

## Digestion of concentrates in sheep

### 3.\* Effects of rumen fermentation of barley and maize diets on protein digestion

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1. The effect of the extent of ruminal fermentation of starch on the postruminal digestion of starch and protein was investigated in sheep, differences in rumen fermentation being obtained by giving diets based on barley, maize, or maize plus sodium chloride. The mean percentages of starch ( $\alpha$ -linked glucose polymers) fermented in the rumen were 91, 79 and 78 respectively with these diets, but there was considerable variability, particularly with the maize diets.

2. When large amounts of starch escaped fermentation in the rumen, substantial quantities passed the terminal ileum and were mostly fermented in the large intestine; the greatest amount of starch found in the faeces was 2% of intake.

3. A decrease in the extent of rumen fermentation of starch was associated with a decrease in the concentration of crude protein and of diaminopimelic acid (DAPA) in the abomasal fluid, but there was no difference in quantity of crude protein disappearing from the small intestine. The concentration of DAPA was greater with the barley diet than with the maize diet, indicating that more dietary protein, originating from maize than from barley, escaped the rumen undegraded. This conclusion was supported by a greater similarity between the amino acid composition of the abomasal fluid and the diet when maize was given.

In previous work (Ørskov, Fraser & Kay, 1969) it was shown that when sheep were given barley or flaked maize the extent of rumen fermentation of starch was almost invariably over 90%, but when they received uncooked maize only 80–85% of the starch was fermented in the rumen. The observations made with barley agreed with those of other workers (Topps, Kay & Goodall, 1968; Thivend & Journet, 1968; MacRae & Armstrong, 1969; Nicholson & Sutton, 1969). The fact that large quantities of starch passed beyond the rumen with ground maize was noted by Karr, Little & Mitchell (1966) and Thivend & Journet (1970) and the difference we found between ground and flaked maize has recently been confirmed by Beever, Coehlo Da Silva & Armstrong (1970).

Since microbial protein synthesis is likely to be related to the quantity of carbohydrate fermented (Hungate, 1966), Ørskov *et al.* (1969) suggested that the extent of rumen fermentation would influence the amount of microbial protein available to the animal. Starch digested in the small intestine would not contribute to microbial synthesis, and if starch were fermented in the large intestine the microbial protein thus formed would not be digested subsequently. It was later shown (Ørskov & Foot, 1969) that starch fermentation in the caecum increased faecal nitrogen output and that this nitrogen was the result of an increased production of microbial protein (Ørskov, Fraser, Mason & Mann, 1970). It is therefore possible that when large

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quantities of starch are digested postruminally there may be less microbial protein available to the host animal. The present experiment was conducted to examine the effect of the extent of rumen fermentation on the protein digested in various segments of the gut. Maize or barley was offered at a high level of feeding. In an attempt to decrease rumen fermentation by increasing the rate of passage through the rumen, a third treatment was used, in which salt was added to a maize diet (Potter, 1968).

## EXPERIMENTAL

### *Animals*

Three female North-Country Cheviot sheep aged about 4 months at the start of the experiment were used. They were fitted with cannulas in the abomasum and in the terminal ileum as described by Ørskov *et al.* (1969), and the disappearance of digesta in various segments of the gut was determined with chromic oxide as a marker.

### *Design and treatments*

A 3 × 3 Latin square design was used. The length of each period was 14 d, during the last 24 h of which samples were obtained of abomasal and ileal contents and of faeces.

The treatments were: (1) rolled barley + urea to give 15% crude protein; (2) kibbled maize + urea to give 15% crude protein; (3) kibbled maize + urea + 2% NaCl to give 15% crude protein.

