Pasture Intake and Substitution Rate Effects on Nutrient Digestion and Nitrogen Metabolism during Continuous Culture Fermentation

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ABSTRACT

A continuous culture system was used to investigate ruminal digestion in response to increased pasture intake and three different substitution rates (SR) in a 4 × 4 Latin square design. The treatments were 1) low pasture (55 g dry matter (DM)/d, 2) medium pasture (MP, 65 g DM/d), 3) high pasture (75 g DM/d), and 4) pasture (45 g DM/d) plus concentrate (PC, 30 g DM/d). Treatments were designed to produce a low (0.33), medium (0.67), and high (1.00) SR (g of pasture/g of concentrate) by contrasting the low, medium, and high pasture intake treatments with the pasture plus concentrate treatment, respectively. Pasture was fed at 0630, 1000, 1730, and 2100 h, and concentrate at 0600 and 1700 h. Digestibility of DM and neutral detergent fiber were not affected by the amount of pasture. As the amount of pasture increased, pH decreased linearly, and total volatile fatty acid and NH₃-N concentrations, and nonammonia N and bacterial N flows increased linearly. Concentrate supplementation did not affect DM digestibility at high SR but increased DM digestibility at low SR. Concentrate supplementation reduced pH and NH₃-N concentrations at the three SR. Concentrate supplementation reduced the ratio of rumen degradable N to rumen degradable organic matter; however, the mechanism depended on the SR. High SR, concentrate supplementation reduced rumen degradable N, which reduced NH₃-N concentration without affecting bacterial N flow. At low SR, concentrate supplementation increased rumen degradable organic matter, which reduced NH₃-N concentration and increased bacterial N flow. Based on these results, at low SR, concentrate supplementation may enhance animal performance because of higher total DM intake and synthesis of microbial protein.

(**Key words:** pasture, substitution rate, nutrient digestion, N metabolism)

Abbreviation key: BN = bacterial N, HP = high pasture, LP = low pasture, MP = medium pasture, NI = N intake, OMI = OM intake, PC = pasture plus concentrate, RDN = rumen degradable N, RDOM = rumen degradable OM, SR = substitution rate, TN = total N.

INTRODUCTION

High-producing dairy cows on pasture need supplemental energy to reach their genetic potential for total DMI and milk production (Kolver and Muller, 1998). However, providing supplemental feed to grazing dairy cows will result in substitution of pasture by supplement. This has been reported in early studies with low-producing dairy cows grazing ryegrass (Stakelum, 1986) and in recent studies with high-producing dairy cows grazing orchardgrass (Bargo et al., 2002a). Substitution rate (SR) is calculated as: SR (kg pasture/kg supplement) = (pasture DMI in the unsupplemented treatment – pasture DMI in the supplemented treatment)/supplement DMI. An SR close to 1 kg of pasture per kilogram of supplement will result in little increase in total DMI, while a SR lower than 1 kg of pasture per kilogram of supplement will result in an increased total DMI by cows fed a supplement. Substitution rate is one of the major factors explaining the variation in milk response to supplementation (Kellaway and Porta, 1993). Stakelum (1986) indicated the importance of minimizing the SR to obtain high milk response to supplementation. Bargo et al. (2002a) reported that high-producing dairy cows supplemented with a corn-based concentrate and grazing at a low pasture allowance had a lower SR than cows grazing at a high pasture allowance (0.26 vs. 0.55 kg of pasture/kg of concentrate). The lower SR resulted in higher total energy and DMI and milk response to supplementation (1.36 vs. 0.96 kg of milk per/kg of concentrate), which was partially attributed to a higher microbial protein synthesis (Bargo et al., 2002a).

Previous in vivo (O'Mara et al., 1997; García et al., 2000) and continuous culture (Kolver et al., 1998; Bach

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et al., 1999) studies have reported the effect of concentrate supplementation of pasture diets on bacterial protein synthesis and digestion when concentrate replaced pasture at a SR close to 1 kg of pasture/kg of concentrate. García et al. (2000) reported that corn or barley supplementation at a SR of 1 kg of pasture/kg of grain reduced N intake and rumen $\text{NH}_3\text{-N}$ concentration but did not affect passage of NAN or bacterial N to the small intestine compared to a pasture-only diet. At an SR of 0.85 kg of pasture/kg of supplement, rumen and duodenal cannulated Friesian cows consuming ryegrass pasture and 3 kg/d of molassed beet pulp had a similar total DMI and microbial N flow to the small intestine as the unsupplemented cows (O'Mara et al., 1997). In a continuous culture study, Kolver et al. (1998) replaced orchardgrass pasture at a SR of 1 with 7.9, 19.6, and 23.3% of starch. Intake of N, $\text{NH}_3\text{-N}$ concentration, and total N and $\text{NH}_3\text{-N}$ flows were reduced linearly as starch was increased; however, bacterial N and NAN flows were similar among treatments (Kolver et al., 1998). Another continuous culture study (Bach et al., 1999) compared pasture-only vs. pasture plus different energy supplements (45% of the total diet as beet pulp with molasses, cracked corn, or soybean hulls) at a SR of 1 g of pasture/g of supplement and reported that $\text{NH}_3\text{-N}$ concentration was reduced by supplements, but bacterial N flow was similar among treatments. In summary, previous in vivo and continuous culture studies that have replaced pasture by supplement at a SR of 1 kg of pasture/kg of supplement reported that bacterial N synthesis was not increased, and the only benefit was a reduction in $\text{NH}_3\text{-N}$ concentration, more a result of a dilution in N intake than an improvement in capture of $\text{NH}_3\text{-N}$ by ruminal microorganisms.

