Journal of Animal and Veterinary Advances 6 (8): 986-990, 2007
ISSN: 1680-5593

Influence of Intraruminal Infusion of Acetate on Ruminal Characteristics and NDF Digestion in Feedlot Steers

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Abstract: Four Holstein steers (406 kg) with cannulas in the rumen and proximal duodenum were used in a 4×4 Latin square design to evaluate the influence of acetate infusion on characteristics of ruminal and total tract digestion. The basal diet contained 51.3% steam-flaked corn and 33.8 % of forage (alfalfa and sudangrass hay). Dry matter intake was restricted to 2.0% of BW (8.24 kg d^{-1}). Acetic acid was dissolved in distilled water to produce four treatment solutions of 0, 0.333, 0.666and 1.000 M. Treatment solutions were infused into the rumen via the ruminal cannula at the rate of 11.1 mL min^{-1} (16 L d^{-1}). There were no treatment effects ($p>0.20$) on ruminal pH, lactate and ammonia concentrationand estimated methane production. Acetate infusion increased (linear effect, $p<0.10$) total ruminal VFA molar concentrationand molar proportion of acetate, but decreased molar proportion of butyrate (linear effect, $p<0.01$) and valerate (linear effect, $p<0.05$). There was a quadratic effect of acetate infusion on ruminal tonicity ($p<0.05$) and molar proportion of isovalerate, being maximal with the 0 and 960 mL d^{-1} acetate infusion rate. There were no treatment effects ($p>0.20$) on ruminal microbial efficiency and ruminal and total tract digestion of N . However, acetate infusion decreased (linear effect, $p<0.05$) ruminal and total tract NDF digestion. Ruminal VFA concentration explained 48% ($p<0.01$) of the variation in ruminal NDF digestion. In contrast, ruminal pH had limited value ($r^2= 0.03$; $p>0.20$) as a predictor of NDF digestion.

Key words: Ruminal pH, VFA, acetic acid, NDF digestion, steers

INTRODUCTION

Feeding high-concentrate diets to ruminants often reduces fiber digestion, presumably due to greater ruminal acid production and associated effects on ruminal pH (Hoover, 1986). As pH decreases, bacteria extrude H^+ in order to maintain intracellular equilibrium against the proton flux, thus maintaining intracellular anion concentration (Booth, 1985). Acid tolerant ruminal bacteria can allow their intracellular pH to decrease as the extracellular pH drops, affording resilience to the increasing proton gradient (Russell and Wilson, 1996; Takehiro *et al*., 1997). Ruminal fibrolytic bacteria appear to be particularly sensitive to declining pH. Russell (1991) proposed that this sensitivity may be due to limitations in maintaining the proton gradient across the cell wall, leading to intracellular anion toxicity. However, the direct role of pH on growth rate of acid-sensitive bacteria is not completely understood. For example, Roe *et al*. (1998) observed that acetate treated *Escherichia coli* cultures exhibit greater inhibition than would be expected simply from changes in the inoculum pH.

The purpose of this study, was to re-examine the influence of ruminal pH and ruminal VFA concentration on NDF digestion in a conventional receiving diet (23% NDF) for feedlot cattle.

MATERIALS AND METHODS

Four Holsteins steer (406 Kg) with cannulas in the rumen and proximal duodenum (Zinn and Plascencia, 1993) were used in a 4×4 Latin square experiment to evaluate the influence of acetate infusion on characteristics of ruminal and total tract digestion.Steers were maintained in slotted-floor pens (6.2 m^2) with ad libitum access to water. Dry matter intake was restricted to 8.24 kg d^{-1}, fed in equal portions at 0800 and 2000 daily. Composition of the basal diet is shown Table 1.Chromic oxide (0.4 % DM) was included in the complete mixed diets as a digesta marker. Acetic acid (Glacial; Certified ACS PLUS; Assay 99.9%; Cat# A38P-20; Fisher Scientific, Pittsburgh, PA 15275-9952) was dissolved in distilled water to produce four treatment solutions of 0, 0.333,

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Table 1: Composition of experimental diets fed to steers

