orthogonal contrasts testing main effects and interactions were made for individual trials and pooled data. Another set of orthogonal contrasts tested the following effects: HPHD vs. all other treatments, HPMD vs. MPHD and MPMD, and MPHD vs. MPMD. Because no significant differences (P>.10) were observed for the latter two comparisons, probabilities of differences are not presented. Analysis of pooled performance data from the three trials was according to the following model:

$$Y_{ijkl} = tr_i + B\ (X - X) + t_k + e_{ijkl}$$

where tr = trial, B = regression coefficient of pretreatment, and t = treatment. Rumen and blood data were pooled for Trials 1 and 3 and analyzed as a least squares ANOVA (8).

TABLE 2. Crude protein percentages and estimated protein degradabilities of diets for the different trials.

	Diet[1]			
Item	HPHD	HPMD	MPHD	MPMD
Crude protein, % of DM				
Trial 1	19.2	19.9	16.5	16.6
Trial 2	17.1	17.1	15.1	15.1
Trial 3	18.9	18.6	16.6	16.7
Mean	18.4	18.5	16.1	16.1
Degradability in the rumen, % of CP[2]				
Trial 1	64.6	57.4	64.7	59.6
Trial 2	65.0	58.9	64.8	60.0
Trial 3	66.0	58.5	65.0	61.3
Mean	65.3	58.3	65.0	60.3

[1]HP = High protein; MP = medium protein; HD = high degradability; MD = medium degradability.

[2]Calculated from undegradable protein estimates (25).

RESULTS AND DISCUSSION

Ingredients of concentrates and nutrient content of feed used are in Table 1. Because the alfalfa and concentrate were fed separately, nutrient composition of diets is an arithmetic composite of the two components based on chemical analyses and percents used. Mean CP of the three trials approached predicted values and is given in Table 2. Degradable protein as determined by ficin assay (27) averaged 60, 40, 57, and 40% for total diets, which is lower than when calculated from NRC (25) based on ingredient composition (65, 58, 65, and 60%). However, degradability values determined by ficin assay were within the range of in vivo values reported in ruminant protein usage (26) for similar mixed diets fed to dairy cows.

Because of a possible interaction of heat stress with dietary K and Na (2, 30), concentrations of these minerals were calculated from dietary ingredients (24). Range for K was 1.20 to 1.39% with highest concentrations for the HPHD diet and lowest for HPMD. Dietary Na averaged .28% with no difference between diets. Mean concentrations of K and Na were approximately 30% higher than NRC recommendations (25).

Weekly averages of maximum and minimum ambient temperatures for the three trials were 35.2 and 18.2°C, respectively, with somewhat different patterns for the different trials