Using continuous culture fermentation, the objectives of this study were to evaluate: 1) the effect of increasing the amount of pasture DMI and 2) the effect of different SR on nutrient digestion and N metabolism. We hypothesize that concentrate supplementation would reduce the rumen degradable N (**RDN**) to rumen degradable OM (**RDOM**) ratio but act through different mechanisms to improve the utilization of dietary N.

MATERIALS AND METHODS

Experimental Design

A dual flow continuous culture system designed to simulate ruminal digestion and solid and liquid outflow to the small intestine was used in this experiment. Four pasture-based diets were compared in a 4×4 Latin square design to study the ruminal digestion of increased pasture DMI and three levels of SR (pasture DMI reduction by concentrate supplementation). The

four dietary treatments were (Table 1) 1) low pasture (**LP**, 55 g DM/d), 2) medium pasture (**MP**, 65 g DM/d), 3) high pasture (**HP**, 75 g DM/d), and 4) pasture plus concentrate (**PC**, 45 g DM/d pasture plus 30 g of DM/d of concentrate). The ruminal response to increased pasture DMI was studied comparing treatments from 1 to 3. The response to different levels of SR was studied comparing the PC treatment against each of the pasture-only treatments. The SR was 0.33, 0.67, and 1.00 when LP, MP, and HP treatments were compared with the PC treatment, respectively (Table 1).

Fermenters were fed in an attempt to simulate a grazing dairy cow at different pasture allowances (LP, MP, and HP) and a grazing dairy cow supplemented twice daily after each milking in two equal concentrate feedings (PC). For the PC treatment, half of the daily concentrate DM was fed at 0600 h and the other half at 1700 h. For all the treatments, pasture was fed four times in equal feedings during the day at 0630, 1000, 1730, and 2100 h in an attempt to simulate the grazing pattern. Pasture used in the study had an average botanical composition of 50% smooth brome grass (*Bromus inermis* L.), 33% orchardgrass (*Dactylis glomerata* L.), 7% Kentucky bluegrass (*Poa pratensis* L.), and 10% weeds and dead material. During the early spring of 2001, pastures samples were plucked by hand to the approximate height that cows grazed and were freeze-dried. A corn-based concentrate (Table 2) was used as supplement for the PC treatment. Pasture and concentrate samples were ground using a Wiley mill through a 1-mm screen.

Continuous Culture Operation

A four-unit dual flow continuous culture system similar to that described by Hoover et al. (1989) with the following modifications: a 50-g stirring bar replaced the magnetic impeller assembly unit; pH was not controlled; neither pasture nor concentrate were pelleted; concentrate was fed separately from the pasture; and urea was added to the mineral buffer solution (Weller and Pilgrim, 1974) at a rate of 0.4 g/L.

Approximately 3 h after feeding, ruminal inoculum was obtained from a lactating Holstein cow consuming a TMR ad libitum (approximately 50% concentrate:50% forage). Each of the four 1000-ml fermenters was inoculated with rumen fluid siphoned with a hand pump from the ventral/dorsal rumen. In addition, approximately 30 g of whole ruminal digesta was added to each fermenter. Solids and liquid dilution rates were adjusted daily to approximate 4 and 11%/h, respectively, by regulation of buffer input and filtrate removal rates. Although the total amount of DM fed to the fermenters differed among treatments, the same solids

Table 1. Dietary intake of nutrients and chemical composition.

	Treatments ¹				SEM
	LP	MP	HP	PC	
DM, g/d					
Concentrate	—	—	—	30	—
Pasture	55	65	75	45	—
Total	55	65	75	75	—
SR, ² g pasture/g concentrate	0.33	0.67	1.00	—	—
N, g/d					
Concentrate	—	—	—	0.62	0.002
Pasture	2.22	2.63	3.03	1.82	0.01
Total	2.22	2.63	3.03	2.44	0.01
NSC, g/d					
Concentrate	—	—	—	17.3	0.12
Pasture	9.9	11.7	13.5	8.1	0.09
Total	9.9	11.7	13.5	25.4	0.10
NDF, g/d					
Concentrate	—	—	—	4.3	0.01
Pasture	20.6	24.4	28.1	16.9	0.13
Total	20.6	24.4	28.1	21.2	0.14
Chemical composition					
OM, % of DM	90.4	90.4	90.4	91.7	0.03
CP, % of DM	25.3	25.3	25.3	20.3	0.22
NSC, % of DM	18.0	18.0	18.0	33.9	0.12
NDF, % of DM	37.5	37.5	37.5	28.2	0.22
ADF, % of DM	23.2	23.2	23.2	16.3	0.13
NE _L , ³ Mcal/kg	1.46	1.46	1.46	1.63	—

¹LP = Low pasture (55 g DM/d), MP = medium pasture (65 g DM/d), HP = high pasture (75 g DM/d), PC = pasture (45 g DM/d) plus concentrate (30 g DM/d).

²Substitution rate = (pasture in the pasture-only diet – pasture in the PC diet)/concentrate in the PC diet. For the LP treatment, SR = (55 g – 45 g)/30 g = 0.33 g/g; for the MP treatment: SR = (65 g – 45 g)/30 g = 0.67 g/g; for the HP treatment: SR = (75 g – 45 g)/30 g = 1.00 g/g.

³Estimated using values of NRC (2001).

and liquid dilution rate were used in all the treatments. Values for solids and liquid dilution rates were chosen based on recent studies conducted with high-producing cows on pasture (Reis and Combs, 2000a, 2000b; Reis et al., 2001) that reported increased total DMI with concentrate supplementation but no change in solids or liquid dilution rate. Fermenters were constantly purged with N₂ gas (40 ml/min) to preserve anaerobiosis and temperature was maintained at 39°C.

