Effects of Time at Suboptimal pH on Rumen Fermentation in a Dual-Flow Continuous Culture System

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ABSTRACT

Ruminal pH varies considerably during the day, achieving values below 6.0 when cows consume large amounts of concentrates. Low ruminal pH has negative effects on ruminal fermentation. However, previous studies have indicated that rumen bacteria may resist short periods of low ruminal pH, and it is not clear how long this period may be before rumen microbial fermentation is negatively affected. Seven dual-flow continuous culture fermenters (1,320 mL) were used in 3 replicated periods with the same diet (97 g of dry matter/d of a 60:40 forage-to-concentrate diet, 18.3% crude protein, 35.9% neutral detergent fiber), temperature (39°C), and solid (5%/h) and liquid (10%/h) dilution rates to study the effects of increasing time at suboptimal pH on rumen microbial fermentation and nutrient flow. Treatments were a constant pH of 6.4 and 6 different intervals of time during the day (4, 8, 12, 16, 20, 24 h) at suboptimal pH (5.5), with the rest of the day being at pH 6.4. Polynomial equations were derived using the Mixed procedure of SAS, and linear, quadratic and cubic terms were left in the equation if $P < 0.10$. True organic matter digestion decreased with increasing time at suboptimal pH and was best described by a cubic regression ($\text{TOMD} = 58.5 - 2.15x + 0.16x^2 - 0.0037x^3$; $R^2 = 0.74$). Digestion of NDF ($\text{DNDF} = 55.1 - 1.00x$; $R^2 = 0.75$) and digestion of ADF ($\text{DADF} = 56.2 - 1.33x$; $R^2 = 0.78$) decreased linearly with increasing time at suboptimal pH. Total VFA had a cubic response ($\text{VFA} = 112.7 - 2.09x + 0.17x^2 - 0.0054x^3$; $R^2 = 0.82$). The proportion of acetate decreased linearly ($\text{acetate} = 58.7 - 0.61x$; $R^2 = 0.79$). The propionate proportion increased ($\text{propionate} = 17.6 + 2.09x - 0.044x^2$; $R^2 = 0.85$) and branched-chain VFA decreased ($\text{BCVFA} = 4.45 - 0.51x + 0.014x^2$; $R^2 = 0.75$) quadratically. The ammonia N concentration ($\text{NH}_3\text{-N} = 5.85 - 0.13x$; $R^2 = 0.46$) and flow ($\text{NH}_3\text{-N flow} = 0.18 - 0.0039x$; $R^2 = 0.43$) decreased linearly as the time at suboptimal pH increased. Crude

protein degradation ($\text{CPd} = 41.9 - 1.60x + 0.060x^2$; $R^2 = 0.71$), efficiency of microbial protein synthesis ($\text{EMPS} = 26.6 - 0.33x + 0.021x^2$; $R^2 = 0.77$), microbial N flow ($\text{MN flow} = 1.38 - 0.036x + 0.0015x^2$; $R^2 = 0.77$), and dietary N flow ($\text{DN flow} = 1.49 + 0.041x - 0.0015x^2$; $R^2 = 0.65$) had a quadratic response. The flow of essential, nonessential, and most individual AA increased linearly with increasing time at suboptimal pH. The effects of pH on rumen fermentation appear to start as soon as pH drops to suboptimal pH.

Key words: acidosis, pH, rumen fermentation

INTRODUCTION

High-producing dairy cows require high-concentrate diets to meet their productive potential. The intake of large amounts of concentrate decreases ruminal pH, which has negative effects on rumen microbial fermentation. Low pH affects rumen fermentation and microbial growth (Russell and Dombrowski, 1980; Mould and Ørskov, 1983; Hoover, 1986), but most current feeding systems for dairy cattle (INRA, 1989; AFRC, 1993; NRC, 2001) do not include the effect of pH in their models. Others (Dijkstra et al., 1992; Pitt et al., 1996) are able to predict pH changes and include the effects of the decrease in ruminal pH on microbial fermentation, but they use a threshold average pH below which microbial fermentation and nutrient digestion are affected and do not consider the effects of diurnal pH fluctuations. For example, the Cornell Net Carbohydrate and Protein System (Pitt et al., 1996) uses an average pH of 6.2 as the threshold below which the growth of fiber-utilizing microbes and the rates of structural carbohydrate digestion are depressed, and it assumes that structural carbohydrates are not degraded below pH 5.8. The experiment reported here is part of a larger project that evaluates the effects of ruminal pH and its fluctuations on rumen microbial fermentation in vitro. Previous studies (Cardozo et al., 2000, 2002) have evaluated the effect of pH and the type diet on rumen microbial fermentation and have identified ruminal pH of 6.4 and 5.5 as being optimal and suboptimal, respectively. Calsamiglia et al. (2002) evaluated the effects of pH fluctuations and observed that constant low pH