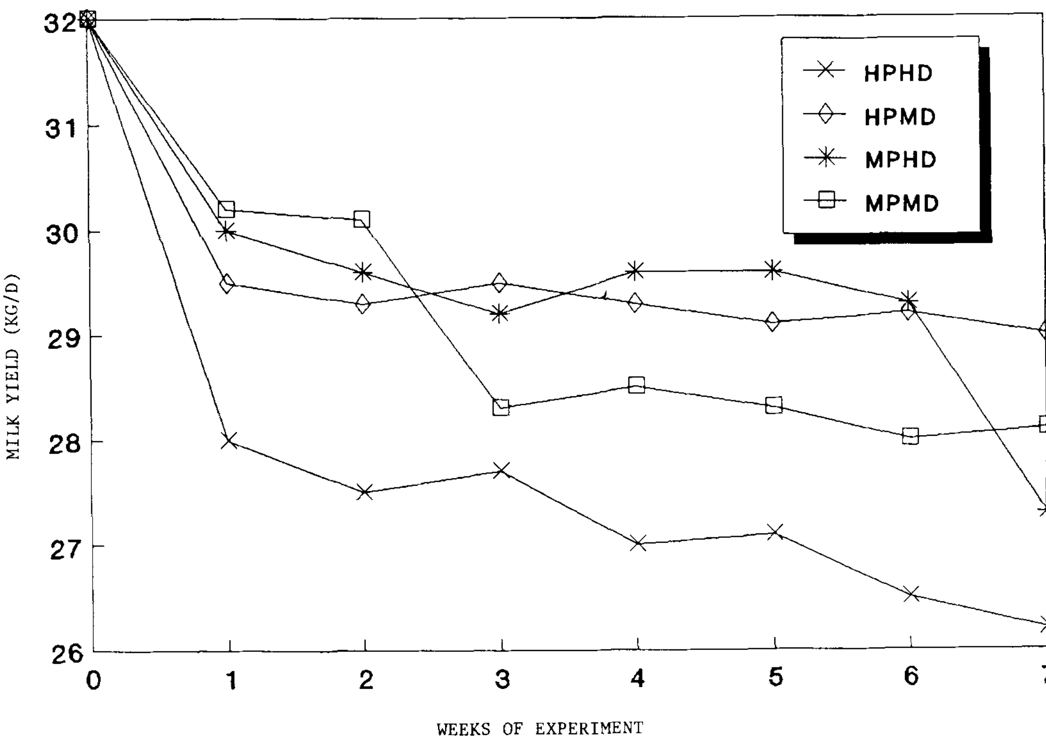

Figure 1. Weekly milk production of cows fed rations varying in high (HP) or medium (MP) protein level and high (HD) or medium (MD) degradability during warm weather (data pooled from Trials 1, 2, and 3).

(Table 3). During Trial 1, there was a warming trend in early summer, whereas a cooling trend was observed for Trials 2 and 3, which were during late summer. However, all maxima exceeded the thermal neutral zone for dairy cattle (22). Even though Trial 2 extended into early October, maximum temperatures during the last week reached as high as 36°C.

Maximum daily temperatures frequently exceeded 40°C during all trials with 55% of days over 35°C and 84% over 32°C. Hourly temperature data, available only for Trial 3, showed that ambient temperatures exceeded 30°C (considered above the thermal neutral zone for cattle [22]), an average of 10 h/d. The temperature-humidity index (THI) was also calculated (17) for Trial 3. The high maximum THI (ranging from 77.6 to 82.6) confirm that cows were thermally stressed. Johnson and Vanjonack (17) suggested milk yields started to decline at a THI above 73. Data in Table 3 show that THI exceeded 73 from 12 to 22 h/d during all of Trial 3.

Except for the HPHD cows in Trial 1, rectal temperatures were not affected by protein treatment and reflect the hot environmental temperatures, particularly during Trial 3. In a recent study involving 46 cows at the same location, managed under similar conditions, Gomez-Alarcon (10) observed mean weekly temperatures of 38.4°C during February, March, April, and 39.4°C during June, July, August. In 11 of 12 comparisons, treatment means for the summer months exceeded 38.4°C. Highest water intakes were shown for cows fed HPHD, which might have had a depressing effect on rectal temperatures. Moreover, mean rectal temperatures in our trials were in the upper range (39.4°C) given for dairy cows by Swenson (28).

Respiration rates were not affected by treatment, but were two- to threefold greater than suggested as normal for adult cows (28). The higher values in Trial 3 than 1 and 2 reflect slightly higher ambient temperatures and higher relative humidities.

Water intakes in Trials 1 and 2 corresponded to the hot ambient temperatures to which cows

TABLE 3. Mean weekly ambient temperatures, relative humidities, temperature-humidity index, and heat-stress indices for cows in Trials 1, 2, and 3.

	Ambient temperatures						Relative humidity				THI[1]	
	Maximum			Minimum			Trial 2		Trial 3		Trial 3	
Week of trial	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	0500	1700	0500	1700	High	Hours >73
	(°C)						(%)					
1	30.6	39.4	38.9	11.7	23.0	20.5	56	26	49	8	80.1	14
2	33.1	37.9	37.6	13.8	21.3	23.5	65	24	56	16	81.4	19
3	35.5	33.5	35.8	13.9	15.6	23.8	54	20	80	31	81.7	22
4	33.4	35.7	37.3	14.1	17.2	23.7	49	23	76	25	82.6	22
5	39.3	29.8	32.8	18.9	13.2	21.8	57	20	86	46	79.5	15
6	40.6	30.4	34.5	18.3	13.8	20.9	61	30	87	26	79.3	15
7	. . .	32.3	35.4	. . .	13.3	22.1	50	24	50	22	77.6	12

