
THE PROCEEDINGS OF THE
NEW ZEALAND SOCIETY
OF
ANIMAL PRODUCTION
VOLUME 62 2002

SOME RECENT RESULTS OF RESEARCH IN RUMEN MICROBIOLOGY

D. E. WRIGHT*

Summary

Studies on the foaming properties of rumen liquor taken from cows fed daily on red clover have suggested that both foaming and anti-foaming substances are present. The ability of penicillin to reduce bloat has indicated that microbiological factors are involved possibly by inhibiting metabolism of chloroplast fat.

Both protozoa and rumen bacteria have been shown to hydrogenate the unsaturated fatty acids present in chloroplasts. Preliminary experiments on microbial lipase activity has demonstrated the presence of enzymes capable of hydrolysing various lipid substrates.

RESEARCH in ruminant physiology has received much attention since the early 1940's, as it is recognized that ruminants are not only of sufficient biological interest themselves to justify a comprehensive study, but improvements in animal husbandry can best be accomplished by understanding more completely the ruminant's metabolic processes.

The rumen microbial population is composed of bacteria and also much larger organisms, the ciliated protozoa, which possess some degree of cellular organization. While the protozoa are not considered essential to maintain a healthy rumen, they undoubtedly play an important role in the degradation of plant material since the protozoon protein is similar in quantity to the bacterial protein.

Several disorders of ruminants are the consequence of the uncontrollable nature of the rumen fermentation, bloat being one such disorder. It is now generally accepted that bloat in cattle grazing on legumes arises from the trapping of the fermentation gases in a foaming rumen ingesta with the result that the animal cannot release this gas by the normal eructation mechanism. Considerable effort has been directed towards the elucidation of the processes whereby the rumen contents of bloating cows form this stable foam, but, in spite of the mass of information which has accumulated, the factor or combination of factors responsible for bloat are still open to doubt.

*Plant Chemistry Division, Department of Scientific and Industrial Research, Palmerston North.

It is considered that animal, microbiological and plant factors are all involved. Amongst the latter, plant cytoplasmic protein, saponins, pectic substances, muscle poisons, and variations in the level of plant fat have been suggested as possible factors. Of these, plant cytoplasmic protein seems to possess properties similar in some respects to that of rumen liquor. Mangan (1959) has examined the foaming properties of rumen liquor and plant protein and found that these samples had similar relationships between pH and foam stability. However, since there is no significant day-to-day variation in the level of plant protein which can be correlated with the bloaty or non-bloaty nature of red clover, obviously the presence of plant protein in rumen liquor is not the sole reason for the formation of a stable foam.

The strength of foam will depend not only on the nature and concentration of foaming substances, of which protein is probably an important one, but also on the presence of anti-foaming agents such as various fats, the use of which in preventing bloat is widespread. It has been observed at Plant Chemistry Division that centrifuging rumen liquor at high speed is accompanied by an increase in the foam strength of the clarified fluid, suggesting that the particulate matter in the rumen liquor, consisting of bacteria, protozoa and plant fragments, possessed considerable anti-foaming activity. Since chloroplasts contain a high concentration of lipid (30 to 35 per cent. of the dry weight), it is possible that the fat contained in these bodies has surface-active properties of significant proportion in the rumen. This view is supported by the fact that defatted chloroplasts have no anti-foaming properties but instead have a foam stabilizing effect.

Mangan, Johns and Bailey (1959) have examined the effect of penicillin on the foaming properties of rumen contents and found great differences in the properties of rumen liquor taken from a cow dosed with penicillin when compared with that from a control undosed animal.

From Table 1 it can be seen that the foam stability and foam expansion values of the liquor from the penicillin-dosed animal were much lower than in the control. This is surprising, since the soluble protein was more than four times as great in the fluid from the treated cow. This result can best be explained by assuming that the anti-foaming properties of the rumen liquor from the dosed cow were sufficient to overcome the high protein content of the fluid.