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reduced OM, fiber, and protein degradation, whereas transitory decreases of pH below the optimal level had either small or insignificant effects. These results agree with those of Sauvant et al. (1999) and de Veth and Kolver (2001b), who indicated that rumen bacteria might resist short periods of low ruminal pH without affecting overall fermentation. However, it is necessary to determine for how long the pH may be suboptimal before rumen microbial fermentation is negatively affected. In fact, the effects of pH fluctuations on microbial fermentation and nutrient flow have been identified as one of the research needs to improve the prediction of nutrient digestion in the rumen (de Veth and Kolver, 2001b; Calsamiglia et al., 2002). The objective of this study was to determine the effect of transitory reductions of pH to a suboptimal level (5.5) on rumen microbial fermentation and nutrient flow from a dual-flow continuous culture system.

MATERIALS AND METHODS

Seven 1,320-mL dual-flow continuous culture fermenters (Hoover et al., 1976) were used in 3 replicated periods of 8 d. Fermenters were inoculated with ruminal fluid obtained from a rumen-fistulated lactating dairy cow fed a 60% forage and 40% concentrate diet. Temperature (38.5°C), and liquid (10%/h) and solid (5%/h) dilution rates were held constant and were monitored using a personal computer and LabView software (FieldPoint; National Instruments, Austin, TX). Anaerobic conditions were maintained by infusing N₂ at a rate of 40 mL/min. Artificial saliva (Weller and Pilgrim, 1974) was continuously infused into flasks and contained 0.4 g/L of urea to simulate recycled N. Treatments were a constant optimal pH (6.4), a constant suboptimal pH (5.5), and 5 different intervals of time (4, 8, 12, 16, 20 h) at a suboptimal pH (5.5) during each day. During the remainder of each day, pH was controlled at pH 6.4. Ruminal pH was controlled at either optimal pH (6.4 ± 0.05) or suboptimal pH (5.5 ± 0.05) by automatic infusion of 3 N HCl or 5 N NaOH. Consumption of NaOH averaged 19.5 mL/d and was not affected by treatment, and consumption of HCl (mL/d) increased linearly with increasing time at suboptimal pH ($y = 20.7 + 1.03x$). All fermenters were fed 97 g of DM/d of a 60:40 forage-to-concentrate diet (18.4% CP, 35.0% NDF, and 21.0% ADF, DM basis) added in equal portions at 0800, 1600, and 2400 h. The diet consisted of (DM basis) 38.0% alfalfa hay, 20.4% ground corn grain, 17.5% ground corn silage, 14.6% soybean meal, 8.8% ground barley grain, 0.22% white salt, and 0.48% of a vitamin and mineral mix, and was designed to meet or exceed current nutrient recommendations for a Holstein cow (650 kg of BW) producing 30 kg of milk

(NRC, 2001). The vitamin and mineral mix contained (in 1 kg) 7 mg of Co, 167 mg of Cu, 33 mg of I, 2,660 mg of Mn, 27 mg of Se, 660 mg of Zn, 1,000 kIU of vitamin A, 200 kIU of vitamin D₃, 1,330 mg of vitamin E, 2.67 g of urea, 67 g of NaCl, 33 g of S, and 300 g of MgO.