Sample Collection and Analyses

The study was conducted during four 10-d periods, the first 7 d for adaptation followed by 3 d for sample collection. Effluent was collected into 5-L plastic jugs, which were submerged approximately one-third of the way into a water bath (4°C) starting on the fourth day of each period. The solid and liquid effluent weights were recorded daily at 1800 h and discarded until the last 3 d of each period. On the last 3 d, the solid and liquid effluent portions were mixed, homogenized using a 3-L Waring blender (Waring, New Hartford, CT), and a 600-ml subsample was collected. The 600-ml subsample was kept at 4°C until the last sampling day of each period and three subsamples were then composited for

each fermenter. A 500-ml subsample was collected for DM analysis. The remaining effluent composite sample (1300 ml) was freeze-dried and ground through a 1-mm screen. In addition, a 50-ml sample of effluent was collected at 0, 0.5, 1, 2, 4, and 6 h starting at 0600 h before concentrate feeding for analysis of NH₃-N and VFA (Yang and Varga, 1989) on each sample day. The last 3 d of each period, pH was recorded hourly using a Cole Palmer pH/mV per degree Celsius meter with RS – 232 and Recorder output. On the last day of each period, bacteria were isolated from the fermenter contents (Kolver et al., 1998), freeze-dried, and ground through a 1-mm screen.

Samples of pasture, concentrate, and effluent were analyzed for DM, CP, and ash (AOAC, 1990), NDF (Ankom Daisy II, ANKOM Technology Corp., Fairport, NY), and NSC (Smith, 1981; modified to use potassium ferricyanide as the colorimetric indicator). Samples of pasture and concentrate were also analyzed for ADF (Ankom Daisy II, ANKOM Technology Corp., Fairport, NY) and soluble CP, degradable CP, ether extract, and minerals by wet chemistry (Dairy One, Forage Analysis Laboratory, Ithaca, NY). Bacterial samples were analyzed for ash and CP (AOAC, 1990). Purine concentrations (Zinn and Owens, 1986) in effluent and bacterial

Table 2. Ingredient and chemical composition (mean $\pm$ SD) of concentrate and pasture used in dietary treatments.

	Concentrate	Pasture
Ingredient composition		
Dry corn	71.7	—
Wheat midds	14.5	—
Molasses	3.4	—
Protein blend ¹	3.2	—
Animal fat	1.6	—
Canola meal	1.1	—
Limestone	1.0	—
Dicalcium phosphate	0.9	—
Salt	0.9	—
Corn gluten meal	0.7	—
Dynamate 22S	0.40	—
Magnesium oxide	0.36	—
PS TM premix	0.12	—
Selenium premix	0.07	—
Vitamins A, D, E	0.05	—
Chemical composition		
OM, % of DM	93.8 $\pm$ 0.2	90.4 $\pm$ 0.1
CP, % of DM	12.9 $\pm$ 0.2	25.3 $\pm$ 0.7
Soluble CP, % of CP	16.1 $\pm$ 0.4	40.7 $\pm$ 0.9
Degradable CP, % of CP	31.7 $\pm$ 0.5	84.2 $\pm$ 1.1
NSC, % of DM	57.7 $\pm$ 1.6	18.0 $\pm$ 1.4
NDF, % of DM	14.3 $\pm$ 0.2	37.5 $\pm$ 2.1
ADF, % of DM	5.9 $\pm$ 0.2	23.2 $\pm$ 1.4
Ether extract, % of DM	6.7 $\pm$ 0.3	4.5 $\pm$ 0.7
NE _L , Mcal/kg ²	1.89	1.46
Ca, % of DM	1.24 $\pm$ 0.40	0.44 $\pm$ 0.30
P, % of DM	0.74 $\pm$ 0.10	0.42 $\pm$ 0.07
Mg, % of DM	0.52 $\pm$ 0.08	0.16 $\pm$ 0.04
K, % of DM	0.44 $\pm$ 0.05	3.67 $\pm$ 0.45
Na, % of DM	0.54 $\pm$ 0.06	0.02 $\pm$ 0.01

¹One-third of each fish meal, feather meal, and blood meal.²Estimated using values of NRC (2001).

isolates were used to partition effluent N flow into bacterial and nonbacterial fractions and to calculate true DM and OM digestibility and flows.

Statistical Analyses

Data were analyzed as 4×4 Latin square design using the PROC MIXED procedure of SAS (1999). The model included the fixed effects of treatments and period, the random effect of fermenter, and the residual error. Least squares means and SEM are reported for all data. Significance was declared at $P < 0.05$. To accomplish the first objective, linear and quadratic contrasts were conducted within the pasture-only treatments. Most of the variables measured did not show significant quadratic effects; therefore, only linear effects are presented. To accomplish the second objective, the following contrasts were conducted: 1) low SR: PC vs. LP, 2) medium SR: PC vs. MP, and 3) high SR: PC vs. HP.

RESULTS AND DISCUSSION

Diets

The concentrate, based on dry ground corn (72%, on DM basis), had 12.9% CP and 1.89 Mcal of NE_L/kg DM (Table 2). The pasture was of high quality with low NDF (37.5%) and high CP (25.3%) and reflected the vegetative, early spring stage of growth at harvest (Table 2). Previous studies (Bargo et al., 2002a, 2002b) conducted using the same pasture reported higher NDF content ($\geq 50\%$); however, in those studies samples were taken during the entire grazing season from late April to early October.

Compared to the PC treatment, the SR (reduction in pasture DMI by the concentrate DMI) was 0.33, 0.67, and 1.00 g of pasture/g of concentrate for the LP, MP, and HP treatments, respectively (Table 1). On the pasture-only diets, intake of N, NSC, and NDF linearly increased as the amount of pasture DMI increased from 55 to 75 g/d. Concentrate supplementation reduced the intake of N and NDF but increased the intake of NSC.

Table 3. Nutrient digestibility of pasture-only and pasture plus concentrate diets during continuous culture fermentation.

