Degradation in the rumen of starch and nitrogen contents in chemically treated barley

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SUMMARY

Different chemical treatments of barley i.e. formaldehyde alone and in combination with wood sugar, acetic acid and isobutyric acid were ivestigated for their effects on rumen degradation of dry matter (D.M.), nitrogen and starch in barley. Four Jersey cows, equipped with rumen fistula were used for rumen incubation of chemically treated barley samples and control (untreated barley) contained in dacron bags. Following incubation, samples of all treatments and control were analysed for dry matter, nitrogen and starch. Some of the formaldehyde containing treatments were apparently more effective than others.

In conclusion, formaldehyde combined with wood sugar, acetic acid and isobutyric acid gives more protection of barley starch and nitrogen than formaldehyde alone, or formaldehyde combined with wood sugar. combination of formaldehyde with acetic acid or with two acids (acetic and isobutyric acids) appear to enhance the protection of nitrogen and starch in barley, and that all of these treatments were more effective than the one containing no formaldehyde.

INTRODUCTION

It is generally accepted that carbohydrate entering the reticulo-rumen is digessted by both bacteria and protozoa, the process being referred to as microbial degradation. The end products of such microbial degradation are VFAs (acetate, propionate and butyrate), carbon dioxide and methane. Part of carbohydrate escaping rumen degradation (if any) can be digested in the small

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intestine.

Proteins are degraded in the rumen to peptides, amino acids and ammonia by rumen micro-organisms. Some diets contain proteins which are considerably resistant to degradation in the rumen, for example fishmeal (Mehrez et. al., 1980), however, most of the plant proteins are highly degraded in the rumen. Protein can be protected from rumen degradation by various methods, the most important being formaldehyde treatment. Protein is protected to avoid the synthesis of lower quality microbial protein from high quality protein and to save the energy which would be used in synthesis and excretion of urea from excess ammonia. Similarly starch can be protected to reduce the energy cost of converting propionate to glucose by gluconoegenesis. Many advantages can be gained from protection of starch as well as protein.

The objective of the experiments to be described was to obtain information on the extent to which barley grain - pre - treated with various combinations of formaldehyde, wood sugar, acetic and isobutyric acids was protected in the rumen from fermentation therein. The dacron bag technique was used in combination with four rumen fistulated heifers fed a ration of hay/ barlely. (See Meherz and Orskov, 1977).

MATERIALS AND METHODS

The initial experimental work, involving the incubation of dacron bags in the rumen of Jersey cows, was carried out at the University Farm, Cockle Park. The subsequent analysis of bag contents were performed in the Department of Agricultural Biochemistry and Nutrition in the School of Agriculture, University of Newcastle upon Tyne.

Animals:

Four Jersey cows, (238.0 ± 11.0 kg L.Wt.) equipped with rumen fistulae (5 cm diameter) were used for the experiments. Each animal was offered daily a ration of 2.6 kg hay, 2 kg grass nuts, 2 kg rolled barley and 0.6 kg soya.

The animals were fed twice daily at 08.00 hours and 16.00 hours and were housed in individual loose boxes.

Description of grain samples:

Samples of various chemically treated whole barley were provided by Far-

mos Group Ltd., Finland. Each chemical treatedment had been applied at three levels.

Treatment	Chemical composition
1	Wood sugar, formaldehyde, acetic acid, isobutyric acid.
2	Wood sugar, formaldehyde.
3	Wood sugar, acetic acid, isobutyric acid.
4	Formaldehyde.
5	Formaldehyde, acetic acid, isobutyric acid.
6	Formaldehyde, acetic acid.
7	Wood sugar.
8	Acetic acid, isobutyric acid.
Control	Untreated barley.

The three level of application of the treatments (level 1, 2 and 3) contained the same relative proportions of components within each treatment. For the treatments containing formaldehyde, level 1 contained 1 g formaldehyde per 100 g crude protein (CP), level 2 contained 2 g formaldehyde per 100 g CP and level 3 contained 3 g formaldehyde per 100 g CP. For the treatments not containing formaldehyde, level 1 contained the same concentration of components as were present in treatment 1, level 1. Similarly, level 2 and 3 of these treatments (not containing formaldehyde) contained the same concentration of components as were present in level 2 and 3, respectively of treatment 1.

