Ruminal Digestion and Fermentation of High-Producing Dairy Cows with Three Different Feeding Systems Combining Pasture and Total Mixed Rations

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ABSTRACT

Six multiparous Holstein cows fitted with rumen cannulas were used to study the effect of three feeding systems combining pasture and total mixed rations (TMR) on ruminal digestion in a 21-wk repeated measures experiment. The three treatments were: 1) pasture plus concentrate (PC), 2) pasture plus partial TMR (pTMR), and 3) TMR (nonpasture). Ruminal $\text{NH}_3\text{-N}$ concentration was lower on both the pTMR and TMR treatments (10.2 ± 0.5 mg/dL) than on the PC treatment (19.9 ± 0.5 mg/dL). Ruminal pH was not affected by treatments and averaged 5.87. Neither total volatile fatty acid concentration (137.5 mmol/L) nor individual volatile fatty acid proportions (63.1, 20.6, and 12.0 mol/100 mol for acetate, propionate, and butyrate, respectively) differed among treatments. The pTMR treatment reduced the total potentially degradable fraction of dry matter (85.5 vs. 82.3%) and the potentially digestible fraction of neutral detergent fiber (82.1 vs. 74.9%) of pasture compared to the PC treatment. Ruminal $\text{NH}_3\text{-N}$ losses were reduced when combining pasture and TMR; however this combination decreased the ruminal digestion of pasture, indicating the presence of associative effects in the rumen.

(**Key words:** pasture, partial total mixed ration, rumen fermentation, in situ digestion)

Abbreviation key: ED = effective degradability, PC = pasture plus concentrate, pTMR = pasture plus partial TMR.

INTRODUCTION

When pasture plus concentrate is fed to high genetic merit cows, milk production is lower than typically

found in confinement feeding systems based on a nutritionally balanced TMR where DMI and milk production are maximized (White, 2000; White et al., 2001; Bargo et al., 2002b). Studies with high producing dairy cows on pasture (Reis and Combs, 2000; Soriano et al., 2000; Bargo et al., 2002a) reported that energy supplementation with 8 to 9 kg/d of corn-based concentrates resulted in total DMI of 22 kg/d and milk production of 30 kg/d. This lower animal performance compared with non-grazing systems may be related to changes in ruminal fermentation and digestion when cows grazed good-quality pasture. Pasture-based diets with high-quality pastures (<50% NDF) and concentrate supplementation are often characterized by reduced rumen pH (<6.0), low acetate/propionate ratio, high $\text{NH}_3\text{-N}$ concentration, and high rate of passage (Bargo et al., 2001; Holden et al., 1994; Rearte and Santini, 1993).

Supplementing a pasture-based feeding system with a TMR may improve performance through changes in rumen digestion and fermentation. This system is called partial TMR (pTMR) because the pasture grazed by the cows is not physically part of the TMR. Feeding a pTMR result in higher performance because of higher total DMI and milk production with a higher fat and protein contents (Bargo et al., 2001b). Potential positive effects on rumen fermentation could include a higher or a more stable rumen pH and a reduction in rumen $\text{NH}_3\text{-N}$ concentration because of the inclusion in the diet of forage sources higher in effective fiber and lower in CP content than pasture. However, with the combination of pasture and TMR, associative effects may occur in the rumen such as a reduction in NDF digestion of pasture (Dixon and Stockdale, 1999).

Previous studies have compared animal performance of high-producing dairy cows on TMR vs. pasture as the only feed (Kolver and Muller, 1998; Kolver et al., 2000), TMR vs. pasture plus concentrate (White, 2000; White et al., 2001), or TMR vs. TMR plus pasture (Soriano et al., 2001). However, those studies did not evaluate rumen digestion. The objective of this study was to compare rumen digestion and fermentation of high-producing dairy cows fed pasture plus concentrate,

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Table 1. Chemical composition of hand-plucked pasture samples during the four intake measurement periods.

	May	June	August	September	$\bar{X}$	SEM
DM, %	19.9 ^a	17.0 ^{ab}	15.7 ^b	17.1 ^{ab}	17.4	1.0
OM, % of DM	93.2 ^a	92.9 ^a	90.9 ^b	91.1 ^b	92.1	0.2
CP, % of DM	25.5 ^a	26.2 ^a	29.5 ^b	23.9 ^a	26.3	0.9
Soluble CP, % of DM	6.4	6.3	7.2	6.2	6.5	0.5
NSC, % of DM	13.0 ^a	14.1 ^{ab}	13.5 ^{ab}	14.9 ^b	13.9	0.6
NDF, % of DM	53.5 ^a	52.1 ^a	46.5 ^b	47.5 ^b	49.9	1.0
ADF, % of DM	25.9 ^{ab}	27.4 ^a	25.0 ^b	26.1 ^{ab}	26.1	0.6
IVDMD, % of DM	57.6	57.5	58.3	58.5	58.0	1.2

^{a,b,c}Least square means in the same row with different superscripts differ ($P < 0.05$).

pTMR, and TMR corresponding to feeding regimens in a companion study (Bargo et al., 2002b).

MATERIALS AND METHODS

Cows and Treatments

Six multiparous Holstein cows fitted with ruminal cannulas [BW, 653 ± 79 kg; milk yield, 43.7 ± 9.2 kg/d; parity, 3.0 ± 0.9 ; DIM, 115 ± 24 (mean $\pm$ SD)] were used in a 21-wk trial to study the effect of three feeding systems on rumen digestion and fermentation. Cows were selected from The Pennsylvania State University Dairy Cattle Research and Education Center (University Park, PA), which averaged 11,436 kg of milk and 363 kg of protein per cow per lactation in 1999. Cows were grouped in two groups of three by lactation number and DIM, and randomly assigned to three dietary treatments: 1) pasture plus concentrate (PC), 2) pasture plus TMR or partial TMR (pTMR), and 3) TMR (nonpasture). The performance of 45 cows used in a lactation study is reported in a companion paper (Bargo et al., 2002b).

