

Digestion of concentrates in sheep

1. The effect of increasing the concentration of soya-bean meal in a barley diet on apparent disappearance of feed constituents along the digestive tract

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1. Four sheep were given four diets containing proportions of rolled barley and soya-bean meal varied to provide 10.3, 13.3, 16.1 and 19.9% crude protein in the dietary dry matter; the treatments were given according to a 4 × 4 Latin square design. The mean daily intake was 989 g dry matter. The apparent disappearance of protein, ash, ether extractives and carbohydrate before the abomasum, between the abomasum and terminal ileum and between the terminal ileum and rectum was measured.

2. The amount of non-ammonia crude protein (Y_1 , g/d) disappearing from the small intestine increased with protein intake (X , g/d) according to the equation $Y_1 = 2.12X - 0.0057X^2 - 83$, reaching a maximum when there was about 19% crude protein in the dry matter of the diet.

3. The treatments had no significant effects on the disappearance of starch, ether extractives or ash. About 93% of starch disappeared in the rumen and 6% in the small intestine. The total mean daily intake of ether extractives was 21 g; 9 g were added in the rumen, 24 g disappeared from the small intestine and 6 g were excreted in the faeces. The total mean daily intake of ash was 67 g; 26 g were added in the rumen, 37 g disappeared from the small intestine, 9 g from the large intestine and 47 g were excreted in the faeces.

The extent of the digestion of dry matter and starch in various parts of the alimentary tract of ruminants has been studied in some detail and the literature has recently been reviewed (Ørskov, 1969; Armstrong & Beever, 1969). While it is generally recognized that extensive deamination of protein occurs in the rumen (McDonald, 1948; Chalmers & Synge, 1954), very few quantitative data are available on the extent to which increments in the protein concentration of a feed influence the amount of protein digested in the small intestine. Hogan & Weston (1967) have shown that the amounts of non-ammonia crude protein (NACP) passing the abomasum were similar whether the diet contained 7.8 or 19.8% crude protein. On the other hand, Clarke, Ellinger & Phillipson (1966) have shown that when an increasing amount of soya protein was added to a diet the amount of α -amino acids disappearing from the small intestine increased. Also, in a recent experiment with young lambs it was found that, when the protein concentration of the diet was increased at high constant energy intakes, the growth rate and the retention of nitrogen in the body increased (Andrews & Ørskov, 1970a, b).

The series of experiments reported here was done to provide more quantitative information about the digestion of protein, and at the same time to obtain additional information about the disappearance of other feed constituents along the digestive tract; the possible association of these factors with protein formation and digestion was also investigated. A short account of this work has been published (Ørskov & Fraser, 1969).

EXPERIMENTAL

Animals

Three female and one male sheep were used; they were Cheviot or Suffolk Crosses. The sheep weighed about 35 kg each at the beginning of the experiment and were about 9 months old. The animals were fitted with abomasal and terminal ileal cannulas similar to that described by Ørskov, Fraser & Kay (1969).

Design and treatments

The design was a 4×4 Latin square with feeding periods of 14 d. During the last 24 h of each period, samples of abomasal, ileal and rectal contents were obtained.

In all of the four treatments rolled barley was used, and was supplemented with soya-bean meal to supply 10.0, 13.3, 16.6 and 19.9% crude protein in the diets given in treatments 1-4 respectively.

Feeding level and feed composition

The level of feeding was fixed initially in relation to live weight according to the formulas used to estimate near *ad lib.* intake (Andrews & Ørskov, 1970a). The intake was then kept constant throughout the 2 months duration of the trial. A chromic oxide mixture was prepared by mixing one part of chromium sesquioxide and four parts of wheat flour into a paste with water. The paste was then dried at 100°, after which it was ground in a laboratory hammer mill. This procedure was used to ensure homogeneous mixing into the concentrate where the chromium sesquioxide was used as an indigestible marker to estimate disappearance of digesta along the digestive tract.

The diets were all pelleted through a 4.6 mm die. The average chemical composition of each diet is given in Table 1.