Sample Collection and Processing

Each experimental period consisted of 5 d for adaptation and 3 d for sampling. During sampling days, collection vessels were maintained at 4°C to impede microbial action, solid and liquid effluents were mixed daily and homogenized for 1 min, and a 500-mL sample was removed via aspiration. Upon completion of each period, effluents from the 3 d of sampling were composited and mixed within the fermenter and were homogenized for 2 min. Samples were taken for total N, ammonia N, and VFA analyses. The remainder of the sample was lyophilized. Dry samples were analyzed for DM, ash, NDF, ADF, and purine contents. Bacterial cells were isolated from fermenter flasks on the last day of each period by a combination of several procedures selected to obtain the maximum detachment without affecting microbial cell integrity (Whitehouse et al., 1994). One hundred milliliters of a 0.2% methylcellulose solution and small marbles (30 at 2 mm and 15 at 4 mm in diameter) were added to each fermenter, incubated in the same fermenter flask at 39°C for 1 h to remove attached bacteria, and refrigerated at 4°C for 24 h. After refrigeration, fermenter contents were agitated for 1 h to dislodge loosely attached bacteria. The content was filtered through cheesecloth and washed with saline solution. Bacterial cells were isolated by differential centrifugation at 1,000 × *g* for 15 min to eliminate feed particles, and at 20,000 × *g* for 20 min to isolate the bacterial pellet. Pellets were rinsed twice with saline solution and recentrifuged at 20,000 × *g* for 20 min. The last rinse was done with distilled water to prevent contamination of bacteria with ash. Bacterial cells were lyophilized and analyzed for DM, ash, nitrogen, and purine contents. Digestion of OM, NDF, ADF, and CP, and flows of total, nonammonia, microbial, and dietary N were calculated as described by Stern and Hoover (1990).

Chemical Analyses

Effluent DM was determined by lyophilizing 200-mL aliquots in triplicate with subsequent drying at 103°C in a forced-air oven for 24 h. The DM contents of diets and bacterial samples were determined by drying samples for 24 h in a 103°C forced-air oven according to the AOAC method (AOAC, 1990). Dry samples were

Table 1. Effect of time (h) at suboptimal pH (5.5) on true OM and fiber digestion in continuous culture of rumen fluid

Digestion, %	Treatment ¹							SEM	Regression equation ²	R ²	RMSE ³
	H	L4	L8	L12	L16	L20	L24				
True OM	57.6	54.9	48.0	48.6	50.7	50.8	47.6	1.59	$y = 58.5 - 2.15x + 0.16x^2 - 0.0037x^3$	0.74	2.51
NDF	57.7	52.6	39.9	43.4	40.8	36.3	30.9	4.83	$y = 55.1 - 1.00x$	0.75	5.83
ADF	58.2	54.3	36.8	39.4	38.4	30.9	23.7	5.81	$y = 56.2 - 1.33x$	0.78	7.29

¹Treatments: H = constant pH 6.4; L4 = pH at 6.4 except for 4 h at 5.5; L8 = pH at 6.4 except for 8 h at 5.5; L12 = pH at 6.4 except for 12 h at 5.5; L16 = pH at 6.4 except for 16 h at 5.5; L20 = pH at 6.4 except for 20 h at 5.5; L24 = constant pH at 5.5.

²Linear, quadratic, or cubic regression was considered valid when the term of higher order was statistically significant ($P < 0.10$).

³RMSE = root mean square error.

ashed overnight at 550°C in a muffle furnace. Fiber components of diets and effluents were analyzed sequentially by the detergent system (Van Soest et al., 1991) using thermostable α -amylase and sodium sulfite. Total N contents in feed, effluents, and bacterial samples were determined by the Kjeldahl method (AOAC, 1990). Ammonia N was analyzed in 4-mL subsamples of filtered fluid that were acidified with 4 mL of 0.2 N HCl and frozen. Samples were centrifuged at 25,000 $\times g$ for 20 min, and the supernatant was analyzed for ammonia N by colorimetry (Chaney and Marbach, 1962). Samples for VFA were prepared as described by Jouany (1982) using 4-methylvaleric acid as an internal standard. The analysis was performed by GLC (model 6890; Hewlett-Packard, Palo Alto, CA) using a polyethylene glycol terephthalic acid-treated capillary column (BP21; SGE, Europe Ltd., Buckinghamshire, UK). Dry effluent and mixed bacterial cells were analyzed for purine bases content by HPLC (Beckman Instruments, Palo Alto, CA) according to the procedure of Balcells et al. (1992). Amino acids were analyzed by hydrolyzing samples (25 mg) with 1,000 μ L of 6 N HCl containing 0.5 μ L/mL of mercaptoethanol at 110°C for 22 h, 45 min in sealed, evacuated tubes. Derivatization was conducted using the AccQ-Tag AA analysis method (Waters Co., Milford, MA). This involved derivatizing samples with 30 μ L per tube of Waters AccQFluor reagent (6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate) at 55°C for 9 min, 20 s. Amino acid analysis was performed by reversed-phase HPLC (HP1100, AccQ-Tag AA analysis column, 3.9 $\times$ 150 mm silica base bonded with C₁₈; Agilent, Santa Clara, CA) with UV-visible detection using procedures of the Waters AA analysis method, modified to ensure separation of derivatized AA. Norleucine was used as the internal standard.