Management and feeding of cows on the PC, pTMR, and TMR treatments were described by Bargo et al. (2002b). The chemical composition of the pasture grazed by cows on the PC and pTMR treatments is shown in Table 1. The chemical composition of the concentrate used to supplement the cows on the PC and pTMR treatments, and the TMR fed to the cows on the pTMR and TMR treatments was previously reported (Bargo et al., 2002b).

Experimental Measures and Sample Analyses

Dry matter intake was measured during wk 2 (May 15), 6 (June 12), 14 (August 7), and 19 (September 11) using the Cr_2O_3 method as described by Bargo et al. (2002b). Pasture samples were analyzed for DM, CP, soluble CP, ADF, NDF, NSC, and ash using the procedures detailed in Bargo et al. (2002b).

Ruminal fluid samples were collected on wk 3 (May 26), 7 (June 23), 15 (August 18), and 20 (September 22) at 0, 4, 8, 12, 16, and 20 h beginning at 0500 h on each of those 4 d. The concentrate was red at 0600 and 1800 h for the PC treatment, and at 0600 h for the pTMR treatment. The TMR was fed at 0730 h for the TMR treatment, and at 1800 h for the pTMR treatment. At the 4 (0900 h), 8 (1300 h), 16 (2100 h), and 20 h (0100 h) sampling times, the two cows on the PC treatment were sampled in the pasture paddock using a temporary corral made with mobile gates. At the 4 (0900 h) and 8 h (1300 h) sampling times, the two cows on the pTMR treatment were sampled in the pasture with the PC treatment. At the 0 (0500 h) and 12 h (1700 h), cows on the PC and pTMR treatments were sampled indoors because these two sampling times coincided with the time of milking. At the 16 (2100 h) and 20 h (0100 h) sampling times, the two cows on the pTMR treatment were also sampled indoors. Cows on the TMR treatment were sampled indoors at all sampling times. Ruminal digesta samples were taken from the dorsal, ventral, and caudal areas of the rumen and squeezed through four layers of cheesecloth. The pH of the filtered ruminal fluid was measured immediately using a portable digital pH-meter, and a 15-mL aliquot preserved with 3 mL of 25% metaphosphoric acid and 3 mL of 0.6% 2-ethyl butyric acid (internal standard). These samples were stored at -20°C and subsequently analyzed for $\text{NH}_3\text{-N}$ and VFA (Yang and Varga, 1989).

The in situ technique was used on wk 3 (May 25), 7 (June 22), 15 (August 17), and 20 (September 21) to estimate ruminal disappearance of DM, CP, and NDF of pasture, concentrate, and TMR on the different treatments. Pasture and concentrate samples were incubated in the cows on the PC and pTMR treatments. Samples of TMR were incubated in the cows on the TMR treatment. Hand-plucked pasture, concentrate, and TMR samples were taken the day before the bags were incubated. To simulate cows chewing, fresh pasture samples were cut into lengths of approximately 1 cm, while concentrate and fresh TMR samples were

processed using a meat-grinding machine for 1 min. Approximately 5 g of DM of cut pasture, processed concentrate, and TMR samples were placed in polyester bags (22.9 cm × 10.2 cm; Marvelier White, Strauss Co., New York, NY) with a mean pore size of 52 μ m. Before use, the bags were dried in a forced-air oven at 55°C for 48 h. Filled bags were closed with a plastic tie 2 cm below the top, resulting in an effective surface area of 20.9 cm × 10.2 cm. Bags were tied to the end of a 100-cm nylon line and incubated in the rumen of the six rumen-cannulated cows for 0, 4, 8, 12, 16, 20, 24, and 48 h after being soaked in 39°C distilled water for 15 min beginning at 0500 h. Bags were inserted in reverse order, and the 24, 20, 16, 12, 8, and 4 h incubation times coincided with rumen fluid sampling times. Before incubation, the filled bags were maintained at 4°C. Duplicate bags were incubated at all times, except at 24 and 48 h when triplicate bags were incubated. An alfalfa hay standard (4-mm ground) was incubated in duplicated bags at 4, 24, and 48 h and was used to monitor variation within and among cows. All standards were within a previously established range (coefficient of variation <10%) and no time points required correction. Duplicate bags with no sample (blanks) were incubated at 4, 24, and 48 h and used to adjust for material entering the bags. After retrieval at 0 h, bags were immediately washed in a washing machine (three cycles; 2-min fill and 2-min rinse per cycle, followed by a final spin; Lykos and Varga, 1995) and brought to the laboratory in an insulated thermos. Bags were then opened, and the contents were washed with cold running water inside the bags with gentle agitation for 1 min and squeezed. Bags were dried at 55°C for 48 h, weighed to determine DM disappearance, and the residue ground through a 1-mm screen (Wiley Mill; Thomas Scientific, Philadelphia, PA). Duplicate or triplicate (24 and 48 h bags) residues at each time point were composited within cow and analyzed for DM and CP (AOAC, 1990) and NDF (Ankom Daisy II, ANKOM Technology Corp., Fairport, NY). Unincubated samples of pasture, concentrate and TMR used to fill the bags were also analyzed for DM and CP (AOAC, 1990) and NDF (Ankom Daisy II).

