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Rumen foam volume and stability in cattle selected for low or high bloat susceptibility

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ABSTRACT

A simplified method to measure foam volume ($V\infty$) and stability ($1/k_2$) was used to investigate the influence of saliva on foam properties and its relationship to bloat susceptibililty. Significant differences in $V\infty$ and $1/k_2$ were observed between samples of rumen fluid (within-sample CVs were 9% and 18%, while between sample CVs were 57% and 70% for $V\infty$ and $1/k_2$, respectively). Mixing bovine saliva with rumen fluid or diluted clover juice resulted in a large increase in $V\infty$ and $1/k_2$ (4.6- and 7.8-fold, respectively). This effect was strongly synergistic as saliva alone produced essentially no foam. The assay was used to quantify the efficacy of a paraffin-based anti-bloat drench ($V\infty$ and $1/k_2$ were decreased by up to 4-fold and 6-fold, respectively). Saliva from non-bloating cattle, four each from herds bred for low- or high-bloat susceptibility, was measured for the effect on the foam parameters of a rumen fluid sample. No significant differences due to herd-of-origin were observed.

Keywords: Saliva; cattle; bloat; susceptibility; foam

INTRODUCTION

Pasture bloat is a metabolic disorder of cattle characterised by a build-up of foam in the rumen, which in severe cases can distend the rumen to the point that breathing and the circulation are impaired. Bloat typically occurs while grazing on lush leguminous pasture, is characterised by a sudden and unpredictable onset, and can rapidly lead to death if left untreated. Bloat can be successfully alleviated or prevented by regular drenching with anti-foaming agents. However, effective bloat management is a significant time and labour cost. It has been estimated that bloat costs the New Zealand dairy industry at least $25-50 M annually in animal deaths, labour costs and lost production (Morris *et al.*, 1997 and Livestock Improvement Corporation, unpublished data 1997).

Individual cattle vary considerably in their susceptibility to bloat (Johns, 1954). This has been shown to be a genetically inherited trait (Cockrem and McIntosh, 1976; Morris *et al.*, 1991), and successive generations of selection at Ruakura for low- or high-bloat susceptibility have resulted in two herds (LS and HS) with a large divergence in their mean bloat susceptibility scores (Morris *et al.*, 1997).

Rumen foam has been the subject of a number of studies, but as yet the primary cause of the disorder is not understood. The stability and strength of foam produced from rumen liquor, plant extracts, and bovine saliva has been measured (Mangan, 1958; Mangan, 1959). The physical properties and chemical makeup of rumen foam has also been investigated (Jones and Lyttleton, 1969; Jones and Lyttleton, 1973; Laby and Weenink, 1966; Waghorn, 1988). A range of physical and biochemical parameters have been studied in an effort to identify markers for bloat susceptibility. These include the composition of saliva (Carruthers and Morris, 1993; Clarke *et al.*, 1974; Jones *et al.*, 1986; McIntosh, 1975; Phillipson and Mangan, 1959; Rajan *et al.*, 1996), intra-rumen pressure and quantity of digesta (Cockrem *et al.*, 1987; Morris and Carruthers, 1991; Waghorn, 1991), physical measurements, and blood and urine constituents (Carruthers and Morris, 1994; McIntosh *et al.*, 1988; Morris and Carruthers, 1991). However, despite these studies, the physical-biochemical mechanisms underlying the propensity of some cattle but not others to bloat has remained elusive.

METHODS

A previously described method for the measurement of foam volume and stability (Laby, 1969) was simplified and used to assess interactions between rumen fluid, pasture juice, and saliva in the production of foam, to quantify the efficacy of a bloat drench in vitro, and to assess the effect of saliva from LS and HS cattle on foam parameters.

Collection of rumen fluid, saliva, and pasture juices

Rumen fluid was collected from Friesian dairy cows each fitted with a rumen fistula, and grazing annual ryegrass/ white clover mixed pastures. These cows were unrelated to the Ruakura bloat-breeding trial described below. A sample of the rumen contents was collected from these cows, within 30 minutes of their leaving pasture, by ladling some digesta from near the top of the rumen through the opened fistula. The collected sample was squeezed through cheesecloth, and the filtrate (rumen fluid) was recovered and stored at -20°C until use.