Statistical Analysis

The experiment was planned as a randomized complete block design. Results were analyzed by the Mixed procedure of SAS (v. 9.1; SAS Institute, Cary, NC). A polynomial regression model, with period as a random

effect and pH as a fixed effect, was used to study the nature of the relationship between the explanatory variable (time at suboptimal pH) and the response variable. Linear, quadratic, and cubic terms were evaluated, and the model was considered valid when the term of higher order was statistically significant. Statistical significance was declared using a significance value of 0.10. The maximum or minimum, in quadratic equations, and the inflexion point, in cubic equations, were calculated using the first derivative.

RESULTS AND DISCUSSION

When the pH was reduced to 5.5 for 24 h, digestion of OM, NDF, and ADF, the concentration of total VFA and ammonia N, the proportion of acetate, and the acetate-to-propionate ratio decreased, and the proportion of propionate and flow of AA increased, as expected (Tables 1 to 4). These results are consistent with previous reports that evaluated the effect of low pH on rumen microbial fermentation, except for changes in CP degradation and dietary N flow, which will be discussed later (Hoover et al., 1984; Shriver et al., 1986; Calsamiglia et al., 2002). However, few studies have evaluated the effects of time at suboptimal pH on rumen microbial fermentation (de Veth and Kolver, 2001b; Calsamiglia et al., 2002). True OM digestion decreased with increasing time at suboptimal pH and was best described by a cubic regression (Table 1), decreasing almost linearly up to an inflexion point at 11 h and 8 min, and remained relatively constant thereafter. This result agrees with de Veth and Kolver (2001b), who also reported a linear reduction of OM degradation between 0 and 12 h at suboptimal pH. In contrast, Calsamiglia et al. (2002) observed no differences in OM digestion between a constant high pH (6.4) and 8 or 12 h at low pH, but the low pH was set at pH 5.7. Similarly, de Veth and Kolver (2001a) found only a small reduction in OM digestion of a high-quality pasture between pH 6.2 and 24 h at 5.8, but the reduction was larger when the pH was 5.4. These results suggest that the effects of pH on OM

Table 2. Effect of time (h) at suboptimal pH (5.5) on total VFA concentration and VFA profile in continuous culture of rumen fluid

Item	Treatment ¹							SEM	Regression equation ²	R ²	RMSE ³
	H	L4	L8	L12	L16	L20	L24				
Total VFA, mM	113.2	105.4	104.6	103.1	100.5	94.7	86.4	2.62	$y = 112.7 - 2.09x + 0.17x^2 - 0.0054x^3$	0.75	2.51
VFA, mol/100 mol											
Acetate	59.2	57.0	53.8	50.2	48.5	44.9	46.3	1.92	$y = 58.7 - 0.61x$	0.79	2.89
Propionate	18.5	24.4	30.1	36.9	39.4	43.4	40.9	2.40	$y = 17.6 + 2.09x - 0.044x^2$	0.85	4.03
Butyrate	14.7	13.1	11.5	8.7	8.0	8.2	8.8	0.93	$y = 15.2 - 0.71x + 0.018x^2$	0.79	1.48
Isobutyrate	0.66	0.59	0.53	0.30	0.26	0.15	0.15	0.09	$y = 0.67 - 0.024x$	0.67	0.15
Valerate	2.83	2.97	3.70	3.69	3.66	3.31	3.87	0.35	$y = 3.03 + 0.034x$	0.41	0.53
Isovalerate	4.03	1.95	0.31	0.12	0.12	0.00	0.00	0.62	$y = 3.77 - 0.47x + 0.014x^2$	0.73	0.98
BCVFA ⁴	4.69	2.53	0.83	0.41	0.38	0.15	0.15	0.68	$y = 4.45 - 0.51x + 0.014x^2$	0.75	1.01
Acetate:propionate	3.30	2.45	1.86	1.37	1.24	1.03	1.14	0.25	$y = 3.28 - 0.22x + 0.0053x^2$	0.82	0.40

