The effect of three techniques of rumen pH assessment on measured pH of rumen fluid and digesta in an *in vitro* artificial rumen system

A.M. BROOMFIELD and S.J. GIBBS*

Faculty of Agriculture and Life Sciences, P.O. Box 84, Lincoln University, Lincoln 7647, New Zealand
*jim.gibbs@lincoln.ac.nz

ABSTRACT

Rumen pH assessment by three techniques was compared using an *in vitro*, artificial rumen system. Rumen digesta from dairy cows fed kale, fodder beet or ryegrass and clover pasture was used in an *in vitro* artificial rumen system. The digesta pH was measured by obtaining rumen fluid by centesis, by direct probe insertion, and by removal and filtration of fluid through cloth. This was done at different time intervals and at different heights from the base of the artificial rumen. There were significant differences in mean diet group pH. There was a significant difference between mean pH of all three techniques at the first time interval, and similarly between centesis and both the other techniques at the second and third time intervals. There was no significant difference between collection site height at any time interval. For dairy cows fed these forage diets, the technique of pH assessment of *in vitro* rumen digesta significantly influenced the pH measured, with centesis associated with higher mean pH than direct probe assessment. Given the importance of accurate rumen pH assessment, and the implications of differences between rumen fluid and rumen digesta pH in forage based ruminants, further research using the methodology with *in vivo* models is warranted.

Keywords: pH; rumen fluid; rumen digesta; rumenocentesis.

INTRODUCTION

Ruminants transfer energy from the diet principally through bacterial fermentation of carbohydrates to organic acids, with the rumen as the site of greatest microbial activity. Rumen pH is one of the important physical parameters of that environment for optimal function of the micro-organisms that inhabit it. Rumen pH in cattle depends on nutrient concentration of ration, total intake, intake patterns, acid removal, salivary and buffer inflow and other factors (Krause & Oetzel, 2006; Enemark, 2008). Abnormally low rumen pH, referred to as acidosis, has been reported to affect both rumen function and systemic health (Nagaraja & Lechtenberg, 2007).

There has been little published work on continuous rumen pH measurement in contemporary grass based systems (Gibbs *et. al.,* 2007), but where total mixed rations (TMR) are fed, cattle have been reported at greater prevalence of acidosis (Nordlund, 2003; Krause & Oetzel, 2006). For TMR fed dairy cattle a rumen fluid pH value above 5.5 has been considered clinically normal (Garrett *et al*., 1999; Kleen *et al*., 2003; Krause & Oetzel, 2005), with lower rumen pH categorised as acidosis and considered undesirable. Rumen pH may be measured directly in rumen fistulated ruminants, but in commercial herds extraction of rumen fluid via oro-ruminal intubation with cloth filtration of contents or percutaneous rumenocentesis have been widely used for clinical and production assessment of rumen pH (Kleen *et al*., 2004). However, both methods sample rumen fluid rather than digesta, and both have significant logistical and clinical limitations (Duffield *et al*., 2004; Strabel *et al*., 2007). In addition, there are no existing formal studies of the similarity of rumen fluid pH with donor rumen digesta pH in forage based systems to enable confident use of rumen fluid pH as the optimal measure of rumen pH.

In this trial the effect of two techniques used to obtain rumen fluid using cloth filtration and centesis, from rumen digesta on pH measurement was compared against direct measurement of pH in rumen digesta *in vitro*. Dairy cows on three forage diets were used as donors for rumen digesta used with simple, artificial rumen systems. Different sites of collection at three heights from the base of the artificial rumen were used to imitate sampling at different rumen sites in cattle.

MATERIALS AND METHODS

Nine non-lactating, rumenally cannulated dairy cows were allocated to three equal groups on different diets: kale (*Brassica oleracea*) and cereal straw; fodder beet (*Beta vulgaris*) and ryegrass hay (*Lolium perenne*); and ryegrass and clover (*Trifolium repens*) pasture and cereal straw. The trial was run on the Lincoln University Research Farm at Springston, Canterbury. The cows were fed at industry standard rations in each diet to gain 12-25 kg live weight across the winter period from June until early August 2010.

