Diurnal Variation in Ruminal pH on the Digestibility of Highly Digestible Perennial Ryegrass During Continuous Culture Fermentation

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ABSTRACT

Dairy cows grazing high-digestibility pastures exhibit pronounced diurnal variation in ruminal pH, with pH being below values considered optimal for digestion. Using a dual-flow continuous culture system, the hypothesis that minimizing diurnal variation in pH would improve digestion of pasture when pH was low, but not at a higher pH, was tested. Four treatments were imposed, with pH either allowed to exhibit normal diurnal variation around an average pH of 6.1 or 5.6, or maintained at constant pH. Digesta samples were collected during the last 3 d of each of four, 9-d experimental periods. A constant pH at 5.6 compared with a constant pH of 6.1 reduced the digestibility of organic matter (OM), neutral detergent (NDF), and acid detergent fiber (ADF) by 7, 14, and 21%, respectively. When pH was allowed to vary (averaging 5.6), digestion of OM, NDF, and ADF were reduced by 15, 30, and 36%, respectively, compared with pH varying at 6.1. There was little difference in digestion parameters when pH was either constant or varied with an average pH of 6.1. However, when average pH was 5.6, maintaining a constant pH significantly increased digestion of OM, NDF, and ADF by 5, 25, and 24% compared with a pH that exhibited normal diurnal variation. These *in vitro* results show that gains in digestibility and potential milk production can be made by minimizing diurnal variation in ruminal pH, but only when ruminal pH is low (5.6). However, larger gains in productivity can be achieved by increasing average daily ruminal pH from 5.6 to 6.1.

(Key words: ruminal pH, pasture, diurnal variation, digestibility)

Abbreviation key: DOMD = organic matter digestibility of DM, C5.6 = constant pH of 5.6, C6.1 = constant pH of 6.1, ME = metabolizable energy, V5.6 = normal

diurnal variation at an average pH of 5.6, V6.1 = normal diurnal variation at an average pH of 6.1, respectively.

INTRODUCTION

Understanding the influence of ruminal pH on digestibility is important when devising feeding strategies. Recent studies suggest a different relationship exists between ruminal pH and digestion parameters when cows are fed fresh pasture compared with TMR. A study of 121 pasture-based diets explored relationships between ruminal fluid pH and measurements of rumen fermentation of pasture and its effect on animal production (Kolver and de Veth, 2002). Mean daily ruminal fluid pH varied from 5.6 to 6.7. Low ruminal pH was associated with a higher microbial N flow from the rumen, higher milk yield, and lower concentrations of milk fat. Milk yields were highest in the pH range 5.8 to 6.2. These pH levels are considered below optimal for digestion of TMR, but the *in vivo* and *in vitro* studies suggest a greater tolerance of low pH when low-starch pasture diets with high fiber fermentability are fed (de Veth and Kolver, 2001a; Kolver and de Veth, 2002). Although de Veth and Kolver (2001a) reported that digestion of pasture was optimized at pH 6.35 in an *in vitro* continuous culture system, digestion and synthesis of microbial protein were largely insensitive to pH across a broad range of pH from 5.8 to 6.6.

In addition to having a low average daily ruminal pH, grazing dairy cows offered highly digestible pasture often have pronounced diurnal variation in ruminal pH, with pH ranging from 5.5 to 6.8 over a 24-h period (Carruthers et al., 1997; Kolver and de Veth, 2002; Wales and Doyle, 2003). What is unclear is the extent to which diurnal variation vs. average daily pH affects ruminal digestion and microbial protein synthesis.

Variation in ruminal pH can be explained in part by the diurnal grazing pattern of the cow and periodicity of DMI and subsequent rumination (Wales and Doyle, 2003). Ruminal pH is the net effect of rates of production and absorption of acidic fermentation products and the addition of buffers to the rumen via saliva. Williams

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et al. (2001) reported that the average daily ruminal pH of grazing dairy cows was lower when DMI was high and was lower when Persian clover (*Trifolium resupinatum* L.) was consumed compared with perennial ryegrass (*Lolium perenne* L.). This research appears to represent the lowest average daily ruminal pH (5.6) reported for grazing dairy cows.

For cows consuming concentrate/forage diets, Hoover (1986) suggested that cyclic and short duration reductions in culture pH below 6.2 would cause a moderate, transient reduction in fiber digestion. However, further pH reductions in the range 5.0 to 5.5 for longer periods would cause severe reductions in OM and fiber digestion through reduced growth rates and numbers of cellulolytic organisms. This is supported by experiments of Stewart (1977), who showed that in vitro digestion of cellulose was completely inhibited when pH was 5.2.