	Treatments ¹				SEM	P, $P \leq^2$	SR, $P \leq^3$		
	LP	MP	HP	PC		Linear	Low	Medium	High
Apparent digestibility									
DM, %	47.5	50.6	49.0	52.2	1.29	0.33	0.02	0.33	0.08
OM, %	61.5	64.2	62.5	65.2	1.16	0.51	0.04	0.53	0.12
NDF, %	76.1	78.7	80.2	75.1	2.30	0.25	0.74	0.29	0.15
NSC, %	77.9	78.9	78.1	85.1	1.24	0.81	0.01	0.01	0.01
True digestibility									
DM, %	62.7	68.8	70.9	72.4	3.02	0.14	0.09	0.49	0.78
OM, %	77.9	83.8	86.1	86.4	3.15	0.15	0.14	0.62	0.96

¹LP = Low pasture (55 g DM/d), MP = medium pasture (65 g DM/d), HP = high pasture (75 g DM/d), PC = pasture (45 g DM/d) plus concentrate (30 g DM/d).

²Preplanned orthogonal contrast for increasing the amount of pasture (P): Linear = linear contrast for LP, MP, and HP.

³Preplanned orthogonal contrast for levels of substitution rate (SR): Low SR: PC vs. LP, Medium SR: PC vs. MP, High SR: PC vs. HP.

Total diets averaged 25.3% CP, 18.0% NSC, 37.5% NDF, and 1.46 Mcal of NE_L/kg DM for the three pasture-only treatments, and 20.3% CP, 33.9% NSC, 28.2% NDF, and 1.63 Mcal of NE_L/kg DM for the PC treatment (Table 1).

Digestibility of Nutrients

Effect of increased pasture DMI. Apparent digestibility of DM and OM were not affected by the amount of pasture DMI and averaged 49.0 and 62.7%, respectively (Table 3). These DM and OM digestibilities are in general agreement with those of deVeth and Kolver (2001), who reported DM and OM digestibilities of 55.9 and 58.2%, respectively, for a ryegrass-only diet fermented in continuous culture. Both true DM and OM digestibility tended to increase ($P < 0.15$) within the pasture-only diets as the amount of pasture DMI increased (Table 3), which is probably associated with the reduction in pH observed as pasture DMI increased (Table 4). Other continuous culture studies reported lower true DM and OM digestibility for legume/grass mix (Bach et al., 1999) or orchardgrass (Kolver et al., 1998) pastures of higher NDF content.

Apparent digestibility of NDF was not affected by the amount of pasture DMI (Table 3) and averaged 78.3%, indicating the high quality of the pasture. Previous studies reported lower apparent digestibility of NDF (28.1%, Bach et al., 1999; 43.5%, Kolver et al., 1998), probably because the pastures had higher NDF content (>45%). Apparent NDF digestibility during continuous culture fermentation of a ryegrass, with a similar NDF content (37.9%) to our pasture averaged 71.3% at pH above 6.2 (deVeth and Kolver, 2001). Apparent digestibility of NSC did not differ among the pasture-only treatments and averaged 78.3% (Table 3). deVeth and

Kolver (2001) reported an apparent NSC digestibility of 83.4% for ryegrass only diets. Others (Kolver et al., 1998; Bach et al., 1999) indicated apparent NSC digestibility of pasture-only diets between 40.8 and 64.9%.

Effect of different SR. Concentrate supplementation did not change apparent or true DM digestibility at the high SR (PC vs. HP) but increased ($P < 0.05$) or tended to increase ($P < 0.10$) apparent and true DM digestibility at the low SR (PC vs. LP; Table 3). Similar values of apparent (52.7%) and true (70.1%) digestibility of DM were reported during continuous culture fermentation when pasture and a corn-based concentrate were fed twice daily (Hongerholt et al., 1998). In a continuous culture study, Kolver et al. (1998) reported a similar true digestibility of DM (68.3%) for a pasture plus starch diet with a SR of 1. Bach et al. (1999), however, found that supplementation with cracked-corn at a SR of 1 increased true digestibility of DM compared to pasture only (57.5 vs. 41.2%) during continuous culture fermentation. The lower values for digestibility in that study compared to this current study could be attributed to the higher NDF content (47.4%) and the lower NSC content (10.6%) in the pasture, the use of cracked instead of ground corn, and the higher passage rate of digesta (7 vs. 4%/h). Apparent and true digestibility of OM followed the same tendency as observed for DM digestibility with similar values at high SR (PC vs. HP) but with significantly higher values with concentrate supplementation at low SR (PC vs. LP; Table 3). The similar OM digestibility between pasture-only and pasture plus supplement diets at a high SR is in agreement with the results of Kolver et al. (1998), who replaced orchardgrass pasture at a 1:1 basis in a continuous culture.

Concentrate supplementation numerically but not significantly reduced the NDF apparent digestibility at

Table 4. Fermentation of pasture-only and pasture plus concentrate diets during continuous culture fermentation.

	Treatments ¹				SEM	P, $P \leq^2$		SR, $P \leq^3$	
	LP	MP	HP	PC		Linear	Low	Medium	High
pH ⁴	6.98	6.89	6.55	5.97	0.05	0.01	0.01	0.01	0.01
VFA, ⁵ mmol/L									
Total	61.7	81.8	96.1	113.2	2.7	0.01	0.01	0.01	0.01
Acetate (A)	39.7	51.6	59.3	70.5	1.7	0.01	0.01	0.01	0.01
Propionate (P)	13.3	15.6	20.7	25.7	0.7	0.01	0.01	0.01	0.01
Butyrate	5.3	9.8	10.8	12.2	0.5	0.01	0.01	0.01	0.01
Isobutyrate	0.60	0.79	0.85	0.69	0.06	0.01	0.23	0.15	0.04
Valerate	1.60	2.55	2.87	2.56	0.14	0.01	0.01	0.96	0.02
Isovalerate	1.15	1.41	1.58	1.56	0.11	0.01	0.02	0.35	0.93
A/P	3.11	3.38	2.90	2.87	0.07	0.02	0.01	0.01	0.76

¹LP = Low pasture (55 g DM/d), MP = medium pasture (65 g DM/d), HP = high pasture (75 g DM/d), PC = pasture (45 g DM/d) plus concentrate (30 g DM/d).