Table 1. *Ingredients and composition of the four diets given to sheep and their energy content expressed on a dry-matter basis*

Diet	Rolled barley (%)	Soya-bean meal (%)	Crude protein (%)	Ether extractives (%)	Starch (%)	Ash (%)	Heat of combustion (kcal/g)
1	89.5	4.5	10.3	2.4	62.0	6.3	4.242
2	85.2	8.8	13.3	1.7	59.8	6.5	4.266
3	75.6	18.4	16.1	2.3	60.9	7.2	4.242
4	68.0	26.0	19.6	2.3	60.0	7.5	4.298

* Each diet contained in addition 1.4% Fuller's earth (to facilitate pelleting), 0.6% Adisco (1000 i.u. vitamin A/g and 200 i.u. cholecalciferol/g; Isaack-Spencer and Co. Ltd, Aberdeen), 2.5% steamed bone flour, 0.25% sodium chloride, 0.25% calcined magnesite and 1% of a mixture of chromic oxide and wheat flour. Of trace minerals, the diets were supplemented with 0.43 mg $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2 mg KIO_3 , 141 mg $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ and 191 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ /kg.

Management and sampling procedure

The feeds were weighed for each period and analysed. The sheep were offered half their feed at 20.00 hours and the remainder at 08.00 hours. Uneaten feed, if any, was weighed daily and dried to constant weight at 100°. The sheep were kept on slats in individual pens and water was freely available.

On the last day of each period, samples of abomasal, ileal and rectal contents were taken at 2 h intervals. Each sample of abomasal contents was about 40 ml. The ileal and rectal samples were difficult to standardize and on several occasions no samples could be obtained from either site, but during the 24 h about 500 g of faeces and 300 ml of ileal contents were obtained. The samples for the 24 h period were pooled and stored at -20° until they were required for analysis. About 38 g dry matter were removed during the 24 h sampling period from the abomasum and 30 g from the terminal ileum. No digesta were returned and it was assumed that the removal of these quantities did not influence markedly the digestion of the remainder.

For convenience in describing the results, the disappearance of digesta between the mouth and the abomasal sampling point has been referred to as disappearance in the rumen. Similarly, disappearances between the abomasal and ileal sampling points, and between the latter and the rectum, have been referred to as disappearances in the small intestine and the large intestine respectively.

Analytical procedure

Total nitrogen in liquid digesta and feeds was measured by the Kjeldahl method of the Association of Official Agricultural Chemists (1960) with mercuric oxide as catalyst. The amount of ammonia formed was determined colorimetrically as the indophenol blue complex in a Technicon AutoAnalyzer (Technicon Instruments Co. Ltd, Hanworth Lane, Chertsey, Surrey).

Ammonia in abomasal and ileal contents was determined by the method of Conway (1957).

Starch was analysed in feed, abomasal and ileal contents as α -linked glucose polymer by the method described by MacRae & Armstrong (1968). Ether extractives were determined by Soxhlet extraction of freeze-dried material; samples were ashed at 600° . 'Carbohydrate' was defined as dry matter less ash, ether extractives and crude protein. The chromium sesquioxide in the ash was brought into solution by acid digestion and its concentration determined in a Technicon AutoAnalyzer using a modification of the method by Stevenson & Clare (1963) described by Mathieson (1970).

The heats of combustion of the diets and faeces were determined in an adiabatic bomb calorimeter.

RESULTS

Statistical analysis

Results for one sheep in one period were excluded from the analysis, as there had been difficulties with the abomasal cannula and the values obtained were clearly anomalous. In the analysis of variance the treatment effects were separated into linear, quadratic and cubic components.

Feed intake

The mean daily intakes of feed over the last 3 d of each period (Table 2) have been taken as the basis for all the calculations. Sampling was not found to influence the

intake. The occasional feed refusals, which occurred especially with low-protein diets, resulted in slightly lower intakes of dry matter with treatments 1 and 2 than with the other two treatments and, as a result, intakes of ether extractives, ash and digestible energy were also lower.