	Heat-stress indices				
	HPHD	HPMD	MPHD	MPMD	SE
Rectal temperature, °C					
Trial 1	38.4[a]	38.9[b]	38.8[b]	38.8[b]	.17
Trial 2	39.0	39.0	39.0	39.0	.15
Trial 3	39.0	39.3	39.3	39.5	.16
Respiration rate, breaths/min					
Trial 1	57.7	53.0	57.8	55.2	2.30
Trial 2	63.0	60.4	58.2	64.1	2.90
Trial 3	90.2	87.9	90.9	94.7	3.43
Water intake, L/d					
Trial 1	149.3	125.1	137.8	129.7	. . .
Trial 2	141.9	122.3	133.6	118.9	. . .

[a,b]Means in rows not sharing similar superscripts differ ($P<.05$).

[1]THI = Temperature-humidity index calculated according to Howard et al. (16). Thermal stress in lactating cows commences when the THI exceeds about 73 (16).

TABLE 4. Least square means for milk yields, milk persistencies, milk composition, dry matter intakes (DMI), and feed efficiencies of cows fed rations varying in protein level and degradability.

	Treatment[1]					Orthogonal contrasts			
	HPHD	HPMD	MPHD	MPMD	SE	HP vs. MP	HD vs. MD	IA[1]	HPHD vs. all others
						P			
Trial 1,[2] 5 cows/treatment									
Milk, kg/d	28.3	30.0	31.5	31.1	1.2	.08	.58	.41	.08
3.5% FCM, kg/d	25.5	29.0	29.9	29.5	1.1	.04	.16	.07	.01
Milk fat, %	2.9	3.3	3.2	3.2	.2	.61	.32	.22	.12
Milk protein, %	3.0	3.1	3.2	3.1	.1	.60	.94	.54	.55
DMI, kg/d	22.4	23.3	26.3	25.6	1.1	.01	.95	.44	.05
Milk persistency[3]	84.0	88.5	93.2	92.2	3.3	.07	.60	.39	.07
Feed efficiency, milk/DMI	1.1	1.2	1.1	1.2	.1	.48	.34	.40	.53
Trial 2,[2] 6 cows/treatment									
Milk, kg/d	24.5	28.1	25.9	26.1	1.4	.85	.21	.25	.21
3.5 FCM, kg/d	19.7	23.9	22.3	22.5	1.7	.72	.20	.23	.11
Milk fat, %	2.4	2.6	2.6	2.7	.2	.50	.41	.18	.27
Milk protein, %	2.9	2.8	2.9	3.0	.1	.26	.64	.40	.92
DMI, kg/d	21.2	22.6	22.4	22.9	.8	.36	.28	.51	.14
Milk persistency[3]	84.6	97.2	90.2	90.5	4.9	.92	.20	.22	.17
Feed efficiency, milk/DMI	.9	1.1	1.0	1.0	.1	.90	.34	.30	.28
Trial 3,[2] 4 cows/treatment									
Milk, kg/d	27.5	28.2	28.7	28.9	1.9	.63	.82	.97	.62
3.5% FCM, kg/d	25.9	27.4	27.4	26.4	2.1	.91	.91	.52	.65
Milk fat, %	3.1	3.3	3.2	3.0	.2	.64	.86	.33	.85
Milk protein, %	3.2	3.3	3.3	3.4	.1	.37	.74	.76	.40
DMI, kg/d	20.2	18.6	20.5	20.0	.7	.26	.19	.41	.56