### *Level of feeding and composition of diets*

The amount of feed offered in the first period to each sheep was calculated according to its live weight by means of a formula obtained from experiments with other sheep to give near *ad lib.* intake. In successive periods the amount of feed offered was

Table 1. *Ingredients of the diets (percentage by weight) and chemical composition (percentage of dry matter)*

Treatment	Composition											
	Ingredients				Crude					Ether	Energy	
	Barley	Maize	Urea	Minerals*	protein	Starch	Ash	extrac- tives	kcal/g	(kJ/g)		
Barley	92.8	—	2.6	4.5	15.1	58.9	5.0	1.7	4.20	(17.6)		
Maize	—	93.3	2.1	4.6	15.3	77.7	3.5	2.4	4.29	(17.9)		
Maize + NaCl	—	91.3	2.1	6.6	15.1	76.9	5.7	2.6	4.23	(17.7)		

\* Consisting of 1% Cr<sub>2</sub>O<sub>3</sub> + flour (1:4), 0.6% vitamin mix (1000 i.u. vitamin A palmitate and 200 i.u. cholecalciferol/g), 2.5% steamed bone flour, 0.25% NaCl, 0.25% MgO. The maize + salt diet contained in addition 2% NaCl. Trace minerals were added to all three diets to supply 0.43 mg CoSO<sub>4</sub>·7H<sub>2</sub>O, 141 mg MnSO<sub>4</sub>·4H<sub>2</sub>O, 191 mg ZnSO<sub>4</sub>·7H<sub>2</sub>O and 0.2 mg KIO<sub>3</sub>/kg air-dry feed.

increased by 100 g. The mean daily dry-matter intakes over all periods were 1017, 1074 and 1077 g for treatments 1, 2 and 3 respectively.

Each diet was pelleted through a 7.7 mm die. Their ingredients and chemical composition are given in Table 1.

*Management and sampling procedure*

As in the previous work (Ørskov, Fraser & McDonald, 1971 *a, b*), the feeds were weighed out for each period and the lambs were given half the feed at 08.00 hours and the remainder at 20.00 hours. The amounts of uneaten feed were recorded daily and the uneaten feed was dried to constant weight. Water was available at all times and the lambs were kept on slats in individual pens. Small samples of abomasal and ileal contents and of faeces were obtained at 2 h intervals (Ørskov *et al.* 1971 *a*) during the last 24 h of each period. As in previous work, the disappearances between mouth and abomasum, between abomasum and terminal ileum and between terminal ileum and rectum were taken to represent the disappearances from the rumen, small intestine and large intestine, respectively. Disappearance was calculated from the changes in concentration of chromium at the various sites.

*Analytical procedure*

Total nitrogen was measured by the Kjeldahl method and ammonia in abomasal and ileal fluids by the method of Conway (1957). After hydrolysis of abomasal contents, the hydrolysates were subjected to amino acid analysis on a modified Technicon amino acid analyser (Davidson & Hepburn, 1970). DAPA was determined by the method of Mason (1969).

Chromic oxide was determined by a modification of the method of Stevenson & Clare (1963) described by Mathieson (1970). Ether extractives were estimated by Soxhlet extraction and starch by the method of MacRae & Armstrong (1968). The calculations of volatile fatty acid (VFA) production were made as in our previous work (Ørskov *et al.* 1971 *b*). The VFA proportions used in the calculations reported here were those observed with a similar kibbled maize diet given *ad lib.* to eight other sheep. The mean molar proportions of VFA measured with the kibbled maize diet were: acetic 38.7, propionic 38.5, isobutyric 1.8, butyric 14.2, isovaleric 2.1, and valeric 4.6%.

## RESULTS

It had been hoped that the addition of salt to the maize diet might have produced a substantial increase in water intake. If this had happened the addition of salt might have had various indirect effects on rate of passage of digesta and on rates of absorption. In fact the effect on water intake was small and not statistically significant, the mean water intakes being 2.0, 2.3 and 2.7 l/d for the barley, maize, and maize plus salt diets respectively. We have therefore assumed that the two maize diets were largely equivalent, except in respect of measurements such as ash intake and disappearance where salt would have a direct effect, and in Tables 2-5 we have compared the mean values for the two maize diets with the corresponding results for the barley diet. The standard errors of the differences are based on only 2 degrees of freedom, so relatively large ratios of difference to standard error were required to establish statistical significance.