Item	(%)
Ingredient composition,% (DM basis)	
Steam flaked corn	51.33
Alfalfa hay	19.30
Sudangrass hay	14.47
Cane molasses	9.55
Yellow grease	3.55
Urea	0.71
SALT	0.46
Dicalcium phosphate	0.23
Chromic oxide [a]	0.40
Laidlomicyn propionate	++
Nutrient composition, DM basis [b]	
NE_m, $Mcal^{-1}kg$	2.03
NE_g, $Mcal^{-1}kg$	1.38
Crude protein %	12.73
Ether extract, %	7.00
Ash,%	6.71
NDF, %	22.87
eNDF, % NDF	57.50
Calcium, %	0.77
Phosphorus, %	0.32
Potassium, %	1.31
Magnesium, %	0.20
Sulfur, %	0.23

[a]Added as a digesta marker, [b]Based on tabular values for individual feed ingredients (NRC, 1996)

0.666 and 1.000 M. Treatment solutions were infused into the rumen via the ruminal cannula at the rate of 11.1 mL min^{-1} (16 L d^{-1}) using a variable flow peristaltic pump (Variable Flow Mini-Pump II, VWR Scientific Products, West Chester, PA). Infusion tubing (3.1 mm d.i. ×5.1 m Tygon®; Norton, Akron OH 44309-3660) were moored at the ceiling above each pen using 1 m lengths of 6.3 mm latex tubing to provide constant tension, thus permitting the free movement of steers during treatment infusion. Experimental periods consisted of 7 d for treatment adjustment followed by a 3-d collection period. During the colletion period, duodenal and fecal samples were take from all steers twice daily as follows: d1, 0520, 0920 and 1320; d2, 0640, 1040 and 1440 and d3, 0800, 1200 and 1600 h. Individual samples consisted of approximately 700 mL of duodenal chyme and 200 g (wet basiss) of fecal material. Samples from each steer and within each collection period were composited for analysis. During the final day of each collection period, ruminal samples were obtained from each steer at 4 h after feeding via the the ruminal cannula. Ruminal fluid pH was measured on fresh samples. Samples were strained through four layers of cheesecloth. Two mililiter of freshly prepared 25 % (wt vol^{-1}) meta-phosphoric acid was added to 8 mL of strained ruminal fluid. Samples were then centrifuged (17,000×g for 10 min) and supernatant fluid stored ar-20°C for VFA analysis. Samples were subjected to all or part of the following analysis:DM (oven drying at 105°C until no further weinght loss); ash, Kjeldahl N, ammonia N (AOAC, 1984); NDF (ash-corrected; Chai and Uden, 1998); purines (Zinn and Owens, 1986); chromic oxide (Hill and Anderson, 1958); starch (Zinn, 1990) and VFA concentration of ruminal fluid (gas chromatography; Zinn, 1988). Duodenal flow and fecal excretion of DM were calculated using chromic oxide as digesta marker. Organic matter fermented in the rumen was determined as OM intake minus difference between the amount of total OM reaching the duodenum and microbial OM reaching the duodenum. Feed N escape to the small intestine was considered equal to total N leaving the abomasum minus ammonia N and microbial Nand thus includes any endogenous contributions. The trial was analyzed as a 4×4 Latin square. Treatment effects were tested for linear, quadratic and cubic components by means of orthogonal polynomials (Hicks, 1973).

Animal handling and space were in accordance to the Guide For the Care and Use of Agricultural Animals in Agricultural Research and Teaching (1st Revised Edition, January 1999).

RESULTS AND DISCUSSION

Influence of acetate infusion on ruminal parameter are shown in Table 2. There were no treatment effects ($p > 0.20$) on ruminal pH, averaging 5.87 ± 0.32. Based on NRC (1996) Level 2 model, observed ruminal pH was in very close agreement with expected (5.93) for the basal diet. The lack of effect of acetate infusion on ruminal pH was surprising. More often (Sheperd and Combs, 1998; Williams *et al*., 2004) ruminal VFA infusion has decreased ruminal pH. Even so, absence of effects on ruminal pH as consequence of ruminal infusion of VFA has been observed previously (Noziere *et al*., 2003; Schroeder *et al*., 2006). Variation in response is likely due to differences in duration, concentration and rate of acid infusion. For example, Peters *et al*. (1992) observed a marked drop in ruminal pH (from 6.74 to 6.16) when acetate entry into the rumen was increased by an average of 0.61 M^{-h}. However, they administered the acetic acid into the rumen in a single pulse dose. Sheperd and Combs (1998) observed cessation of rumination and a rapid decline in ruminal pH in dairy cows infused with acetic acid at the rate of 1.57 mol $^{-h}$ (236% the maximum rate used in the present study).