Milk persistency[3]	81.0	83.0	84.2	84.7	5.5	.69	.81	.86	.64
Feed efficiency, milk/DMI	1.3	1.5	1.3	1.3	.1	.67	.40	.25	.37
Pooled data,									
15 cows/treatment									
Milk, kg/d	26.5	28.7	28.5	28.4	.8	.32	.24	.20	.04
3.5% FCM, kg/d	23.5	26.8	26.5	26.1	.9	.25	.13	.06	.01
Milk fat, %	2.8	3.1	3.0	3.0	.1	.62	.28	.17	.08
Milk protein, %	3.1	3.1	3.2	3.1	.1	.13	.86	.85	.37
DMI, kg/d	21.2	21.6	23.0	22.8	.5	.01	.85	.54	.04
Milk persistency[2]	83.3	90.4	89.6	89.0	2.6	.34	.21	.17	.04
Feed efficiency, milk/DMI	1.1	1.3	1.2	1.2	0	.45	.10	.07	.11

[1]HP = High protein; MP = medium protein; HD = high degradability; MD = medium degradability; IA = interaction (protein vs. degradability).

[2]Trials 1, 2, and 3 were conducted from May 8 to June 15, 1985, August 22 to October 8, 1985, and July 25 to September 10, 1986, respectively.

[3]Treatment/pretreatment × 100.

were subjected. They averaged 20% higher than for cows on similar treatments in moderate weather (15), which produced about 25% more milk and were 30% higher than values calculated from NRC (25). Even though water intakes were on a group basis, they tended to be increased on diets of higher rumen degradability, a trend also observed by Higginbotham et al. (15).

Overall, milk yields were quite persistent considering the hot ambient temperatures and the advanced stage of lactation of some cows. Treatment trends in milk and FCM production and milk persistency for the three trials were similar, although not significant for all individual trials (Table 4); so data were pooled to understand diet effects more clearly.

Actual milk, 3.5% FCM, and milk persistencies did not differ due to protein concentration and degradability but were lowest ($P<.04$) for cows fed HPHD when contrasted with all other treatments. Other contrasts (HPMD vs. MPHD and MPMD or MPHD vs. MPMD) were not significant. Mean milk yields for HPHD (averaged for the three trials) dropped below other treatments in the 1st wk and continued lower throughout the entire experimental period (Figure 1). Mean body weight changes for the respective treatments were not different ($P>.10$) (.07, .02, .38, and .11 kg/d), but tended to be greater for medium protein, perhaps because the higher intakes on medium protein resulted in greater fill at weighing.

Even though ingredients used to attain high protein degradability in Trials 1 and 2 (barley and soybean meal vs. milo and corn gluten meal) increased rumen starch fermentability (14), this change should not have contributed to lower milk yields and intakes of the HPHD group. Hassan and Roussel (13) showed that combining protein and starch sources, which degraded rapidly in the rumen, resulted in higher milk yields than combining sources of slower degradability or of differing degradabilities.

Decreases in milk yields on the HPHD diet contradict reports (13, 19, 32) where high crude or degradable protein fed during heat stress increased milk yields. However, cows in our study were subjected to higher ambient temperatures than in other studies. Moreover, degradabilities of CP were not varied (13, 19) and protein intakes were probably in greater excess

TABLE 5. Rumen VFA, NH_4, and blood serum urea nitrogen (BUN), glucose, triiodothyronine (T_3), thyroxine (T_4), and cortisol of cows fed varying protein concentrations and degradabilities during hot weather.[1]

Item	Treatment				SE
	HPHD	HPMD	MPHD	MPMD	
Rumen					
Acetate (A), mol/100 mol	63.0	62.0	63.7	60.3	1.8
Propionate (P), mol/100 mol	21.2	17.2	21.4	24.1	2.3
Butyrate, mol/100 mol	12.1	15.8	11.0	12.1	1.3
A:P	3.0	3.6	3.0	2.5	.4
NH_4, mg/dl[2]	13.4	16.6	11.2	8.0	1.9
Blood serum					
BUN, mg/dl[3]	14.0	10.3	9.8	7.2	.7
Glucose, mg/dl	58.5	63.8	60.8	60.5	4.6
T_3, ng/ml	1.06	.94	1.02	.96	.08
T_4, ng/ml	38.6	32.0	37.8	36.7	3.34
Cortisol, ng/ml	1.10	2.73	2.10	1.83	.68

[1]Mean of seven cows per treatment (three in Trial 1, four in Trial 3). HP = High protein, MP = medium protein, HD = high degradability, MD = medium degradability.

