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QUANTITATIVE STUDIES OF DIGESTION IN THE RETICULO-RUMEN

III. FLUCTUATIONS IN THE NUMBERS OF RUMEN PROTOZOA AND THEIR POSSIBLE ROLE IN BLOAT

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SUMMARY

The evidence for possible connections between rumen micro-organisms and bloat in cattle is reviewed.

The concentration and total numbers of protozoa and of individual species have been measured in cows fed on red clover, using samples and measurements obtained by the bailing technique. Results expressed on a concentration basis can give a misleading picture of the changes in protozoal numbers occurring during a feeding experiment. Holotrich numbers may fall during feeding and the occurrence of this fall appears to depend on the previous feeding. From these and other results it is suggested that fluctuations in protozoa in the rumen may be a factor in determining the bloat susceptibility of cattle.

RUMEN MICRO-ORGANISMS, both bacteria and protozoa, play a vital rôle in rumen function and without doubt contribute to dysfunction. Research work at the Plant Chemistry Division in the field of rumen microbiology has included studies of the rumen population in the normal animal, while keeping in mind that the recognition of abnormalities in the microbial pattern may contribute to an explanation of such ailments as bloat. It has been realized that, although descriptive studies of the organisms and investigations of their biochemical activities *in vitro* help in understanding the digestive process, they can only be applied to the *in vivo* situation if placed on a quantitative basis. With the protozoa in particular, several workers (*e.g.*, Oxford, 1955; Hungate, 1955; Warner, 1962) have gone part way towards this ideal by measuring the numbers of organisms on a concentration basis (number of organisms/ml of rumen liquor). This approach does not allow for changes in the total mass of rumen contents during digestion. The present paper describes measurements of the variation in the total number of protozoa in the rumen and suggests how these, and other results, point to a correlation between the behaviour of certain rumen protozoa and bloat. However, before presenting these results, it is desirable to review evidence from previous work which points to a causal relationship between rumen micro-organisms and bloat.

RUMEN MICRO-ORGANISMS AND BLOAT

Unpublished observations from this laboratory have indicated that, when animals are taken from rough feed and fed on clover, a bloating state usually takes several days to develop. With feedlot bloat (also caused by foaming) the period of adaptation to a bloating condition can take 1 to 4 weeks on a diet of concentrates with a relatively small amount of legume hay (Lindahl and Davis, 1954). One explanation for this change to a bloating state is that time is required for the development of the necessary micro-organism population. Support for this suggestion is provided by an unpublished experiment in which the rumen contents of a non-bloating cow were removed and replaced with those from a bloating animal. The original non-bloater bloated for several days before returning gradually to the non-bloating state.

The fact that antibiotics such as penicillin have been shown to control bloat for periods of up to three days, followed by a development of resistance to the treatment with repeated dosings, indicates a rôle for micro-organisms in the aetiology of bloat. However, Bryant *et al.* (1961), from their studies on the flora and fauna in the rumen of steers fed a feedlot bloat-provoking ration, with and without penicillin, concluded that bloat can occur in animals with widely differing numbers and kinds of bacteria and protozoa. They considered that feedlot bloat is not correlated with the occurrence or numbers of any of the individual groups of bacteria so far cultured.

If it is postulated that differences in the microbial population account for the different tendencies of various individual animals to bloat, the question arises as to what sort of changes in rumen populations might be expected to produce bloat. One hypothesis has been that the population becomes more vigorous and forms more gas than can be eliminated by the host. (A greater gas production is now considered only to increase the severity of the condition rather than be the cause of the ailment.) A second is that different products are formed in the fermentation, while a third suggests that the substances attacked by the micro-organisms differ in bloating and non-bloating animals.