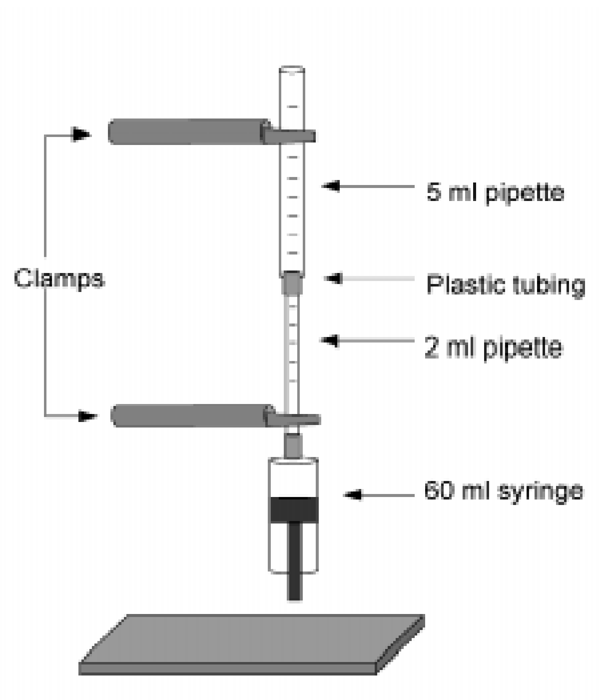
Saliva was collected from cattle from the Ruakura LS and HS herds using a mouth bit and mild vacuum as previously described (McIntosh *et al.*, 1988). After collection, EDTA was added to a final concentration of 10 mM, and phenylmethylsulphonylfluoride was added to a final concentration of 1 mM in order to inhibit proteases. The saliva was then centrifuged at 1500g for 10 min to pellet debris, and stored at -20°C.

White clover leaves or ryegrass were collected from pasture and macerated by blending in a food processor. The material was then placed in a cheesecloth bag and squeezed to extract the juice, which was stored at -20°C.

Measurement of foam volume and stability

The plunger was removed from a 60 ml plastic syringe that was then held in an upright position. A silicone rubber tube was connected to the tip and clamped to prevent leakage. A standard volume of 15 ml of rumen fluid (or other test solution) was placed in the syringe and was mechanically agitated for 15 sec at 8000 rpm using an Ultra-Turrax T25 (Janke & Kunkel, Staufen, Germany) tissue homogeniser fitted with an 18 mm probe. The plunger was then inserted into the syringe, which was then inverted. The free end of the tube was connected to the bottom end of a calibrated vertical column constructed from a 2 ml and a 5 ml plastic syringe (see Fig. 1). The foam was then forced into the column using the plunger, until the foam-liquid interface coincided with a convenient zero point on the gradations of the 2 ml pipette. At exactly one minute after cessation of the mechanical agitation, the tubing connecting the column to the plunger was pinched off, and the level of the foam-liquid interface was measured and at 30 sec intervals thereafter for the next 15 min as it rose due to the ongoing collapse of the column of foam above it.

FIGURE 1.
Diagram of the apparatus used to measure foam parameters.



The rate of collapse of the foam follows the second order rate equation $dv/dt = k_2(V_\infty - v)^2$ as previously described (Laby, 1969), where v is the volume of fluid drained from the foam at time t, and V∞ is the volume of liquid released from the foam at time infinity. V∞ is a measure of the total amount of foam produced, while the value $1/k_2$ is directly proportional to the foam stability. For a foam stability measurement, the assay was performed in triplicate for each condition, the results for V∞ and $1/k_2$ were averaged, and the standard error of the mean was calculated.

RESULTS

Interactions between rumen fluid, pasture juice, and saliva

Rumen fluid by itself produced a variable amount of foam (range of V∞ was 0 to 4.8 ml), depending on the rumen fluid sample tested. Rumen fluid was obtained from a single cow on five different days. The within-sample coefficient of variation on triplicate analyses of the samples was 9% for V∞ and 18% for $1/k_2$, whereas the coefficient of variation between the samples was significantly higher (57% for V∞ and 70% for $1/k_2$). The effect of pasture type on foam parameters was assessed. Rumen fluid samples taken from cows grazed on clover, but not showing signs of clinical bloat, had on average a 4-fold increase in V∞ and 5-fold increase in $1/k_2$ compared with cows grazed on grass (results not shown).