²Preplanned orthogonal contrast for increasing the amount of pasture (P): Linear = linear contrast for LP, MP, and HP.

³Preplanned orthogonal contrast for levels of substitution rate (SR): Low SR: PC vs. LP, Medium SR: PC vs. MP, High SR: PC vs. HP.

⁴Mean of 24 sampling times at each hour.

⁵Mean of 6 sampling times at 0, 0.5, 1, 2, 4, and 6 h starting at 0600 h.

the high SR (PC vs. HP; 75.1 vs. 80.2%) with similar NDF apparent digestibility at the low SR (PC vs. LP; Table 3). Supplementation with starch (Kolver et al., 1998) or cracked-corn (Bach et al., 1999) did not reduce apparent digestibility of NDF. Apparent digestibility of NSC was significantly higher with concentrate supplementation at the three levels of SR (85.1 vs. 78.3%; Table 3). This agrees with previous continuous culture studies that showed higher apparent NSC digestibility of pasture plus corn (79.0 vs. 64.9%; Bach et al., 1999) or pasture plus starch (76.5 vs. 40.8%; Kolver et al., 1998) compared to pasture only.

pH and Volatile Fatty Acids

Effect of increased pasture DMI. As pasture DMI increased from 55 to 75 g/d, mean pH was reduced ($P < 0.05$) linearly from 6.98 to 6.55 (Table 4), which is consistent with feeding restricted or ad libitum pasture. Ad libitum feeding of ryegrass/white clover pasture to dairy cows resulted in lower ruminal pH than restricted feeding (6.13 vs. 6.35; Mackle et al., 1996). Bargo et al. (2002a) also reported lower rumen pH (6.40 vs. 6.57) for dairy cows grazing at a high pasture allowance compared to a low pasture allowance. Higher pH with lower amounts of pasture found in this current study were expected; however, the actual values were higher than those reported by studies with dairy cows (Mackle et al., 1996; Bargo et al., 2002a). The type of buffer used in the fermenters may partially explain the high values. Bach et al. (1999) reported a pH of 6.10 for a legume/grass pasture-only diet in continuous culture fermentation. The three pasture-only treatments followed the

same pattern of pH fluctuations over 24 h (Figure 1) with the two highest peaks before the 0630 and 1730 h pasture feeding and two smaller peaks before the 1000- and 2100-h pasture feedings. None of the pasture-only treatments had a pH lower than 6.2 (Figure 1). This diurnal pattern in ruminal pH is consistent with those reported for grazing dairy cows (Bargo et al., 2002a).

Total VFA concentration in the pasture-only treatments followed the inverse pattern compared with that of pH, with a linear increase from 61.7 to 96.1 mmol/L as pasture DMI increased (Table 4). This agrees with

Figure 1. Diurnal variation in ruminal pH during fermentation of low pasture (LP), medium pasture (MP), high pasture (HP), and pasture plus concentrate (PC) diets. Black arrows indicate time of concentrate feeding (0600 and 1700 h) and gray arrows indicate time of pasture feeding (0630, 1000, 1730, and 2100 h).

results from dairy cows grazing at a high pasture allowance that had higher total VFA concentration than cows grazing at a low pasture allowance (129.8 vs. 116.3 mmol/L; Bargo et al., 2002a). The total VFA concentration found in the LP treatment (65 g DM/d) was similar to that reported for a ryegrass only diet fed at 60 g DM/d in continuous culture (62.3 mmol/L; deVeth and Kolver, 2001). During continuous culture fermentation, higher values of total VFA concentration (82.6 to 126.4 mmol/L) were reported for pasture-only diets (Kolver et al., 1998; Bach et al., 1999) fed at 75 g DM/d, which equates to the level of pasture DMI used in the HP treatment. All the individual VFA concentrations (acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate) increased linearly as pasture DMI increased (Table 4). Bargo et al. (2002a) also reported higher ruminal concentrations of acetate and butyrate in dairy cows as pasture allowance increased.

Effect of different SR. Concentrate supplementation reduced ($P < 0.05$) pH at the high, medium, and low SR (PC vs. HP, MP, and LP; 5.97 vs. 6.55, 6.89, and 6.98, respectively; Table 4). Bach et al. (1999) also reported in a continuous culture study that cracked-corn supplementation at a SR of 1 reduced pH compared to pasture-only (6.02 vs. 6.10). All three pasture-only treatments had a higher ruminal pH during the 24-h sampling period than the PC treatment (Figure 1), with the HP treatment recording a pH that was closer to the PC treatment. The highest pH peaks for the PC treatment were at 0600 and 1700 h before concentrate feeding. Concentrate feeding reduced pH to minimum values at 1100 and 2200 h. This type of pattern with the highest pH values before concentrate feeding and the lowest pH values a couple of hours later has been previously described in continuous culture fermenters fed with pasture and concentrate (Hongerholt et al., 1998) but also in studies with dairy cows grazing similar pasture (Bargo et al., 2002a).