Examples of these three types of action of rumen micro-organisms are plentiful in the literature. For instance, Quin (1943) attached great importance to an organism which was thought to be a yeast, probably *Selenomonas* (McGaughey and Sellars, 1948). He ascribed bloat to rapid gas production and considered this organism to be largely responsible for a very rapid fermentation rate. Polysaccharide slimes or gums produced by rumen bacteria have been suggested as possible foaming or foam-stabilizing agents (Hungate *et al.*, 1955). However, the studies of Bailey (1959) could not demonstrate their presence in the rumens of bloating cows and, when taken in conjunction with the finding of Mangan (1959) that the foaming properties of crude rumen liquor increase with centrifugation, cast considerable doubt on any possible connection between bloat and rumen polysaccharides. Another hypothesis to explain the difference between the ingesta of bloated and unbloated animals was that certain types of micro-organisms in the non-bloating animals rapidly attack or modify the foaming agents (saponins) from the feed, thereby destroying their foam-stabilizing properties (Hungate, 1955). This has not been verified.

A somewhat similar hypothesis is that certain protozoa can remove antifoaming agents from the rumen ingesta and thus increase the degree of foaming. Mangan (1959) demonstrated that clover chloroplasts, which contain a high proportion of lipids, have antifoaming properties. Oxford (1958) observed during studies on the culture and metabolism of the ciliate protozoa, *Epidinium*, that it ingested plant starch and whole or damaged chloroplasts. The ingestion of whole chloroplasts by epidinia would lower the lipid concentration in the rumen and hence increase the foaming potential. The finding of Oxford (1958) that *Epidinium* was the predominant oligotrich protozoa in cows fed on fresh clover and that it became less dominant when grass or hay was fed, suggested that the presence of this ciliate in sufficient numbers might influence the incidence of bloat. Subsequent work by Clarke (1964) has not, however, demonstrated any significant difference in the mean counts of epidinia between days on which cows did or did not bloat. A connection between rumen protozoa and bloat in sheep was suggested by Koffman (1937) who claimed to have demonstrated decreased numbers of the larger species of protozoa in bloating animals.

Since a greater part of the rumen contents during bloating exists in the form of foam, and since the distribution of the large protozoa between the foam and the liquid under it is not uniform, any reported difference in microfauna between bloating and non-bloating animals may be more apparent than real. This highlights the fact that one of the major difficulties in attempting to correlate the numbers of particular bacteria or protozoa in the bloated animal, or indeed any non-bloating condition, is that of obtaining representative samples from a mass which is not homogeneous. As already mentioned, the second problem is the need to obtain an expression of the total numbers of the bacteria and protozoa in the rumen at various times. This can only be obtained by the complete removal of the rumen contents from the animal to give a determination of the total amount of rumen contents; at the same time, this does enable a truly representative sample to be collected.

RESULTS

The technique described by Reid (1965) was used to obtain samples to measure the variation in total numbers, and numbers of individual species, of protozoa in the rumens of identical twin cows on different diets. The counting technique was that of Clarke (1965a). A comparison between total counts and concentration of protozoa is shown in Fig. 1. It can be seen that the concentration figures

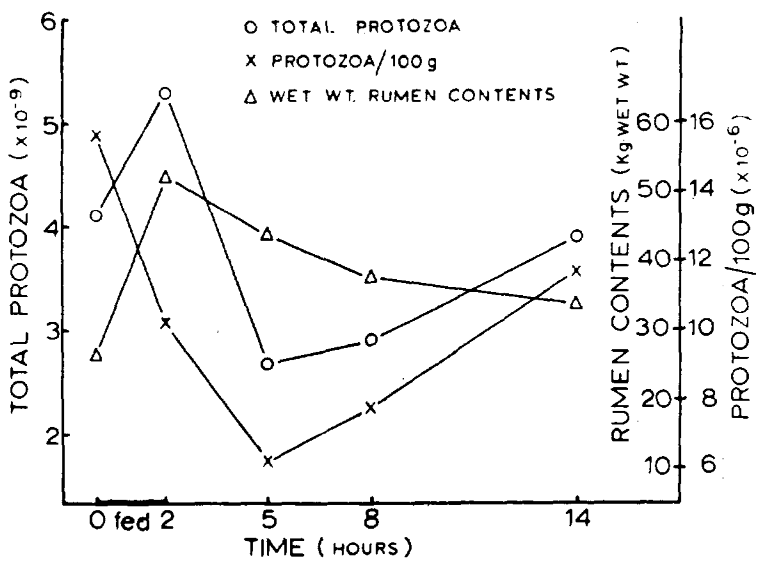

Fig. 1: Weight of rumen contents; total number of protozoa and number/100 g (net wt.) in the rumen of a cow on a diet of fresh red clover, fed once daily.