There were no treatment effects ($p>0.20$) on ruminal lactate and ammonia concentrationand estimated methane production. Both methane production and ruminal ammonia concentration are sensitive to ruminal pH (Lana *et al*., 1998). Ammonia is a weak base with a PK_a of 8.8 at 40°C.Thus, ammonia absorption is influenced by ruminal pH. As pH increases more ammonia is unionized

Table 2: Influence of acetate infusion on ruminal characteristics

	Acetate infusion[a], mL[-d]				
Item	0	320	640	960	SD
Ruminal tonicity, mOsm/kg [b]	384	350	360	372	18
Lactate, mg dL^{-1}	70	69	58	69	21
Ruminal pH	6.02	5.6	5.89	5.96	0.32
Ruminal VFA, mmol L^{-1}[c]	82.42	92.37	87.9	96.5	20.9
Molar %					
Acetate [d]	62.3	65.4	68.1	70.7	4.9
Propionate	19.4	21.6	20.4	18.0	4.1
Butyrate [e]	14.0	10.3	8.4	8.1	1.5
Isobutyrate	0.90	1.04	0.85	0.89	0.22
Isovalerate [f]	1.64	1.16	1.21	1.36	0.28
Valerate [d]	1.62	1.37	1.02	0.98	0.31
Acetate : propionate	3.24	3.35	3.38	4.07	0.98
Methane production [g]	0.35	0.34	0.34	0.36	0.32

[a]Source of acetate: Acetic Acid, Glacial; Certified ACS PLUS; Assay 99.9%; Cat# A38P-20; Fisher Scientific, Pittsburgh, PA 15275-9952, diluted in 15,680, 15,360 and 15,040 mL for 320, 640 and 960 treatment. [b]Quadratic effect, $p<0.05$. [c] Linear effect, $p<0.10$. [d] Linear effect, $p<0.05$. [e]Linear effect, $p<0.01$. [f]Quadratic effect, $p<0.10$. [g] Methane, mol/mol glucose equivalent fermented in the rumen

form (NH_3), which is more readily absorbed through the ruminal wall than is the NH_4^+ form (Rihani *et al*., 1993). The lack of response of ruminal ammonia to acetate infusion has been reported previously (Williams *et al*., 2004).

Consistent with previous studies (Peters *et al*., 1992; Seals and Parker, 1994; Lopez *et al*., 2003) acetate infusion increased (linear effect, $p<0.10$) ruminal total VFA concentrationand molar proportion of acetate. Acetate infusion did not affect ($p>0.20$) molar proportion of propionate, or the acetate: Propionate molar ratio. However, molar proportion of butyrate decreased (linear effect, $p<0.01$) with acetate infusion. Likewise, Sheperd and Combs (1998) also observed no effect of acetate infusion ruminal propionate molar proportion. There was a quadratic effect ($p<0.05$) of acetate infusion on ruminal tonicity ($p<0.05$) and molar proportion of isovalerate, being greater at the 0 and 960 mL^{-d} acetate infusion levels. Ruminal fluid tonicity fluctuated between 308 to 406 $mOsm^{-kg}$. These values are within the normal range (Marshall *et al*., 1992; Montano *et al*., 1999). Seals and Parker (1994) observed increased ruminal tonicity with increasing ruminal VFA concentration. Whereas, in the present study tonicity was greater for the control than for the acetate infused steers.

The influence of acetate infusion on characteristic of ruminal and total tract digestion are shown in Table 3. There were no treatment effects ($p>0.20$) on ruminal microbial efficiency and ruminal and apparent total tract N digestion. Acetate infusion decreased (linear effect, $p<0.05$) ruminal and total tract ash-free NDF digestionand increased (linear effect, $p<0.10$) ruminal starch digestion. Decreased fiber digestion with VFA infusion has been reported previously (Williams *et al*., 2004; Schroeder *et al*., 2006).