Dacron bag procedure:

All the samples of treated and untreated barley were milled using a laporatory hammer mill with 1 mm screen, before they were subjected to the dacron bag technique. The bags containing the samples were incubated in the rumen of each of the four cows for 4, 8 and 24 hours, except for the level 2 samples of treatments 2, 3, 4, 5, 6, 7 and 8, which were only incubated for 8 hours. Samples of treatment 6 level 1 and 3 were also incubated for 8 hours only.

Analytical methods:

Analysis of dry matter, nitrogen content and linked glucose polymer was done for all samples. The latter analysis was determined according to the

method described by MacRae and Armstrong (1969).

Statistical analysis:

The data for all treatments obtained were investigated using one - way analysis of variance, to show any significant difference in the loss of dry matter (D.M.), nitrogen (N) and starch from dacron bags incubated at different lengths of time between various treatments and control.

RESULTS AND DISCUSSION

The starch and nitrogen (N) contents of the original barley (mg/ g D.M.) were determined as 634 and 19.6 respectively.

For the purpose of clarity in discussing the essential results the mean data obtained for each of the 8 treatments plus control at each of 3 application levels for periods of incubation in the rumen of 4 hours, 8 hours and.24' hours are shown in tables 1, 2 and 3 respectively. It was felt that relative to conditions in the fed animal the most significant data would be that for 8 hours of rumen incubation. However, it was felt valuable to also consider the 4 and 24 hours results.

From table 1 (results for 4 hours incubation) and with reference to D.M. loss it can be seen that when the level of application of formaldehyde or other additive was at least (level 1) then none of the treatments gave values significantly different from the control. With reference to N loss at level 1 it can be seen that all the treatments containing formaldehyde did reduce N loss compared with the control but that was only significant ($p < 0.05$) for the complete additive i.e. formaldehyde, wood sugar, acetic acid and isobutyric acid (treatment 1). The mean data for starch loss shown in table 1 for level 1 like D.M. there were no significant differences for individual treatments over control. Table 1 indicates that at the highest level of application of additives all treatments containing formaldehyde had significantly ($p < 0.05$) lower losses of D.M. and N than control. However, starch loss, though lower than for the control for the treatments containing formaldehyde was not significantly so for any of the treatments.

Table 2 details the mean results for D.M., N and starch loss when the samples were incubated for 8 hours in the rumen. With reference to the data for loss of D.M. it can be seen that none of the treatments free of formalde

Table 1: Percentage of dry matter (D.M.), nitrogen (N) and starch from bags during rumen incubation of samples (at 4 hours incubation).

Treatment (Treat. No.)	% D.M. Loss Treat. Level			% N Loss Treat. Level			% Starch Loss Treat. Level		
	1	2	3	1	2	3	1	2	3
Control (9)	56.2	56.2	56.2	42.5	42.5	42.5	73.1	73.1	73.1
HCHO (4)	58.1		36.3*	27.7		01.1*	85.2		53.1
HCHO + wood sugar (2)	46.8		38.0*	21.8		03.1*	80.5		54.1
HCHO + acetic add (6)									
HCHO + acetic + isobutyric acids (5)	55.1		40.9	21.5		14.0*	76.5		55.6
HCHO + wood sugar + acetic + isobutric acids (1)	48.4	41.7	29.5*	16.9*	02.2*	03.7*	82.5	64.1	51.4
Wood sugar (7)	69:7		66.3	43.6	-	38.4	91.6		90.3
Wood'sugar + acetic + isbutric acids (3)	62.4	-	62.8	35.4		37.0	85.2		87.7
Acetic + isobutyric adds (8)	66.2		68.0	38.9		50.3	89.5		92.1

** indicates significant different ($p < 0.05$) betwee4 value and that of control.*

Table 2: Percentage of dry matter (DM.), nitrogen (N) and starch from bags during rumen incubation of samples (at 8 hours incubation).

