

# New Zealand Society of Animal Production online archive

This paper is from the New Zealand Society for Animal Production online archive. NZSAP holds a regular annual conference in June or July each year for the presentation of technical and applied topics in animal production. NZSAP plays an important role as a forum fostering research in all areas of animal production including production systems, nutrition, meat science, animal welfare, wool science, animal breeding and genetics.

An invitation is extended to all those involved in the field of animal production to apply for membership of the New Zealand Society of Animal Production at our website [www.nzsap.org.nz](http://www.nzsap.org.nz)

[View All Proceedings](#)

[Next Conference](#)

[Join NZSAP](#)

---

The New Zealand Society of Animal Production in publishing the conference proceedings is engaged in disseminating information, not rendering professional advice or services. The views expressed herein do not necessarily represent the views of the New Zealand Society of Animal Production and the New Zealand Society of Animal Production expressly disclaims any form of liability with respect to anything done or omitted to be done in reliance upon the contents of these proceedings.

---

This work is licensed under a [Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License](#).



You are free to:

**Share**— copy and redistribute the material in any medium or format

Under the following terms:

**Attribution** — You must give [appropriate credit](#), provide a link to the license, and [indicate if changes were made](#). You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use.

**NonCommercial** — You may not use the material for [commercial purposes](#).

**NoDerivatives** — If you [remix, transform, or build upon](#) the material, you may not distribute the modified material.

<http://creativecommons.org.nz/licences/licences-explained/>

# THE RELEASE OF PLANT CELL CONTENTS AND ITS RELATION TO BLOAT

A. M. BRYANT\*

*Plant Chemistry Division, D.S.I.R., Palmerston North*

## SUMMARY

The release of plant soluble protein and galactolipid resulting from the chewing of ingested red clover has been investigated. The proportion of soluble protein released was greater than the proportion of galactolipid released and was highest with young succulent material. The proportion of galactolipid released was found to be consistently low. Using two cows, a between-animal difference in the release of soluble protein was found and its relation to the recorded occurrence of bloat in the two animals is considered.

IT IS NOW generally accepted that the primary cause of bloat is excessive foaming of the rumen contents. The large amount of gas produced by the fermentation becomes trapped as a stable foam, preventing normal eructation.

Opinion is divided concerning the factors responsible for foaming but it seems reasonable that the generation of a foam would require:

- (a) The presence of foaming agents in a suitable form and concentration.
- (b) Conditions suitable for foam production, including the absence of antifoaming agents.
- (c) A vigorous gas production.

Of the various foaming agents that have been suggested, including saponin, pectin and cytoplasmic protein from the plant, saliva and bacterial extracellular polysaccharides from the animal, a strong case can be made for plant cytoplasmic proteins (Mangan, 1959; Cole and Boda, 1960; Reid, 1960). Comparison of bloating and non-bloating legumes have failed to reveal any striking differences in amounts of possible foaming agents. More convincing, perhaps, has been the failure of workers to find any relationship between bloating potential of the clover as determined with animals and the foaming properties of clover extracts as measured by various laboratory procedures (Mangan and Johns, 1957).

The concept that plant lipids can act as antifoaming agents is a comparatively new development following the discovery that fat-rich chloroplasts possess considerable antifoaming activity. Mangan (1959) found that, even when containing adequate amounts of foaming agent, crude

\* Seconded from Ruakura Agricultural Research Centre, Hamilton.

rumen liquor foamed poorly *in vitro*. In order to obtain a stable foam it was necessary for the chloroplasts to be removed by centrifugation. The effectiveness of antibiotics in bloat control, while partly due to a general retardation of fermentation, may also be due to the inhibition of microbial attack on the chloroplast fat. Thus, the antifoaming activity of the rumen contents is maintained and the formation of a stable foam is prevented. As with cytoplasmic protein, no differences in various lipid fractions between bloating and non-bloating clover have been found. Again this is supported by the *in vitro* foaming tests of Mangan and Johns mentioned earlier.

