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Components of foam and liquor from the rumen of bloating and non-bloating cows

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ABSTRACT

Most cows fed fresh forages containing clover or lucerne have a foamy rumen digesta. When the foam becomes persistent (stable), gasses from fermentation become trapped and the cow bloats. Samples of foam and rumen liquor from bloated and non-bloated cows fed freshly cut lucerne or red clover were analysed to determine differences that may account for foam stability.

In a bloated cow the density of rumen digesta falls from about 950 to 600 or 700 g/ℓ and the foam has a density of 200 to 300 g/ℓ. Analysis of foams showed no difference between bloat and non-bloat samples in dry matter (DM)(7.8%) nitrogen (94 mg/g DM) or chlorophyll (7.4 mg/g DM), but bloat foams had a higher lipid concentration (57 mg/g DM) than non-bloat foams (6 mg/g DM). Number of particles between 9 and 75 μm in diameter showed little difference between bloat and non-bloat foams (40 x 10^7/gDM). The significance of these observations are discussed in relation to foam stability.

Keywords Bloat; foam; cows; lucerne; red clover; rumen; nitrogen; chlorophyll; particle size.

INTRODUCTION

Cows fed fresh pastures containing upwards of 15% dry matter (DM) as legume are prone to bloat. Bloat occurs in only some cows and not others and on some days and not others, but when pasture supply is lush and plentiful most cows will have a foamy rumen digesta. When the foam becomes persistent (stable), then the gas which is produced at 1 to 2 l/min from rumen fermentation, accumulates in small bubbles and cannot be eructated, and bloat occurs. Unstable foam, common to many unbloated cows grazing lush pasture, breaks down rapidly to release gas, which is eructated. This study is an attempt to characterise the principal components of foam and liquor considered likely to be important in the aetiology of bloat.

METHODS

Cows and Diets

Eleven cows were used for determining foam and liquor DM percentage, density of rumen contents and other physical characteristics of rumen contents *in situ*. Four cows fed fresh lucerne (*Medicago sativa* L.) (FL) were used to determine the concentration of nitrogen (N), lipid, pectin, chlorophyll and mineral elements in both foam and liquor when bloated (Grade 2 - 3; Johns, 1954) and not bloated. Similar measurements were undertaken in 2 cows fed red clover (*Trifolium pratense*) (RC). Measurement of small particulate DM was undertaken in 5 cows fed RC. All cows were fed freshly cut lucerne or red clover, and were held indoors in stalls for ease of sampling.

Sample Collection

Cows were sampled 60 to 80 min after the onset of eating. Foam samples were obtained by squeezing 2 to 3 kg rumen digesta through cheese-cloth and scooping it off the liquor. The foam was either frozen or analysed directly, but in all cases drainage of liquid had occurred so that only the foam (and not entrapped liquor) were analysed. Samples of rumen liquor were also taken for analysis. We take the view that foam is generated from rumen liquor *in vivo*, and that this can be achieved artifically by bubbling gases into rumen liquor (Mangan, 1959; Jones and Lyttleton, 1973).

Analytical

Chemical determinations made on freeze dried foam and liquor included N (Williams and Twine, 1967), pectin (Blumenkrantz and Asboe-Hansen, 1973) and mineral elements by inductively coupled plasma emission spectrometry (Lee, 1981). Determinations on fresh material included chlorophyll (Arnon, 1949) and lipids after chloroform:methanol (2:1) extraction.

Particles between 9 and 75 μm in diameter were measured in fresh liquor and foam using a Coulter Counter (Coulter Electronics Ltd, Harpenden, England) fitted with a 280 μm orifice. Although strained foam and liquor contained very few particles larger than 75 μm, they were washed through a 75

TABLE 1 Concentration of components (mg/g DM) in rumen foam and liquor of bloating (grade 2 - 3) and non-bloating cows fed either fresh lucerne (FL) or red clover (RC). Data are means of 4 cows ± SE fed FL, or means of 2 cows fed RC.

Component		Foams		Liquor	
		Bloat	Non-bloat	Bloat	Non-bloat
Nitrogen	FL	96.3 ± 4.2	92.6 ± 3.6	86.4 ± 10.6	85.6 ± 3.8
	RC	96.5	90.9	91.4	76.2
Chlorophyll	FL	7.5 ± 0.7	7.1 ± 0.2	3.8 ± 1.3	4.1 ± 0.5
	RC	4.0	4.5	2.3	2.7
Pectin	FL	5.5 ± 0.7	4.6 ± 0.4	4.9 ± 0.8	4.0 ± 0.2
	RC	4.3	4.4	4.3	4.5
Lipid[1]	RC	56.6 ± 7.8	5.9 ± 1.7		

[1] 6 Bloat and 4 non-bloat cows.