Dilutions (1/15) of saliva in water consistently produced no measurable foam. In contrast, 1/15 dilutions of plant juice produced a significant amount of relatively stable foam. A mixture of 14 ml of rumen fluid and 1 ml of clover juice resulted in a similar V∞ to clover juice alone. When 1 ml saliva was mixed with 1 ml clover juice and 13 ml water, the V∞ was 3.8-fold greater, and stability of the foam produced was 25-fold greater than clover juice alone. These data are summarised in Table 1.

TABLE 1: V∞(ml) and stability of foam (min.ml) produced by rumen fluid, pasture juice and saliva

Fluid	**V_∞(SEM)**	**$1/k_2$(SEM)**
Rumen fluid	0.00 (0.00)	n.a*
Clover juice	0.92 (0.00)	1.7 (0.0)
Grass juice	0.64 (0.02)	1.1 (0.1)
Saliva	0.00 (0.00)	n.a.
Rumen fluid + Clover juice	0.88 (0.04)	1.1 (0.2)
Saliva + clover juice	3.47 (0.45)	43.5 (11.6)

*n.a. not applicable

The interaction of saliva and rumen fluid was tested using saliva from three cattle and aliquots of a single rumen fluid sample from one of them. In this case, the rumen fluid alone produced some foam of moderate foaminess (V∞ = 0.35 ml) and stability ($1/k_2$ = 2.5 min.ml). When 14 ml of this rumen fluid was mixed with 1 ml of saliva from one of the three animals, a 4- to 5-fold increase in V∞ and a 6- to 10-fold increase in $1/k_2$ was observed, depending on the saliva sample used (Table 2). The magnitude of these changes was dependent on the volume of saliva added, with maximal foam stability achieved by adding 0.25 ml saliva (results not shown).

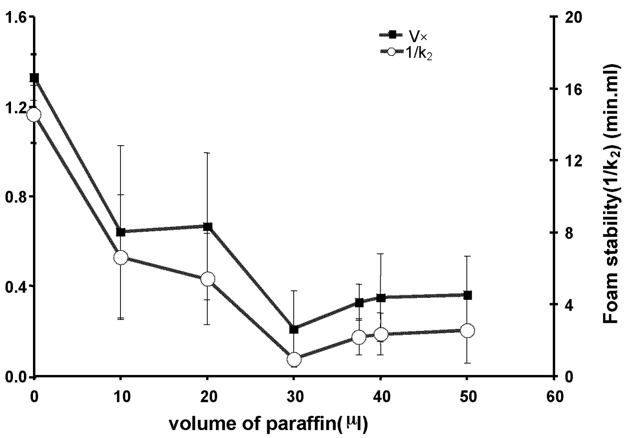
TABLE 2: Interaction between rumen fluid and saliva in altering the foam parameters, V∞(ml) and $1/k_2$ (min.mil).

Rumen fluid	**V_∞(SEM)**	**$1/k_2$(SEM)**
alone	0.35 (0.03)	2.5 (0.6)
+ saliva a	1.57 (0.13)	25.0 (5.7)
+ saliva b	1.76 (0.13)	18.9 (3.3)
+ saliva c	1.55 (0.07)	16.4 (1.9)

In vitro measurement of the efficacy of an anti-foaming agent

The addition of 40 ml of paraffin, equivalent to its standard dose as an anti-bloat drench, to the foam assay was found to decrease the V∞ of a 1/15 dilution of clover juice by 1.6-fold and rumen fluid by 4-fold. This dose of paraffin also decreased the foam stability by 3.6-fold (clover juice) or 6.6-fold (rumen fluid) (results not shown). The concentration of paraffin required for its maximum inhibitory effect on foam production and stability was determined. Increasing volumes of paraffin decreased the V∞ and the foam stability until a plateau was reached at approximately 30 ml, at which a 4.5-fold decrease in V∞ and 5-fold decrease in foam stability was observed (Fig. 2).

FIGURE 2. Values for V∞ (filled squares) and $1/k_2$ (open circles) obtained from 15 ml aliquots of a single rumen fluid sample to which increasing volumes of liquid paraffin had been added. The values are the mean of three independent measurements on the same sample. The error bars represent the standard error of the means.