This explanation is supported by the remarkable increase in foam stability and expansion when this liquor was centrifuged,

TABLE 1: EFFECT OF 500,000 UNITS OF PENICILLIN GIVEN 16 HOURS PREVIOUSLY ON FOAMING PROPERTIES OF RUMEN LIQUOR 2 HOURS AFTER COMMENCEMENT OF FEEDING.

Cow	*pH*	*Bloat Score*	*Foaming Properties*				*Soluble protein*
			Crude		*Centrifuged*		
			F.E.(cm)*	*F.S.†*	*F.E.*(cm)*	*F.S.†*	*mg.N/100ml.*
Control	6.19	II	45	0.64	45	0.31	10.0
Treated	6.30	0	4	Nil	45	36.6	45.2

* Foam expansion † Foam strength

thus removing the particulate matter. The clarified solution produced a strong foam consistent with its high protein content. Obviously, the anti-foaming agents were present amongst the centrifuged precipitate.

The fact that penicillin exerted such a pronounced effect suggested that a microbiological factor is involved whereby the anti-foaming substances present are subjected to less microbial attack than normal. As a result of these findings, an investigation is being made of various aspects of lipid breakdown in the rumen, a field of rumen metabolism which has been virtually unexplored.

Until recently, it had been thought that plant lipids were mostly comprised of triglycerides as in animal fats. However, last year, Benson, Wintermans and Wiser (1959) in the U.S.A. and Weenink (1959) at the Fats Research Laboratory, Wellington, showed that red clover chloroplast fat was quite different in composition. The American workers found that these lipids consist mainly of galactosyl-glycerol esters of long chain fatty acids, linolenic acid with three unsaturated bonds being the principal acid. Three different types have been isolated and identified. These are galactopyranosyl-glyceryl-fatty acid, (galactopyranosyl 6-sulphate)-glyceryl-fatty acid, and digalactopyranosyl-glyceryl-fatty acid. These structures are most unusual and the presence of large hydrophilic groups on the long chain fatty acid suggests that their surface activity behaviour could be most interesting and may possess anti-foaming properties.

Apart from these features connected with bloat research, two other fields in which a knowledge of plant lipids and their metabolism is of importance are white muscle disease and atherosclerosis. Unsaturated fatty acids have been suggested as involved in both these conditions, in white muscle disease by having an anti-vitamin E effect, and in preventing atherosclerosis by lowering the cholesterol content of blood.

The metabolism of lipids can be separated into three divisions, hydrogenation, hydrolysis and metabolism of the hydrolysis products.

Hydrogenation

Reiser (1951) first drew attention to the ability of rumen contents to hydrogenate unsaturated oils. This work was confirmed by several others (Shorland, Weenink, Johns and McDonald, 1957; Hoflund, Holmberg and Sellmann, 1955; Garton, Hobson and Lough, 1958) using sheep, cows or goats and extended to include a wide range of unsaturated lipids. While the fact is well known that ruminants do modify their ingested fats more than non-ruminants, no attempts have been made until recently to determine the types of micro-organisms responsible for this effect. Usually, it has been assumed that the rumen bacteria carry out the hydrogenation, the ciliated protozoa apparently being ignored.

At Plant Chemistry Division, Oxford (1958) has found that, in cows fed red clover, the ciliated protozoon, *Epidinium ecaudatum,* was the predominant oligotrich in the rumen. Since these organisms have been observed to ingest whole chloroplasts, it seemed possible that they may play a part in bloat owing to their removal of anti-foaming agents.

The role of rumen protozoa in hydrogenation was examined by preparing thick suspensions which were virtually bacteria-free, and incubating them overnight in the presence of antibiotics with a preparation of chloroplasts isolated from red clover. A control flask in which the protozoa had been killed by heating was incubated concurrently. The samples were freeze dried and the lipids extracted by boiling with organic solvents. The fatty acids were isolated and their methyl esters analyzed by gas-liquid chromatography at the Fats Research Laboratory. The results in Table 2 show considerable hydrogenation of the fatty acids from chloroplast lipid.

TABLE 2: RESULTS OF INCUBATION OF PLANT CHLOROPLASTS WITH LIVE AND DEAD CILIATES, EXPRESSED AS PERCENTAGE OF TOTAL C_{18} ACIDS.