The concentrations of non-ammonia crude protein (NACP) and of ammonia in the abomasal and ileal dry matter are given in Table 2, together with the concentrations

of DAPA in the abomasum. The difference in concentration of crude protein in the faeces between the maize and barley diets was significant ( $P < 0.05$ ). The concentration of ammonia in abomasal fluid was also higher with the maize than with the barley diets ( $P < 0.05$ ), and the concentration of DAPA was significantly greater with the barley than with the maize diets ( $P < 0.05$ ).

Table 2. Concentration of diaminopimelic acid (DAPA) and non-ammonia crude protein (NACP) in abomasal and ileal fluids, crude protein in faeces and ammonia in abomasal and ileal fluids of sheep

(Each value is the mean of three observations)

Treatment	DAPA	NACP in abomasum (% of DM)	NACP in ileum (% of DM)	Crude protein in faeces (% of DM)	Ammonia in abomasum (mg N/ 100 ml)	Ammonia in ileum (mg N/ 100 ml)
	(g/16 g non- ammonia N in abomasal fluid)					
Barley	0.686	25.4	17.8	20.0	13.2	37.0
Maize	0.446	23.1	20.0	21.3	15.3	35.7
Maize (+NaCl)	0.426	25.8	19.1	21.9	18.0	28.2
Mean difference, with SE and level of significance, barley—(combined) maize	0.250 ± 0.046 $P < 0.05$	0.9 ± 1.9 NS	-1.8 ± 0.64 NS	-1.6 ± 0.20 $P < 0.05$	-3.5 ± 0.71 $P < 0.05$	5.0 ± 6.0 NS

DM, dry matter; NS, not significant.

Table 3 gives the level of intake and apparent disappearance of protein along the digestive tract. The differences between treatments in the quantities of NACP disappearing from the digestive tract were small and not significant, and the only difference which was significant was the apparent disappearance of crude protein from the rumen, which was greater with the maize diets ( $P < 0.05$ ) than with the barley diet. We should point out that the maize plus salt diet was largely responsible for this difference, and that the difference between the results for the two maize diets was also statistically significant ( $P < 0.05$ ), but we have no explanation to offer for this difference.

The intake of starch, its concentrations in the abomasum and ileum fluids and its disappearance along the digestive tract are shown in Table 4. The concentration of starch in the feed was greater with the maize diets, so that the intake of starch was greatest with those diets. The percentage of starch in the abomasal fluid was greater with the maize than with the barley diet ( $P \div 0.01$ ) and the percentage of the starch intake that disappeared in the small intestine was also greater with the maize diets ( $P < 0.05$ ).

Table 5 gives the values for the intake and disappearance of the ether extractives and ash in the various segments of the gut. With all three diets, the amount of ether extractives disappearing in the small intestine was greater than that consumed. The ash intake and its disappearance from the rumen were greatest with the diet containing salt ( $P < 0.001$ ).

Table 3. Intake of dry matter (DM) and crude protein (CP), disappearance of non-ammonia crude protein (NACP) in the small intestine, and crude protein disappearance in the large intestine and excretion in faeces of sheep

(Each value is the mean of three observations)

Treatment	DM intake (g/d)	CP intake (g/d)	CP dis- appearance in rumen (g/d)	NACP passing abomasum (g/d)	NACP dis- appearance in small intestine (g/d)	NACP passing ileum (g/d)	CP dis- appearance from large intestine (g/d)	CP excreted in faeces (g/d)
Barley	1017	154	11.0	143	95	48	9	40
Maize	1074	164	16.7	148	99	50	13	38
Maize (+NaCl)	1077	163	28.7	134	88	47	14	33
Mean difference, with SE and level of significance, barley—(combined) maize		-9	-11.7±2.1 P < 0.05	2±8.1 NS	2±3.0 NS	0±6.4 NS	-5±6.3 NS	5±3.7 NS

NS, not significant.