Effect of saliva from LS and HS cattle on the foam parameters of rumen fluid

Saliva from four animals from each of the Ruakura LS and HS herds was added to separate 15 ml aliquots of a single standard rumen fluid sample. The volume of saliva added was that required for the addition of 0.5 mg of total salivary protein, which ranged from 0.4 to 1.1 ml. Measurements of the V∞ and foam stability were made (Table 3). In all cases, the addition of saliva resulted in a significantly increased V∞ and foam stability over the rumen fluid alone, confirming the synergistic interaction between saliva and rumen fluid. The magnitude of the synergism varied between the individual saliva samples, but there was no discernible difference between saliva from HS versus LS animals. Additional experiments were performed using 0.25 mg salivary protein, and with a standard volume of 0.5 ml. These also resulted in no discernible herd-of-origin effect on the V∞ and foam stability (results not shown).

TABLE 3: Effect of saliva from LS and HS cattle on foam parameters of rumen fluid

Rumen fluid	V_∞(SEM)	$1/k_2$(SEM)
alone	1.20 (0.14)	14.5 (2.9)
+ LS saliva d	1.63 (0.15)	18.9 (1.8)
+ LS saliva e	1.56 (0.23)	19.6 (2.2)
+ LS saliva f	1.74 (0.22)	27.0 (3.4)
+ LS saliva g	1.77 (0.26)	23.3 (3.6)
+ HS saliva h	1.74 (0.18)	26.3 (1.8)
+ HS saliva i	1.86 (0.29)	23.8 (3.5)
+ HS saliva j	2.06 (0.33)	32.3 (6.4)
+ HS saliva k	1.56 (0.28)	19.6 (4.1)

DISCUSSION

The apparatus and procedure we have described here to quantify the volume of liquid retained in the foam (V∞) and the foam stability ($1/k_2$) of a range of fluids, is considerably simplified compared with previous studies. The data presented confirm that the estimates of V∞ and $1/k_2$ are repeatable, with coefficients of variation for V∞ and $1/k_2$ of 9% and 18% respectively and we have shown that the assay can be used to quantify the efficacy of anti-bloat agents in vitro.

Several aspects of the procedure were explored for their potential to introduce variability. The method of rumen fluid collection and foam production can influence the results, however, these were minimised through standardisation of the procedure of rumen fluid collection and foam production. Significant differences were observed between samples collected at different times or after changes in feeding routine. These effects are the likely reason for the variability in the V∞ and $1/k_2$ between the different rumen fluid samples, and shows that the foaming properties of rumen fluid are not constant either within or between non-bloating animals. No differences in V• and $1/k_2$ were observed between freshly prepared rumen fluid, saliva, and plant juices and the same samples after freezing and re-thawing (results not shown).

Pasture juices alone readily produced a large amount of stable foam, confirming earlier studies with pasture extracts (Jones and Lyttleton, 1969; Mangan, 1959). We found that clover juice produced more foam of greater stability than juice from ryegrass (results not shown). Bloat-potent forages have been reported to contain a higher concentration of total protein as well as a protein fraction with high foaminess compared with non-bloat potent forages (McArthur and Miltimore, 1966; McArthur and Miltimore, 1969).

We found that saliva alone produces essentially no foam. Previous studies on the foaming properties of whole saliva and its subfractions are contradictory. Mangan (1959) reported that salivary mucoprotein readily produces foam, while Bartley and Yadava (1961) reported that salivary mucoprotein inhibits foaming of plant extracts. We observed a significant synergy between saliva and rumen fluid or plant juice, which has not been previously reported. These results show that despite its lack of inherent foaminess in our assay, saliva is an important contributor to the production of foam in the rumen, and that the makeup of saliva might influence the degree of susceptibility to bloat. Although significant differences were observed between the saliva from HS and LS cattle in their ability to produce foam when added to rumen fluid, there was no correlation with the herd of origin. Therefore, these results by themselves do not support a role for saliva in determining bloat susceptibility. However, it is possible that the experimental design has masked an effect of saliva on bloat due to the rate of saliva secretion or differences in saliva composition.

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