μm sieve prior to counting to prevent blockage. Coulter analysis required a 2 ml aliquot of electrolyte containing about 0.4 mg DM to be counted. This was repeated 10 times (in triplicate) over the 9 to 75 μm range of particle sizes measured. Sample DM percentage was also determined.

RESULTS AND DISCUSSION

Physical Characteristics of Digesta

The rumen contents of a bloated cow contains no free liquid and there is no clear *rafting* of digesta which is common to non-bloated forage fed cattle. The density of rumen contents is usually around 600 to 700 g/ℓ (Grade 2 bloat) but is likely to be lower in more severe bloat. Density of rumen digesta in non-bloated cows fed forages is about 900 to 950 g/ℓ.

Foam from which the liquor is allowed to drain has a density of about 300 g/ℓ for FL and 220 g/ℓ for RC. The mean DM content (±SE) of drained foams from 11 non-bloated cows was 7.8 ± 0.48% (FL) and 10.2 ± 0.64% (RC). When bloat occurred the foam DM content was similar for both forages (8.6 ± 0.18%). Bloat foams are able to hold a large amount of liquid and also solids prior to squeezing through cheese-cloth. In non-bloated cows the quantity of foam is small, with a low liquid holding capability, and a low compressive strength and persistence (Jones and Lyttleton, 1973) compared to bloat foams.

The DM content of rumen liquor from 11 non-bloated cows was 3.47 ± 0.14% (FL), 3.35 ± 0.29% (RC) and from bloated cows 3.45 ± 0.40% for both forages. DM content of whole rumen contents of cows fed FL or RC is usually 10 to 13%. More detailed information DM content and particle size distribution of rumen digesta in non-bloated cows fed FL or RC is given by Waghorn (1986).

Nitrogen and Lipid

The N content of foam and liquor DM was similar, and not affected by presence or absence of bloat (Table 1). If N is expressed as crude protein (CP), then DM in both foam and liquor contains about 56% CP for both FL and RC diets. This is much higher than plant CP concentration in FL (19-22%) or RC (22-27%) suggesting N rich plant components such as chloroplasts as well as bacteria are concentrated in the rumen liquor and foam components, however the extent of this concentration appears unrelated to bloat.

Lipid concentration was determined in foam of bloated and non-bloated cows fed RC. Lipid was concentrated 10-fold in bloat foam (Table 1), but data are not available for concentration in rumen liquor. These observations support those of Laby and Weenink (1966), and Reid *et al.* (1975) where yellow bubbles were present in increasing amounts in rumen contents of bloated cows fed RC.

Pectin and Carbohydrate

Pectin is a gelling agent and has potential to stabilise and add strength to foam. This is particularly true of low methoxyl pectins which can be esterified with Ca^{++} ions forming cross linkages between polymers and a high gel strength (The Copenhagen Pectin Factory Ltd., 1980). As legumes contain higher concentrations of pectin in DM than grasses, pectin concentration was determined in both foam and liquor. The effects of pectin addition to the rumen were also investigated in relation to bloat.

Pectin concentration was 6% of DM for RC and 8 to 9% for FL fed in this experiment, but the pectin concentration in the foam and liquor of bloated and non-bloated cows fed either diet was only 0.3 to 0.7% of DM, and therefore too low to affect foam stabilisation (Table 1).

On 3 occasions 200 g low methoxyl pectin (GENU PECTIN LM-101 AS, The Copenhagen Pectin Factory Ltd, Denmark) in ethanol, to aid dispersion, was added with 10 g calcium (as $CaCl_2.2H_2O$) to the rumen of cows immediately before feeding FL. In all cases mild bloat was evident within 40 min of feeding, but the bloat was short lasting and the foam unstable. Bloat may have been aided by the presence of pectin but may also have occurred without pectin addition. Pectin is readily fermented by rumen microorganisms and probably lost within minutes of release from the plant under normal feeding conditions. It is therefore unlikely to influence foam stability and will not be a significant factor in the aetiology of bloat.

Neutral detergent fibre (NDF) concentrations in 4 foam samples ranged between 2 and 12% of foam DM, and were unrelated to bloat. NDF comprised 43% cellulose, 38% hemicellulose and 19% lignin. The amount of NDF present is probably a reflection of the amount of material entrapped in foam rather than a source of stability.

