Digestion of concentrates in sheep

2.* The effect of urea or fish-meal supplementation of barley diets on the apparent digestion of protein, fat, starch and ash in the rumen, the small intestine and the large intestine, and calculation of volatile fatty acid production

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1. Diets of rolled barley supplemented with urea or fish meal at four different levels were given in a change-over experiment to four sheep with cannulas in the abomasum and in the terminal ileum.

2. Estimates were made of the disappearance of protein, ether extractives, starch, and ash in the various segments of the alimentary canal, and of the production of volatile fatty acids when the urea supplements were given.

3. The disappearance (Y, g/d) of non-ammonia crude protein from the small intestine increased with increasing protein intake (X, g/d) on the fish-meal diets according to the equation $Y = 0{\cdot}37X + 44$. There was no increase in the disappearance with the urea supplements.

4. In agreement with earlier work, it was shown that faecal nitrogen excretion was influenced to a much greater extent by fermentation in the large intestine than by that in the rumen. There was an apparent synthesis of ether-extractable lipid in the rumen at rates of 21 and 18 g/d with the urea and the fish-meal diets respectively.

5. The energy of the volatile fatty acids produced when the urea diets were given was estimated to be 59% of the digestible energy consumed.

In a previous communication (Ørskov, Fraser & McDonald, 1971), it was demonstrated that, when a barley diet for sheep was supplemented with increasing amounts of soya-bean meal, large increases occurred in the disappearance of non-ammonia crude protein (NACP) from the small intestine. The increases tapered off at very high intakes of protein, suggesting that the rate of deamination of amino acids in the rumen increased progressively. Contrary to results reported by Hogan & Weston (1967), who showed no difference in the amount of NACP reaching the duodenum when the diet contained 7·8 or 19·8% crude protein, the results here showed a substantial increase when the concentration of protein in the diet was increased from about 10 to 20%. The results suggest that the increases might be due to increases in the amounts of dietary protein escaping rumen fermentation and not to increased production of microbial protein, since microbial protein is likely to be related to the amount of carbohydrate fermentation (Hungate, 1966). The experiments reported here were conducted to supply more information on this point. It was thought that, if the increases consisted of microbial nitrogen, similar increases would occur as a result of urea supplementation. The results of adding urea supplements to barley–concentrate diets have therefore been compared with the results of giving increments of fish meal.

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EXPERIMENTAL

Animals

Four female sheep were used; they were Suffolk and Cheviot Crosses and each sheep weighed approximately 45 kg at the start of the experiment. All the animals were fistulated in the abomasum and terminal ileum as described by Ørskov, Fraser & Kay (1969).

Level of feeding and feed composition

The sheep were offered the same quantity of feed/d as in the previous work (Ørskov *et al.* 1971) which was based on a near *ad lib.* intake, and the amount was fixed in relation to live weight immediately before the experiment began.

The ingredients and composition of the concentrate mixture are given in Table 1. All the diets were pelleted through a 7·7 mm die.

Table 1. *Ingredients and proximate composition on a dry-matter basis of the diets given to sheep*

Treatment	Barley (%)	Urea (%)	Fish meal (%)	Minerals and vitamins* (%)	Crude protein (%)	Starch (%)	Ash (%)	Ether extractives (%)	Energy (kcal/g)
Control	95·4	—	—	4·6	9·5	61·9	5·0	2·1	4·253
Urea level 1	94·5	0·9	—	4·6	12·1	58·8	5·2	1·8	4·285
Urea level 2	93·4	2·0	—	4·6	15·4	62·8	5·0	1·9	4·329
Urea level 3	92·2	3·2	—	4·6	18·9	62·7	5·0	2·1	4·269
Urea level 4	91·2	4·3	—	4·6	23·4	59·8	4·7	1·9	4·165
Fish meal level 1	91·6	—	3·8	4·6	11·3	53·6	5·8	1·8	4·294
Fish meal level 2	86·5	—	8·9	4·6	14·4	56·9	6·8	2·1	4·341
Fish meal level 3	81·3	—	14·1	4·6	18·9	47·1	9·6	2·2	4·283
Fish meal level 4	76·2	—	19·2	4·6	21·5	46·3	9·8	2·3	4·326