Dry matter, CP, and NDF disappearance of pasture and TMR samples, and DM and CP disappearance of concentrate at each incubation time were calculated from the DM, CP, and NDF residues in each bag. Kinetic parameters of DM, CP, and NDF in situ disappearance of pasture, concentrate, and TMR were estimated by the Marquardt nonlinear procedure (NLIN) of SAS (1999). If a delay on the initiation of disappearance of DM, CP, or NDF was observed, a model including a lag time parameter was used.

No lag was observed in the disappearance of DM and CP in pasture, concentrate, and TMR samples; therefore data were fitted to the model of Ørskov and McDonald (1979):

$$D = A + B (1 - e^{-kd t})$$

where

D = DM or CP disappearance

A = soluble fraction, %

B = insoluble potentially degradable fraction, %

kd = fractional degradation rate, %/h

t = time, h

Effective degradability (**ED**) of DM and CP of forages was calculated in previous studies (Hoffman et al., 1993; Kolver et al., 1998; Elizalde et al., 1999) as $ED = A + B (kd/(kd + kp))$, assuming a single passage rate (kp) of 6%/h. Others (Waldo et al., 1972; Coblenz et al., 1998) suggested that the use of a single passage rate for different forages is not appropriate. Because in this study, cows received pasture and conserved forage that differed in rate of passage (Holden et al., 1994), ED of DM and CP was not calculated.

A lag was observed in the disappearance of NDF in pasture and TMR samples; therefore data were fitted to the model of Mertens and Loften (1980):

$$FR = P e^{-kd(t-L)} + U, \text{ when } t > L; \\ \text{and } FR = P + U, \text{ when } 0 < t < L$$

where

FR = NDF remaining at time t

P = potentially digested fraction (100 - U) at fractional rate kd, %

kd = fractional degradation rate, %/h

U = fraction undigested at 48 h, %

L = lag time, h

t = time of incubation, h

Milk production was recorded daily from d 1 (May 8) to 147 (October 1). Milk samples were collected weekly during the 21 wk of the experiment and preserved with 2-bromo-2-nitropropane-1,3 diol. Milk fat, true protein, and lactose were analyzed by infrared spectrophotometry (Foss 605B Milk-Scan; Foss Electric, Hillerød, Denmark) by the Pennsylvania DHIA milk testing laboratory. Cows were weighed after the p.m. milking on two consecutive days every 2 wk starting on wk 1. During the same weeks, the body condition of the cows was scored by two experienced independent observers using

the five-point BCS scale (1 = thin, 5 = fat; Wildman et al., 1982).

Statistical Analyses

Rumen digestion data were analyzed as repeated measures using the PROC MIXED procedure of SAS (1999). The model included the fixed effects of treatments, week, week $\times$ treatment interaction, hour, hour $\times$ treatment interaction, hour $\times$ week interaction, hour $\times$ week $\times$ treatment interaction, the random effect of cows nested within treatment, and the residual error.

For each variable analyzed, cow nested within treatment was subjected to three covariance structures: compound symmetry, autoregressive order 1, and unstructured covariance. The covariance that resulted in the smallest Akaike's Information Criterion and Schwarz Bayesian Criterion was used. Least squares means and SEM are reported for all data. When significant ($P < 0.05$) effects due to dietary treatments were detected, mean separation was conducted by the PDIF option in SAS (1999).

RESULTS AND DISCUSSION

Animal Performance

The objective of presenting these data is to demonstrate that the rumen cannulated cows were similar in performance to the cows on the same diets in the larger companion study (Bargo et al., 2002b). If the rumen-cannulated cows had similar trends in DMI, milk production and composition, and BW, extrapolation of rumen digestion data to the companion study with a greater number of cows ($n = 45$) is valid. Cows on the TMR treatment consumed 17% more total DMI than cows on the PC treatment, and 2% more than cows on the pTMR treatment. On a DM basis, cows on the PC treatment consumed 22.6 ± 1.4 (mean $\pm$ SEM) kg/d of a diet composed of 39% concentrate and 61% pasture, cows on the pTMR treatment consumed 25.7 ± 1.4 kg/d of a diet composed of 10% concentrate, 29% pasture, and 61% TMR, and cows on the TMR treatment consumed 26.3 ± 1.4 kg/d of a diet composed of 100% TMR. Total DMI and diet composition were similar to those reported in the performance study (Bargo et al., 2002b): 21.6, 25.2, and 26.7 kg/d for the PC, pTMR, and TMR treatments, respectively.

Differences in milk production among treatments were similar to the companion study (Bargo et al., 2002b), which reported 28.5, 32.0, and 38.1 kg/d of milk for the PC, pTMR, and TMR treatments, respectively. In this study, the cows on the TMR treatment produced 39% more milk than those on the PC treatment (39.7 vs. 28.5 kg/d; ± 4.2 SEM) and 25% more milk than cows

on the pTMR treatment (39.7 vs. 31.7 kg/d; ± 4.2 SEM). Similar trends were found for milk fat and true protein content to those reported in Bargo et al. (2002b). The cows on the pTMR and TMR treatments had higher milk fat percentage compared to cows on the PC treatment (3.33 vs. 3.10% ; ± 0.10 SEM). The highest true protein was found for the TMR treatment (3.08%) and the lowest for the PC treatment (2.76%), with an intermediate value for the pTMR treatment (2.94%). Milk urea N was higher for cows on the PC treatment than either the pTMR or TMR treatments (15.6 vs. 11.8 mg/dl; ± 2.0 SEM).