give an entirely misleading picture of the variation that occurs in protozoal numbers by neglecting the change in volume of rumen contents that occurs with feeding. These results are not from the same feeding experiments as those described by Reid (1965) and Bailey (1965).

The marked diurnal variation in the total number of ciliate protozoa in the rumen was found to result mainly from changes in the holotrich population (Clarke, 1965a). The change in number, under different feeding regimes, of one of the types of holotrich protozoa (*Isotricha*) is illustrated in Fig. 2.

Compared with a rise in numbers in the two-hour period following the start of feeding on hay, there is no change when clover is fed following a previous regime of one feed per day, while a very definite fall occurs in total numbers on feeding clover following two feeds of clover on previous days. Other results demonstrated a marked fall in numbers

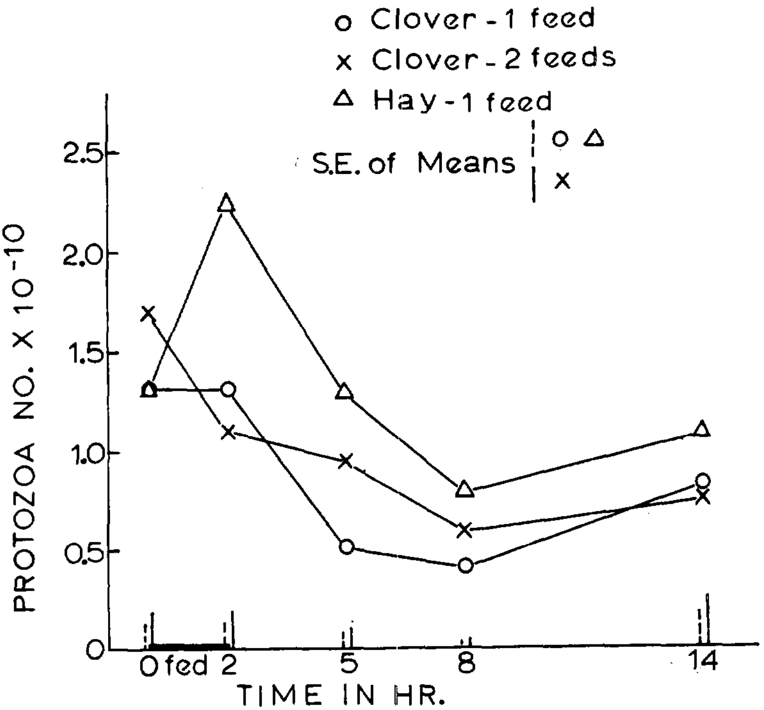

Fig. 2: Mean numbers of isotrich protozoa in the rumens of 2 cows on diets of fresh, red clover, and clover hay.

of another holotrich species (*Dasytricha*) occurring in the 2 to 5 hour period after feeding. This decline in the numbers of holotrichs after feeding is considered to be too great to be solely the result of passage of ingesta out of the rumen and is probably attributable to bursting of the organisms.

Rumen holotrich protozoa have a metabolic abnormality whereby they are unable to control the storage of reserve polysaccharide inside their bodies and in the presence of excess substrate burst as a result of continued synthesis of polysaccharide. This has not only been demonstrated *in vitro* (Sugden and Oxford, 1952; Oxford, 1955) but has also been observed *in vivo* by the writer on numerous occasions in samples from cows feeding on fresh clover. In the case of the *Isotricha* the bursting of the organism is dependent on the availability of substrates which are soluble sugars and starch grains of small size, and sugars in the case of the *Dasytricha*.