¹Treatments: H = constant pH 6.4; L4 = pH at 6.4 except for 4 h at 5.5; L8 = pH at 6.4 except for 8 h at 5.5; L12 = pH at 6.4 except for 12 h at 5.5; L16 = pH at 6.4 except for 16 h at 5.5; L20 = pH at 6.4 except for 20 h at 5.5; L24 = constant pH at 5.5.

²Linear, quadratic, or cubic regression was considered valid when the term of higher order was statistically significant ($P < 0.10$).

³RMSE = root mean square error.

⁴Branched-chain VFA.

degradation started to be important only when the pH dropped below 5.7.

The effects of pH on fiber degradation are well documented (Mould and Ørskov, 1983; Hoover, 1986), but research is limited on the effect on fiber degradation during short periods of time at suboptimal pH. Degradation of NDF and ADF decreased linearly as the time at suboptimal pH increased (Table 1). de Veth and Kolver (2001b) also reported a linear reduction in NDF and ADF digestion when incubation was conducted at increasing periods of suboptimal pH (5.4). Calsamiglia et al. (2002) reported no effects after 8 or 12 h at suboptimal pH on fiber degradation, although trends suggested a linear decrease and pH was set at 5.7. Fibrolytic bacteria do not die at low pH but reduce their microbial activity, because they need to maintain their proton-motive force across the cell membrane, which increases the energy requirements for maintenance, resulting in

a slower growth and eventually leading to the washout of specific microbial populations from the rumen (Strobel and Russell, 1986; Russell and Wilson, 1996). Therefore, it seems reasonable that the longer the time at suboptimal pH, the greater the reduction in fiber digestion.

Total VFA concentration decreased as the time at suboptimal pH increased and was best described by a cubic regression (Table 2). The inflexion point occurred at 11 h and 12 min, and was consistent with changes observed in true OM degradation. The proportion of acetate decreased linearly, and the proportion of propionate increased quadratically (Table 2) and reached the maximum at 23 h and 45 min, resulting in a quadratic response in the acetate-to-propionate ratio, which reached the minimum at 22 h and 6 min. The proportions of butyrate and branched-chain VFA also decreased quadratically with time at suboptimal pH.

Table 3. Effect of time (h) at suboptimal pH (5.5) on N metabolism of rumen microbes in continuous culture

Item	Treatment ¹							SEM	Regression equation ²	R ²	RMSE ³
	H	L4	L8	L12	L16	L20	L24				
N-NH ₃ , mg/d	5.96	5.40	4.68	4.47	3.53	3.10	3.12	0.86	$y = 5.85 - 0.13x$	0.46	1.31
N flow, g/d											
Ammonia	0.18	0.17	0.15	0.14	0.11	0.10	0.10	0.03	$y = 0.18 - 0.0039x$	0.43	0.04
NAN	2.89	2.83	2.91	2.94	2.87	3.07	2.90	0.03			
Bacterial	1.40	1.23	1.21	1.15	1.15	1.29	1.34	0.17	$y = 1.38 - 0.036x + 0.0015x^2$	0.77	0.14
Dietary	1.52	1.61	1.71	1.80	1.72	1.78	1.56	0.12	$y = 1.49 + 0.041x - 0.0015x^2$	0.65	0.14
EMPS ⁴	26.4	24.5	27.6	26.0	24.9	27.9	31.4	3.44	$y = 26.6 - 0.33x + 0.021x^2$	0.77	2.94
CPd ⁵ %	40.9	37.6	33.7	30.2	33.2	31.0	39.5	5.45	$y = 41.9 - 1.60x + 0.060x^2$	0.71	5.34

¹Treatments: H = constant pH 6.4; L4 = pH at 6.4 except for 4 h at 5.5; L8 = pH at 6.4 except for 8 h at 5.5; L12 = pH at 6.4 except for 12 h at 5.5; L16 = pH at 6.4 except for 16 h at 5.5; L20 = pH at 6.4 except for 20 h at 5.5; L24 = constant pH at 5.5.