* Consisting of 1 % Cr_2O_3 + flour (1:4), 0·6 % Adisco (1000 i.u. vitamin A/g and 200 i.u. cholecalciferol/g; Isaack-Spencer and Co. Ltd, Aberdeen); steamed bone flour, 2·5 %; NaCl, 0·25 % and MgO, 0·25 %. Trace minerals were added to give 0·43 mg $CoSO_4.7H_2O$, 141 mg $MnSO_4.4H_2O$, 191 mg $ZnSO_4.7H_2O$ and 0·2 mg KIO_3/kg.

Design and treatments

Four sheep were given a diet of rolled barley supplemented with urea at four levels in four 14 d periods according to a Latin square design. In another four periods the same four sheep were given the rolled barley diet similarly supplemented. In order that the two sets of treatment comparisons should be made under as nearly as possible the same conditions, the urea periods and the fish-meal periods were alternated. In a final period at the end of the trial all four sheep were given the basal diet without any supplement. Details of the diets are given in Table 1.

Management and sampling procedures

The sheep were kept on slats in individual pens and were given half their daily feed at 08·00 hours and half at 20·00 hours. Uneaten feed was weighed each day and dried to constant weight at 100°; water was freely available. During the last 24 h of each treatment period samples of abomasal, ileal, and rectal contents were obtained

at 2 h intervals as described previously (Ørskov *et al.* 1971); water consumption was recorded.

After the main experiment had been completed, two of the sheep were again given a urea-supplemented diet and used to provide samples of rumen contents. The samples were obtained by stomach pump at 2 h intervals and composite 12 h samples were prepared. It would have been desirable for rumen samples to have been obtained at the same times as the other samples, but it was considered unwise to impose this additional stress on the animals. The proportions of volatile fatty acid (VFA) in abomasal content could not be used as they were found to bear little relation to that found in the rumen.

Analytical procedure

The feeds, abomasal and ileal contents and the faeces were analysed for total nitrogen by the Kjeldahl method and for starch by the method of MacRae & Armstrong (1968). Ether extractives were determined by Soxhlet extraction of freeze-dried material, and ash by ashing at 600°. Chromium oxide was measured by a modification of the method of Stevenson & Clare (1963) as described by Mathieson (1970).

Ammonia was determined by the method of Conway (1957). A modified Technicon amino-acid analyser was used to determine amino acid composition from hydrolysates prepared from freeze-dried abomasal samples from which a composite sample had been made for each treatment. The 'carbohydrate' was calculated as (dry matter) −(ash, ether extractives, and crude protein) except with the urea diets, where only the crude protein in the basal feed was subtracted since urea contains no protein.

The technique of gas–liquid chromatography with flame ionization detector (Pye 104) was used to determine the VFA proportions in acidified samples of rumen contents. The molar percentages of acetic, propionic and butyric acids (a, p and b respectively) were used to estimate the efficiency (E) of conversion of carbohydrate into VFA according to the relationship $100\ (0{\cdot}622a+1{\cdot}092p+1{\cdot}560b)/(a+p+2b)$, as derived by Ørskov, Flatt & Moe (1968). The VFA production was then estimated as $E.M/100$, where M (kcal) is the energy of fermented carbohydrate, which was taken as the sum of the carbohydrate disappearing from the rumen and from the large intestine. To derive the production of each acid, the VFA molar proportions were converted to the respective energy proportion using values in kcal/mole of 209, 367 and 524 for acetic, propionic and butyric acids respectively.

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

RESULTS

After having completed two periods, one sheep contracted pneumonia and subsequently died. It was replaced by a similar sheep. In the analysis of variance the results obtained for these two sheep were treated as if they had come from one animal.