Large amounts of gas arise in the rumen as an end product of fermentation and by acidification of salivary bicarbonate. Suggestions that bloat is due to the formation of excessive gas caused by an abundance of substrate such as soluble sugars and organic acids have not been substantiated. Not only has there been a failure to relate soluble sugar and organic acid levels with bloat, but it has also been shown that in the case of severe bloat there is a lower intake of clover and therefore substrate than when no bloat occurred.

The absence of any relationship between foaming tests and bloating potency of clover, led Mangan and Johns (1957) to suggest that the relative rate of release of foaming and antifoaming substances may be important.

The feeding animal systematically chews its food, with the addition of copious amounts of saliva, before swallowing it as discrete boli. The boli pass down the oesophagus and through the cardia into the rumen. As the boli are mixed with the rumen contents, the bolus liquid, consisting of saliva and plant juices released from the plant cells by chewing, rapidly becomes an integral part of the rumen fluid. Substances making up the bolus liquid would thus be readily available for fermentation and participation in the foaming properties of the rumen fluid. The availability of substances still contained within the macerated plant material would depend on subsequent rumination and microbial breakdown.

A rapid release of soluble protein from the plant cells brought about by the chewing of the ingested herbage should increase the likelihood of foam production. On the other hand, released lipid-rich chloroplastic material should repress this effect. The release of soluble protein in greater abundance than chloroplastic material would, other things being equal, be expected to favour foam formation and

therefore bloat. Reid *et al.* (1962) developed methods to examine this suggestion. Using the red-brown colour due to polyphenolic substances in aqueous extracts of boli as an index of soluble protein, they found that soluble protein was liberated from red clover to about 1.8 times the extent of the liberation of chloroplastic material.

In the work reported here, the effect of chewing has been studied, not only with a view to ascertaining whether there is a difference between animals but also whether the maturity of the herbage is of importance. In addition, some attempt has been made to relate the results to bloat.

#### MATERIALS AND METHODS

The methods used in this work were essentially the same as those used by Reid *et al.* (1962).

Freshly cut red clover was presented to rumen fistulated cows and lots of about 15 boli were collected at the cardia by inserting an arm through the fistula and catching the bolus as it emerged from the oesophagus. The time interval between each swallow was sufficient to allow the arm to be removed and the bolus placed in a suitable container. In order to make this collection possible, sufficient of the rumen contents was removed to bring the liquid level well below the cardia.

Five hundred gram samples of boli were fractionated into filtrate and washed residue by gentle elution with water, the filtrate being made up to volume (2 litres). The amounts of soluble protein and galactolipid present in the filtrate were assumed to be those which had been released from the plant cells by the cows' chewing. Conversely, the amounts present in the washed residue were assumed to be those which had not been released. Soluble protein remaining in the residue was extracted by grinding the residue with buffer at 4°C in an end runner mill. Analysis for soluble protein on aliquots of the centrifuged residue extract and filtrate was made by determining the N content, by Kjeldahl procedure, of the precipitate formed by addition of an equal volume of cold 10% TCA solution. Galactolipid in the filtrate and residue was determined by the method of Bailey (1962).

An estimate of the recovery of these constituents was made by adding together the amount of soluble protein (or galactolipid) released by chewing and the amount not released and expressing the total so obtained as a percentage of the actual intake. In estimating the latter, the

problem was one of determining how much feed was represented in a known weight of bolus rather than determining the actual intake.

The procedure of repeatedly offering weighed amounts of food, then within minutes removing it in order to measure weight lost, was rejected in favour of the method using dry matter as a marker (Glenday and Reid, 1962). The amount of feed in a 500 g sample of bolus was found using the formula:

$$F = \frac{b - 0.01 B}{x - 0.01}$$

where

$F$  = wet weight of feed in bolus  
 $B$  = wet weight of bolus sample  
 $b$  = dry weight of bolus sample  
 $x$  = dry matter ratio of clover fed

Values for  $b$  and  $x$  were determined on 4 to 6 samples each of approximately 200 g. Triplicate samples (25 g) for determination of the total content of soluble protein and galactolipid in the clover were taken by cutting into  $\frac{1}{2}$  in. lengths 15 to 20 small grab samples of the feed and mixing well before sampling. Methods of analysis were as outlined above, soluble protein being extracted by grinding as before. Suitable calculations enabled estimates to be made of the amount of soluble protein and galactolipid in any given weight of bolus.