Table 2. *Daily intakes of various dietary constituents by sheep receiving four diets containing different concentrations of crude protein*

Crude protein concentration in feed (%)	Dry matter (g/d)	Starch (g/d)	Crude protein (g/d)	Ether extractives (g/d)	Ash (g/d)	Carbohydrate (g/d)	Digestible energy intake (kcal/d)
10.3	850	527	87	20	54	689	2850
13.3	997	596	133	17	65	782	3507
16.1	1093	666	174	25	79	815	3518
19.6	1016	610	199	23	76	718	3489

Table 3. *Apparent digestibilities (%) of various dietary constituents in sheep receiving different concentrations of crude protein in the diet*

(Mean values for four sheep)

Crude protein concentration in feed (%)	Dry matter	Energy	Protein	Ether extractives	Ash	Carbohydrate
10.3	77.1	78.5	59.8	76.8	25.4	84.1
13.3	80.9	82.1	71.9	65.2	31.4	86.9
16.1	74.7	75.9	65.0	73.2	21.1	82.1
19.6	78.3	79.9	78.0	73.6	33.9	83.1
SE of treatment means	2.5	3.0	3.6	6.7	2.9	2.3

Table 4. *Concentration of non-ammonia crude protein in digesta and faeces (% of dry matter) and ammonia nitrogen in abomasal and ileal contents of sheep receiving four diets containing different concentrations of crude protein*

Crude protein concentration in feed (%)	Non-ammonia crude protein		Ammonia-N (mg/100 ml)		Crude protein in faeces (%)
	Abomasal contents (%)	Ileal contents (%)	Abomasal contents	Ileal contents	
10.3	21.9	17.0	6.8	20.2	18.5
13.3	29.2	18.2	9.2	27.0	19.5
16.1	31.4	20.2	11.7	16.3	22.5
19.6	28.7	20.9	19.8	29.4	19.7
SE of treatment means	1.5	2.6	0.9	3.3	1.2

The apparent digestibilities of the various constituents are given in Table 3. The only significant effect was that apparent digestibility of crude protein increased with protein concentration, an increase which may be represented by a straight line. Table 4 shows the influence of protein concentration on the concentration of ammonia and of NACP in abomasal and ileal contents, and of crude protein in the faeces. As dietary concentration of crude protein increased, abomasal NACP increased sharply

at first and then levelled off, both the linear and quadratic components being significant ($P < 0.05$). In contrast, the increase in the abomasal concentration of ammonia ($P < 0.001$) tended to become steeper as crude protein concentration continued to increase. The linear and quadratic components were both significant ($P < 0.001$ and $P < 0.05$ respectively). There were no significant effects on the concentration of either NACP or ammonia in ileal fluid. There was a small irregular increase in concentration of crude protein in the faeces with increasing concentration in the feed.

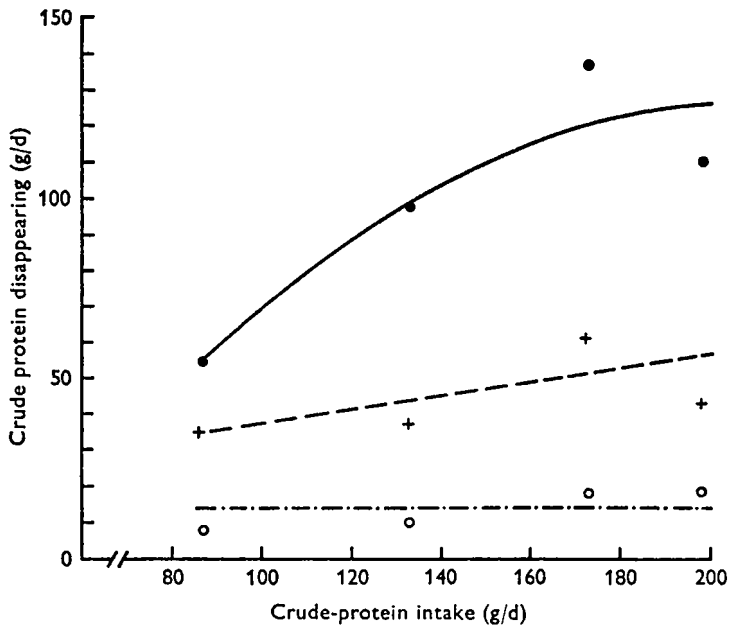


Fig. 1. The effect of crude-protein intake by sheep on the disappearance of crude protein in the small (●—●) and large intestines (○—·—·○) and on crude protein excreted in the faeces (+—+). The points indicate treatment mean values.