It has been demonstrated (Clarke, unpub. data) that the cell contents of burst isotrichs have considerable foam-stabilizing properties and could, therefore, influence the foaming properties of the rumen ingesta. The fact that both the number of isotrich protozoa present and the state of their metabolism will influence the amount of foaming material released into the rumen makes them strong candidates for explaining the adaptive phenomena observed in the development and loss of the bloating condition as outlined earlier in this paper.

Thus, it can be seen from Fig. 2 that, in an animal conditioned to two feeds daily, where the organisms could be expected to contain more storage material after overnight fast, the drop in numbers of protozoa is very much greater than in the animals fed once per day where the loss approximately balanced the gain. The organisms in an animal on a single feed of clover hay, where the soluble sugar and starch level could be expected to be lower than that in fresh clover, showed a net gain in total numbers, presumably owing to a minimum loss of organisms by bursting.

This reasoning receives support from the finding of Clarke (1965b) that there is a positive correlation between the dry weight of protozoa in the rumen before feeding and the subsequent severity of bloat (Fig. 3). This has been shown to be due to the increased dry weight of protozoal cells rather than to an increase in protozoal numbers. When there is a higher initial content of storage material, more isotrichs could be expected to burst at an early stage

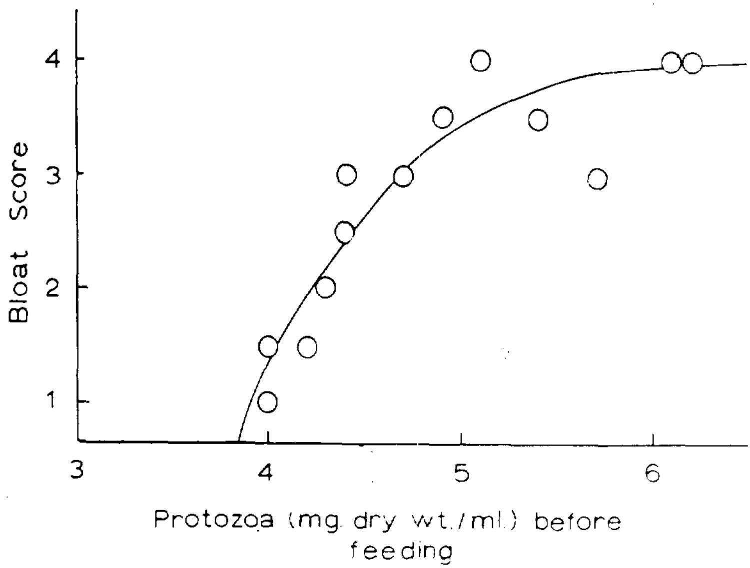

Fig. 3: Relation between severity of bloat and weight of protozoa in rumen liquor before feeding.

of feeding, leading to an increased foaming potential. The results shown in Fig. 3 are from experiments conducted before the bailing out technique was used. They are, however, comparable with the other quantitative results as they are from animals fasted for 18 to 24 hours when the contents are reasonably homogeneous and differences in total amounts are minimal.

DISCUSSION

The emphasis in this paper has been on the possible rôle of protozoa in the development of the bloating condition. This does not mean that they are regarded as the sole cause of the ailment. The foam which causes bloat has been shown to be of the protein type, with the degree of foaming probably dependent on the balance between foaming and antifoaming agents. It is suggested that the cell contents released on bursting may alter the balance of foaming and antifoaming agents normally present in the rumen towards the formation of a very stable foaming state. Evidence has been presented that antifoaming agents in the form of lipids of the plant chloroplasts (Mangan *et al.*, 1959) and foaming agents, plant cytoplasmic (Mangan, 1959) and chloroplastic (McArthur *et al.*, 1964) proteins, do occur in the rumen. However, there is no evidence to suggest that fluctuations in the levels of plant proteins or lipids occur which could explain the variation in bloat incidence on different days or on different pastures. It is also difficult to see how changes in the levels of plant constituents could explain the difference in bloat susceptibility between animals on the same pasture.