Table 4. Intake of starch and concentrations of starch in abomasum and terminal ileum, and its disappearance in various segments of the gut of sheep

(Each value is the mean of three observations)

Treatment	Intake (g/d)	In abomasum (% of DM)	In ileum (% of DM)	Disappearance in rumen (g/d)	Disappearance in rumen (% of intake)	Disappearance in small intestine (g/d)	Disappearance in small intestine (% of intake)	Passing ileum (g/d)
Barley	599	8.8	3.0	546	91.5	46	7.3	8
Maize	835	22.9	10.3	656	80.0	149	16.6	31
Maize (+NaCl)	829	23.1	12.0	645	80.7	151	15.7	34
Mean difference, with SE and level of significance, barley—(combined) maize	-233	-14.2±1.5 P < 0.01	-8.2±3.4 NS	-104±50 NS	11.1±3.0 NS (P < 0.1)	-104±33 NS (P < 0.1)	-8.9±1.8 P < 0.05	-25±13 NS

DM, dry matter; NS, not significant.

The dietary comparisons made in Tables 2-5 are to some extent complicated by the considerable variability which was found between individual determinations of the extent of rumen fermentation with the same diet. However, this variability made it possible to make comparisons on the same diet between instances when little starch and when considerable amounts of starch escaped rumen fermentation. Table 6 gives two of the three sets of individual values for each of the maize diets, the set with intermediate extent of rumen fermentation being omitted for each diet. The first and third columns of values show high concentrations of starch in the abomasum, ileum and faeces, whereas the second and fourth columns show low concentrations. The

Table 5. *Intake and disappearance of ether extractives and ash in various segments of the gut and apparent digestibility in sheep*

(Each value is the mean of three observations)

Treatment	Intake (g/d)	Disappearance in:			Excretion in faeces (g/d)	Apparent digestibility (%)
		Rumen (g/d)	Small intestine (g/d)	Large intestine (g/d)		
			Ether extractives			
Barley	17	-10	21	1.1	5.2	69
Maize	26	-22	39	1.5	6.7	75
Maize (+ NaCl)	27	-11	30	4.0	4.4	84
Mean difference, with SE and level of significance, barley - (combined) maize	-10	6.4 ± 5.8 NS	-13.7 ± 5.8 NS	-1.7 ± 1.7 NS	-0.3 ± 1.1 NS	-10.2 ± 4.6 NS
			Ash			
Barley	51	-5.7	20.1	6.2	30.1	41
Maize	38	-5.2	8.8	9.6	24.6	35
Maize (+ NaCl)	61	15.2	10.4	10.7	24.8	60
Mean difference, with SE and level of significance, barley - (combined) maize	1	-10.7 ± 1.5 <i>P</i> < 0.05	10.5 ± 2.5 NS	-4.0 ± 4.7 NS	5.4 ± 2.3 NS	-6.5 ± 4.5 NS

large amounts of starch escaping rumen fermentation were associated with reduced concentrations of crude protein in the abomasum, lower concentrations of DAPA in the abomasal crude protein, larger faecal excretions of crude protein, and lower VFA productions (as percentages of digestible energy).

The mean DAPA concentrations in Table 2 suggested that the protein in abomasal fluid contained a lower proportion of microbial protein with maize than with barley feeding. To determine whether this was supported by a greater similarity of amino acid compositions of abomasal fluid to that of the diet, amino acid analysis was carried out on samples of abomasal fluid from the sheep given maize. The results are given in Table 7 together with amino acid composition of abomasal samples from sheep given barley + urea diets (Ørskov *et al.* 1971*b*). Also the amino acid composition from sheep given a urea purified diet has been given (Ørskov & Fraser, 1970 and unpublished work). Urea supplied 95% of the nitrogen in that diet, so the protein in the abomasal fluid would be expected to be almost entirely of microbial origin. Since no analysis of

amino acid composition was carried out on the diets, the mean compositions of maize and barley have been quoted from Eggum (1968). The amino acid compositions for the abomasal fluids clearly show much greater similarity between that of the urea purified diet and that of the barley diet than between the former and that of the maize diet. Of the seventeen comparisons for individual amino acids, thirteen point in the direction of that similarity and only two in the other. Similar comparisons between the

Table 6. *Influence of extent of fermentation of a maize diet on digestion of sheep*

