EFFECT OF INTRARUMINAL ACID OR BUFFER INFUSION ON THE RATE OF NEUTRAL DETERGENT FIBRE DIGESTION IN HIGHLY DIGESTIBLE PASTURES IN DAIRY COWS

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SUMMARY
Control, acid or buffer solution was infused intraruminally into cows eating highly digestible grass-based pasture and the rates of neutral detergent fibre (NDF) degradation of 4 highly digestible ryegrasses were determined using the nylon bag technique. The control solution was water, the acid solution was a mixture of acetic and hydrochloric acids and the buffer solution contained sodium hydrogen carbonate and disodium hydrogen orthophosphate. The average daily ruminal fluid pH of cows receiving acid infusion was lower, and the diurnal variation in ruminal fluid pH was greater, than that of cows receiving the control or buffer infusion. Cows receiving acid or buffer infusion had a slower rate of NDF degradation than cows receiving the control infusion. The rate of NDF degradation did not decrease as the proportion of NDF in the grass increased. The composition and structural arrangement of NDF was likely to have differed between pasture sources. The cellulolytic pH threshold for highly digestible pasture in grazing cows remains poorly defined because variations in pH are likely to be as, or more, important than a mean value.

Keywords: rumen pH, fibre degradability, highly digestible pastures, dairy cows

INTRODUCTION
Several studies have shown that a reduction in rumen fluid pH to below 6.0 reduces the digestibility of forages (Stewart 1977), particularly of the neutral detergent fibre (NDF) component (Mould and Ørskov 1983). While pH 6.0 has been described as the 'cellulolytic threshold', there is evidence that the effect varies depending on the quality of the forage (Mould *et al.* 1983), and the threshold may be lower than 6.0 for highly digestible pastures (De Veth and Kolver 2001).

The chemical characteristics of highly digestible pastures vary due to species and cultivar composition and leaf to stem ratios (Doyle *et al.* 2000). Pastures with a low concentration of NDF are usually more degradable than those with a higher NDF concentration. De Veth and Kolver (2001) have shown that the reduction in DM digestibility of perennial ryegrass (*Lolium perenne* L.) when the pH fell below 5.8 was due almost entirely to a decrease in rate and extent of digestion of NDF.

The hypotheses tested were that, when cows consumed a diet of highly digestible grass-based pasture, the rate of NDF degradation 1) would be reduced when the rumen fluid pH was lowered by infusion of acid, and 2) would decrease to a greater degree as the proportion of NDF in the grass increased.

MATERIALS AND METHODS
Six dry, rumen fistulated cows (liveweight (mean ± s.d.) 622 ± 49.0 kg) were used in an incomplete block design involving 3 treatments, and 2 periods. The treatments were:
1. Control – intraruminal infusion of electrolyte solution,
2. Acid – intraruminal infusion of the control solution with acetic and hydrolchloric acids added, and
3. Buffer – intraruminal infusion of the control solution with buffer added.

In each period, each treatment was tested on 2 cows. At the completion of the experiment, 2 of the 3 rumen infusion treatments had been tested on each cow. The periods were 10 days (6 days to stabilise rumen pH with infusion, and 4 days of rumen measurements) separated by 2 days rest. Cows grazed as a group on perennial ryegrass–white clover (*Trifolium repens* L.) pasture for 2 weeks prior to the experiment. They were randomly allocated to the 3 treatments. In each period, 4 ryegrass pasture types were subjected to nylon bag measurements of degradability in each cow in a randomised block design. More complete details of the methods are given in Williams (2003).

The control infusion solution was water plus electrolyte salts based on the mix of salts [potassium chloride, sodium chloride, magnesium chloride hexahydrate and calcium chloride dihydrate (molar ratio 40:40:1.5:1)] used in McDougall's buffer (McDougall 1948). The acid infusion solution was a mix of glacial acetic (technical grade) and hydrochloric acids (30%, commercial grade) in a molar ratio of 4:1 in water plus the same amount of electrolyte as the control solution. The concentration of the acid solution was varied as necessary (0.76-1.75 M) for each cow in an attempt to achieve an average daily ruminal fluid pH of around 5.8. The buffer infusion solution comprised 492 g sodium hydrogen carbonate and 185 g disodium hydrogen orthophosphate dissolved in water plus the same amount of electrolyte as the control solution.

Twenty litres of solution was made up daily for each cow. Solutions were infused by gravity feed from 20 L reservoirs on shelves above the cows, with flow rates adjusted 4 times daily to maintain rates of approximately 15 mL/min. Infusions were through 2 tubes into the dorsal and ventral rumen and were stopped when cows were released from their stalls prior to each feed.