At the three levels of SR (PC vs. HP, MP, and LP), concentrate supplementation increased ($P < 0.05$) total VFA concentration compared to pasture-only (113.2 vs. 96.1, 81.8, and 61.7 mmol/L, respectively; Table 4), which is consistent with the lower pH observed in the PC treatment compared to the three pasture-only treatments. Hongerholt et al. (1998) reported a total VFA concentration of 97.2 mmol/L for PC diets in continuous culture. In agreement with this current study, Bach et al. (1999) found higher total VFA concentration with pasture-plus cracked-corn than with pasture-only (141.9 vs. 126.4 mmol/L), although Kolver et al. (1998) reported no differences in total VFA concentration (85.0 mmol/L) when pasture plus starch and pasture-only diets were compared. A digestion study with ruminally and duodenally cannulated cows also reported no differ-

ences in total VFA concentration (94.7 mmol/L) when cows were fed winter oats only or winter oats plus corn or barley replacing the pasture at a SR of 1 (García et al., 2000). Most of the individual VFA concentrations increased ($P < 0.05$) with the concentrate supplementation at the three levels of SR (PC vs. HP, MP, and LP; Table 4). Kolver et al. (1998) reported no differences in acetate and propionate concentration between pasture-only and pasture-plus starch diets in continuous culture. Proportions of individual VFA (mol/100 mol) did not differ among treatments in this study (data not shown). Supplementation with cracked-corn reduced the proportion of acetate (54.3 vs. 71.4 mol/100 mol) and increased the proportion of propionate (22.1 vs. 17.8 mol/100 mol) compared to pasture only in a continuous culture study (Bach et al., 1999). García et al. (2000) reported for ruminally and duodenally cannulated cows a lower proportion of acetate and a higher proportion of propionate with corn or barley supplementation compared to winter oats-only diets.

Nitrogen Metabolism

Effect of increased pasture DMI. Intake of N increased ($P < 0.05$) linearly from 2.2 to 3.0 g/d (Table 5) as the amount of pasture DMI increased from 55 to 75 g/d. As the amount of N intake increased within the pasture-only diets, $\text{NH}_3\text{-N}$ concentration increased linearly ($P < 0.05$) from 23.4 to 27.1 mg/dl. Kolver et al. (1998) reported a $\text{NH}_3\text{-N}$ concentration of 35.2 mg/dl for a orchardgrass pasture with a CP content of 24.4%. For pastures lower in CP content (<20%) than the one used in this study, lower $\text{NH}_3\text{-N}$ concentrations have been reported (10.3 mg/dl, Bach et al., 1999; 16.0 mg/dl, deVeth and Kolver, 2001).

Flow of total N, NAN, and bacterial N increased linearly ($P < 0.05$) as the pasture DMI increased (Table 5). A significant quadratic increase was reported for bacterial N flow as the pH increased from 5.4 to 6.6 (de Veth and Kolver, 2001). Significant relationships were found between OM intake (OMI, g/d) and flows of total N (TN, g/d), NAN (g/d), and bacterial N (BN, g/d) indicating that an increase in OMI increased the TN flow: $\text{TN} = 0.35 (\text{SE } 0.27) + 0.031 (\text{SE } 0.005) \text{ OMI}$ ($P < 0.05$; $R^2 = 0.81$); NAN flow: $\text{NAN} = -0.25 (\text{SE } 0.49) + 0.029 (\text{SE } 0.008) \text{ OMI}$ ($P < 0.05$; $R^2 = 0.55$); and BN flow: $\text{BN} = -1.26 (\text{SE } 0.51) + 0.041 (\text{SE } 0.009) \text{ OMI}$ ($P < 0.05$; $R^2 = 0.70$). No significant ($P > 0.05$) relationship was found between OMI and flow of dietary N. The positive relationship between OMI and BN flow and the lack of relationship between OMI and dietary N flow indicates that the BN fraction primarily contributed to the increased in flow of NAN as OMI increased. In the review by Clark et al. (1992), significant positive relationships

Table 5. Nitrogen metabolism of pasture-only and pasture plus concentrate diets during continuous culture fermentation.

	Treatments ¹				SEM	P, $P \leq^2$	SR, $P \leq^3$		
	LP	MP	HP	PC		Linear	Low	Medium	High
N intake, g/d	2.22	2.63	3.03	2.44	0.01	0.01	0.01	0.01	0.01
NH ₃ -N, ⁴ mg/dl	23.4	27.5	27.1	15.0	1.4	0.04	0.01	0.01	0.01
CP degradation, %	78.3	91.5	91.0	84.5	5.7	0.22	0.53	0.48	0.50
N flows, g/d									
Total N	1.88	2.13	2.42	2.07	0.05	0.01	0.02	0.36	0.01
NH ₃ -N	0.61	0.82	0.63	0.44	0.09	0.86	0.27	0.03	0.22
NAN	1.27	1.31	1.79	1.63	0.09	0.01	0.05	0.06	0.29
Bacterial N	0.79	1.08	1.53	1.25	0.12	0.01	0.05	0.38	0.19
Dietary N	0.48	0.23	0.27	0.38	0.15	0.43	0.69	0.56	0.67
N flows, % of total N flow									
NH ₃ -N	32.2	38.8	25.8	21.4	4.1	0.36	0.15	0.04	0.52
NAN	67.8	61.2	74.2	78.6	4.1	0.36	0.15	0.04	0.52
Bacterial N	42.3	50.6	62.6	60.3	4.9	0.04	0.06	0.27	0.78
Dietary N	25.5	10.6	11.6	18.3	7.0	0.26	0.54	0.52	0.57
Bacteria efficiency									
g N/kg DM truly digested	22.8	23.9	28.7	23.0	1.79	0.07	0.94	0.74	0.08
g N/kg OM truly digested	20.3	21.8	26.1	21.0	1.71	0.07	0.80	0.79	0.09

¹LP = low pasture (55 g DM/d), MP = medium pasture (65 g DM/d), HP = high pasture (75 g DM/d), PC = pasture (45 g DM/d) plus concentrate (30 g DM/d).

²Preplanned orthogonal contrast for increasing the amount of pasture (P): Linear = linear contrast for LP, MP, and HP.