Sample collection and analysis

In late July and early August, all three cows in each diet group were rumen sampled simultaneously by direct, manual evacuation twice, three days apart. The procedure for each cow was identical. At 09:00 h all cows in one group were placed in a portable yard at the site and ten litres of rumen digesta from each cow was removed to a sealed container via the rumen fistula. The digesta was kept at 39°C in a water bath while transported to a laboratory. After thoroughly mixing the digesta from each cow, nine litres was divided equally between three (A, B and C) sealed, cylindrical, three litre plastic containers. The containers had been modified with a rubber diaphragm to allow insertion of needles. These were then gently shaken and turned end for end to simulate movement within the rumen. They were then placed in a 39°C oven and left for 30 minutes before sampling.

Containers A, B and C each had a single rubber diaphragm 5, 10 and 15 cm from the bottom, respectively. A 14 gauge, 5 cm needle attached to a 25 mL syringe was inserted through the rubber diaphragm horizontally to position the needle point at the mid-diameter of the container. A pH probe (IJ44, Ionode Pty Ltd Brisbane, Australia) was then carefully inserted from the opened surface of the container perpendicularly down to contact the needle point, and the pH of the digesta at that point was measured using a pH meter (HD 2105.1 Delta Ohm, Padova, Italy). Ten mLof rumen fluid was then aspirated under low pressure, and the pH of this was immediately measured using the same pH measurement system. A 50 g (wet weight) sample of digesta was then manually removed from the site of the needle tip. This was manually strained through two layers of muslin cloth by applying moderate pressure by hand; the pH of the filtrate was immediately measured with the same pH measurement system. Each container was then gently shaken and revolved before being returned to the 39°C oven for a further 30 minutes. This procedure was repeated three times for each container.

Statistical analysis

The recorded pH from each container, in each diet group, at each sampling were compared between measurement techniques, diets, at time intervals of 30, 60 and 90 minutes, and site collection heights using general linear model ANOVA with SAS statistical software (v9.1.3, SAS Institute, NC, USA).

TABLE 1: The effect of diet on mean ± standard error of the mean of measured pH of bovine rumen samples at 30, 60 and 90 minutes after the rumen liquor was placed in an artificial rumen.

Time interval (minutes)	Diet			P value
	Fodder beet	Grass	Kale	
30	6.88 ± 0.05^{a}	6.83 ± 0.05^{a}	6.35 ± 0.04^{b}	<0.001
60	6.75 ± 0.06^{a}	6.76 ± 0.05^{a}	6.11 ± 0.05^{b}	<0.001
90	6.55 ± 0.07^{a}	6.72 ± 0.06^{a}	6.00 ± 0.05^{b}	<0.001

Different superscripts within rows indicate values that differ significantly (P <0.05).

TABLE 2: The effect of the three techniques of collecting rumen fluid on mean ± standard error of the mean of measured pH of bovine rumen samples collected 30, 60 and 90 minutes after the rumen liquor was placed in an artificial rumen.

Time interval (minutes)	Collection technique			P value
	Direct	Cloth	Centesis	
30	6.50 ± 0.05^{a}	6.66 ± 0.05^{b}	6.89 ± 0.04^{c}	<0.001
60	6.42 ± 0.06^{a}	6.47 ± 0.05^{a}	6.73 ± 0.05^{b}	<0.001
90	6.33 ± 0.07^{a}	6.32 ± 0.06^{a}	6.62 ± 0.05^{b}	<0.001

Different superscripts within rows indicate values that differ significantly (P <0.05).

TABLE 3: The effect of collection site height (Low, Medium and High) within an artificial rumen on mean ± standard error of the mean of measured pH of bovine rumen samples collected 30, 60 and 90 minutes after the rumen liquor was placed in the artificial rumen.

Time interval (minutes)	Collection site height			P value
	Low	Medium	High	
30	6.68 ± 0.05	6.67 ± 0.05	6.70 ± 0.05	>0.05
60	6.55 ± 0.06	6.53 ± 0.06	6.54 ± 0.06	>0.05
90	6.43 ± 0.06	6.38 ± 0.06	6.46 ± 0.07	>0.05

RESULTS

The mean pH of samples from all three diet groups at the three time intervals examined is displayed in Table 1. There was a significant (P <0.001) difference in mean diet group pH between kale and both fodder beet and grass at all the time intervals.

The mean pH of samples obtained by the three different techniques of rumen fluid or digesta extraction and measurement, across all diet groups and at the three different time intervals, is displayed in Table 2. There was a significant difference (P <0.001) between mean measured pH of all three techniques at the first time interval, and

between centesis and the other techniques at the second and third time intervals. The proportionate fall in mean pH of each diet group across the three time intervals was not similar, with the grass treatment group observed to have the lowest reduction in mean pH across the three time intervals.