In agreement with the results reported in the performance study (Bargo et al., 2002b), initial BW did not differ among treatments but variation among cow was high as expected because of the low number of cows (668 ± 56 kg/d; mean $\pm$ SEM). Numerically, cows on the PC treatment gained less BW during the experiment than the cows on the pTMR or TMR treatments, following the trend reported by Bargo et al. (2002b). Although no significant ($P > 0.05$) differences were detected among treatments in this study for any animal performance variables because of the low number of cows, all these variables followed the same trends reported in the companion animal performance study (Bargo et al., 2002b).

Ruminal Fermentation

Ruminal pH. Ruminal fermentation measures are shown in Table 2. Mean rumen pH did not differ among treatments and averaged 5.87 ± 0.04 ($P > 0.05$). The similar rumen pH for the three treatments could be related to the lower forage-to-concentrate ratio (52:48) on the TMR treatment and the limited intake of fiber and the high rumen digestibility of the pasture on both the PC and pTMR treatments even with a higher forage-to-concentrate ratio (60:40; Bargo et al., 2002b). Satter et al. (1999) reported that the rumen pH of high-producing dairy cows fed diets with 50% of concentrate is often 5.7. Similar values (5.79 ± 0.05 , range: 5.68 to 5.87) to the PC treatment were reported for dairy cows consuming fresh grass-legume forage and supplemented with 10 kg/d of corn (Reis et al., 2001). For lactating dairy cows in early lactation fed a TMR with corn as the main energy source, Dann et al. (1999) reported a higher mean rumen pH (6.33 ± 0.08) than in our study. Although both studies fed a TMR with an approximately 50:50 forage-to-concentrate ratio, the lower rumen pH found in the present study may be partially attributed to the higher DMI (26.3 vs. 21.0 kg/d) and lower NDF content (30.7 vs. 33.9%) compared to the study of Dann et al. (1999).

Table 2. Ruminal fermentation characteristics of dairy cows with three different feeding systems.

	Treatments ¹				Effects, ² <i>P</i> ≤			
	PC	pTMR	TMR	SEM	T	P	T × P	T × H
pH	5.89	5.88	5.83	0.04	0.55	0.05	0.17	0.01
NH ₃ -N, mg/dL	19.96 ^a	10.75 ^b	9.74 ^b	0.48	0.01	0.01	0.01	0.01
Total VFA, mmol/L	140.92	130.05	141.56	3.96	0.22	0.01	0.55	0.03
VFA, mol/100 mol								
Acetate (A)	64.88	63.59	60.71	1.42	0.25	0.06	0.01	0.01
Propionate (P)	19.42	19.74	22.49	1.23	0.29	0.13	0.04	0.42
Butyrate (B)	11.56	12.19	12.37	0.92	0.82	0.01	0.01	0.04
Iso-Butyrate	0.97 ^a	0.87 ^{ab}	0.78 ^b	0.04	0.09	0.01	0.01	0.01
Valerate	1.68	2.22	2.19	0.23	0.31	0.02	0.01	0.02
Iso-Valerate	1.49	1.38	1.44	0.10	0.71	0.01	0.01	0.01
A/P	3.35	3.25	2.74	0.22	0.25	0.48	0.01	0.06
(A+B)/P	3.95	3.87	3.30	0.25	0.28	0.13	0.04	0.31
Rumen content								
Wet fill, kg	78.3	76.1	89.2	7.9	0.53	0.59	0.82	...
Dry fill, kg DM	13.6	12.6	13.7	0.3	0.12	0.46	0.53	...
DM content, %	17.4	17.0	15.3	1.5	0.60	0.90	0.91	...

^{a,b,c}Least square means in the same row with different superscripts differ ($P < 0.05$).

¹PC = Pasture plus concentrate (PC), pTMR = pasture plus partial TMR, TMR = total mixed ration.

²T = treatment, P = period, T × P = treatment × period interaction, T × H = treatment × hour.

A significant treatment × hour interaction was found for rumen pH ($P < 0.05$). This indicates that although the mean rumen pH did not differ, the variations in daily rumen pH patterns differed among treatments (Figure 1). Rumen pH of the PC treatment followed the typical pattern of grazing cows supplemented twice daily after milking (Bargo et al., 2001). The PC treatment had a significant ($P < 0.05$) reduction in rumen

Figure 1. Daily rumen pH variations of dairy cows with three different feeding systems: pasture plus concentrate (PC), partial TMR (pTMR), and total mixed ration (TMR). The two black arrows indicate the time that concentrate was fed (0600 and 1800 h), and the gray arrow the time that TMR was fed (0730 h). The treatment × hour interaction was significant ($P < 0.05$). Significant differences ($P < 0.05$) between PC and pTMR at 0500, 1300, 1700, and 0100 h, between PC and TMR at 0900 and 1700 h, and between pTMR and TMR at 0500, 0900, 1300, and 1700 h. Overall SEM = 0.11.