The effects of increasing protein intake on the apparent absorption of NACP in the small and large intestines and on the faecal excretion of NACP are illustrated in Fig. 1. The relationship between the disappearance of NACP from the small intestine (Y_1 , g/d) and the protein intake (X , g/d) is described by the regression equation $Y_1 = 2.12X - 0.0057X^2 - 83$. The equation had a residual standard deviation (RSD) of ± 24 g/d. The level of protein intake had no significant effect on the disappearance of NACP from the large intestine, and the line shown is therefore drawn through the mean value of 13.6 g/d. The relationship between NACP excreted in the faeces (Y_2 , g/d) and the protein intake is described by the regression equation $Y_2 = 0.15X + 21$, with a RSD of ± 13 g/d. It can be seen from Fig. 1 that the apparent absorption of NACP from the small intestine increased markedly with increasing protein intake. The maximum absorption would appear to have been reached at about 19% crude protein in the diet.

There were no significant differences due to treatment in the apparent disappearance

of ash, ether extractives or starch in various parts of the tract; only the means have therefore been included in Table 5. The negative values for ether extractives and ash in the rumen indicate increases, and so the subsequent disappearances total over 100% of the amounts in the feed. Starch was not determined in the faeces since previous results (Ørskov *et al.* 1969) have shown that only trace amounts were found in faeces when barley was given.

The amino acid compositions of composite samples of abomasal contents were determined but have not been included since there were no systematic changes in amino acid composition with increasing dietary crude protein; there were no differences in the total amounts of amino acids per 16 g nitrogen.

Table 5. *Mean intake and apparent disappearance of starch, ether extractives and ash in various segments of the digestive tract in four sheep receiving rations containing different concentrations of protein*

(Mean values for fifteen observations with standard deviation between observations)

Feed constituent	Intake (g/d)	Disappearance from			Found in faeces (g/d)
		Rumen (g/d)	Small intestine (g/d)	Large intestine (g/d)	
Starch	600	560 ± 65 (93%)	33 ± 13 (6%)	ND	ND
Ether extractives	21	-9 ± 9.6 (-42%)	24 ± 8.1 (115%)	0 ± 2.2 (0%)	6 ± 2.3 (28%)
Ash	68	-29 ± 19.6 (-42%)	37 ± 18.9 (56%)	9 ± 5.4 (13%)	47 ± 10.4 (73%)

ND, not determined.

DISCUSSION

Disappearance of protein in various segments of the gut

The results show that the protein intake significantly affected the amount of protein absorbed from the small intestine. This was apparently not due to increased secretion into the gut as this quantity is small in relation to that passing from the diet (Hogan & Weston, 1967). This finding agrees with observations of Clarke *et al.* (1966), who showed increases in the amount of protein disappearing between the duodenum and the terminal ileum when increasing amounts of soya-bean protein were given.

Hogan & Weston (1967), on the other hand, found no differences between diets containing 7.8 or 19.8% crude protein in the amount of NACP passing the duodenum and showed that the NACP was related to the disappearance of organic matter in the rumen. Microbial nitrogen is more likely to be related to disappearance of carbohydrate than to disappearance of organic matter, and it can be shown in our work that the NACP passing to the abomasum increased from 21 g/100 g of carbohydrate that disappeared in the rumen on diet 1 to 43 g/100 g on diet 4.

The differences between our results and those of Hogan & Weston (1967) are likely to be associated with differences in level of feeding; in our work the mean intake was

about 1000 g dry matter, while in the work of Hogan & Weston the intake was about 450 g dry matter/d, and rate of passage along the digestive tract is likely to affect the amount of dietary protein which escapes fermentation in the rumen. However, solubility of the protein may be more important, and in the work of Hogan & Weston (1967) the high-protein diet was achieved by using lucerne hay and not soya bean as in our experiment.