Treatment (Treat. No.)	% D.M. Loss Treat. level			% N Loss Treat. level			% Starch lop Treat. level		
	1	2	3	1	2	3	1	2	
Control (9)	72.6	72.6	72.6	60.7	60.7	60.7	93.5	93.5	
HCHO (4)	74.0	62.9	41.3*	54.9	32.5*	07.2*	94.4	85.8	60.9*
HCHO . wood sugar (2)•	60.7	61.4	47.6*	30.6	29.2*	14.4*	87.7	84.2	62.3*
HCHO + acetic acid (6)	72.6	54.8*	44.7*	52.5	19.4	06.7*	93.0	72.1*	56.90
HCHO + acetic + isobutyric acids (5)	68.2	57A*	44.2*	45.8	26.5*	10.7	88.9	73A*	58.7*
HCHO + wood sugar + acetic + isobutyric acids (1)	56.3*	46.5*	37.3*	25.8*	04.8*	04.3*	88.0	65.7*	61.5*
Wood sugar (7)	79.5	78.8	73.7	71.4	66.7	53.7	97.4	97.2	95.4
Wood sugar + acetic + isbutpic acids (3)	74.2	76.9	68.8	63.8	67.6	55.1	94.3	96.9	92.8
Acetic + isobutyric acids (8)	74.5	80.0	72.4	55.7	72.6	63.0	95.0	98.0	95.4

** indicates significant different ($p < 0.05$) between value and that of control.*
+ no significant difference ($p > 0.05$) between HCHO alone and any of taeatments containing HCHO.

Table 3: Percentage of dry matter (D.M.), nitrogen (N) and starch from bags during rumen incubation of samples (at 24 hours incubation).

Treatment (Treat. No.)	% D.M. Loss Treat. level			% N loss Treat. level			% Starch loss Treat. level		
	1	2	3	1	2	3	1	2	1
Control (9)	86.4	86.4	86.4	91.4	91.4	91.4	99.4	99.4	99.4
HCHO (4)	86.5		74.9	88.9		44.4*	99.7		96.4
HCHO + wood sugar (2)	80.4+		78.7	80.9		46.9*	99.5		98.1
HCHO + acetic acid (6)	-								
HCHO + acetic + isobutyric acids (5)	85.2	-	73.5	90.5		40.2*	99.7	-	95.4
HCHO + wood sugar + acetic + isobutyric acids (1)	85.4	75.3*	68.4*	83.4	82.8*	29.7*	99.5	96.0	88.0*+
Wood sugar (7)	88.3		86.7	91.0		90.1	99.8		99.7
Wood sugar + acetic + isbutyric acids (3)	87.6		85.4	89.6		90.9	99.4		99.5
Acetic + isobutyric acids (8)	87.4		84.3	91.8		88.6	99.8		99.4

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** indicates significant different ($p < 0.05$) between value and that of control.*
+ indicates significant difference ($p < 0.05$) between HCHO alone and that of any treat. containing HCHO.