There were occasional refusals of feed, the mean dry-matter intakes (g/d) being 1069 on the control diet, 1083, 967, 1109 and 1004 with the four levels of urea, and 1005, 938, 1077 and 1023 with the four levels of fish meal. To allow for the effects of

these variations in intake on quantities of digesta, all such quantities were adjusted to a dry-matter intake of 1025 g/d, assuming proportionality.

Separate analyses of variance were made for the results for the urea and for the fish-meal diets. Because of doubts as to whether variability was homogeneous, the analyses were not combined, and separate standard errors were calculated for the two sets of results. The standard errors do not apply to the results for the control diet, as these were obtained in a separate experimental period outwith the main design.

Table 2. *Mean concentrations of non-ammonia crude protein and of ammonia in abomasal and ileal contents and of crude protein in faeces of sheep*

(Mean values for four animals)

Treatment	Non-ammonia crude protein (% dry matter)		Crude protein (% dry matter)	Ammonia (mg N/100 ml)	
	Abomasum	Ileum	Faeces	Abomasum	Ileum
Control	24·5	17·1	18·9	7·2	12·9
Urea level 1	24·7	19·0	17·8	12·2	8·4
Urea level 2	25·0	19·5	16·9	21·1	13·7
Urea level 3	22·3	19·1	16·7	24·9	12·4
Urea level 4	23·6	21·5	16·5	30·7	16·3
SE* of means of urea treatments	1·4	1·4	0·8	2·5	3·0
Fish meal level 1	23·1	18·9	17·3	11·0	14·3
Fish meal level 2	25·4	17·1	16·1	15·4	16·2
Fish meal level 3	27·4	19·7	16·0	17·9	18·9
Fish meal level 4	32·0	21·3	15·2	17·0	17·1
SE* of means of fish-meal treatments	1·4	0·8	0·6	1·1	2·3

* Not applicable to unsupplemented diet.

Table 3. *Apparent digestibilities* (%) *for sheep of the dietary components*

(Mean values for four animals)

Treatment	Dry matter	Energy	Crude protein	Ether extractives	Ash	Carbo-hydrate
Unsupplemented diet	81·1	80·9	61·6	85·0	39·6	85·7
Urea level 1	79·0	79·3	70·2	80·6	34·2	82·7
Urea level 2	81·0	81·6	79·0	77·8	27·7	85·9
Urea level 3	80·1	80·7	82·5	74·7	14·8	85·9
Urea level 4	82·7	83·4	88·1	80·9	30·3	87·0
SE* of means of urea treatments	1·8	2·1	3·0	3·7	6·9	1·3
Fish meal level 1	78·1	79·6	66·1	74·0	19·2	84·0
Fish meal level 2	80·9	83·1	78·5	79·2	32·1	85·7
Fish meal level 3	80·3	82·3	83·3	83·0	44·2	84·3
Fish meal level 4	80·2	84·2	85·9	79·7	40·1	84·1
SE* of means of fish-meal treatments	0·6	1·0	1·5	1·5	6·8	1·0

* Not applicable to unsupplemented diet.

Values for NACP and ammonia concentrations in abomasal and ileal contents and for crude protein in faeces are given in Table 2. The only significant effect of increasing the urea supplement was a linear increase in ammonia concentration in abomasal

fluid ($P < 0.01$), but both ammonia and NACP concentrations in abomasal contents increased linearly with increasing fish-meal supplementation ($P < 0.01$), and the percentage of crude protein in the faeces fell ($P < 0.05$).

Values for the apparent digestibilities of dietary components calculated from the chromic oxide marker are presented in Table 3. As the level of supplementation increased, either with urea or with fish meal, there were increases in apparent crude-protein digestibility. The linear component of the increase was highly significant ($P < 0.001$) in both instances, and the quadratic component was significant with fish meal ($P < 0.05$), suggesting that the increase was tapering off. Urea had no other significant effect, but the apparent digestibilities of energy and ether extractives were lower with the lowest level of fish meal than with the three higher levels ($P < 0.05$).