Sheep ...	1	2	1	3
Period ...	2	1	3	1
Treatment ...	Maize	Maize	Maize + NaCl	Maize + NaCl
DM intake				
g/d	1213	1002	1303	850
g/d.kg <sup>0.75</sup>	82.6	82.3	84.3	85.8
Digestible energy intake (kcal/d)	3936	3730	4380	3290
Starch				
Intake (g/d)	942	778	1003	654
In abomasum (g/100 g DM)	44.2	4.2	44.9	8.3
In ileum (g/100 g DM)	24.1	0.5	20.1	5.6
In faeces (g/100 g DM)	8.0	—	6.9	—
Fermented in rumen (g/d)	540	757	547	630
Digested in small intestine (g/d)	324	21	378	13
Fermented in large intestine (g/d)	57	—	60	—
Excreted in faeces (g/d)	21	—	17	—
Energy absorbed as volatile fatty acids (% of digestible energy)	38	58	34	64
Crude protein intake (g/d)*	111 + (74)	92 + (62)	117 + (80)	76 + (44)
NACP (% of abomasal DM)	19.3	26.1	17.1	29.2
Crude protein disappearing in rumen (g/d)	10	19	23	31
NACP				
Disappearing in small intestine (g/d)	106	92	91	62
Disappearing in large intestine (g/d)	10	17	27	3
Crude protein excreted in faeces (g/d)	59	27	57	17
Apparent protein digestibility†	46.5	71.7	51.7	77.6
Diaminopimelic acid (g/16 g non-ammonia N in abomasal contents)	0.356	0.495	0.394	0.479

DM, dry matter; NACP, non-ammonia crude protein.

\* Values in parentheses represent urea N × 6.25.

† Urea not taken into account.

amino acid composition of the diet itself and of the corresponding abomasal fluid show differences to be greater with barley for twelve of the amino acids and for maize only in five. These results are not in themselves conclusive, but they add weight to the suggestion from the DAPA results that barley feeding leads to a higher proportion of microbial protein in abomasal fluid than does maize feeding.

Table 7. Comparison of the amino acid compositions (g amino acid/16 g N) of abomasal fluids and those of the diet for sheep receiving rolled barley, maize or a urea purified diet

Amino acid	Composition of abomasal fluid in lambs receiving:						
	Urea purified diet	Rolled barley diet		Maize diet		Composition of	
		Range	Mean (6)	Range	Mean (4)	Barley	Maize
Aspartic acid	7.7	6.5-9.2	8.0	7.6-8.7	8.0	7.0	7.2
Threonine	3.9	3.9-5.2	4.4	4.2-4.5	4.3	3.2	4.0
Serine	4.0	3.8-4.8	4.4	4.5-5.1	4.9	4.2	5.0
Glutamic acid	8.9	10.0-13.1	11.2	13.2-15.3	14.4	25.1	17.5
Proline	2.1	3.1-4.2	4.0	4.3-6.1	5.2	—	—
Glycine	3.9	3.9-4.6	4.3	3.9-4.8	4.4	4.5	4.0
Alanine	6.1	5.5-6.1	5.9	7.1-8.6	7.9	4.6	8.2
Valine	4.9	4.4-5.1	4.9	4.7-5.3	5.1	5.3	5.0
Cystine	1.1	0.8-1.1	1.0	1.3-1.5	1.3	2.3	2.3
Methionine	2.0	1.8-2.3	2.1	2.6-3.0	2.7	1.8	2.3
Isoleucine	4.2	3.5-4.3	4.0	3.8-4.7	4.4	3.7	3.8
Leucine	5.9	5.9-6.6	6.2	9.4-10.2	9.9	7.1	10.6
Tyrosine	3.3	2.6-4.1	3.5	3.8-4.3	4.1	3.7	4.2
Phenylalanine	3.4	3.6-4.3	3.9	4.2-4.4	4.2	4.9	4.5
Lysine	6.2	5.9-6.7	6.3	4.4-5.9	5.2	3.7	2.7
Histidine	1.7	1.7-2.0	1.9	1.6-2.2	2.0	2.2	2.6
Arginine	4.2	2.2-4.9	3.8	3.6-4.5	4.1	5.4	4.3