During the experiment, cows were individually fed 4 times a day (at 0600, 1120, 1640 and 2200 h). They were untethered prior to each feeding time for stalls to be cleaned, and during the rest periods. Fresh perennial pasture, harvested to a residual height of 5 cm at 0800 h daily, was supplemented with dextrose monohydrate. The pasture, comprised of 43% perennial ryegrass and 49% white clover (*Trifolium repens* L.), had an *in vitro* DM digestibility of 82%, and crude protein and NDF concentrations of 220 and 400 g/kg DM, respectively. The pasture was stored loosely packed in plastic bins in a cool room until required for each of the 4 daily feeds. Any pasture that cows refused to eat was weighed daily and a subsample taken for DM determination. Pasture samples were analysed for *in vitro* digestibility (Clarke *et al*. 1982), total nitrogen as determined by a Leco FP-428 (Leco Australia Pty Ltd), and neutral detergent fibre using the method described by Van Soest *et al.* (1991).

Cows were offered pasture at about 1.1 times maintenance energy requirements (between 1.1–1.2% liveweight or 6.6-8.1 kg DM/day) determined using the equation, M_m (MJ/day) = (F+A)/k_m where F (MJ/day) = 1 * 0.53 (liveweight/1.08)$^{0.67}$ and A (MJ/day) = 0.0095 * liveweight (AFRC 1993). For these calculations, the pasture was assumed to have an ME concentration of 10.5 MJ/kg DM and cows were weighed on the first 3 days of each treatment period. Dextrose monohydrate was added to the 1120 and 1640 h feeds to increase readily fermentable carbohydrate in the diet, with the amount fed being approximately 9% of the daily pasture DM intake. Actual ME intake was calculated from pasture intake, amount of dextrose monohydrate fed and amount of acetic acid infused, with the energy available from dextrose monohydrate and acetic acid assumed to be 2.06 and 0.88 MJ/mol, respectively.

Rumen fluid pH was monitored daily by collecting samples from cows at each feeding time. During the last 4 days of each period, ruminal fluid pH was measured at feeding times and at 0200, 0800, 1300, 1800 and 2400 h on 2 of the days and 0400, 1000, 1500 and 2000 h on the other 2 days. The fluid sampled at the 0600, 1300, 1800 and 2400 h on the last day of each period was analysed for ammonia nitrogen colorimetrically by a Flow Injection Analyser (Lachat Instruments: QuickChem Method 18-107-06-1-A), and volatile fatty acids (VFA) by the method of Erwin *et al.* (1961).

Degradability of DM and NDF of 4 highly digestible ryegrasses was measured using the nylon bag technique in the last 4 days of each period. The *in vitro* DM digestibilities of the incubated grassses ranged from 89.3 to 92.5%, with the range in NDF concentration being 342 to 413 g/kg DM. Freeze-dried samples of the grasses were ground through a 3 mm screen prior to incubation for 3, 6, 12, 24, 36, 48 and 96 h. Methods for insertion, removal, washing and analysis of material remaining in bags, and calculation of degradation variables, are described by Williams (2003). Neutral detergent fibre was analysed as for the herbage fed.

Treatment differences were determined using residual maximum likelihood (REML) analysis performed with Genstat Release 4.2. For intake, infusion and rumen fermentation variables, infusion treatment was the fixed effect and cow + period was the random effect. For analysis of nylon bag degradation data, the fixed effects were infusion treatment + pasture type and random effects were cow, period and pasture type. Pairwise differences of treatments were compared by Fisher's test of unrestricted least significant difference.

RESULTS

Cows receiving the acid infusion had lower pasture intake (5.0 v. 6.4 kg DM/day; P<0.05) and estimated total ME intake (73 v. 88 MJ ME; P<0.05) than cows receiving control or buffer infusions. An average of 14.2 moles/day of acid was infused, with the amount of bicarbonate and phosphate infused into the cows on the buffer treatment equivalent to about 41 L of saliva (McDougall 1948).

The average daily ruminal fluid pH of cows infused with acid was lower (6.0 v. 6.7; P<0.05) than that of cows receiving control or buffer infusions. The diurnal variation in ruminal fluid pH was greater for cows infused with acid than in those receiving the control or buffer solutions (Figure 1). Infusion treatment had no effect (P>0.05) on the average daily ruminal fluid ammonia-N (13 mg/100 mL) and total VFA (123 mmol/L) concentrations, the proportion of propionate in the VFA mixture (17.8 %) or the lipogenic to glucogenic VFA ratio (4.5). However, cows receiving the acid had a higher (P<0.05) proportion of acetate (71.4 v. 69.0 %) in the total VFA, and cows receiving the buffer had a higher (P<0.05) proportion of butyrate (9.2 v. 8.4 %) than did the cows in the other treatments.