Table 4. *Crude-protein intake, non-ammonia crude protein (NACP) passing the abomasum and terminal ileum and excreted in the faeces and the amounts disappearing in the rumen, small intestine and large intestine of sheep receiving either urea or fish meal as nitrogen supplement (all in g/d)*

(Values have been adjusted to the mean dry-matter intake of 1025 g/d; mean values for four animals)

	N × 6·25		NACP			N × 6·25	
Treatment	Intake	Absorbed from rumen	Passing abomasum	Absorbed from small intestine	Passing terminal ileum	Absorbed from large intestine	Excreted in faeces
Unsupplemented diet	97	−29	127	77	51	13	37
Urea level 1	123	−6	129	76	54	16	37
Urea level 2	157	28	129	67	63	30	33
Urea level 3	193	53	140	83	57	23	34
Urea level 4	240	109	131	80	50	21	29
SE* of means of urea treatments	—	12	13	11	6	5	3
Fish meal level 1	116	−34	151	95	56	17	39
Fish meal level 2	148	9	140	89	51	20	32
Fish meal level 3	194	20	174	117	57	24	33
Fish meal level 4	220	35	186	132	54	23	31
SE* of means of fish-meal treatments	—	6	4	7	6	5	1

* Not applicable to unsupplemented diet.

Table 4 presents mean daily intakes and faecal excretions of crude protein, estimates of the amounts of protein passing the sampling points, and estimates of protein absorption in the various sections of the alimentary tract. As the level of urea supplementation increased, the apparent absorption of crude protein from the rumen increased correspondingly ($P < 0.001$) and there were no significant changes further along the tract. As the level of fish-meal supplementation increased, the disappearance of crude protein from the rumen increased ($P < 0.001$), but to a lesser extent than with urea, and there were significant changes further along. The amount of NACP passing the abomasum increased linearly ($P < 0.001$), as did the amount disappearing from the

small intestine ($P < 0{\cdot}01$). There were also significant effects ($P < 0{\cdot}01$) on the excretion of crude protein in the faeces, which was less for the three higher levels of addition of fish meal than for the lowest level. The disappearance of NACP from the small intestine (Y, g/d), with the basal and with the fish-meal diets, could be described by the equation $Y = 0{\cdot}37X + 44$ (residual standard deviation = 21 g), where (X, g/d) was the crude-protein intake.

Table 5. *Dietary intakes and faecal excretions of starch, ash and ether extractives, and the amounts of each disappearing in the rumen, small intestine and large intestine of sheep receiving either urea or fish-meal supplements (all in g/d)*

(Means with standard deviations between observations, based on sixteen observations after adjustment to the mean dry-matter intake of 1025 g/d. Values in parentheses are the mean values as percentages of the corresponding intakes)

			Apparent disappearance in			
Nitrogen supplement	Feed constituent	Intake	Rumen	Small intestine	Large intestine	Excretion in faeces
Urea	Starch	625	605 ± 12 (97 %)	13 ± 13 (2 %)	—	—
Fish meal	Starch	522	501 ± 7 (96 %)	14 ± 9 (3 %)	—	—
Urea	Ether extractives	20	−21 ± 8 (−108 %)	37 ± 8 (185 %)	0·3 ± 2 (2 %)	4 ± 2 (21 %)
Fish meal	Ether extractives	22	−18 ± 6 (−83 %)	36 ± 5 (165 %)	−1 ± 2 (−3 %)	5 ± 1 (21 %)
Urea	Ash	51	−29 ± 14 (−57 %)	27 ± 12 (53 %)	15 ± 7 (30 %)	38 ± 14 (74 %)
Fish meal	Ash	82	−21 ± 19 (−26 %)	34 ± 27 (41 %)	16 ± 14 (20 %)	52 ± 14 (64 %)

Measurements of the apparent rates of disappearance in the rumen, small intestine and large intestine and of the rates of excretion in the faeces, of starch, ether extractives, and ash are given in Table 5, together with mean values and standard deviations for the two sets obtained during periods of urea and fish-meal supplementation. Only general means for urea and fish-meal supplementation are given in Table 5 since the only significant effect observed with different levels of supplementation was that the intake of ash, and consequently the amounts disappearing, increased with increasing fish-meal supplementation. It will be seen that only about 5 % of the starch in the diet escaped fermentation in the rumen, but that the amount of ether extractives leaving the rumen was about twice that taken in. Amounts of ash leaving the rumen were also in excess of amounts consumed, as indicated by negative values for the 'disappearance' rates.