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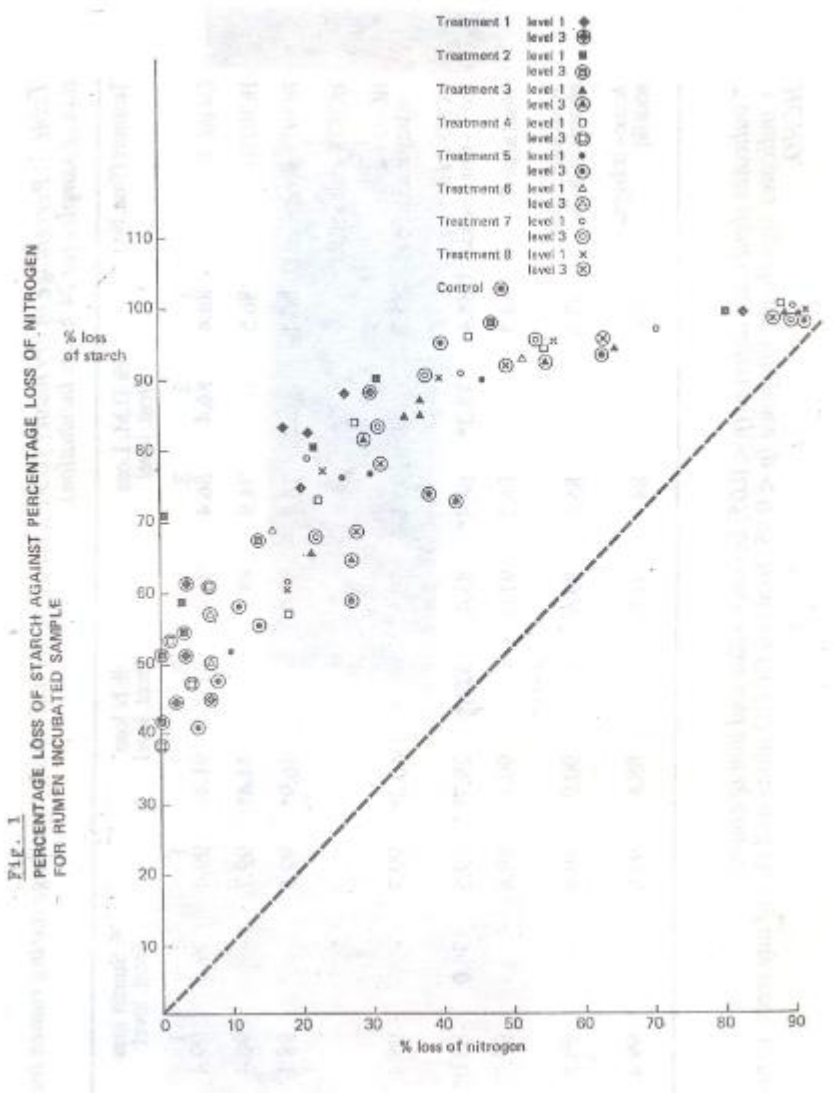

Fig. 1
PERCENTAGE LOSS OF STARCH AGAINST PERCENTAGE LOSS OF NITROGEN FOR RUMEN INCUBATED SAMPLE

hyde had any protective effect and this was also true for N and starch. However, at level 1 application of additive D.M. and N loss were significantly reduced ($p < 0.05$) when the complete additive was used (treatment 1). It can be seen from table 2 that at the intermediate level of additive application formaldehyde and acetic acid (treatment 6) or formaldehyde, acetic and isobutyric acid (treatment 5) as well as the complete set of additive (treatment 1) significantly reduced D.M., N and starch loss. With N the formaldehyde alone (treatment 4) and formaldehyde and wood sugar (treatment 2) also significantly reduce N loss although the value for D.M. and starch loss were lower than the control but not significantly so.

At the highest level of application of additive all treatments containing formaldehyde significantly reduced D.M., N and starch loss. There were, however, no significant differences between treatments in the extent to which they gave protection to D.M. or N or starch.

Table 3 shows the comparable data for the 24 hours incubation period. In this instance there was significant protection of N of the formaldehyde con taining additives, but for D.M. and starch only the complete treatment (1) gave a significant reduction compared with control.

Thus it can be seen that all treatments containing formaldehyde when used at the highest level of addition (level 3) resulted in protection of N (protein); at the 8 hours incubation the same true for starch. The magnitude of the protection of N (protein) was markedly greater than that of starch, although it must be noted that the extent of protection of starch was quite considerable even with formaldehyde alone. Unfortunately, no data are available to show the extent to which the N or starch so protected could be rendered available by host enzyme attack in the abomasum and the small intestine.

It can be seen from the result present in table 2 the use of complete additive at the lowest level of application stil gave a very considerable protection of N. The data shown in table 2 relating to 8 hours incubation indicate that, with respect to N loss at the intermediate level of application of additive, there were no significant differences between any of the treatments containing formaldehyde. However, it is noticeable that the complete additive (treatment 1) gave much lower N loss than the other formaldehyde containing treatments. This was not true nevertheless for starch. Thus there might appear to be some advantage in using the complete additive to achieve the most effective protection of protein.