Viable cellulolytic microbial populations remain present in the rumen as long as ruminal fluid pH is above the required pH threshold for sufficient periods during the day to permit microbial growth at rates equal to or greater than fiber passage rate (Weimer, 1998). However, this threshold is not well defined. De Veth and Kolver (2001b) maintained pH in continuous culture at a suboptimal level (5.4) for 4- to 12-h periods. Results suggested that the period of time that pH was below optimal (pH 6.3) may be more critical for digestion than the relationship between mean daily pH and optimal pH.

Reducing diurnal variation in ruminal pH may provide opportunities to improve digestion and, subsequently, milk production. A review of 35 experiments that have increased feeding frequency demonstrated average increases in milk fat concentrations of 7% and milk fat yield of 8% (Gibson, 1984). Improvements in milk fat concentrations have only been reported for diets that included more than 60% concentrate (Sutton et al., 1985). Compared with these high-starch diets, the average ruminal pH of cows grazing good-quality pasture is approximately 6.15 (Kolver and de Veth, 2002). It might be expected that a more stabilized rumen environment would not improve milk fat concentrations on these diets. However, the impact of diurnal variation on digestibility is uncertain when very fermentable forages, such as clover or ryegrass, being rapidly growing, result in low average daily ruminal pH (5.6; Williams et al., 2001).

The current study tested two hypotheses using dual-flow continuous culture fermenters with a diet of highly digestible perennial ryegrass pasture: 1) digestion of OM, NDF, and ADF will be lower when the average daily culture pH is maintained at a constant pH of 5.6 compared with 6.1, and 2) diurnal variation in culture

Table 1. Metabolizable energy concentrations (MJ/kg of DM) and chemical composition of the perennial ryegrass diet (% of DM).

Composition	
ME ¹	12.3
OM	89.8
CP ²	19.0
NDF	43.2
ADF	23.1
NSC ³	16.3
Crude fat	4.00
Ca	0.48
Mg	0.19
P	0.39
Na	0.21
K	3.12

¹ME = Metabolizable energy = organic matter digestibility of DM $\times$ 0.16.

²CP = % N $\times$ 6.25.

³NSC = Water-soluble carbohydrate and starch.

pH will reduce digestibility when average culture fluid pH is 5.6, but not when culture pH is 6.1.

MATERIALS AND METHODS

Four treatments were tested where highly digestible perennial ryegrass was fed to 4 fermenters twice daily during 4 9-d periods according to a randomized complete block design. Culture pH was either allowed to exhibit normal diurnal variation around an average pH of 6.1 or 5.6 (**V6.1** and **V5.6**, respectively), or maintained at a constant pH equivalent to the average pH of the variable treatments (**C6.1** and **C5.6**). The rationale for selecting the treatments was to represent the diurnal patterns of ruminal pH observed in dairy cows grazing highly digestible pasture and to investigate opportunities to improve digestion by minimizing diurnal pH variation. The pasture fermented was of sufficient quality to result in a mean daily culture pH of 6.1 (**V6.1**). The pH 5.6 treatments were chosen to reflect the lowest mean daily pH reported for a pasture diet (Williams et al., 2001).

Highly digestible, ryegrass-dominant herbage (composition described in Table 1) was freeze-dried and ground through a 1.6-mm screen (Christy Laboratory Mill, Ipswich, UK) and formed into loose pellets (25 mm diameter $\times$ 15 mm long). Portions of these pellets were added to the fermenters in two equal meals per day at 12-h intervals. This feeding regimen was used to mimic the diurnal variation in intake and ruminal pH observed in grazing dairy cows that are offered two fresh allocations of pasture each day. Wales and Doyle (2003) reported that ruminal pH typically varied by $\pm$ 0.5 pH units from the average daily pH for cows grazing

Figure 1. Diurnal variation in rumen fluid pH for pasture only (○), pasture plus 1.6 kg of DM straw (△), pasture plus 4.7 kg of DM grain (■), or pasture plus 5.0 kg of DM grain and 1.7 kg of straw (▼) (Wales and Doyle, 2003).

clover offered at a low herbage allowance (Figure 1). The current *in vitro* study achieved a similar range in pH units (± 0.5) and a similar 2-peak pattern in culture pH (Figure 2).