Table 6 presents estimates of VFA production and fermentation losses for the urea diets. The VFA proportions were acetic acid 46·0 %, propionic acid 39·4 %, isobutyric acid 2·6 %, butyric acid 5·2 %, isovaleric acid 2·0 % and valeric acid 4·8 %. There were no significant treatment differences. Carbohydrate disappearing from the rumen accounted for 91 % of the total carbohydrate fermented, so the error arising from applying the VFA proportions found in the rumen to include the caecum could not

be important as it has been found that the VFA proportions found in the caecum do not differ very markedly from the proportions found in the rumen (Ørskov, Fraser, Mason & Mann, 1970).

Table 6. *Rates of digestible energy intake, carbohydrate fermentation, volatile fatty acid production, and fermentation losses of sheep receiving a diet of rolled barley alone or supplemented with urea at four levels*

(Mean values for four animals, after adjustment to the mean dry-matter intake of 1025 g/d)

Treatment	Digestible energy intake (kcal/d)	Carbohydrate fermented (g/d)	Acetic acid (kcal/d)	Propionic acid (kcal/d)	Butyric acid (kcal/d)	Volatile fatty acids (% of digestible energy)	Fermentation losses methane + heat: kcal	Fermentation losses methane + heat: % of digestible energy
Unsupplemented diet	3529	624	776	1169	222	61·4	437	11·8
Urea level 1	3481	600	742	1116	212	59·6	417	12·0
Urea level 2	3620	661	819	1230	234	63·0	460	12·7
Urea level 3	3530	555	687	1033	196	54·3	386	11·0
Urea level 4	3559	584	722	1086	206	56·6	406	11·4
SE* of treatment means	—	24	30	46	9	2·6	17	0·6
Mean of all observations	3542	605	749	1127	214	59·0	421	11·8

* Not applicable to unsupplemented diet.

Table 7. *Amino acid composition (g amino acid/16 g nitrogen) of abomasal fluid of sheep receiving barley diets supplemented with increasing amounts* (1–4; *see Table* 1) *of urea or fish meal*

Amino acid	Purified diet*	No supplement	Urea supplement 1	Urea supplement 2	Urea supplement 3	Urea supplement 4	Fish-meal supplement 1	Fish-meal supplement 2	Fish-meal supplement 3	Fish-meal supplement 4	Fish meal†
Aspartic acid	7·7	8·0	9·2	8·0	6·5	9·2	7·4	9·0	8·7	9·3	9·0
Threonine	3·9	4·1	5·2	3·9	4·1	4·8	4·0	4·6	4·7	4·9	4·2
Serine	4·0	4·7	4·7	4·1	3·8	4·8	4·7	4·5	4·6	4·8	4·0
Glutamic acid	8·9	13·1	11·1	10·6	11·5	11·0	12·5	11·5	12·0	12·4	13·5
Proline	2·1	3·9	3·7	3·1	4·2	4·9	3·9	4·2	4·6	4·1	—
Glycine	3·9	4·6	4·1	4·2	4·5	4·2	5·2	5·7	5·6	6·0	6·8
Alanine	6·1	5·8	6·1	5·8	6·0	5·5	5·7	6·0	6·1	6·4	6·3
Valine	4·9	5·1	4·9	4·9	4·4	5·1	5·4	5·0	5·2	5·5	5·4
Cystein	1·1	1·1	1·0	0·9	0·8	1·1	1·1	0·8	0·9	1·3	1·6
Methionine	2·0	1·8	2·1	2·1	2·3	2·1	2·2	2·7	2·8	3·2	3·1
Isoleucine	4·2	4·3	4·1	3·7	3·8	4·3	4·1	4·0	4·3	4·6	4·2
Leucine	5·9	6·2	6·6	6·4	6·0	6·3	7·1	6·8	7·3	7·9	7·2
Tyrosine	3·3	3·4	3·7	3·7	3·5	4·1	3·5	3·8	4·5	4·4	3·0
Phenylalanine	3·4	3·8	4·3	3·6	4·0	3·9	3·7	4·2	4·7	4·8	4·0
Lysine	6·2	6·5	6·7	6·2	6·0	5·9	6·8	6·9	6·9	7·5	7·1
Histidine	1·7	1·8	1·8	1·7	2·0	2·0	1·7	2·1	2·2	2·2	2·1
Arginine	4·2	2·2	4·3	4·1	4·3	4·9	5·0	8·2	5·4	5·5	5·8
α-γ-Diaminopimelic acid	0·9	1·9‡	0·8	0·7	0·7	0·5	0·6	0·5	0·6	0·4	—
Total	74·4	82·3	84·4	77·7	78·4	84·6	84·6	90·5	91·1	95·2	—