[2]Protein effect ($P<.05$).

[3]Protein and degradability effects ($P<.01$).

of requirements in our study. A parallel study in moderate weather during May and June 1986 in Provo, UT (15) showed higher milk yields on HPHD when the same protein comparisons were made. However, cows were fed different diet ingredients and were milked three times daily; therefore, protein intakes on HD diets averaged 27% above suggested requirements (25) compared with 34% in this study.

Reduction of 3.5 FCM yield for HPHD compared with all other treatments averaged 3.2 kg/d, which is equivalent to about 2.2 Mcal NE_l (25). For the respective treatments, mean CP intake in excess of requirements (25), determined at initiation of treatment, averaged 31, 37, 18, and 16%, equal to 144, 171, 85, and 80 g N/d. Assuming an additional energy expenditure for urea synthesis of 5.5 Kcal/g N (20), the 62 g/d more N consumed on HPHD than the MP diets would have accounted for only 341 kcal additional energy or about 16% of difference in milk energy produced. The 1.8 kg/d lower DM intakes of HPHD compared with MP cows equalled 2.9 Mcal NE_l and might explain the lower milk yields, but intakes were also decreased on HPMD. Why consumption was lower on HP than MP diets is not clear. Even though initial protein intake on HPMD exceeded calculated requirements more than HPHD did, the HPMD diet did not depress milk yields and resulted in greatest efficiency of feed utilization.

Danfaer et al. (5) reported a reduction in milk energy output of 1.08 Mcal net energy/d when dietary CP increased from 19 to more than 23% of DM. The calculated net energy cost used for synthesizing urea and excreting excess N was 10 Mcal/d. Increased CP in our study comparing medium to high protein diets was approximately 2.4% and might partially account for the depressed milk yields observed for HPHD.

Percentages of fat in milk were somewhat low (Table 3), but other studies have shown decreased fat percentage during hot weather (21, 22). Although not significant, milk fat percentage was lower for HPHD than other treatments, which contributed to larger differences in FCM than in unadjusted milk yields.

Milk protein percent was not different among treatments, which agrees with other studies where protein varied in diets fed lactating cows (6, 16). Hassan and Roussel (13) noted higher milk protein for diets of 21% CP vs. 14% when fed during heat stress, but differences in dietary protein in our study were less.

The HP diet decreased ($P<.10$) intakes in Trial 1 and pooled data (Table 4). Generally, increases in DM intake occurred as the CP in rations increased to about 18% (4, 11, 18, 23) but not always (7, 16). Our results are not in agreement with other studies (13, 32), which noted higher intakes when crude or soluble protein was increased in diets fed heat-stressed cows. Feed efficiency was apparently affected by treatment, tending to be lower for the HPHD diet ($P<.07$).

High protein diets resulted in higher ruminal ammonia ($P<.01$) than from low protein diets (Table 5). Surprisingly, MPMD was numerically higher in ammonia than HPHD. Large variations due to possible salivary contamination of rumen samples, even though initial rumen collections were discarded at each sampling, or varying ammonia at different rumen sampling sites (31) might explain this apparent discrepancy. Protein amount and degradability affected blood urea nitrogen (BUN) ($P<.01$), which is often a more sensitive index of dietary protein than rumen ammonia (18). Hammond et al. (12) also found BUN related to amount and solubility of dietary protein. Relatively low BUN in heat-stressed cows is often attributed to decreased feed intakes (21). However, intakes were relatively high for cows fed MP diets in this experiment but were reduced by HP.

Serum glucose, T_3, T_4, and cortisol were not significantly affected by treatment (Table 4), but the three hormones, often diminished during heat stress (6, 17), were much lower than in cows fed diets of similar protein concentrations and degradabilities in a cooler environment (15).

Results of this study show that high protein diets (18.5%) of high rumen degradability decreased milk yields in midlactation cows subjected to hot summer temperatures compared with high protein diets of lower degradability or lower protein diets (16.1%) of high or medium degradability.

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