De Veth and Kolver (2001a) have described the operation of the *in vitro* dual-flow continuous culture system. At the start of each period ruminal inoculum was obtained from the same lactating, ruminally cannulated Holstein-Friesian cow grazing a highly digestible, perennial ryegrass-based pasture. Eight liters of ruminal fluid and approximately 200 g of digesta were collected

at 1230 h and transferred to the laboratory in pre-warmed, insulated containers. Ruminal fluid was slowly mixed for 30 s in a Rotor bar blender (4 L capacity; Rotor Ptd. Ltd., Neuheiten, Switzerland), and fermenters were inoculated within 25 min of collection. Approximately 5 g of fresh ruminal digesta was added to each fermenter to supply particle-associated bacteria.

Agitation was set at 160 rpm, and the temperature of the culture maintained at 39°C. The culture was continuously infused with N_2 to maintain anaerobiosis. The liquid dilution rate was maintained at 12%/h, and the solid dilution rate was held at 5%/h (equivalent to a retention time of 8.3 and 20 h, respectively) by the regulation of buffer input, acid and alkali input (for C6.1 and C5.6), and filtrate removal rates. The dilution rates were similar to those used in previous continuous culture studies (de Veth and Kolver, 2001a).

Culture pH was kept constant (± 0.05 pH unit) for C6.1 and C5.6 by automatic administration of 5N HCl and 5N NaOH. Treatment C6.1 cows received 21 ± 5.0 mL HCl/d (mean $\pm$ sd) and 23 ± 3.9 mL NaOH/d compared with 39 ± 12.5 mL HCl/d of acid and 17 ± 1.5 mL NaOH/d for C5.6 cows. Cows in 3 of the treatments (C6.1, V6.1, and C5.6) received mineral buffer (Teather, 1990) and cows in the V5.6 group received mineral buffer acidified with HCl at concentrations equivalent to 22 mL of 5N HCl/d. The acidification of the buffer solution was designed to achieve an average culture pH that was equivalent to C5.6. It resulted in a 0.5 pH decrease compared with V6.1 and maintained a variation (± 0.5 pH units) and diurnal pattern similar to those of V6.1 (Figure 2). Culture pH was automatically recorded every 30 min for the duration of the experiment.

Inputs of acid and alkali, temperature, filtration flow, and agitation were recorded immediately prior to each feeding. The solid and liquid effluent (simulating ruminal outflow to the small intestine) were weighed at 1300 h each day to determine dilution rates and discarded until the last 3 d of each period. On the last 3 d of effluent collection, a water bath maintained the effluent at 4°C to limit microbial fermentation in the effluent. During the 3 d of effluent collection, liquid and solid effluent portions were mixed each day and homogenized using a 4-L blender, and a 600-mL subsample was collected and stored at 4°C. Two 25-mL samples of effluent were also squeezed through 2 layers of cheesecloth, with 1 sample being acidified with 0.23 mL of 50% H_2SO_4 . These samples were stored at -20°C and later analyzed for VFA and ammonia N concentrations. The 600-mL subsamples were composited, using a blender, across the sampling days for each treatment. A subsample of the composite (800-mL) was collected for determination

Figure 2. Average daily culture pH ($\pm$ SE) in response to continuous culture pH maintained at a constant value of 6.1 (◆) or 5.6 (◇), or allowed to express normal diurnal variation around an average daily culture pH of 6.1 (■) or 5.6 (□).

Table 2. Digestibility of nutrients of perennial ryegrass in response to continuous culture pH maintained at a constant value of 6.1 (C6.1) or 5.6 (C5.6), or allowed to express normal diurnal variation around an average daily culture pH of 6.1 (V6.1) or 5.6 (V5.6).

Item	Interactions ¹				SED ²
	C6.1	V6.1	C5.6	V5.6	
True digestibility (%)					
DM	63.8 ^a	66.9 ^a	58.2 ^b	55.0 ^c	1.43
OM	66.6 ^a	69.1 ^a	61.9 ^b	59.0 ^c	1.21
Apparent digestibility (%)					
DM	55.7 ^a	57.7 ^a	50.3 ^b	46.1 ^c	1.42
OM	58.8 ^a	60.5 ^a	54.5 ^b	50.6 ^c	1.18
NSC	83.7 ^a	84.4 ^a	83.6 ^a	81.7 ^b	0.96
Fiber digestibility (%)					
NDF	67.6 ^a	65.6 ^a	57.9 ^b	46.2 ^c	3.18
ADF	75.0 ^a	74.7 ^a	59.1 ^b	47.7 ^c	3.72

¹Main effects are not presented as all interactions were significant at $P < 0.05$. Within rows, different letters denote significant differences at $P < 0.05$.