## RESULTS

The results in Table 1 were obtained from feeding the same cow clover from the same stand at various stages of growth, ranging from leaf blades and petioles only to mature plants in full bloom. The effect of chewing on the release of soluble protein was quite dramatic; the proportion released by chewing decreased with increasing maturity.

TABLE 1: EFFECT OF CLOVER MATURITY ON THE RELEASE OF SOLUBLE PROTEIN AND GALACTOLIPID THROUGH CHEWING

Date	Soluble Protein Per cent. D.M.	Galactolipid Per cent. D.M.	
	Released	D.M.	Per cent. Released
Feb. 27, 1963	6.53	58.7 $\pm$ 2.0	0.51
Mar. 7, 1963	5.69	52.7 $\pm$ 2.0	0.49
Mar. 18, 1963	5.69	44.0 $\pm$ 2.0	0.31
Apr. 5, 1963	5.03	31.0 $\pm$ 2.0	0.60
Recovery (%)	103.1 $\pm$ 1.9	104.4 $\pm$ 3.6	

Except for the results of April 5, the release of galactolipid was consistently low although the recovery figures were somewhat erratic. These results are of interest in that it has been shown that there is an association of increasing severity of bloat with decreasing D.M. percentage of the clover (Mangan and Johns, 1957). If the above results give a true indication of the release of foaming and antifoaming principles of clover, then the production of a stable foam is apparently favoured by feeding young succulent clover.

Table 2 gives results obtained by using a member from each of two pairs of identical twins. The figures result from duplicate determinations made on each animal on four different days. Two of the days were in April, 1963, and two in November of the same year. It was intended

TABLE 2: PROPORTIONS OF SOLUBLE PROTEIN AND GALACTOLIPID RELEASED BY TWO ANIMALS THROUGH CHEWING

	<i>Soluble Protein</i>	<i>Galactolipid</i>
Per cent. of D.M. ....	8.35 ± 1.3	0.62 ± 0.13
Per cent. released:		
Cow 29 ....	29.5 ± 1.2	17.3 ± 2.2
Cow 13 ....	37.5 ± 1.2	19.5 ± 2.2
Per cent. recovered ....	96.9 ± 1.1	94.9 ± 2.0

that the measurements made in the early summer (November) would be with clover releasing a large proportion of its soluble protein in order to accentuate any between animal differences. Although the clover used during this period was quite immature, protein release was not high and was possibly due to the dry growth conditions existing at that time. Even so, as the table shows, cow 13 caused significantly more soluble protein to be released than did cow 29. There was no difference in the amounts of galactolipid released. As the partial emptying of the rumen and subsequent boli collection prevented the experimental animals bloating, their twin mates were fed the same clover and the occurrence of any bloat graded using the system of Johns (1954). Using the intact members of the twin sets as indicators of the incidence of bloat it was found that bloat occurred on only two of the four days. On one of these days, cow 14 (the twin mate of cow 13) bloated to Grade 4 whereas cow 30 showed no bloat. Thus this result could be interpreted that the animal releasing the greater

amount of foaming agent during chewing of the ingested herbage was more prone to bloat. However, the next time bloat occurred, the position was reversed — cow 30 bloated to Grade 3 whereas cow 14 showed only a trace, indicating that such a simple theory was probably untenable.

These results are, of course, too few to condemn or confirm with any certainty, but as the between-animal difference in release of soluble protein was quite constant (Table 2) the bloat records for the two twin pairs were examined to see whether cow 13 was more susceptible than cow 29. The use of past records was also considered necessary for, as shown below, the system of using the intact twin member as a control could not be considered satisfactory with these two twin pairs.

For this analysis bloat grades were doubled, and intermediate gradings, + or −, were counted  $\frac{1}{2}$ . Thus  $2+$  =  $2\frac{1}{2}$  and on being doubled became 5. Only those feeding times at which one or more animals showed bloat and where the likelihood of bloat was not affected by any treatment were included, making a total of 138 records covering two seasons. In total bloat grade for the two seasons there was a greater difference between animals of a pair than between the pairs themselves as shown in Table 3.