Mineral Elements

Concentration of mineral elements were similar in bloat and non-bloat foam and liquor DM (Table 2), except for a small ($P<0.05$) reduction in Mg concentration in bloat foam. Mineral elements were not concentrated in foam compared to liquor, and bloat foam did not have elevated Ca, Mg or high Ca to P ratios (Clarke and Reid, 1974.).

There were large variations betwen cows in the mineral concentration of bloat liquor DM, compared to non-bloat liquor and foams. This was especially true for Na where a 3-fold range in concentration within 4 cows was observed, but concentrations of mineral elements in liquor appeared quite unrelated to those in bloat foams.

In cows fed RC a similar lack of clear separation of mineral concentrations between bloat and non-bloat foams and liquors was evident. RC data have been omitted from Table 2 for brevity, but most concentrations in DM were similar to that of FL with large differences between the 2 cows a frequent occurrence.

Chlorophyll and Chloroplasts

Chlorophyll is used as an indicator for chloroplast numbers. Chloroplasts have been considered a factor in foam stabilisation and in the aetiology of bloat (Stifel *et al.*, 1968; Howarth *et al.*, 1982; Majak *et al.*, 1985) especially in relation to their fraction 1 protein content (Jones *et al.*, 1978). Chloroplasts and chloroplast fragments may aid foam stabilisation because they are rich in both protein and lipid and could interact with both hydrophobic and hydrophillic components of foams, and strengthen junctions between foam bubbles (Aubert *et al.*, 1986).

In this study cholorophyll concentration in the lucerne plant was 3.7 to 3.9 g/kg DM, and 2.2 g/kg DM in RC. Individual chloroplasts contain about 2.4 x 10^{-9} mg chlorophyll, and each mg chlorophyll is associated with about 25 μg fresh chloroplast (4 to 8% chlorophyll in chloroplast DM; Hall, 1976). On this basis 5 g fresh lucerne (1 g DM) will contain about 1.7 x 10^{9} chloroplasts.

The number of chloroplasts released into rumen digesta have not been measured, but if 65% of lucerne chlorophyll is released by chewing when cattle are fed FL (Waghorn *et al.*, 1988), and it is only slowly degraded in the rumen (Mangan and West, 1977), then a cow consuming 2.5 kg FL DM in 1h will release about 2.5 x 0.65 x 1.7 x 10^{12} chloroplasts. This is equivalent to 6.63 g chlorophyll in a pool of about 45ℓ(Waghorn, 1986), or 4.3 mg/g liquor DM. Actual concentrations in bloat and non-bloat liquor DM were 3.8 and 4.1 mg/g respectively (Table 1).

Presence or absence of bloat did not affect chlorophyll concentration in liquor or foam, although concentration in foam DM was nearly twice that for liquor DM (Table 1). When RC was fed,

TABLE 2 Mean ± SE of concentration of mineral elements in rumen and liquor of bloating (grade 2-3) and non-bloating cows fed fresh lucerne.

Mineral	Foam Bloat	Foam Non-bloat	Liquor Bloat	Liquor Non-bloat	Lucerne as fed
			(mg/g dry matter)		
Calcium	25.6 ± 1.2	25.9 ± 0.6	27.8 ± 1.2	27.7 ± 0.5	12.9
Postassium	33.0 ± 1.5	38.6 ± 4.4	48.3 ± 4.8	52.6 ± 7.9	31.3
Magnesium	4.45 ± 0.11	4.81 ± 0.37	6.59 ± 0.79	6.72 ± 0.71	2.21
Sodium	26.4 ± 2.0	32.3 ± 4.4	46.8 ± 10.4	42.0 ± 3.0	0.818
Phosphorus	12.2 ± 0.3	12.3 ± 0.2	12.6 ± 0.7	13.1 ± 0.5	3.72
Sulphur	4.98 ± 0.08	4.85 ± 0.12	5.38 ± 0.16	5.14 ± 0.20	3.64
			(μg/g dry matter)		
Copper	28.9 ± 1.0	27.6 ± 1.4	25.4 ± 2.0	27.6 ± 2.7	10.6
Iron	842 ± 151	495 ± 83	611 ± 69	458 ± 88	203
Manganese	133 ± 9	109 ± 3	124 ± 7	114 ± 11	58.8
Zinc	95.1 ± 4.9	85.4 ± 5.4	74.8 ± 5.0	80.4 ± 6.9	34.8

concentrations in the whole plant DM, and in rumen foam and liquor DM were about 60% of that with FL, suggesting concentration *per se* was not important in relation to bloat.