²SED = Standard error of the difference.

of DM concentrations. The remaining effluent was freeze-dried and ground through a 1-mm screen.

On the last day of each period, microorganisms were harvested by mixing the contents of each fermenter in a 4-L Rotor blender at high speed for 30 s. Digesta was strained through 2 layers of nylon cloth (100 μ m) and centrifuged at 1000 $\times g$ for 10 min to remove feed particles. Microorganisms were isolated from the supernatant by centrifuging 3 times at 20,000 $\times g$ for 30 min (Sorvall RC-5C centrifuge, Dupont, Newton, CT). Isolated microbial material was prepared for analyses by freeze-drying and grinding through a 1-mm screen.

Concentrations of VFA were determined according to Playne (1985) by automated GLC (5890A, Hewlett Packard, Avondale, PA). Concentrations of ammonia-N were determined by autoanalyzer (Hitachi 717, Mannheim, Germany) using an ammonia kit UV method. Microbial samples were analyzed for ash and N (AOAC, 1990). Purine concentrations (Zinn and Owens,

1986) of microbial and effluent samples were determined. The ratio of N:purine in effluent and microbial samples was used to calculate microbial N flow from the fermenter and true DM and OM digestibility (corrected for microbial DM and OM, respectively). Pasture and effluent were analyzed for ash, N, NDF, ADF (Van Soest et al., 1991), and NSC (Dubois et al., 1956). The soluble N, crude fat, and mineral concentrations of the diet were also determined (AOAC, 1990). The pasture was analyzed for OM digestibility of DM (**DOMD**) by near infrared reflectance spectroscopy (Ulyatt et al., 1995). Metabolizable energy (**ME**, MJ/kg DM) was calculated from DOMD, (ME, MJ/kg of DM = DOMD $\times$ 0.16; ARC, 1980). A sample of the mineral buffer solution was taken on each collection day and combined for DM and ash determination. True and apparent nutrient digestibilities (%) were defined as intake minus effluent divided by intake, with the effluent corrected for buffer DM.

Data was analyzed by ANOVA according to a 2×2 factorial, with level of average daily pH (6.1 or 5.6) and pattern of diurnal pH (variable or constant) as main effects, employing a randomized complete block experimental design using Genstat (Oxford, UK). Data from only 3 periods contributed to the analysis of C5.6; as in period 4, this treatment required approximately 3 times the acid to maintain pH as the same treatment in the other periods, and we suspected that fermentation had ceased some time prior to 5 d after inoculation. Main effects only are presented when interactions were not significant. Significance was determined at $P = 0.05$.

RESULTS

There were significant interactions between level of average daily culture pH and pattern of diurnal culture pH for nutrient digestibility parameters (Table 2). The true digestibility of OM and DM were highest ($P < 0.05$) for the treatments with pH 6.1 (C6.1 and V6.1), inter-

Table 3. Concentrations of total VFA, molar proportions of important individual acids and ratios of molar proportions of lipogenic:glucogenic VFA when culture pH averaged either pH 6.1 or 5.6, or when the diurnal pattern of pH was maintained at a constant value or allowed to express normal diurnal variation.

Item	Main effects				SED ¹
	pH		Diurnal pH		
	6.1	5.6	Constant	Variable	
Total VFA (mmol/L)	66.7 ^a	49.9 ^b	61.7 ^a	54.9 ^b	2.23
Acetate (mmol/mol total VFA)	572 ^a	495 ^b	541	526	11.2
Propionate (mmol/mol total VFA)	218 ^b	249 ^a	235	232	8.6
<i>n</i> -Butyrate (mmol/mol total VFA)	146 ^b	176 ^a	158	164	12.3
A/P ²	2.64 ^a	2.00 ^b	2.32	2.31	0.090

¹SED = Standard error of the difference. Within rows for each main effect, different letters denote significant differences at $P = 0.05$.

²A/P = Molar proportions, where A is acetate and P is propionate.

Table 4. Ammonia-N concentrations, true CP digestibility (CP digestibility adjusted for microbial N), flows of nitrogen, and efficiency of microbial-N production when daily fluid pH averaged either pH 6.1 or 5.6 or when the diurnal pattern of pH was constant or allowed to exhibit normal diurnal variation.