Table 3: Influence of acetate infusion on characteristic of ruminal and total tract digestion

	Acetate infusion[a], mL[-d]				
Item	0	320	640	960	SD
Intake, g d^{-1}					
DM	8241	8241	8241	8241	
OM	7710	7710	7710	7710	
Starch	2577	2577	2577	2577	
NDF	1698	1698	1698	1698	
N	160	160	160	160	
Duodenal g d^{-1}					
OM	3902	3812	3776	3712	22
Starch [b]	605	543	498	460	90
NDF [c]	862	959	989	1032	87
N	156	154	151	160	10
Nonammonia N	150	148	144	154	10
Microbial N	97.1	97.5	95.5	98.2	5.9
Feed N	53.1	50.3	48.8	55.5	6.9
Rumen digestion, %					
OM	62.0	63.2	63.4	64.5	2.7
Starch [c]	76.6	79.0	80.5	81.9	3.6
NDF [b]	49.2	43.5	41.7	39.7	4.8
Feed N	66.8	68.6	69.5	65.0	4.3
Microbial efficiency [d]	20.4	20.3	19.6	19.7	1.7
Nitrogen efficiency [e]	0.93	0.92	0.90	0.96	0.10
Ruminal ammonia, mg dL^{-1}	9.4	10.4	11.2	10.8	2.0
Fecal excretion, g d^{-1}					
OM	1624	1710	1764	1678	98
Starch	89.1	80.7	96.8	94.3	31.1
NDF [c]	787	828	890	881	54
N	47.2	47.8	48.7	48.2	2.7
Postruminal digestion,% duodenal					
OM [b]	58.3	55.0	53.3	54.7	2.2
NDF	8.4	13.2	9.7	12.1	7.3
Starch	84.3	85.1	80.4	79.4	6.1
N	69.46	68.9	67.71	69.97	1.26
Total digestion, %					
OM	78.9	77.8	77.1	78.2	1.3
NDF [b]	53.5	51.1	47.5	48.6	3.3
Starch	96.5	96.8	96.2	96.4	1.2
N	70.5	70.2	69.6	69.7	1.7

[a]Source of acetate: Acetic Acid, Glacial; Certified ACS PLUS; Assay 99.9%; Cat# A38P-20; Fisher Scientific, Pittsburgh, PA 15275-9952, diluted in 15,680, 15,360 and 15,040 mL for 320, 640 and 960 treatment. [b]Linear effect, $p<0.05$. [c]Linear effect $p<0.10$. [d]Grams microbial N kg^{-1} OM fermented. [e]Grams nonammonia N entering the small intestine/gram N intake

It has been generalized (Peters *et al*., 1992) that the depression in fiber digestion is largely a function of ruminal anion concentration (pH). Russell and Wilson (1996) observed that fiber digestion decreases because cellulolytic bacteria cannot adequately control their intracellular anion gradient as pH declines. Grant and Weidner (1992) further verified this concept in vitro by fermenting various forages in cultures of ruminal fluid wherein pH was controlled experimentally by means of citrate buffers. They observed that both lag phase and rate of fiber digestion were sensitive to sustained culture pH below 6.2. Nevertheless, in the present study (wherein ruminal pH for all treatments was less than 6.2), pH did not explain ($r^2 = 0.03$) the observed variation in ruminal NDF digestion.

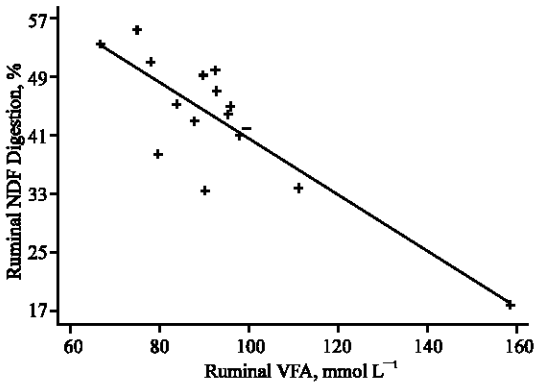

Fig. 1: Relationship between ruminal VFA concentration and NDF digestion

In contrast, we observed a strong (r^2 = 0.48) relationship between ruminal VFA concentration and ruminal ash-free NDF digestion (Fig. 1; ruminal digestible NDF = 92.8-0.555 ruminal VFA; $p<0.01$; SD = 6.9). Thus, our study reveals that VFA concentration, per se, may be a more important determinant of growth-rate of cellulolytic bacteria than pH. This concept is supported by Roe *et al.* (1998) who observed that increasing acetate concentration caused greater inhibition of growth rate in *Escherichia coli* cultures than would be expected simply from associated changes in pH.

CONCLUSION

Total ruminal VFA concentration may be a more important determinant of growth rate of cellulolytic bacteria and NDF digestion than pH, alone.

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