pH at 0900 h (5.80) and at 2100 h (5.76), after cows were fed supplement and moved to the pasture. Significant increases in rumen pH on this treatment were found at 1700 h (6.08) and at 0500 h (6.09), after noon and midnight when cows have a low grazing activity and the concentrate was likely digested. The pTMR treatment had significantly ($P < 0.05$) higher rumen pH than the PC treatment at 1300 h (6.14 vs. 5.79) and 1700 h (6.46 vs. 6.08), which could be attributed to a higher forage-to-concentrate ratio between the a.m. and p.m. milking. At the morning feeding, cows on the pTMR treatment consumed 2.2 kg DM of concentrate and 7.5 kg DM of pasture (forage-to-concentrate ratio of 77:23), while cows on the PC treatment consumed 4.4 kg DM of concentrate and 7.0 kg DM of pasture (forage-to-concentrate of 61:39; Bargo et al., 2002b). A substantial reduction in rumen pH was observed with the pTMR treatment after 1700 h when cows remained indoors and consumed the TMR (6.46 at 1700 h vs. 5.69 at 2100 h; $P < 0.05$). On the TMR treatment, the rumen pH increased in the early morning (5.98 at 0500 h vs. 6.17 at 0900 h), then decreased (5.85 at 1300 h, 5.79 at 1700 h, and 5.57 at 2100 h) which coincided with cows starting to eat the TMR that was fed once daily around 0730 h. Among the three treatments, the pTMR resulted in the greatest variations in pH (a minimum of 5.48 at 0100 h and a maximum of 6.46 at 1700 h); while the TMR treatment presented the most constant rumen pH pattern with a maximum of 6.17 at 0900 h and a minimum of 5.57 at 2100 h.

A significant overall period effect was detected for rumen pH ($P < 0.05$; Table 3). Comparing rumen pH

Table 3. Period effects on ruminal fermentation characteristics of dairy cows with three different feeding systems.

	Treatments ¹			SEM ³	Effects, ² P≤	
	PC	pTMR	TMR		P	T × P
pH						
Period 1 (May)	6.07 ^{A,a}	5.88 ^{ab}	5.81 ^b	0.10	0.05	0.17
Period 2 (June)	5.70 ^{B,a}	5.87 ^{ab}	5.69 ^a	0.10	...	...
Period 3 (August)	5.88 ^{AB}	5.85	5.91	0.10	...	...
Period 4 (September)	5.91 ^A	5.90	5.91	0.10	...	...
NH ₃ -N, mg/dl						
Period 1 (May)	10.34 ^{A,a}	6.34 ^{A,b}	6.94 ^{A,b}	1.52	0.01	0.01
Period 2 (June)	19.19 ^{B,a}	12.74 ^{B,b}	8.99 ^{AB,c}	1.52	...	...
Period 3 (August)	28.75 ^{C,a}	11.98 ^{B,b}	10.52 ^{B,b}	1.52	...	...
Period 4 (September)	21.56 ^{B,a}	11.94 ^{B,b}	12.53 ^{B,b}	1.52	...	...

^{A,B,C}Least square means in the same column with different superscripts differ ($P < 0.05$).

^{a,b,c}Least square means in the same row with different superscripts differ ($P < 0.05$).

¹PC = pasture plus concentrate (PC), pTMR = pasture plus partial TMR, TMR = total mixed ration.

²P = period, T × P = treatment × period interaction.

³Standard error of the differences of least square means.

across periods and within treatments, cows on the PC treatment had a significantly lower pH during period 2, whereas cows on the pTMR and TMR treatment exhibited a more constant pH. This could be related to a decreased forage-to-concentrate ratio on the PC treatment during period 2 because of a lower pasture DMI (11.3 vs. 14.9 kg/d) and a similar concentrate DMI (8.9 kg/d) compared to period 1 (Bargo et al., 2002b). In the companion study (Bargo et al., 2002b), cows on the PC treatment had a lower forage-to-concentrate ratio during period 2 compared to periods 1, 3, and 4 (56:44 vs. 61:39). Comparing within periods and across treatments, cows on the PC treatments had higher rumen pH than cows on the pTMR and TMR treatments during period 1. In the other three periods, mean rumen pH did not significantly differ among treatments ($P > 0.05$).

Volatile fatty acid concentrations. Total VFA concentration did not differ among treatments and averaged 137.5 mmol/L ($P > 0.05$; Table 2). Previous studies with dairy cows on pasture and supplemented with corn-based concentrate reported a total VFA concentration of >130 mmol/L (Bargo et al., 2002a; Reis et al., 2001). For early lactation dairy cows fed a TMR of a 50:50 forage-to-concentrate ratio, Dann et al. (1999) reported a lower total VFA concentration (129 mmol/L) than that found in this study (141.6 mmol/L) which is consistent with the higher rumen pH reported by Dann et al. (1999). Individual VFA molar proportions did not differ among treatments ($P > 0.05$). The three principal individual VFA proportions averaged 63.1, 20.6, and 12.0 mol/100 mol for acetate, propionate, and butyrate, respectively. The acetate/propionate ratio (3.11) and the (acetate + butyrate)/propionate ratio (3.71) did not differ among treatments ($P > 0.05$).

Rumen NH₃-N concentrations. Rumen NH₃-N concentration was significantly higher for the PC treatment than for both the pTMR and TMR treatments (19.9 vs. 10.2 mg/dl; $P < 0.05$, Table 2). This result is consistent with the significantly higher milk and plasma urea N on the PC treatment reported in the animal performance study (Bargo et al., 2002b). Combining pasture and TMR on the pTMR treatment increased efficiency of N utilization because of the reduction in N losses and the increase in milk protein yield with a similar total N intake (Bargo et al., 2002b). Similar rumen NH₃-N concentration (16.9 mg/dl) was reported for high-producing dairy cows supplemented with 10 kg/d of different types of corn and consuming a fresh-cut grass-legume pasture with 21.4% of CP (Reis et al., 2001). Bargo et al. (2002a) reported lower NH₃-N concentration (8.9 mg/dl) for dairy cows grazing a grass pasture and supplemented with a corn-based concentrate, however the pasture CP content in that study was lower (19.9%) than in the current study (26.3%). Dann et al. (1999) reported a mean NH₃-N concentration of 10.1 mg/dl for dairy cows on a TMR diet, a similar value to 9.7 mg/dl found for the TMR treatment (9.7 mg/dl).