²Linear, quadratic, or cubic regression was considered valid when the term of higher order was statistically significant ($P < 0.10$).

³RMSE = root mean square error.

⁴Efficiency of microbial protein synthesis: g of N/kg of OM truly digested.

⁵CP degradation.

Table 4. Effect of time (h) at suboptimal pH (5.5) on amino acid flows in continuous culture of rumen fluid

Item	Treatment ¹							SEM	Regression equation ²	R ²	RMSE ³
	H	L4	L8	L12	L16	L20	L24				
Flow, mg/d											
Ser	676	678	831	759	742	828	819	48.6	y = 693 + 5.72x	0.62	63.9
Thr	679	629	713	694	708	735	733	26.5	y = 659 + 3.30x	0.51	39.4
Gly	606	579	558	637	657	663	673	22.8	y = 599 + 3.30x	0.50	35.4
Ala	827	780	884	854	874	904	904	34.3	y = 812 + 4.21x	0.61	44.3
Arg	635	571	683	688	671	743	748	34.3	y = 605 + 6.00x	0.50	58.7
Pro	577	556	663	646	652	701	718	29.6	y = 570 + 6.26x	0.67	44.3
Val	784	749	853	828	844	884	887	34.6	y = 772 + 5.12x	0.59	48.2
Met	207	198	244	187	224	203	218	13.2	NS		
Ile	718	683	782	755	772	807	809	32.9	y = 707 + 4.48x	0.57	45.7
Leu	1,079	1,037	1,211	1,184	1,176	1,275	1,294	58.3	y = 1064 + 9.66x	0.61	83.6
Phe	672	656	743	733	734	788	817	34.8	y = 661 + 6.15x	0.63	48.7
Lys	656	614	713	696	681	758	782	42.3	y = 632 + 5.65x	0.54	60.3
His	221	222	246	265	254	283	277	15.1	y = 221 + 2.67x	0.64	20.7
Tyr	473	456	519	501	525	535	533	23.5	y = 469 + 3.08x	0.56	32.3
Essential, ⁴ g/d	5.65	5.36	6.19	6.03	6.06	6.48	6.56	0.27	y = 5.53 + 0.04x	0.61	0.38
Nonessential, g/d	3.16	3.05	3.55	3.39	3.45	3.63	3.65	0.12	y = 3.14 + 0.02x	0.62	0.19

¹Treatments: H = constant pH 6.4; L4 = pH at 6.4 except for 4 h at 5.5; L8 = pH at 6.4 except for 8 h at 5.5; L12 = pH at 6.4 except for 12 h at 5.5; L16 = pH at 6.4 except for 16 h at 5.5; L20 = pH at 6.4 except for 20 h at 5.5; L24 = constant pH at 5.5.

²Linear, quadratic, or cubic regression was considered valid when the term of higher order was statistically significant ($P < 0.10$).

³RMSE = root mean square error.

⁴Essential AA: Thr, Arg, Val, Met, Ile, Leu, Phe, Lys, His; nonessential AA: Ser, Gly, Ala, Pro, Tyr.

de Veth and Kolver (2001b) observed that increasing the time at suboptimal pH (up to 12 h) resulted in a decrease in total VFA and acetate concentrations and an increase in the propionate concentration in response to increasing time at suboptimal pH, although time at suboptimal pH had no effect on the butyrate concentration. Calsamiglia et al. (2002) reported similar trends when incubations were conducted at suboptimal pH for 8 or 12 h, although differences were not always significant. Changes in the proportions of VFA are likely related to the reduction in fiber digestion. The reduction in branched-chain VFA with increasing time at suboptimal pH is likely due to a reduced deamination of AA at low pH, and agrees with the lower ammonia N concentration reported. Because branched-chain VFA are essential growth factors for fibrolytic bacteria (Slyter and Weaver, 1971), the negative effect of low pH on fibrolytic bacteria may have been aggravated by the limited availability of branched-chain VFA, which was less than 1 mM when pH was 5.5 for 8 h or more.