## DISCUSSION

*The effect of diet on the extent of rumen fermentation*

The results presented here fully confirm our earlier work (Ørskov *et al.* 1969), which showed that diets containing uncooked maize cereals were fermented in the rumen to a much lesser extent than were barley diets. An attempt was made to speed up the rate of passage from the rumen by including additional salt with the maize, so as to achieve a further increase in the amount escaping fermentation. From the results presented it is apparent that this was not achieved, probably because the amount of salt added was not great enough to stimulate much increase in water intake. The work also confirms the observation (Ørskov *et al.* 1969) that  $\alpha$ -linked glucose polymers passing the abomasum are not always completely digested in the small intestine but substantial quantities may disappear through fermentation in the large intestine, thus affecting the type of fermentation and microbial nitrogen synthesis at that site (Ørskov *et al.* 1970).

An interesting observation was the degree of variability in rumen fermentation measured with the same diet. It can be calculated from values in Table 6 that starch digested in the small intestine varied from about 2 to 37%, depending on the amount entering from the rumen. Under conditions in which large quantities passed through the rumen but were not fermented in it, about 6% disappeared in the large intestine and 2% was excreted in the faeces. This is likely to affect utilization, since much smaller losses due to the production of heat and gases during fermentation will be incurred. It is, however, not only of relevance to total utilization for, if a similar variability occurred with lactating animals, it would undoubtedly affect the production



of milk fat markedly because of an increased absorption of glucose (McClymont & Vallance, 1962). The quantities of starch absorbed from the small intestine may also have important implications as far as enzyme adaptation is concerned and the results obtained here may suggest that the ruminant is capable of becoming adapted to secrete larger amounts of amylase than has hitherto been realized. This aspect has been discussed earlier (Ørskov, 1969), when attention was drawn to the fact that Clary, Mitchell & Little (1967) reported an increase in amylase secretion as a result of an increase in post-ruminal digestion of starch.

*The effect of type of cereal and extent of rumen fermentation  
on protein digestion*

The lack of a technique which can accurately separate microbial protein from dietary protein in digesta prevents any conclusive evidence being obtained, and the mean values shown in Table 3 do not indicate much effect from post ruminal digestion of starch on the amounts of protein passing the abomasum or disappearing from the small intestine. Only the individual values in Table 6 showed that the percentage of protein in the abomasal fluid was much lower when large amounts of starch escaped the rumen fermentation.

There are, however, some indications from the measurements of DAPA concentration and amino acid composition in abomasal fluid which suggest:

(1) That more dietary protein escaped the rumen undegraded with the maize diets than with the barley diet. This is supported by the lower DAPA concentration obtained when the lambs were given maize in place of barley, and also by the tendency of a closer similarity between the amino acid composition of the diet and that of the abomasal fluid for maize-fed than for barley-fed lambs. It may be noted that maize protein is relatively rich in zein which has been shown to be degraded very slowly in rumen fluid (McDonald, 1952; Annison, 1956).

(2) That the amount of microbial protein formed will depend on the extent of rumen fermentation. As discussed earlier, this would be expected on theoretical grounds (Hungate, 1966). If the concentration of DAPA can be taken as some measure of the proportion of microbial protein, then the values in Table 6 certainly support the hypothesis.

The two observations where the largest quantities of starch passed the terminal ileum (sheep 1, periods 2 and 3, Table 6) and where substantial quantities were absorbed in the large intestine gave increases in faecal crude protein and a decreased apparent protein digestibility. The trend was similar to that observed when starch was infused into the caecum (Ørskov & Foot, 1969) but part of the increase in faecal nitrogen observed here is likely to have been of dietary origin since there were also large increases in the amount passing the terminal ileum.

In agreement with previous work (Ørskov *et al.* 1971 *a, b*), it has been found here that an increase in the amount of ether-extractable lipids occurred in the rumen over that in the diet, presumably owing to microbial lipid synthesis. The work again emphasizes that with the ruminant the apparent digestibility of dietary constituents

such as ash, ether extractives and protein may bear little relation to the amounts available.

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