TABLE 3: BLOAT SUSCEPTIBILITY OVER TWO YEARS OF MEMBERS OF THE TWO TWIN PAIRS AS SHOWN BY TOTAL BLOAT GRADE

Cow		Total Bloat Grade*
13	....	$276 \pm 27$
14	....	$303 \pm 27$
29	....	$266 \pm 27$
30	....	$356 \pm 27$

\* After Johns (1954).

Besides indicating that animals of a pair were not very similar in their bloating behaviour, the data also show that the two animals used in the chewing experiments were quite similar. Although these two animals differed in "chewing effectiveness", the difference was not sufficient to result in a difference in bloating behaviour. From this negative result it cannot be inferred that chewing is not a factor contributing towards animal difference in bloat susceptibility. The choice of animals was unfortunate in that any attempt to gain evidence for a theory accounting for between-animal differences would be bound to produce

a negative result because of their similarity in bloat susceptibility. It is possible that the difference in release of soluble protein between animals, although statistically different, was not of sufficient magnitude to result in physiological differences.

Table 3 also suggests that the relationship between bloat grades of these related animals was surprisingly low in spite of the tendency to bloat being an inherited characteristic (Johns, 1954). This suggestion was tested by correlation analysis which gave correlation coefficients for the related animals between bloat grades of:

$$13/14 \ r = +0.389 \text{ for } 136 \text{ d.f. } (P < 0.001)$$

$$29/30 \ r = +0.379 \text{ for } 136 \text{ d.f. } (P < 0.001)$$

These coefficients, although highly significant, mean that only approximately 0.38<sup>2</sup> is the proportion of the variation of bloat grade in any animal associated with the bloat grade of its twin. This index is only 14.5%, so that only a small fraction of the performance of any animal could be estimated from its twin's performance. In view of this, using the untreated member of one of these two twin pairs as a control could not be considered satisfactory. It is emphasized that this can be said only of the two twin pairs used in this work and may not apply to other pairs of animals.

#### DISCUSSION

The results reported here are in agreement with those of Reid *et al.* (1962) and show that the chewing of the ingested clover causes a rapid release of soluble protein, the amount, whether expressed in absolute or relative terms, being partly dependent on the condition of the clover. On the other hand, the proportion of chloroplastic material released, measured as galactolipid, remains fairly constant, the absolute amount depending on the amount contained in the herbage. Although no comprehensive measurements of readily fermentable material were made, the few determinations of total soluble sugars that were made indicated that the proportion released by chewing was as great if not greater than soluble protein.

Although no between-animal difference was found in the effect of chewing on the release of galactolipid, some results presented by Bailey (1964) are relevant. When comparing twin pair 13/14 with pair 29/30, he found that galactolipid was more rapidly released from the solid rumen ingesta in cows 13 and 14 than in the other pair.

This suggests that, in comparison with cow 29, the more effective chewing of cow 13, as indicated by soluble protein release, was sufficient to precondition the plant material in such a manner as to facilitate a more rapid release of galactolipid once the bolus had reached the rumen.

In an effort to gain some knowledge of the cause of these differences, microscopic examination of some bolus material was made. Gross damage such as torn and broken leaf blades, petioles and stems was evident. Cells bordering these damaged areas were, as well as could be judged, completely disrupted and devoid of the cytoplasm and cellular inclusions. Most of the leaf blade material was crushed and darkened in appearance, indicating that the underlying cells were disrupted and the intervening air spaces had become filled with cytoplasmic fluid. Although soluble protein and other compounds in true solution would then be able to escape through an occasional rupture of the epidermis and through the stomata, sectioning of the damaged areas revealed that many of the chloroplasts were retained within the cell. Their size presumably prevented their escape. Petioles and stalks were also extensively damaged, but, being metabolically inactive in comparison with leaf tissue, contain lower levels of protein and chloroplastic material. With immature clover, not only is there more cytoplasmic material hence soluble protein, but it is also assumed that the less fibrous nature of the material results in a greater proportion of the cells being disrupted by chewing, allowing the release of a greater percentage of the total cytoplasmic material.