Sample	*Saturated C_{18} acids*	*Unsaturated C_{18} acids* Monoene	Diene	Triene
Live	17.5	10.6	27.6	44.3
Dead	10.7	16.2	2.8	70.3

The fatty acids in the heated sample are very similar to those found in chloroplasts, linolenic acid being predominant. Overnight incubation has resulted in a decrease of triene and monoene acids with increases in the diene and saturated acids. There has been little conversion of diene to monoene which suggests that the mechanisms involving hydrogenation show a considerable degree of specificity towards the position of the double bond in the unsaturated fatty acid substrate. A similar situation exists with the conjugated double bonds of carotene which Shorland *et al.* (1957) reported to be resistant to hydrogenation in the rumen.

Similar experiments were done using either washed suspensions of rumen bacteria suspended in phosphate buffer containing sodium sulphide or rumen bacteria suspended in rumen liquor. In these experiments the protozoa and plant debris were removed by centrifuging.

The results (Table 3) show marked hydrogenation by the bacteria in rumen liquor but only a slight effect by the washed suspension. The low degree of hydrogenation by the latter sample is presumably the result of either harmful effects caused in the handling and preparing the suspensions, or the presence of some unknown factors in rumen liquor which stimulates hydrogenation. The pattern of hydrogenation is similar to that found for protozoa with a build-up of diene acid, although some diene has been further converted to monoene and saturated acids.

These experiments have shown that both bacteria and protozoa are able to hydrogenate unsaturated fatty acids. The build-up of diene acids in both cases can be regarded as atypical

TABLE 3: HYDROGENATION OF CHLOROPLAST FAT BY RUMEN BACTERIA. RESULTS GIVEN AS PERCENTAGE OF TOTAL C_{18} ACIDS.

Sample + chloroplasts + glucose	*Saturated C_{18} acid*	*Unsaturated C_{18} acid*		
		Monoene	*Diene*	*Triene*
Boiled bacteria + phosphate buffer	4.0	4.0	10.0	82.0
Live bacteria + phosphate buffer	4.1	11.0	7.6	77.3
Difference	+0.1	+7.0	—2.4	—4.7
Boiled bacteria in rumen liquor	5.0	6.5	5.0	83.5
Live bacteria in rumen liquor	9.4	24.1	62.2	4.3
Difference	+4.4	+17.6	+57.2	—79.2

since Shorland *et al.* (1957) found that linolenic acid is converted mainly to monoene acids. The lack of hydrogenation of the dienes may be due to either the comparatively short time of these experiments, 18 hours as compared with 2 days in the experiments of Shorland *et al.*, or a fall in pH of the medium, or the appearance of toxic substances, all of which are possible factors in reducing the activity of the micro-organisms.

It is possible that the extent of hydrogenation may vary according to the species of protozoa and bacteria present in the rumen. The results of Mayhead and Barnicoat (1956), which show a seasonal variation in the iodine values of milk fat, could well be explained on this basis since at least one species of protozoa, *Epidinium ecaudatum* shows a seasonal variation in population. According to Oxford (1958), during November, December and January when iodine values of milk fat are low, the rumen ciliates consist largely of epidinia, whereas during February and March this species is present in greatly decreased numbers.

Hydrolysis

The enzymes which split fats are known as lipases and their action, which is relatively unspecific, releases from triglycerides a mixture of diglycerides, monoglycerides, fatty acids and glycerol. Garton, Hobson and Lough (1958) at the Rowett Research Institute have shown that rumen contents taken from sheep possess high lipase activity, 24 hours incubation with linseed oil resulting in 75 per cent. of the total lipid recovered at the end of the experiment being free higher fatty acids.

Similar experiments at Plant Chemistry Division have shown less evidence for lipase activity towards triglycerides. Only once in several attempts has it been possible to demonstrate breakdown of linseed oil, 20 per cent. of the recovered lipid being free fatty acid. Using as substrates the water soluble esters of lauric and oleic acid, Tween 20 and Tween 80 respectively, between 30 and 40 per cent. of these were hydrolysed in 24 hours. Tributyrin, which cannot be considered a typical triglyceride substrate, is readily split by the rumen micro-organisms, both bacteria and protozoa containing tributyrinase enzymes.