Variations in the numbers and state of the rumen protozoa could explain the known variations in bloat incidence, particularly when it is realized that animals on the same feed do not necessarily have the same rumen flora and fauna (Moir, pers. comm.). This is evidently influenced by both the feed and by the host.

However, the hypothesis involving the holotrich protozoa has yet to be confirmed by following the changes in numbers in bloating animals. The major practical problem which remains to be overcome is that of bailing out bloated animals and obtaining a representative sample of the rumen contents.

REFERENCES

BAILEY, R. W. (1959): *N.Z. J. agric. Res., 2*: 355.

——— (1965): *Proc. N.Z. Soc. Anim. Prod., 25*: 85.

BRYANT, M. P.; ROBINSON, I. M.; LINDAHL, I. L. (1961): *Appl. Microbiol., 9*: 511.

CLARKE, R. T. J. (1964): Ph.D. thesis: *Studies on the Flora and Fauna of the Bovine Rumen*. Massey University, Palmerston North.

——— (1965a): *N.Z. J. agric. Res.* (In press).

——— (1965b): *Nature* [Lond.] (In press).

HUNGATE, R. E. (1955): In *Biochemistry and Physiology of Protozoa*. (Ed. S. H. Hutner and A. Lwoff), *2*: 159–99. Academic Press, New York.

HUNGATE, R. E.; FLETCHER, R. W.; DOUGHERTY, R. W.; BARRENTINE, B. F. (1955): *Appl. Microbiol., 3*: 161.

KOFFMAN, M. (1937): *Skand. Vet. Tidsskr., 27*: 45.

LINDAHL, I. L.; DAVIS, R. E. (1954): *J. anim Sci., 13*: 1024.

MANGAN, J. L. (1959): *N.Z. J. agric. Res., 2*: 47.

MANGAN, J. L.; JOHNS, A. T.; BAILEY, R. W. (1959): *N.Z. J. agric. Res., 2*: 342.

MCARTHUR, J. M.; MILTMORE, J. E.; PRATT, M. J. (1964): *Can. J. anim. Sci., 44*: 200.

MCCAUGHEY, C. A.; SELLARS, K. C. (1948): *Nature* [Lond.], *161*: 1014.

OXFORD, A. E. (1955): *Exp. Parasitol., 4*: 569.

——— (1958): *N.Z. J. agric. Res., 1*: 809.

——— (1959): *N.Z. J. agric. Res., 2*: 365.

QUIN, J. I. (1943): *Onderstepoort J. vet. Sci. anim. Ind., 18*: 91.

REID, C. S. W. (1965): *Proc. N.Z. Soc. Anim. Prod., 25*: 65.

SUGDEN, B.; OXFORD, A. E. (1952): *J. gen. Microbiol., 7*: 145.

WARNER, A. C. I. (1962): *J. gen. Microbiol., 28*: 129.

DISCUSSION

DR J. B. HUTTON: *Were estimates made of the variation in percentage D.M. between grab samples taken from within the animal as well as from the total digesta following removal, and if so, what was the extent of this variation?*

DR C. S. W. REID: Direct comparisons were not made in the present work. Previous observations have shown that the problem of sampling total solids from the animal arises from the tendency for solid plant residues to accumulate in the upper layers of the rumen contents. The dry matter in such regions can reach 15 to 20%. In contrast, the more liquid contents of the ventral regions commonly have a dry matter of 3 to 5%. The amount of this latter liquid fraction varies and one does not know what proportions of contents from the upper and lower regions must be taken, at any time, to produce a representative, pooled sample.

DR D. E. WRIGHT: *Has tritiated water been used as a marker for measuring water volume in the rumen?*

DR REID: Tritiated water has been used to estimate the fluxes of water across the rumen wall (von Engelhardt, 1963): *Pflügers Archiv., 278*: 141; 152). Radiation risks would have to be considered in using radioactive markers in bailing experiments.