Item	Main effects				SED ¹
	PH		Diurnal pattern		
	6.1	5.6	Constant	Variable	
Ammonia-N, mg/100 mL	15.5 ^a	13.5 ^b	14.5	14.4	0.73
True CP digestibility,%	76.6 ^a	58.3 ^b	68.4	66.4	2.62
Flows, g/d					
Total N	1.56 ^b	1.82 ^a	1.64 ^b	1.74 ^a	0.035
Ammonia N	0.60 ^a	0.52 ^b	0.57	0.56	0.028
Dietary N	0.43 ^b	0.76 ^a	0.58	0.62	0.048
Microbial N	0.53	0.54	0.50 ^b	0.57 ^a	0.022
Nonammonia N	0.96 ^b	1.30 ^a	1.07 ^b	1.19 ^a	0.050
Flows, % of nonammonia N flow					
Dietary N	44.5 ^b	58.6 ^a	52.4	50.7	2.99
Microbial N	55.5 ^a	41.4 ^b	47.6	49.3	2.99
Microbial efficiency					
g of N/kg of DM truly digested	13.4 ^b	15.9 ^a	13.6 ^b	15.7 ^a	0.69
g of N/kg of OM truly digested	13.3 ^b	15.3 ^a	13.2 ^b	15.4 ^a	0.65

¹SED = Standard error of the difference. Within rows for each main effect, different letters denote significant differences at $P = 0.05$.

mediate for C5.6, and lowest for the V5.6 treatment. Apparent digestibility of NSC was lowest ($P < 0.05$) on V5.6 treatment, and was higher and not different on the other three treatments. The digestibility of NDF and ADF was highest ($P < 0.05$) and not different for C6.1 and V6.1, intermediate for the C5.6, and lowest for V5.6.

There was a reduction ($P < 0.001$) in the concentrations of total VFA when the average daily culture pH was reduced from 6.1 to 5.6 (Table 3). A lower culture pH also altered the molar proportions of VFA. Acetate and n-butyrate were significantly reduced ($P < 0.05$) and propionate was significantly increased ($P < 0.01$) when culture pH decreased from 6.1 to 5.6. As a result, the ratios of the molar proportions of the lipogenic to glucogenic VFA, (expressed as acetate:propionate) were significantly reduced ($P < 0.001$) when culture pH was reduced from 6.1 to 5.6.

Concentrations of total VFA in culture were higher ($P < 0.01$) when the pattern of diurnal pH was held constant compared with when it was allowed to vary (Table 3). However, the molar proportions of acetate, propionate, and n-butyrate, and the ratio of the molar proportions of lipogenic:glucogenic VFA were not influenced by the diurnal pattern of pH.

The concentrations of ammonia N and the true digestibility of CP were significantly reduced ($P < 0.05$) when the average daily culture pH decreased from 6.1 to 5.6 (Table 4). The flow of total N increased when culture pH decreased ($P < 0.001$) from 6.1 to 5.6. This increase in total N flow was partly due to an increase ($P < 0.001$) in the flow of nonammonia N but was partly offset by a small decrease ($P < 0.05$) in the flow of ammonia N.

When pH was 6.1, microbial protein synthesis was less ($P < 0.01$) efficient than at a culture pH of 5.6.

The concentrations of ammonia N and the true digestibility of CP were not influenced by the diurnal pattern of culture pH (Table 4). However, flow of total N was reduced ($P < 0.05$) when the diurnal pattern of culture pH was constant compared with variable diurnal pattern, with the differences partly attributed to a decrease ($P < 0.01$) in the flow of microbial N. Flows of dietary, ammonia and nonammonia N were not influenced by the pattern of diurnal culture pH. Microbial production was less ($P < 0.05$) efficient when the diurnal pattern of culture pH was constant compared with when it was variable.

DISCUSSION

This experiment quantified the effect of diurnal variation in ruminal culture pH as being separate from the general effect of average daily pH. Digestion parameters were not different when culture pH was either allowed to vary normally throughout the day about 6.1 in response to twice daily feeding or maintained at a constant pH of 6.1. However, when average culture pH was 5.6, maintaining a constant pH significantly increased digestion of OM, NDF, and ADF compared with a culture exhibiting normal diurnal variation in pH. Digestion at pH 5.6 (C5.6) was significantly lower than digestion at pH 6.1 (C6.1), which is consistent with previous research (de Veth and Kolver, 2001a; Kolver and de Veth, 2002).