The mean pH of samples obtained from each of the three sites of collection (low, medium and high), across all diet groups and techniques of rumen fluid and digesta extraction and measurement, and at three time intervals, is displayed in Table 3. There were no significant differences ($P > 0.05$) between sites of collection at any time interval.

DISCUSSION

In this study rumen fluid obtained by centesis, across all diet groups and time series, was of significantly higher measured pH than either rumen fluid obtained by cloth filtration of digesta or *in situ* probe measurement of digesta (Table 2). For rumen contents measured directly at the first time interval, rumen digesta was also significantly lower in pH than either cloth filtration or centesis treatments. These trends were present in each group despite a significant difference ($P < 0.001$) in overall mean measured pH between kale and both fodder beet and grass diet groups (Table 1). In contrast, Duffield *et al*. (2004) compared the pH measurement using the rumenocentesis and oro-ruminal intubation techniques in dairy cows, and reported samples obtained by rumenocentesis measured mean values more than 0.3 pH units lower than simultaneous samples obtained by oro-ruminal intubation. The authors attributed this to the varying salivary contamination in the oesophagus and reticulum. Garrett *et al.* (1999) compared low volume syringe and needle rumenocentesis (3 mL) with high volume, site matched, rumen fluid sampling (200 mL) accessed by a fistula, and reported a mean 0.28 pH unit increase in measurements from the latter. However, Nordlund (2003) cited unpublished data of a 0.1 pH reduction with an indwelling pH sensor compared to rumen fluid sample obtained manually via the fistula.

In this study design, differential salivary contamination of samples obtained by any method was excluded, and rumen fluid volumes collected by cloth filtration were low (<20 mL) and obtained from digesta directly sited at the needle point. Given that 200 mL of free flowing rumen fluid requires approximately 800g of donor rumen digesta, it is apparent that in the study of Garrett *et al.* (1999) they did not maintain the same site specificity of fluid collection as this study. This may explain the difference in pH measurements between the rumenocentesis and manual extraction techniques in that study, as rumen fluid would flow into the capture jar from the digesta around the collection site. It should also be noted that there are reports of significant differences in rumen pH between ventral sac and reticular sites accessed via fistula (Lane *et al.*, 1968). These are the sites from which the Duffield *et al*. (2004) obtained samples in their study, suggesting the differences recorded in that study could also be attributed to reasons other than salivary contamination of the oro-ruminal tube during oesophageal passage.

There were no significant differences in mean pH, across all diet groups and time intervals, between the three collection site heights (Table 3). Previous studies have suggested a difference in measured pH between the top and bottom of the rumen in cattle (Lane *et al*. 1968). One explanation for the lack of stratification of pH reported in this current study is that there was no active fluid movement except by gravity in these artificial rumens, which may reduce pH stratification, and the difference in heights between the sites of collection were perhaps insufficient to produce any differentiation in these diets. Although this was a surprising finding, there are no available studies to determine if there is indeed *in vivo* stratification of rumen pH in the forage diets examined in this study.

In the current study, the methodology of inserting a pH probe to the needle tip made it apparent that in each diet group the use of a needle to extract rumen fluid from digesta routinely led to rumen feed particles gathering at the needle tip, and rumen fluid drawn into the syringe was filtered through this. Reports in the literature on rumenocentesis confirm this occurrence in cattle fed other diets (Nordlund, 2003). It is notable that across all diet groups in this study mean pH was highest in the samples obtained by needle (Table 2). This sample might be expected to have the lowest proportion of solid matter and be lowest in direct digesta measurement. No differences in mean pH for cloth filtered samples were recorded between these samples except at the third time interval. Considered with the clear finding that compared to *in situ* pH assessment by the probe, both other techniques had highly significant increased mean pH in each diet group at the first time interval, this indicates that whole rumen digesta has a lower pH than rumen fluid for dairy cattle fed these diets. This finding, if validated *in vivo*, has implications for future rumen pH assessment of pasture based cattle.