Fergusson and Terry ((1955) pointed out that, as bloat can develop 15 to 30 min after commencement of feeding, the bloat-provoking principle(s) in the plant must rapidly diffuse out of the plant material. As they considered that the amount of material released from the herbage by chewing would be small and of no consequence, they favoured protein or protein degradation products in the rumen before feeding as being more important than the protein in the bloat-provoking herbage. In view of the results reported here and those of Reid *et al.* (1962), such a view is no longer tenable, for it is apparent that chewing results in extensive liberation of soluble plant cytoplasmic material. However, the condition of the rumen contents as well as that of the feed may be important, for Johns *et al.* (1957) have observed that animals prone to bloat have rumen contents of a more frothy consistency before feeding than those which do not bloat.

Although there is evidence that the glycolipids possess surface active properties, it is not to be inferred from this work that galactolipid is necessarily the antifoaming principle present in clover. It has been used only as an index of chloroplastic material for it is known that it is mainly associated with chloroplasts which do have antifoaming properties. A further point of importance is that a method of quantitative determination suitable for routine use has been developed (Bailey, 1962). Such an analysis is more desirable than cumbersome procedures measuring poorly defined fractions once commonly encountered in lipid chemistry.

It is not suggested that the release of cell contents brought about by chewing is a primary cause of bloat. That bloat can be produced on feeding hay or concentrates and that a stand of clover can be bloat provoking one day and not the next, militate against any such suggestion. What is more likely is that bloat is caused by a complex of plant and animal factors which may vary in individual importance from day to day. The effect of chewing is but one which may help to explain the differences between animals in their susceptibility to the ailment.

Finally, these findings are not only of possible interest to bloat but must obviously be of importance when considering the rumen digestion of succulent foods. Because of the effect of chewing, the food constituents are not presented to the rumen microorganisms simultaneously and the easily fermentable compounds in true solution would be the first to be attacked. Hence, on these grounds alone, it is not surprising that the rumen metabolism of fresh and dried grass has been found to be different (Christian and Williams, 1957).

#### ACKNOWLEDGEMENTS

The author wishes to thank P. Evans for microscopic examination of the bolus material, A. C. Glenday for some of the statistical analyses, and Miss S. Murrell for help with the chemical analyses.

#### REFERENCES

- Bailey, R. W., 1962: *Anal. Biochem.*, 3: 178.  
\_\_\_\_\_, 1964: *N.Z. J. agric. Sci.* In press.  
Christian, K. R., Williams, V. J., 1957: *N.Z. J. Sci. Tech.*, 38A: 1003.  
Cole, H. H., Boda, J. M., 1960: *J. Dairy Sci.*, 43: 1585.  
Fergusson, W. S., Terry, R. A., 1955: *J. agric. Sci.*, 46: 257.  
Glenday, A. C., Reid, C. S. W., 1962: *N.Z. J. agric. Res.*, 5: 247.

- Johns, A. T., 1954: *N.Z. J. Sci. Tech.*, 36A: 289.  
Johns, A. T., Mangan, J. L., Reid, C. S. W., 1957: *N.Z. vet. J.*, 5: 115.  
Mangan, J. L., 1959: *N.Z. vet. J.*, 2: 47.  
Mangan, J. L., Johns, A. T., 1957: *N.Z. J. agric. Res.*, 2: 342.  
Reid, C. S. W., 1960: *Proc. 8th Internat. Grassl. Congr.*, p. 668.  
Reid, C. S. W., Lyttelton, J. W., Mangan, J. L., 1962: *N.Z. J. agric. Res.*, 5: 237.

### DISCUSSION

*Q: Could fistulation have any effect on the capacity of a cow to bloat?*

A. M. BRYANT: The effects are not great. Cows prone to bloat before fistulation remain so after their rumens have been fistulated.

DR C. S. W. REID: Where cows have been under observation beforehand, we have seen no great change in bloating behaviour following fistulation. Differences in surgical technique when fistulating a pair of animals may result in differences in leakage, the extent of adhesions, or of denervation of the stomach. These may be factors contributing to the kind of differences described by Mr Bryant. It is of interest that the same two pairs of animals used in Mr Bryant's experiments were also used in rumen volume studies: in this last respect, the members of each set were closely similar.