Effect of Ruminal pH on Digestibility when pH Is Held Constant

The reduced true OM digestibility of pasture (7%) when culture pH was kept constant at 5.6 compared with constant at pH 6.1 supported our first hypothesis; however, the reduction in digestibility was less than the reduction observed with high-starch diets. With mixed diets over a similar range in pH, digestion of OM was reduced by more than 19% (Hoover et al., 1984; Shriver et al., 1986), and it is generally considered that a pH below 6.2 is suboptimal for microbial growth (Pitt et al., 1996). In the current experiment, the size of the reduction in OM digestibility was consistent with an analysis of datasets with grazing cows. Kolver and de Veth (2002) identified relationships between ruminal pH and measurements of ruminal fermentation and animal production variables from 121 diets based on fresh pastures from 23 experiments. Mean daily ruminal fluid varied from pH 5.6 to 6.7, and they demonstrated that low ruminal pH was associated with a higher microbial N flow from the rumen, higher milk yields, and lower concentrations of milk fat. The highest milk yields occurred in the pH range of 5.8 to 6.2, with only one data point below pH 5.8. These results support the use of this *in vitro* system as a model of *in vivo* digestion and provide further evidence that the digestion of herbage is less compromised than mixed forage—concentrate diets within the pH range of 5.8 to 6.2. The critical pH for maintenance of acceptable digestibility appears to be about 5.8, as the current experiment showed reduced digestibility at 5.6.

Effect of Ruminal pH on Digestibility when pH Is Allowed to Express Normal Diurnal Variation

Dairy cows grazing pastures have pronounced diurnal ruminal fluid pH patterns (Wales and Doyle, 2003). Generally, this variation is observed as 2 rapid declines in pH coinciding with access to a fresh allocation of herbage following each milking. However, average daily pH from cows grazing pastures varies significantly with amount of pasture consumed and nutritive characteristics of the pasture (Williams et al., 2001). The variable diurnal pH treatments imposed in the current experiment mimicked those observed in dairy cows grazing highly digestible pasture and illustrated the potential improvement in digestibility if diurnal variation could be minimized.

Digestion coefficients of OM, NDF, and ADF were reduced by 15, 30, and 36%, respectively, when average daily culture pH was allowed to vary throughout the day in response to twice-daily feeding at pH 5.6 vs. with 6.1 (V5.6 vs. V6.1). The lower pH is at the extreme reported for grazing dairy cows occurring when large

amounts of highly digestible pasture are consumed. This suggests that digestibility of fiber fractions, in particular, may be considerably reduced *in vivo*. Approximately two-thirds of the 15% reduction in the digestion of OM was attributed to a reduction in the digestibility of NDF, and one-third was attributed to a reduced ADF digestibility. It appears that there was a proportional reduction in the digestibility of both the hemicellulose and ADF fractions.

Impact of Reduced Digestibility on Milk Production

Reductions in digestibility of the magnitude observed in our experiment would be expected to have a measurable impact on milk production. If a similar postruminal digestibility between treatments was assumed, a decrease in ruminal pH from 6.1 to 5.6 would reduce OM digestibility by 10 percentage units. This would represent a decrease in energy supply of approximately 2.0 MJ of ME/kg of DM and equates to approximately 6.5 kg of milk for a grazing dairy cow consuming 18 kg of DM. If the diurnal variation in ruminal pH can be removed, additional energy equivalent to 2 kg of milk would be expected at a ruminal pH of 5.6 as a result of a 3 percentage unit increase in OM digestibility.

The larger, negative impact of a variable ruminal pH when pH was low (5.6) compared with a pH of 6.1 supported our second hypothesis. This suggests that feeding strategies, such as more frequent feeding that minimize diurnal variation in ruminal pH, will give significant benefits when daily pH is low (5.6). In the review of Gibson (1984), feeding more frequently than twice daily increased milk fat concentrations by 7%. However, within experiments, significant responses to increased frequency of feeding have only occurred when milk fat concentrations were originally depressed. There is little information on the impact of feeding frequency on milk fat concentrations for dairy cows grazing pasture. Dalley et al. (2001) reported no milk yield benefits to increasing the feeding frequency of pasture from once daily to 6 times per day. Although data on ruminal fluid pH were not reported, milk fat concentrations averaged 4% for Holstein-Friesian cows receiving a single daily allocation of pasture. These cows were clearly not milk fat depressed; therefore, benefits of frequent feeding were unlikely to be realized. Based on findings of the present experiment, only those cows consuming diets of highly digestible herbage resulting in low ruminal pH (e.g. 5.6), with pronounced diurnal variation, are likely to respond to more frequent feeding. Milk fat concentrations could be used to indicate whether a response to frequent feeding might be expected.