The ammonia N concentration and flow decreased linearly as the time at suboptimal pH increased (Table 3). These results agree with those of Calsamiglia et al. (2002), who reported a lower ammonia N concentration when incubations were conducted at suboptimal pH for 12 h, compared with constant pH at 6.4. The reduction in ammonia N concentration at low pH may be the result of a reduction in dietary protein degradation. However, CP degradation was best described by a quadratic regression, reaching the minimum of 31.2% at

13 h and 18 min, and slowly recovering up to 39.8% at 24 h. The flow of dietary N was best described by a quadratic regression, reaching the maximum at 13 h and 21 min. The initial decrease in CP degradation and increase in dietary N flow agrees with the observations of de Veth and Kolver (2001b), who also observed a reduction in CP degradation and an increase in dietary N flow when incubations were conducted at suboptimal pH for increasing periods up to 12 h, although their response was linear. However, Calsamiglia et al. (2002) observed that protein degradation was further decreased as the time at suboptimal pH increased from 12 to 24 h, and other reports have also indicated lower protein degradation after incubations were conducted at a constant suboptimal pH (Hoover et al., 1984; Shriver et al., 1986). The increase in protein degradation between 12 and 24 h in the present trial was not expected. Although one could speculate that the increase in protein degradation may reflect a shift toward amylolytic bacteria, which tend to be more proteolytic than cellulolytic bacteria (Wallace and Cotta, 1989), this was unlikely if we consider that most research has reported a reduction in CP degradation at low pH (Hoover et al., 1984; Cardozo et al., 2000; Calsamiglia et al., 2002), and it is inconsistent with the observed reduction in ammonia N and branched-chain VFA concentrations. Therefore, we have no clear explanation for the increase in CP degradation as time at suboptimal pH increased from 12 to 24 h. Microbial nitrogen flow was best described by a quadratic equation (Table

3), reaching the minimum at 12 h at suboptimal pH. de Veth and Kolver (2001b) also observed a decrease in bacterial N flow (g/d) when incubations were conducted at increasing periods of suboptimal pH (5.4), up to 12 h. However, they observed a linear relationship, which could have occurred because they maintained fermenters at suboptimal pH from 0 to 12 h and did not evaluate longer periods. We hypothesize that the recovery in bacterial N flow in the present trial could be due to a shift toward strains of bacteria that grow more efficiently at low pH. Other authors did not observe differences in bacterial N flow between a constant high pH and a low pH (Calsamiglia et al., 2002; Wales et al., 2004). The efficiency of microbial protein synthesis was best described by a quadratic equation, with the lowest point occurring at 7 h and 53 min. de Veth and Kolver (2001b) also observed a reduction in efficiency of microbial protein synthesis (EMPS) as incubations were conducted at increasing periods of suboptimal pH (5.4), but they reported a linear reduction up to 12 h and did not report effects of longer periods. The recovery in EMPS in the constant suboptimal pH (5.5) treatment to similar levels compared with the constant optimal pH (6.4) treatment is in agreement with the results of Calsamiglia et al. (2002) and Cardozo et al. (2000), who did not find significant differences in EMPS between a constant pH of 6.5 and a constant suboptimal pH. However, others have reported that when incubations are conducted at a constant suboptimal pH, the EMPS increases compared with pH above 6.0 (Shriver et al., 1986; de Veth and Kolver, 2001a; Wales et al., 2004).

Increasing the time at suboptimal pH resulted in a linear increase in the flow (mg/d) of individual (except Met), essential, and nonessential AA (Table 4). This is in agreement with Calsamiglia et al. (2002), who reported a higher flow of total and essential AA, Gly, Lys, Leu, His, and Arg when incubations were conducted at a constant pH of 5.7 compared with a constant pH of 6.4. It is likely that a marginal acidosis may be beneficial for dairy cattle from the standpoint of protein nutrition.

CONCLUSIONS

Low pH affected rumen microbial fermentation and nutrient flow in continuous culture fermenters. The effects of time at suboptimal pH were different depending on the factor measured. Overall, the effects of pH on rumen fermentation appeared to take place as soon as the pH started to become suboptimal. The largest effects occurred within the first 12 h of suboptimal pH and, in most cases, longer periods had only small additional effects. From the standpoint of protein nutrition,

low pH resulted in an improved flow of total and essential AA.

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