Table 2 indicates that N loss was significantly reduced ($p < 0.05$) as the level of formaldehyde in the treatment increased. This was true for formaldehyde alone, formaldehyde and acetic acid and formaldehyde, acetic and isobutyric acids. It would also appear from table 2 that there was some mrit in using the4 complete additive as distinct from one containing formaldehyde alone or with wood sugar or with the acids singly or together. However, as already indicated the data in table 2 show no significant difference in the value and hence this cannot have any statistical validity.

Table 2 summarises the mean data for starch loss at 8 hours incubation for each of the 3 level of application of additives. It can be seen that with formaldehyde alone or formaldehyde and wood sugar, only the highest level of treatment gave a significant lower loss than level **1 or 2. However, the** presence of acetic acid with formaldehyde or acetic and isobutyric acids with formaldehyde resulted in a significantly lower loss of starch at level 2 and 3 application. It would thus appear that starch protection is more effectively achieved by the presence of an acid such as acetic with formaldehyde. However, there seems little of adding to formaldehyde any constituent Other than acetic acid.

It was felt that it might be useful to plot the loss of starch against the loss of nitrogen from dacron bags incubated in the rumen, in order to see if there were any effects of protein protection on starch and whether the protection of protein conveyed a protection to the starch (see fig. **1).**

The diagram shows that starch losses are far greater than nitrogen losses since, if they were of the same magnitude, the point would lie along a straight line at 45° to the axis and passing through the origin. The overall shape of the plot is a curve which shows that the loss of starch per unit loss of N is greatest where the amount of nitrogen already lost is small, and least where the amount of nitrogen lost is large. All the treatments free of formaldehyde follow the same curved pattern although the curve which they produce is shifted further from the axis than curves of the other treatments containing formaldehyde. this implies that **the basic relationship between starch loss and nitrogen loss is independent of the effectiveness of the treatment.**

Because of the presence of a layer of protein surrounding each starch granule in the barley grain, it seems likely that the starch cannot be released, or attacked by enzymes and digested, until the protein layer has been reptured. Thus as the susceptibility of the protein to rumen degradation is lowered, the

amount of starch escaping rumenal breakdown would increase.

From the results it is apparent at the lowest level of application none of the treatments gave any effective protection of starch, although protein protection was observed. This may be due to the concentration of the chemicals at the lowest level of application being insuffient to penetrate into the endosperm of the grain, and protection being restricted to the components of the peripheray of the grain. On the other hand the highest level of treatment application appear to be very effective in protection of protein and to a considerable extent for starch protection.

It must be emphasised that there is need for a considerable further work before such treatment of grain could be recommended in practice. There are a number of matters that must be considered. Firstly, is the protein and also the starch which appear to be protected capable of being digested under the host enzymes of the small intestine of the ruminant animal. Clearly if this is not so then all the treatment of the grain would have succeeded in doing with to reduce the substrate i.e. starch available for microbial fermentation in the rumen and equally reduce the potential supply of amino acid nitrogen to the animal. Secondly, it is important to apppreciate that there is need to maintain an efficient microbial fermentation in the rumen and this might be seriously effected if all or a major part of the grain fed was so treated. It is thus clear further research work must be done on these materials, before theri role in ruminant livestock production can be assessed.

It is clear that all the treatments containing formaldehyde at the 3 levels of application provided and effective means of protection of barley grain protein, while treatments free of formaldehyde and control show no significant effect on protecting protein or starch from rumen degradation. Although no apparent protection of starch was afforded by any of the treatments at the lowest of application considerable protection of starch was observed at the two highest level (2 and 3) of application for treatments containing formaldehyde.

No research into the postruminal digestion of the treated barley samples was carried out, but before any conclusion can be drawn about barley, research into their postruminal digestion and absorption is necessary.

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