Particulate Dry Matter

Because small particles have been shown to increase the persistence of rumen foams generated *in vitro* (Jones and Lyttleton, 1973), small particulate material between 9 and 75μm in cross sectional diameter was measured in rumen liquor and foam of bloated and non-bloated cows fed RC. Analyses were carried out in 5 non-bloating cows on 2 consecutive days where rumen liquor and foam was obtained before and after 75 min of eating (about 3 kg DM consumed). Samples were obtained from 2 mildly bloated cows (Grade 1-2) on the day prior to, and from 1 medium bloated cow (Grade 3) on the day following the non-bloat collections.

Results (Table 3) have been grouped into 3 fractions (9-23, 34-45, 45-75μm) for ease of presentation. Most apparent are the large numbers of particles in the smallest (9-23μm) size range, although they only account for 30 to 40% of the mass of particles between 9 and 75 μm in diameter.

When bloat occurred there was a small increase ($P<0.05$) in the proportion of 45 to 75 μm particles in both foam and liquor, and a small reduction ($P<0.05$) in 9 to 23 μm particles in liquor, however the 45 to 75 μm fraction contains only a few particles (Table 3) and small increases are unlikely to affect foam stability.

Comparisons between cows in the distribution of DM between size fractions for foam and liquor were non-significant, but major differences were evident for liquor between days (9-23 μm fraction, $P<0.01$) and in relation to eating. Eating increased the proportion of 9 to 23 μm DM in liquor from 29.1% to 37.9% of the total ($P<0.01$) with an equivalent reduction in the 23 to 45 μm fraction. Foam was unaffected.

Although the amounts and proportions of DM between 9 and 75 μm in size were relatively unaffected by bloat, feeding and cows, it is interesting to quantitate the particles measured in relation to total DM passing a 75 μm sieve. Measured particles account for 20 to 30% of DM, so that 70 to 80% of liquor and foam DM must be either smaller than 9 μm diameter, or in solution. Smaller particles include bacteria, plant structural fragments and chloroplasts. Earlier estimates of chloroplast numbers suggest about 1.8 x 10^6/mg DM in rumen liquor.

CONCLUSION

This paper has summarised some aspects of rumen foam and liquor compostion in relation to bloat in cows. Crude protein was the principal component of foam and liquor DM (approximately 56% of DM), and CP, lipid, NDF, pectin and mineral elements combined to account for at least 70% of DM in foam and liquor. Although only the lipids showed large differences between bloat and non-bloat foams, and require further investigation, other components should not be dismissed on the basis of data reported here. For example N concentrations do not distinguish between NPN or different protein fractions. Salivary proteins and fraction 1 plant (chloroplast) proteins have been strongly implicated with bloat and foam stabilisation (Mangan, 1959; Clarke and Reid, 1974); Jones *et al.*, 1978). Chloroplasts themselves have also been implicated in foam stabilisation (Howarth *et al.*, 1982) and Canadian workers are breeding lucerne with a slow initial rate of leaf rupture and chloroplast release with a view to reducing bloat potency of lucerne (Majak *et al.*, 1985). Data comparing FL with RC suggest absolute concentration of chloroplasts may not be critical because both forages are potent inducers of bloat, yet RC contains only 60% of the chlorophyll in lucerne.

Future work should examine more closely the sources of N in foams, the degree of chloroplast rupture and the type of lipid present in bloat and non-bloat foams and liquor. When clear differences are elucidated, then the cause of bloat in some cows and not others, and on some days and not others, may begin to become apparent.

TABLE 3 Percentage and numberical distribution of dry matter (DM) between 9 - 23 μm, 23 - 45 μm and 45 - 75 μm diameter fractions in rumen liquor, foam liquor and foam residues of bloaty and non-bloaty cows fed fresh red clover.

Component	Particle size (μm)					
	Bloat			Non-bloat		
	9-23	23-45	45-75	9-23	23-45	45-75
Percentage DM distribution:						
Foam	31.3	30.5	38.2	42.2	32.7	25.1
Liquor	30.3	27.8	41.9	39.0	27.5	33.5
Number of particles per mg DM:						
Foam	38440	3910	673	86560	4570	526
Liquor	38580	3060	606	38770	2740	507

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