Although rumenocentesis is currently the most common technique for rumen pH assessment used in commercial veterinary practice (Gianesella *et al.,* 2010), the finding that rumen fluid pH is not the same as rumen digesta pH in the three forage diets examined in this study suggests it may have more significant limitations as a tool for physiological

assessment than have been previously identified. Given that rumenocentesis is a surgical technique only to be carried out by veterinarians, requires chemical and physical restraint in most cases, and has documented health risks such as localised abscesses or peritonitis (Kleen *et al.*, 2004; Strabel *et al*., 2007), conclusive evidence that the technique does not accurately represent rumen pH *in vivo* in forage diets would significantly weaken grounds for routine use in cattle of pasture based systems.

CONCLUSION

This experiment showed a significant ($P < 0.001$) difference between the pH of rumen samples obtained by cloth filtration, needle centesis and direct digesta measurement, with centesis reported as the highest pH value across all diet treatments and time intervals. This indicates that for the forage diets of this experiment, rumen fluid is a different pH to rumen digesta *in vitro*. Previous reports (Duffield *et al.,* 2004; Kleen *et al*., 2004) suggested rumenocentesis, as a better field technique for rumen pH assessment in intact cows than oro-ruminal intubation, as the technique of choice in commercial herds. However, if further research using *in vivo* models validates a consistent difference between rumen fluid pH and rumen digesta in forage based ruminants, alternative techniques of rumen pH assessment may be required.

REFERENCES

Duffield, T.; Plaizier, J.C.; Fairfield, A.; Bagg, R.; Vessie, G.; Dick, P.; Wilson, J.; Aramini, J.; McBride, B. 2004: Comparison of techniques for measurement of rumen pH in lactating dairy cows. *Journal of Dairy Science* **87(1)**: 59-66.

Enemark, J. 2008: The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. *The Veterinary Journal* **176(1)**: 32-43.

Garrett, E.F.; Pereira, M.N.; Nordlund, L.E.; Goodger, W.J.; Oetzel. G. 1999: Diagnostic methods for the detection of subacute ruminal acidosis in dairy cows. *Journal of Dairy Science* **82(6)**: 1170-1178.

Gianesella, M.; Massimo, M.; Stelleta, C.; Ravarotto, L.; Guidice, E.; Van Saun, R.J. 2010: Evaluating the effects of rumenocentesis on health and performance in dairy cows. *Acta Veterinaria Brno* **79:** 459-468.

Gibbs, S.J.; Laporte-Uribe, J.; Trotter, C.; Noel, J. 2007: Rumen function and lameness in pasture based dairy cows of the South Island of New Zealand. *Journal of Dairy Science* **90(Supplement):** 655.

Kleen, J.L.; Hooijer, G.A.; Rehage. J.; Noordhuizen, J.P.T. 2003: Subacute ruminal acidosis (SARA): A review. *Journal of Veterinary Medicine A Physiology, Pathology, Clinical Medicine* **50**: 406-414.

Kleen, J.L.; Hooijier, G.A.; Rehage, J.; Noordhuizen, J.P.T. 2004: Rumenocentesis: a viable instrument in herd health diagnosis? *Deutsche Tierarztliche Wochenschrift* **111**: 458-462.

Krause, M.K.; Oetzel, G.R. 2005: Inducing subacute ruminal acidosis in lactating dairy cows. *Journal of Dairy Science* **88**: 3633-3639.

Krause, M.K.; Oetzel, G.R. 2006: Understanding and preventing subacute ruminal acidosis in dairy herds: a review. *Animal Feed Science and Technology* **126**: 215-236.

Lane, G.T.; Noller, C.H.; Colenbrander, V.F.; Cummings, K.R.; Harrington, R.B. 1968: Apparatus for obtaining ruminoreticular samples and the effect of sampling location on pH and volatile fatty acids. *Journal of Dairy Science* **51**: 114-116.

Nagaraja, T.G.; Lechtenberg, K.F. 2007: Acidosis in feedlot cattle. *Veterinary Clinics of North America: Food Animal Practice* **23(2)**: 333-350.

Nordlund, K.V.M. 2003: Herd-based diagnosis of subacute ruminal acidosis. *Proceedings of the 36th Annual Conference of the American Association of Bovine Practitioners,* 15-17 September 2003. Columbus, Ohio, USA.

Strabel, D.; Ewy, A.; Kaufmann, T.; Steiner A.; Kirchhofer, M. 2007: Rumenocentesis: A suitable technique for analysis of rumen juice pH in cattle. *Schweiz Arch Tierheilkd* **149**: 301-306.