A significant treatment × hour interaction was detected for NH₃-N concentration ($P < 0.05$), indicating that the pattern of variation over a 24-h period differed among treatments (Figure 2). During the six sampling times, rumen NH₃-N concentration was significantly higher for the PC treatment than for both the pTMR and TMR treatments (Table 2). The pattern of rumen NH₃-N concentration on the PC treatment followed a typical pattern for grazing cows (Bargo et al., 2001), with two daily peaks corresponding to ingestion of high

CP pasture after cows were moved to the paddock (20.7 mg/dl at 1300 h, and 25.8 mg/dl at 2100 h). The two lowest rumen $\text{NH}_3\text{-N}$ concentrations on this treatment were found after midnight (14.2 mg/dl at 0500 h; $P < 0.05$) and noon (17.3 mg/dl at 1700 h; $P < 0.05$). Rumen $\text{NH}_3\text{-N}$ concentration steadily increased on the pTMR treatment during the morning (6.2 mg/dl at 0500 h, 7.1 mg/dl at 0900 h, and 9.7 mg/dl at 1300 h). An increase in rumen $\text{NH}_3\text{-N}$ concentration was detected on the pTMR from 1700 to 2100 h due to the ingestion of TMR when cows were kept indoors overnight (9.3 vs. 20.8 mg/dl; $P < 0.05$), with a latter reduction at 0100 h indicating that cows stopped eating TMR (20.8 vs. 11.3 mg/dl; $P < 0.05$). Although the mean rumen $\text{NH}_3\text{-N}$ concentration was similar between the pTMR and TMR treatments, the patterns differed during the 24-h period. The TMR treatment had a peak of rumen $\text{NH}_3\text{-N}$ concentration at 0900 h (13.2 mg/dl) and then remained relatively constant (10.8 mg/dl at 1300 h and 12.6 mg/dl at 1700 h; $P > 0.05$). After 1700 h, rumen $\text{NH}_3\text{-N}$ concentration decreased reaching the lowest values at 0100 h (6.1 mg/dl) and 0500 h (5.4 mg/dl). The cows fed pTMR treatment exhibited the greatest variations in rumen $\text{NH}_3\text{-N}$ concentration over the 24-h period (minimum 6.2 mg/dl of at 0500 h, maximum of 20.8 mg/dl at 2100 h), while the cows on the TMR treatment had the most constant pattern of rumen $\text{NH}_3\text{-N}$ concentration (minimum 5.4 mg/dl of at 0500 h, maximum of 12.6 mg/dl at 1700 h).

Figure 2. Daily rumen $\text{NH}_3\text{-N}$ variations of dairy cows with three different feeding systems: pasture plus concentrate (PC), partial TMR (pTMR) or TMR. The two black arrows indicate the time that concentrate was fed (0600 and 1800 h), and the gray arrow the time that TMR was fed (0730 h). The treatment $\times$ hour interaction was significant ($P < 0.05$). Significant differences ($P < 0.05$) between PC and pTMR and TMR at 0100, 0500, 0900, 1300, 1700, and 2100 h, and between pTMR and TMR at 0100, 0900, and 2100 h. Overall SEM = 1.8.

Table 4. In situ DM, CP, and NDF degradation kinetics (mean $\pm$ SEM) of the concentrate and the TMR.

	Concentrate	TMR
DM degradation ¹		
A, %	27.83 $\pm$ 1.23	39.27 $\pm$ 0.76
B, %	62.31 $\pm$ 0.84	43.00 $\pm$ 2.05
TD, %	90.13 $\pm$ 0.46	82.27 $\pm$ 1.96
kd, %/h	7.41 $\pm$ 0.31	4.15 $\pm$ 0.42
CP degradation ¹		
A, %	24.78 $\pm$ 0.96	41.99 $\pm$ 4.49
B, %	57.69 $\pm$ 3.01	45.52 $\pm$ 2.58
TD, %	82.47 $\pm$ 2.98	87.51 $\pm$ 6.21
kd, %/h	5.16 $\pm$ 0.95	3.03 $\pm$ 1.01
NDF degradation ²		
L, h	...	5.58 $\pm$ 1.23
S, %	...	2.22 $\pm$ 0.75
P, %	...	70.54 $\pm$ 14.18
U, %	...	27.24 $\pm$ 14.21
kd, %/h	...	3.32 $\pm$ 1.83

¹A = soluble fraction, B = insoluble potentially degradable fraction, TD = total potentially degradable fraction (A + B), kd = fractional degradation rate (Ørskov and McDonald, 1979).

²L = lag time, S = soluble fraction (100 - P - U), P = potentially digested fraction, U = undigested fraction at 48 h, kd = fractional degradation rate (Mertens and Lofton, 1980).

A significant period and treatment $\times$ period interaction ($P < 0.05$) was found for rumen $\text{NH}_3\text{-N}$ concentration (Table 3). Comparing across periods and within treatments, cows on the PC treatment had lowest $\text{NH}_3\text{-N}$ during period 1, intermediate during periods 2 and 4, and a remarkably high peak in period 3. The highest rumen $\text{NH}_3\text{-N}$ concentration on the PC treatment during period 3 corresponded to the highest CP content in the grazed pasture (29.5%; Table 1). In contrast, both the pTMR and TMR treatments had an increase in $\text{NH}_3\text{-N}$ concentration from period 1 to 2 and then remained relatively constant through periods 3 and 4 (Table 3). Comparing within periods and across treatments, $\text{NH}_3\text{-N}$ concentration was always highest on the PC treatment compared to both the pTMR and TMR treatments.