³Preplanned orthogonal contrast for levels of substitution rate (SR): Low SR: PC vs. LP, Medium SR: PC vs. MP, High SR: PC vs. HP.

⁴Mean of 6 sampling times at 0, 0.5, 1, 2, 4, and 6 h starting at 0600 h.

were reported between OMI and flow of NAN and BN based on a database from more than 36 experiments with duodenally cannulated cows. The increase in bacterial N flow can be partially attributed to the larger amount of energy supplied by the larger amount of OM fermented (Clark et al., 1992). For the pasture-only diets, a significant relationship was found between BN flow (BN, g/d) and intake of OM digested (OMD, g/d): $BN = -0.78 \text{ (SE 0.25)} + 0.039 \text{ (SE 0.005) OMD}$ ($P < 0.05$; $R^2 = 0.86$).

Significant relationships were also detected between N intake (NI, g/d) and flows of TN (g/d), NAN (g/d), and BN (g/d) within the pasture-only treatments. This indicates that increasing N intake increased the TN flow: $TN = 0.35 \text{ (SE 0.24)} + 0.68 \text{ (SE 0.09) NI}$ ($P < 0.05$; $R^2 = 0.85$); NAN flow: $NAN = -0.20 \text{ (SE 0.49)} + 0.63 \text{ (SE 0.18) NI}$ ($P < 0.05$; $R^2 = 0.54$); and BN flow: $BN = -1.12 \text{ (SE 0.54)} + 0.86 \text{ (SE 0.20) NI}$ ($P < 0.05$; $R^2 = 0.64$). No significant ($P > 0.05$) relationship was found between NI and flow of dietary N. A positive relationship between NI and passage of NAN to the SI was reported by Clark et al. (1992).

Flows of NH₃-N (0.69 g/d) or dietary N (0.33 g/d) did not differ among the pasture-only treatments (Table 5). When expressed as a percentage of total N flow, flows of NH₃-N, NAN, and dietary N were not affected by pasture DMI. Flow of bacterial N, as percentage of total N flow, was linearly increased ($P < 0.05$) from 42.3 to

62.6% as pasture DMI increased, which is in agreement with the significant relationship between OMI and bacterial N flow and the lack of a significant relationship between OMI and dietary N flow. Efficiency of bacterial protein synthesis per kilogram of DM or OM truly digested within the pasture-only diets tended to increase linearly ($P < 0.07$) as pasture DMI increased. A quadratic increase in efficiency of bacterial protein synthesis (g of N/kg of OM truly digested) when pH increased from 5.4 to 6.6 has been reported by de Veth and Kolver (2001). Other continuous culture studies (Kolver et al., 1998; Bach et al., 1999) reported similar values of efficiency of bacterial protein synthesis for pasture-only diets.

Effect of different SR. Intake of N was significantly lower with concentrate supplementation at the high and medium SR (PC vs. HP and MP) but was significantly higher at the low SR (PC vs. LP; Table 5). Concentrate supplementation reduced ($P < 0.05$) NH₃-N concentration at the three levels of SR (PC vs. HP, MP, and LP; 15.0 vs. 27.1, 27.5, and 23.4 mg/dl). Previous continuous culture studies reported a reduction in NH₃-N concentration from 35.2 to 14.2 mg/dl with starch supplementation (Kolver et al., 1998) and from 10.3 to 2.1 mg/dl with cracked-corn supplementation (Bach et al., 1999). Studies with ruminally cannulated cows on pasture also reported reductions in NH₃-N concentration with supplementation of corn-based concentrate

(8.9 vs. 15.3 mg/dl; Bargo et al., 2002a) or with corn or barley grains (22.9 vs. 29.0 mg/dl; García et al., 2000).

At the high SR (PC vs. HP), concentrate supplementation reduced ($P < 0.05$) flow of total N but did not affect flows of $\text{NH}_3\text{-N}$, NAN, BN, or dietary N (Table 5). However, at the low SR (PC vs. LP), concentrate supplementation increased ($P < 0.05$) flows of total N, NAN, and BN without affecting flows of $\text{NH}_3\text{-N}$ and dietary N (Table 5). Previous continuous culture studies (Bach et al., 1999; Kolver et al., 1998), where pasture was supplemented at a SR of 1, reported no differences in bacterial N flow. None of the N fractions differed at the high and low SR (PC vs. HP and LP), when expressed as percentage of total N flow. Efficiency of bacterial protein synthesis per kilogram of DM or OM truly digested tended to be reduced by concentrate supplementation at the high SR (PC vs. HP; $P < 0.09$), but efficiency was not changed compared at the low SR (PC vs. LP). This agrees with Bach et al. (1999), who found that efficiency of bacterial protein synthesis was reduced (27.7 vs. 21.2 g N/kg OM truly digested) when pasture was supplemented with corn at a SR of 1.

Rumen Degradable Nitrogen to Rumen Degradable Organic Matter Ratio

The RDN:RDOM ratio and the intake of RDN and RDOM are shown in Figure 2. As we hypothesized, concentrate supplementation reduced ($P < 0.05$) the RDN:RDOM ratio compared to all the three pasture-only treatments (34.7 vs. 47.0 g/kg). Kolver et al. (1998) reported a reduction in the RDN:RDOM from 50.5 to 33.0 g/kg when an orchardgrass pasture was supplemented with starch in continuous culture. However, the reduction in the RDN:RDOM ratio by concentrate supplementation occurred for different reasons in each of the different pasture-only diets. At the high SR (PC vs. HP), concentrate supplementation resulted in a significantly lower RDN:RDOM ratio because of a reduction in RDN (2.06 vs. 2.76 g) with no effect on RDOM (58.9 g; Figure 2). The reduction in RDN was associated with a significantly lower $\text{NH}_3\text{-N}$ concentration (15.0 vs. 27.1 mg/dl; Table 5) but had no effect in the flow of bacterial N (1.39 g/d; Table 5). At the low SR (PC vs. LP), concentrate supplementation resulted in a significantly lower RDN:RDOM ratio because of an increase in RDOM (38.7 vs. 59.4 g) with no effect on RDN (1.90 g; Figure 2). The increase in RDOM resulted not only in a significantly lower $\text{NH}_3\text{-N}$ concentration (15.0 vs. 23.4 mg/dl; Table 5) but also in a significant increase in the flow of BN (1.25 vs. 0.79 g/d; Table 5). The increase in flow of bacterial N as RDOM increased is further described by the significant relationship found between