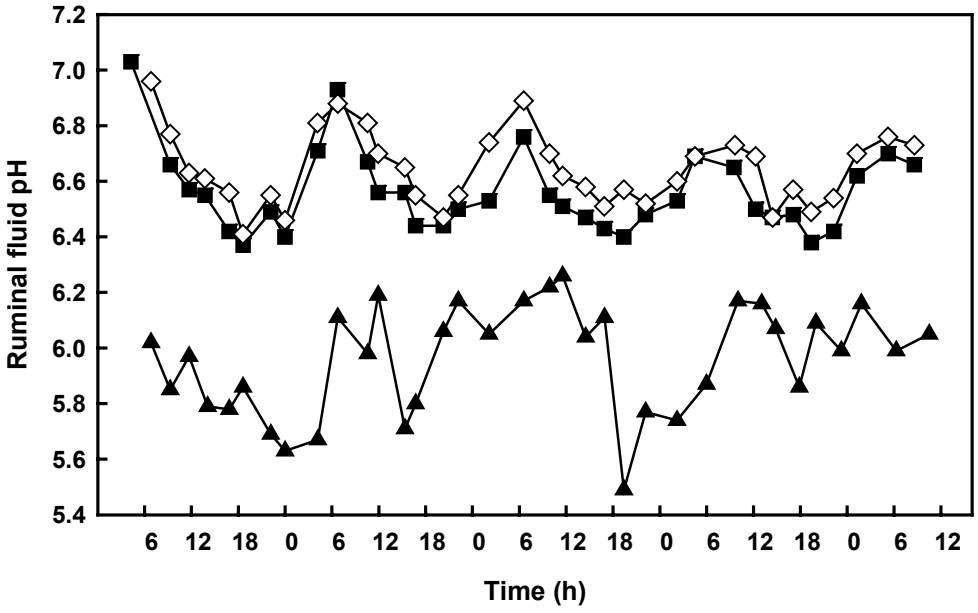

Figure 1. Pattern of ruminal fluid pH during 4 days of nylon bag incubations for cows infused with control (■), acid (▲) or buffer (◇) solutions. Periods 1 and 2 have been averaged. Each day = 0 – 24 h.

The slowly degradable fraction of NDF in the incubated grasses was smaller (89.2 v. 92.0 %; P<0.05) in cows receiving the acid compared with those on the control and buffer treatments. Cows infused with acid or buffer had slower (7.0 v. 8.9 %/h; P<0.05) rates of NDF degradation than those on the control treatment. At an outflow rate of 2%/h, the effective degradability of NDF was lowest (0.65; P<0.05) for cows infused with acid and highest (0.72; P<0.05) for cows infused with the control solution, with the buffer solution being intermediate. However, variation in the NDF concentration of the herbage had no significant effect (P>0.05) on any of the degradation parameters.

DISCUSSION

Infusion of acid decreased ruminal fluid pH compared with the other treatments, and this was associated with a decreased rate of NDF degradation in highly digestible ryegrass compared with the control treatment. De Veth and Kolver (2001) found that in a continuous culture system, digestion of NDF for this type of pasture was not reduced until pH was below 5.8. In the current experiment, the ruminal fluid pH of the acid infused cows averaged above 5.8, but the rate of NDF degradation was reduced none-the-less.

Diurnal variation in pH of 0.5 units existed in the control cows, but the acid infusions increased the diurnal variation, due in part to the need to continually vary infusion amounts to ensure animal health.

Wales *et al.* (2004) reported that NDF digestion in continuous culture was depressed more when average pH fluctuated than when it remained constant throughout the day. This effect of diurnal variation in pH was greater at an average pH of 5.6 compared with 6.1. Hence, the period of time that pH is below optimal, and the extent of diurnal variation, may be more critical for NDF digestion than mean daily pH.

The reduced NDF degradation rate with acid infusion may not have been solely due to the lower average pH and increased variation in pH. Acids can inhibit rumen motility and stimulate saliva secretion (Leek and Harding 1975). Reduced rumen motility may reduce outflow rates and the reduced intake of cows on the acid treatment would decrease turnover. The reasons for the reduction in rate of NDF degradation in pasture when cows received the buffer are unclear. It might have been expected that the NDF degradation rate in cows receiving the buffer infusion would be no different to that in cows receiving the control infusion since average ruminal fluid pH and the daily fluctuations were no different between these treatments. The observed effects of buffer on rumen fermentation may be due to increased rumen fluid dilution rate (Thompson *et al.* 1978; Russell and Chow 1993), and alterations in fluid and small particle outflow rates.

Contrary to expectations, over the range of NDF concentration tested in this experiment, the rate of NDF degradation in highly digestible grass incubated in nylon bags in the rumen did not decrease as the proportion of NDF in the herbage increased. The basis for this expectation was that De Veth and Kolver (2001) showed that the reduction in DM digestibility of highly digestible ryegrass when the pH fell below 5.8 was due almost entirely to a decrease in digestibility of NDF. However, it is apparent that the composition and structural arrangement of the NDF was likely to have differed between pasture sources due to variation in the proportion of leaf and stem, growing conditions, species differences and stage of growth, and simple relationships may not be robust.

In conclusion, infusion of acid into the rumen of cows consuming highly digestible pasture was associated with a decreased rate of degradation of NDF in highly digestible ryegrasses. However, the cellulolytic pH threshold for highly digestible pasture in grazing cows remains poorly defined because variations in pH are likely to be as, or more, important than a mean value. To further test this conclusion would require cows with greater intake potential and, hence, a wider range in basal rumen fluid pH. Characterisation of the populations of bacteria present in the rumen at the low and variable ruminal fluid pH observed when cows graze highly digestible pastures is also required.

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