Since no analytical method, physical or chemical, could reliably separate dietary protein from microbial protein in our samples, the results here cannot conclusively show whether the increased absorption came from increases in microbial nitrogen or from dietary protein which escaped fermentation. It is apparent from Fig. 1 that the digestibility of the NACP entering the small intestine increased with increasing protein concentration in the diet. In the light of estimates that show bacterial protein to be less digestible (approximately 65 % according to Reed, Moir & Underwood, 1949) than soya-bean protein, which is almost completely digestible (Mason, 1969), this would lend support to the contention that the increments consisted mainly of dietary protein which escaped fermentation undegraded. The amino acid composition did not show differences between treatments, probably because soya-bean protein is not sufficiently different in composition from microbial protein. The same observations were made by Potter, Little & Mitchell (1969), who could not detect differences in the amino acid composition of abomasal fluid of sheep receiving urea or soya-bean meal in the diet, and this will be discussed in a subsequent paper (Ørskov, Fraser & McDonald, 1971).

There were no significant differences due to treatment in the amount of nitrogen disappearing from the large intestine. The mean value was 13.6 g NACP/d or 2.2 g nitrogen, which agrees well with observations reported by Goodall & Kay (1965). The nitrogen is likely to have been largely in the form of ammonia arising from deamination of nitrogenous compounds. The amount of nitrogen disappearing from the large intestine is likely to depend upon the amount of substrate being fermented there, as shown by Ørskov, Fraser, Mason & Mann (1970). The influence of treatment on faecal nitrogen was comparatively small (Fig. 1), and this observation agrees with that of Mason (1969) that faecal nitrogen consists mainly of microbial residues. Only a small proportion would consist of indigestible dietary nitrogen.

While increasing protein concentration led to large increases in disappearance of protein from the small intestine, it is also clear from Fig. 1 that substantial losses of dietary protein occurred in the rumen, particularly with the very high concentration of dietary protein. A large increase in the concentration of ammonia nitrogen in abomasal fluid as the dietary protein was increased supports this observation and is in agreement with the observations of McDonald (1948) and of Chalmers & Synge (1954), who showed that there was extensive deamination of protein in the rumen.

Digestion of starch and carbohydrate

The α -linked glucose polymer was almost completely fermented in the rumen (93 %). This agrees with earlier work of Ørskov *et al.* (1969) on starch digestion, which showed that very little barley starch escaped rumen fermentation. It also agrees with

the results of MacRae & Armstrong (1969) and Nicholson & Sutton (1969). Carbohydrates other than those determined as α -linked glucose polymers apparently disappeared in substantial quantities from the small intestine. A mean of 18% of digested carbohydrate was apparently digested in the small intestine, compared with only 6% of the α -linked glucose polymers. This agrees with the results of MacRae & Armstrong (1969) and Karr, Little & Mitchell (1966), who showed that substantial amounts of total reducing sugar disappeared from the small intestine. In our work the total carbohydrate was determined as dry matter less ether extractives, ash, and crude protein. The amount of carbohydrate thus calculated to have been fermented in the rumen is likely to have been a slight underestimate because the factor 6.25 was applied to abomasal NACP, which contains quantities of secretions with a nitrogen content greater than that of protein.

Disappearance of ash and ether extractives

With both ash and ether extractives there were increases in the rumen; they averaged 29 g for ash and 9 g for ether extractives. The amount of ash was highly variable and this was most likely due to addition of salt via saliva. The addition of ether extractives was undoubtedly a result of microbial lipid synthesis. It is possible that a similar synthesis took place in the large intestine also, since the ether extractives determined by the Soxhlet method are likely to underestimate lipids in faeces (Freeman, Holme & Annison, 1968). It is abundantly clear, however, that the apparent digestibility of ash and ether extractives, and indeed of protein, can be very misleading in estimating the amount available to the animal.

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