In Situ Rumen Digestion of Concentrate and TMR

In situ rumen digestion of the concentrate supplemented to cows on both the PC and pTMR treatments, and of the TMR fed to cows on both the pTMR and TMR treatments are shown in Table 4. This information is provided to characterize the feedstuffs. In situ degradation data of the concentrate were obtained from two cows on the PC treatment and two cows on the pTMR treatment. None of the in situ DM or CP kinetics measures were affected by treatment ($P > 0.05$); thus the overall mean of the four cows are presented in Table 4. The concentrate averaged 27.8, 62.3, and 7.4%/h for the soluble fraction, insoluble potentially degradable fraction, and degradation rate of DM, respectively. Sim-

Table 5. In situ DM, CP, and NDF degradation kinetics of pasture grazed by dairy cows with two different feeding systems.

	Treatments ¹		SEM	Effects, ² $P \leq$		
	PC	pTMR		T	P	T × P
DM degradation ³						
A, %	8.71	6.79	1.57	0.48	0.01	0.57
B, %	76.81	75.54	1.58	0.63	0.01	0.53
TD, %	85.53	82.33	0.38	0.03	0.07	0.12
kd, %/h	5.68	6.03	0.44	0.63	0.01	0.14
CP degradation ³						
A, %	6.83	5.81	1.05	0.55	0.01	0.15
B, %	82.99	81.14	2.38	0.64	0.01	0.16
TD, %	89.82	86.48	1.82	0.31	0.16	0.52
kd, %/h	7.77	8.23	0.08	0.06	0.08	0.60
NDF degradation ⁴						
L, h	5.41	6.48	0.23	0.08	0.52	0.81
S, %	1.67	0.89	0.49	0.37	0.05	0.63
P, %	82.05	74.95	0.66	0.02	0.02	0.33
U, %	16.28	24.16	0.66	0.01	0.02	0.33
kd, %/h	6.51	7.86	0.68	0.29	0.08	0.68

¹PC = pasture plus concentrate (PC), pTMR = pasture plus partial total mixed ration.

²T = treatment, P = period, T × P = treatment × period interaction.

³A = soluble fraction, B = insoluble potentially degradable fraction, TD = total potentially degradable fraction (A + B), kd = fractional degradation rate (Ørskov and MacDonald, 1979).

⁴L = lag time, S = soluble fraction (100 – P – U), P = potentially digested fraction, U = undigested fraction at 48 h, kd = fractional degradation rate (Mertens and Loftén, 1980).

ilar values for those respective fractions (28.4, 70.2, and 6.4%/h for OM digestion) were reported for a corn-based concentrate (Kolver et al., 1998). The soluble fraction, insoluble degradable fraction, and degradation rate of CP averaged 24.8, 57.7, and 5.2%/h, respectively. Kolver et al. (1998) reported similar values for the soluble fraction and rate of degradation (28.2 and 4%/h) but higher values for the insoluble degradable fraction (73.2%) of CP for a corn-based concentrate containing 14.4% CP.

In situ DM, CP, and NDF, degradation of TMR was determined in two cows on the TMR treatment. This resulted in substantial variation between cows and large SEM. The TMR averaged 39.3, 43.0, and 4.2%/h for the soluble, insoluble potentially degradable, and degradation rate of DM, respectively; and 42.0, 45.5, and 3.0%/h for the soluble, insoluble potentially degradable, and degradation rate of CP, respectively. A 5.6-h lag time was observed for the in situ NDF digestion of the TMR. The potentially digestible fraction of NDF averaged 70.5%, and the degradation rate of NDF averaged 3.3%/h.

In Situ Rumen Digestion of Pasture

The in situ DM, CP, and NDF digestion of pasture grazed by cows on the PC and pTMR treatments are presented in Table 5. The main goal of the in situ rumen digestion data of pasture was to determine the effects

of feed interactions in the rumen of the cows on the pTMR treatment in comparison with the PC treatment.

In situ DM digestion. The soluble fraction of DM was not affected by treatment ($P > 0.05$) and averaged 7.8% (Table 5). For brome grass and orchardgrass at second node maturity stage (incubated dried and ground), Hoffman et al. (1993) reported a soluble fraction of DM of 36.3 and 39.0%, respectively. The soluble fraction of DM decreased to 32.7 and 34.0% for brome grass and orchardgrass when maturity stage advanced to boot stage (Hoffman et al., 1993). Differences between Hoffman et al. (1993) and this study are likely related to sample processing (ground vs. 1-cm cut, respectively) and soaking time (30 vs. 15 min, respectively). Soluble fraction of DM for fresh brome grass at tillering averaged 24.3% and decreased to 18.6% at stem elongation (Elizalde et al., 1999); no information about sample processing was provided in that study. When a predominantly orchardgrass pasture was incubated in situ using the same procedure (fresh forage cut to 1-cm) used in this study, a soluble fraction of OM of 13.0% was found (Kolver et al., 1998). However, when an orchardgrass-brome grass pasture was freeze-dried and ground to 4-mm (Hongerholt and Muller, 1998), the soluble fraction of DM was higher and averaged 24.4%.

Neither the insoluble potentially degradable fraction (76.2%) nor the degradation rate (5.9%/h) of DM was affected by inclusion of TMR on a pasture-based diet. For pasture-based diets supplemented with concen-

trate, Kolver et al. (1998) reported a degradation rate of OM of orchardgrass pasture of 7.3%/h, and Hongerholt and Muller (1998) reported a degradation rate of DM of orchardgrass-bromegrass pasture of 7.6%/h. Elizalde et al. (1999) found a significant reduction in degradation rate of DM of bromegrass from 11.5 to 6.6%/h as maturity stage advanced from tillering to stem elongation. Hoffman et al. (1993) reported degradation rate of DM of bromegrass between 6 to 5%/h and of orchardgrass between 10 to 8%/h for second node to boot stage of maturity.