The resistance of triglycerides to hydrolysis by rumen contents from clover-fed cows is unexpected but may be due to their different diet when compared with the animals used by Garton *et al.* which received a diet containing concentrates at least one of which included linseed oil. If the lipases in the rumen are adaptive enzymes, the low activity might be explained. This possibility is being studied by including linseed oil in the diet of a cow and examining its rumen contents for lipase action.

Apart from microbial lipase activity, it is very likely that the ingested plant material will itself contain lipase enzymes. No lipases can be demonstrated in saliva collected either from sheep or cattle.

Metabolism of the Hydrolysis Products

The fermentation of glycerol has been studied by Johns (1953) who showed it was slowly metabolized to propionic acid, the rate of disappearance being far less than that found for glucose. It is possible that some glycerol will pass unchanged through the rumen and be absorbed lower down the alimentary tract.

Galactose is utilized by rumen bacteria and protozoa, forming carbon dioxide, hydrogen, acetic acid, butyric acid, formic acid and lactic acid.

The dietary fatty acids modified by hydrogenation are absorbed lower down the alimentary tract, synthesized mostly into triglycerides and laid down as depot fat or used for synthesizing milk fat.

While a promising start has been made to a study of the role of rumen micro-organisms in the degradation and modification of fat, several aspects are still not clear. Perhaps the most interesting of these is the mechanism of hydrolysis of the galactosylglycerol fatty acid esters, the unusual structure of which may mean the presence of enzymes whose properties should be worth investigating.

While the role of plant lipids in bloat is largely speculative, it is obvious that they are of considerable interest to the biochemist from the point of view of their synthesis and role in plant metabolism, their physico-chemical properties and the mechanisms whereby they are degraded in the rumen. The information gathered from these studies should throw some light on what is currently a neglected field of research.

Literature Cited

Benson, A. A., Wintermans, J. F. G. M., Wiser, R. (1959): *Plant Physiol.*, *34* : 315.

Garton, G. A., Hobson, P.N., Lough, A. K. (1958): *Nature*, *182* : 1511.

Hoflund, S., Holmberg, J., Sellmann, G. (1955): *Cornell Vet.*, *45* : 254.

JOHNS, A. T. (1953): *N.Z. J. Sci. Tech.*, *35A* : 262.
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.
MAYHEAD, J. W., BARNICOAT, C. R. (1956): *J. Dairy Res.*, *23* : 238.
OXFORD, A. E. (1958): *N.Z. J. Agric. Res.*, *1* : 809.
REISER, R. (1951): *Fed. Proc.*, *10* : 236.
SHORLAND, F. B., WEENINK, R. O., JOHNS, A. T., MCDONALD, I. R. C. (1957): *Biochem. J.*, *67* : 328.
WEENINK, R. O. (1959): *N.Z. J. Sci.*, *2* : 273.

DISCUSSION

Q.: *Have you any information on the relative importance of protozoa as against bacteria with respect to hydrogenation?*

A.: From the tables it might be thought that the bacteria were responsible for most of the hydrogenation. However, since the protozoa were incubated in an artificial medium and the bacteria in rumen liquor the two sets of results cannot be fairly compared. What their relative importance *in vivo* is, I do not know.

Q.: *Since the rumen can be considered a reasonably stable environment, why should there be a seasonal fluctuation in rumen flora?*

A.: This can be answered with some degree of certainty for the protozoon, *Epidinium ecaudatum*, which seems to require clover starch for its food. Since there is considerable variation in the starch content of red clover, it is probable that the population of this ciliate will vary accordingly.

Q.: *Has any work been done on the seasonal variation of plant lipids?*

A.: Russian workers have examined the chloroplast lipids from red clover taken at different times of the year and found a fairly constant total lipid content. However, they noted fluctuations in the amount of free lipid and what they termed combined lipid. As far as I know, nothing has been done in New Zealand on these lines.