As there were no differences between the digestion characteristics of the two treatments averaging pH 6.1, we can conclude that there is little advantage, in terms of improvements in digestion of OM or NDF, in attempting to minimize diurnal variation of ruminal pH on most pasture diets. This conclusion is supported by in vitro research with TMR that showed no differences in digestion between a constant pH 6.4 and one that fluctuated to pH 5.7 for 4 h, 3 times daily (Calsamiglia et al., 2002).

Reduced pH Affects Fiber Digestibility

The lower true OM digestibility as a result of a lower pH (5.6) when pH was constant was a direct result of a reduction in fiber digestibility. Approximately three-fourths of the 7% reduction in OM digestibility was attributed to a reduction in NDF digestibility. Cellulolytic microorganisms are less tolerant of low pH than are amylolytic and saccharolytic microorganisms; therefore, their contribution to the microbial pool is decreased at low pH, leading to reduced fiber digestion (Russell and Dombrowski, 1980). However, we are speculating that there was a shift toward strains of prominent fiber-digesting bacteria that tolerate a pH of less than 6.0 because there was only a relatively small reduction in OM digestibility and no changes in flow of bacterial N. There is evidence that some fiber-digesting bacteria are not affected by prolonged periods of ruminal pH below 6.0. For example, Weimer et al., (1999) demonstrated that three important cellulolytic bacteria (*Ruminococcus albus*, *Ruminococcus flavefacians*, and *Fibrobacter succinogenes*) temporarily suspended growth during the period immediately after feeding when ruminal pH was relatively acidic, and resumed growth on recovery of pH.

The observation in this experiment that water-soluble carbohydrate and starch digestion was not affected by pH is supported by other research (Hoover et al., 1984; Shriver et al., 1986; de Veth and Kolver, 2001a). These studies showed that NSC was highly digestible over a wide range in pH (pH 4.5 to 7.5) and concluded that microorganisms participating in digestion are largely insensitive to pH change.

The 25% decrease in total VFA concentrations as pH decreased from 6.1 to 5.6 is in agreement with other continuous culture studies (Hoover et al., 1984; Shriver et al., 1986). However, our results are greater than the 13% reduction reported by de Veth and Kolver (2001a) over the same range. The decrease in VFA concentrations at low pH was associated with reduced microbial growth (expressed as flow of microbial N as a proportion of nonammonia N) and the reduced digestibility of the fiber fractions. The large reduction in the molar propor-

tion of acetate and smaller, though significant, increases in propionate and butyrate at low pH (5.6), are in accordance with previous studies (Hoover et al., 1984; de Veth and Kolver, 2001a).

Total VFA concentrations in the current study was reduced by 11% when diurnal patterns of pH were applied, a result in broad agreement with other studies of diurnal variation in ruminal pH (Hoover et al., 1984; Shriver et al., 1986; Russell, 1998; de Veth and Kolver, 2001b). The lack of an effect of diurnal variation on the *M* proportions of individual VFA indicates that the changes in these proportions are due solely to changes in pH throughout the day.

In vitro studies that control culture pH at or above 6.1 are useful for examining the effects on digestion observed in grazing cows that generally express pronounced diurnal variation in pH. However, using these systems to study low pH (5.6) may not adequately reflect digestion that occurs when pH varies during the day.

CONCLUSIONS

This experiment has quantified the separate effects of diurnal variation of culture pH, and the effect of average daily culture pH on the digestion of ryegrass in continuous culture. Digestion parameters were similar when culture pH was either maintained at a constant pH 6.1, or when pH varied about pH 6.1 throughout the day. However, when culture pH was 5.6, maintaining a constant pH significantly increased digestion of OM, NDF, and ADF compared with a culture in which pH expressed normal diurnal variation. Feed digestibility and milk production may be improved with feeding strategies that minimize diurnal variation at low ruminal pH (5.6). However, larger improvements in productivity will occur by using feeding strategies that result in a higher pH (6.1).

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