The total potentially degradable fraction of pasture DM was significantly reduced (85.5 vs. 82.3%; $P < 0.05$) with the inclusion of TMR in the diet, indicating possible associative effects in the rumen on this treatment. The actual value of this fraction depends on the length of in situ rumen incubation. Studies where pasture was incubated in situ for 48 h (Hongerholt and Muller, 1998; Kolver et al., 1998) reported that this fraction ranged from 82.2 to 84.7%. Other studies using a final incubation time of 72 h (Hoffman et al., 1993; Elizalde et al., 1999) reported total potentially degradable fraction of DM of orchardgrass and bromegrass forage between 85.3 to 88.3%. Although it has been recently proposed that forages should be incubated in situ for 72 h (NRC, 2001), the difference in total potentially degradable fraction of DM for this type of pasture between 48 or 72 h incubations was not large.

In situ CP digestion. Neither the soluble, nor the insoluble potentially degradable, nor the total potentially degradable fractions of pasture CP were affected by treatments ($P > 0.05$; Table 5). Similar to results found for pasture DM digestion, pasture grazed in this study had very low soluble fraction of CP (6.3%), which was likely related to the physical form of incubation of the pasture samples. In situ data from Kolver et al. (1998), using the same procedures as used in this study, reported for an orchardgrass-based pasture a soluble fraction of CP of 11.4%. Higher values were reported by Elizalde et al. (1999) for bromegrass (27.1 to 32.3%), by Hoffman et al. (1993) for orchardgrass (46.1 to 51.5%) and bromegrass (45.1 to 46.1%), and by Hongerholt and Muller (1998) for orchardgrass-bromegrass (19.0%) pastures. All these studies, however, used different sample processing (e.g., grinding as in Hongerholt and Muller, 1998) and procedures (e.g., soaking the 0-h bags for a longer period of time as in Hoffman et al., 1993) in comparison to this study. The total potentially degradable fraction of pasture CP averaged 88.2%, which agrees with previously reported orchardgrass (90.1%; Kolver et al., 1998) and orchardgrass-bromegrass (94.3%; Hongerholt and Muller, 1998) pastures. Other studies (Hoffman et al., 1993; Elizalde et al., 1999) reported greater values for this fraction (92.2 to 96.1%),

which could be attributed to longer incubation time (72 h). Degradation rate of CP tended to be slightly greater in the pTMR treatment (8.2 to 7.8%/h; $P < 0.06$). Hongerholt and Muller (1998) reported a degradation rate of CP of 7.6%/h, while Kolver et al. (1998) found a degradation rate of CP of 10.9%. Others also reported greater degradation rate of CP of bromegrass (11.8%/h; Elizalde et al., 1999) and orchardgrass (15.0%/h; Hoffman et al., 1993).

In situ NDF digestion. In situ pasture NDF digestion was lower on the pTMR treatment than on the PC treatment (Table 5). The inclusion of TMR on the pTMR treatment tended to increase the lag time (5.4 vs. 6.5 h; $P < 0.08$) and significantly reduced the potentially digested fraction (82.1 vs. 75.0%; $P < 0.05$) of pasture NDF. The reduction in NDF digestion of pasture is in agreement with the lower total potentially degradable fraction of DM previously discussed. This also indicates the presence of possible associative effects in the rumen of cows on the pTMR treatment, which may be related with the higher rumen pH daily variations (Figure 1) and lower $\text{NH}_3\text{-N}$ concentrations at 0500, 0900, 1300, and 1700 h (Figure 2) compared with the PC treatment. Degradation rate of NDF did not differ between treatments and averaged 7.2%/h.

None of the previous in situ studies that used similar procedures (Kolver et al., 1998) or similar forages (Elizalde et al., 1993; Hongerholt and Muller, 1998) as used in this study reported data on NDF digestion. Hoffman et al. (1993) studied in situ NDF degradation of orchardgrass and bromegrass forages at different stages of maturity. Orchardgrass averaged 3.2, 73.1, and 23.7%, and bromegrass averaged 1.9, 77.4, and 20.7% for the soluble fraction, the potentially digested fraction, and the undigested fraction at 72 h, respectively (Hoffman et al., 1993). These values are similar to those found in our study. Also in agreement with our study, Hoffman et al. (1993) reported a degradation rate of NDF for orchardgrass from 7 to 8%/h and for bromegrass from 5 to 6%/h.

CONCLUSIONS

The three different feeding systems combining pasture and TMR had similar rumen pH (5.87). Neither total VFA concentration (137.5 mmol/L) nor the molar proportion of the major individual VFA were affected by feeding system. Rumen $\text{NH}_3\text{-N}$ concentration was higher for the PC treatment (19.9 mg/dl) than for both the pTMR and TMR treatments (10.2 mg/dl). The inclusion of TMR in the pTMR treatment reduced the total potentially degradable fraction of DM (85.5 vs. 82.3%) and the potentially digested fraction (82.1 vs. 74.9%) of pasture NDF.

Dietary N utilization was improved when combining pasture and TMR compared to pasture plus concentrate. Both feeding systems including TMR resulted in lower rumen $\text{NH}_3\text{-N}$ concentrations than with only pasture plus concentrate. Rumen pH was not affected by the inclusion of TMR for grazing cows. Combining pasture with TMR resulted in associative rumen effects resulting in reduced potential digestion of pasture DM and NDF.

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