Figure 2. Rumen degradable N (RDN) to rumen degradable OM (RDOM) ratio of low pasture (LP), medium pasture (MP), high pasture (HP), and pasture plus concentrate (PC) treatments. Means with different superscripts differ ($P < 0.05$).

intake of RDOM (g/d) and BN flow (g/d): $\text{BN} = -0.46 (\text{SE } 0.26) + 0.032 (\text{SE } 0.005) \text{RDOM}$ ($P < 0.05$; $R^2 = 0.74$).

The changes in RDN and RDOM when pasture is supplemented with concentrate are in agreement with previous continuous culture (Kolver et al., 1998; Bach et al., 1999) and dairy cows (O'Mara et al., 1997; García et al., 2000; Bargo et al., 2002a) studies. Kolver et al. (1998) replaced pasture with starch at a SR of 1 g/g and did not find changes in the flow of BN (1.13 g/d). Another continuous culture study (Bach et al., 1999) did not report differences in flow of BN (0.83 g/d) when

pasture was replaced by cracked-corn at a SR of 1 g/g. Two studies with duodenally cannulated cows also used a SR close to 1 kg/kg when pasture diets were supplemented with barley or corn (García et al., 2000) or molassed beet pulp (O'Mara et al., 1997). Passage of BN to the small intestine did not differ (99.8 g/d) between diets of winter oats pasture or winter oats plus corn or barley (García et al., 2000). García et al. (2000) concluded that greater flows of BN and animal response to supplementation might be expected when each unit of supplement substitutes less than one unit of pasture. Supplementation with molassed beet pulp to a ryegrass pasture did not increase passage of BN postruminally (261 g/d; O'Mara et al., 1997). O'Mara et al. (1997) concluded that the lack of a significant increase in the flow of microbial N to the duodenum was due to the high SR of supplement for pasture. When concentrate supplementation to high producing dairy cows grazing an orchardgrass pasture at a low pasture allowance resulted in low SR (0.26 kg pasture/kg concentrate), rumen $\text{NH}_3\text{-N}$ concentration was reduced (9.1 vs. 15.2 mg/dl) and microbial protein synthesis (estimated by the allantoin:creatinine ratio in urine) was increased (Bargo et al., 2002a). In the current study a significant negative relationship was found between SR (g of pasture/g of concentrate) and the increase in bacterial N (g/d) by concentrate supplementation: $\text{BNi} = 0.85 \text{ (SE } 0.21) - 1.09 \text{ (SE } 0.29) \text{ SR}$ ($P < 0.05$; $R^2 = 0.59$).

IMPLICATIONS

Substitution rate is a key determinant of the milk yield milk response to supplementation (Stakelum, 1986; Kellaway and Porta, 1993; Bargo et al., 2002a). Bargo et al. (2003) reviewed the literature on supplementation to high-producing dairy cows on pasture and found a negative relationship between milk response to supplementation and SR, i.e., the lower the SR the higher the milk response per kilogram of supplement. High-producing dairy cows grazing an orchardgrass pasture at a low pasture allowance had lower SR (0.26 vs. 0.55 kg pasture/kg concentrate) and higher milk response (1.36 vs. 0.96 kg milk/kg concentrate) than dairy cows grazing at a high pasture allowance (Bargo et al., 2002a). Kellaway and Porta (1993) concluded that the milk response increased from 0 to 0.6 kg of milk/kg of concentrate when pasture management changed from ad libitum to restricted pasture feeding. The current study showed that when SR is high, concentrate supplementation reduced $\text{NH}_3\text{-N}$ concentration but did not increase flow of BN. Based on these data, the milk yield response to supplementation would be expected to be negligible at a high SR because total DMI and synthesis of microbial protein is not increased

compared to pasture-only diets. At low SR, concentrate supplementation not only reduced $\text{NH}_3\text{-N}$ concentration but also increased the flow of BN. The milk yield response to supplementation, when SR is low, would be high because of the increase in total DMI, improved utilization of ruminal N, and greater synthesis of microbial protein. The typical SR found with high producing dairy cows is 0.4 to 0.6 kg of pasture/kg of supplement (Bargo et al., 2003), similar to the lower SR used in this study.

CONCLUSIONS

Increased pasture DMI did not affect DM, OM, or NDF digestibility of pasture-only diets. As pasture DMI increased, pH was linearly reduced and total VFA and $\text{NH}_3\text{-N}$ concentration were linearly increased. Flows of total N, NAN, and bacterial N increased linearly as pasture DMI increased. Concentrate supplementation did not affect DM or OM digestibility at high SR but increased DM and OM digestibility at low SR. Digestibility of NDF was not affected by concentrate supplementation at any of the three levels of SR. Concentrate supplementation reduced pH and $\text{NH}_3\text{-N}$ concentration at low, medium, and high SR. Concentrate supplementation also reduced the RDN:RDOM ratio in all of the pasture-only diets, but the mechanism depended on SR. At high SR, concentrate supplementation reduced RDN, which reduced $\text{NH}_3\text{-N}$ concentration without affecting BN flow. At low SR, concentrate supplementation increased RDOM, which reduced $\text{NH}_3\text{-N}$ concentration and increased BN flow.

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