DR WRIGHT: *The dangers in using concentration measurements because of changes in volume in rumen fill have been emphasized. Since the experimental design is such as to accentuate this danger, might not pasture-fed animals give more reliable results?*

DR REID: It depends on the aim of the investigation. Single feed experiments enable one to follow changes occurring after the ingestion of a known amount of feed. Principles can be established which may be of value when seeking to interpret the changes occurring during grazing. It should be pointed out that grazing is discontinuous and during the intervals between grazings changes similar to those occurring after a single feed are likely to take place.

A. W. F. DAVEY: *To what extent are the speakers committed to the complete removal of rumen contents rather than the taking of representative samples which would lend itself to more routine methods?*

DR REID: Bailing, weighing, mixing and return is admittedly a laborious procedure. However, if one requires an accurate measure of the contents of the reticulo-rumen, a truly representative sample of the solids, or desires to follow quantitatively the process of digestion and degradation in the reticulo-rumen, then this is the best available method at the moment.

DR A. H. CARTER: *In view of the possible danger of the bailing technique affecting the digestion process, has the effect of the technique on gross digestion or rate of passage of feed constituents been studied?*

DR REID: No. The fact that dry matter does not accumulate during the experiment suggests that the system has not been grossly distorted by the sampling procedures.

L. R. KINGSBURY: *What are the relative changes in volume of rumen contents immediately after feeding in comparison with the volume 22 hours after feeding?*

DR R. W. BAILEY: In these experiments the changes have been of the order of 1.5 to 2.0 times.

K. MOLLER: *Has bailing out been done in animals which had both rumen fistulae and partial exteriorizations (using the technique of Reid, C. S. W. (Proc. N.Z. Soc. Animal Prod., 23: 169)). If so, what effect did the bailing out have on the ruminal movements?*

DR REID: We have not done this yet but have prepared for it.

PROF. I. L. CAMPBELL: *Would the speakers care to speculate on the nature of the differences in fermentation which might occur between the dry cows in the present experiments, fed once a day, and a grazing cow eating intermittently throughout the 24 hours? For example, would the intermittent addition of soluble carbohydrates slow the digestion of the less soluble constituents?*

DR BAILEY: We are particularly interested in the rates of loss of components such as cellulose under *ad libitum* or grazing conditions. So far as cellulose loss is concerned, one suspects that factors other than soluble carbohydrate fermentation must be relevant under these conditions.

J. D. J. SCOTT: *It was claimed that when cattle are changed from non-bloating to bloating feed there is a time lapse before bloat develops. Experience at Ruakura this year was that bloat occurred on the first morning cattle were introduced to a red clover sward in January after being grazed on non-bloating pastures.*

ANSWER*: The time lapse will depend on the type of non-bloating feed. We mentioned a time lapse of several days when changing from rough feed to bloating feed. If the animals are pastured on feed which already contains a fair proportion of clover and is reasonably succulent, then one would expect that a change to bloating pasture would produce bloat soon after introduction into the new pasture.

E. D. ANDREWS: *I am not sure whether I have grasped the purport of Dr Clarke's paper. Are differences in protozoal populations and hence susceptibility to bloat related to different feeding histories between animals before being placed on a bloating dietary regime?*

ANSWER: There appear to be two major factors influencing the number and state (nearness to bursting) of the protozoa in the animal's rumen:

(a) Some unknown animal factor or factors and

(b) The chemical composition of the ingested feed.

There can be differences in the microbial population between animals on the same feed which can perhaps lead to a difference in susceptibility of animals to bloat. However, there are occasions when the plant composition is such as to be the major, over-riding influence in determining the microbial flora and in making all animals susceptible.

DR D. G. EDGAR: *Do identical twins tend to have populations of protozoa more alike than unrelated animals?*

ANSWER: In general, yes: thus the results in Figs. 1 and 2 are means from identical twins which gave substantially similar results.

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* In Dr Clarke's absence, questions on his paper were answered by Dr R. W. Bailey and Dr C. S. W. Reid.