* Abomasal fluid from a purified diet in which urea supplied 95% of the dietary nitrogen.
† Fish meal with similar nitrogen content (Harvey, 1970).
‡ Difficult to separate accurately and likely to be an overestimate.

The results of amino acid analyses made on a single composite sample of freeze-dried abomasal contents for each treatment are given in Table 7. There do not appear to have been any trends in the results with increasing amounts of urea, but the concentration of several amino acids, for example methionine, and also the total amino acid content per 16 g nitrogen increased with increasing fish-meal supplementation.

The mean water intakes with the four levels of urea were 3·5, 3·9, 4·7 and 4·6 l/d (SE of differences, ± 1·0 l/d). With the four levels of fish meal they were 2·4, 2·7, 3·6 and 2·8 l/d (SE of differences, ± 1·0 l/d). There were significant differences from animal to animal in water intake, but there was no apparent association between the water intake and the rates of disappearance of digesta in the different parts of the tract.

DISCUSSION

The results demonstrate that increased fish meal in the diet leads to a substantially increased disappearance of protein from the small intestine, and they agree with the results of Clarke, Ellinger & Phillipson (1966). Our previous results with soya-bean meal (Orskov *et al.* 1971) were similar, except that the rate of disappearance levelled off at the higher levels of soya-bean meal. The regression equation shows that the additional absorption amounted to 0·37 g/d for each additional g/d of crude protein taken in. It appears from the results that more than half of the increase in crude-protein intake was degraded in the rumen, although the possibility of direct absorption of some amino acids from the rumen cannot, perhaps, be ruled out. The earlier findings of McDonald (1948) and Chalmers & Synge (1954) regarding the extensive destruction of protein in the rumen are supported by the observed increase in ammonia concentration in the abomasal fluid as the amount of fish meal was increased. It was also shown recently (Ørskov, Fraser & Corse, 1970) that the efficiency of utilization of fish meal could be improved by about 30% by giving it in liquid form in such a way that it by-passed the rumen.

There are several reasons for believing that the extra protein passing the abomasum consisted of fish-meal protein that had escaped degradation in the rumen rather than of additional microbial protein. The increased amounts were almost completely digestible, for there were no increases in the amount passing the terminal ileum; this is in keeping with the greater digestibility of fish meal compared with microbial protein (Reed, Moir & Underwood, 1949). The changes in amino acid composition of abomasal fluid, with increasing levels of fish meal, also support this belief, since fish meal has a higher content of the sulphur-containing amino acids, and of total amino acids per 16 g nitrogen, than have rumen micro-organisms (Hungate, 1966; Harvey, 1970).

When urea was added to the diet there were no significant increases in NACP passing the abomasum or absorbed from the small intestine. This observation would suggest that rumen microbial growth was not limited by shortage of protein in the basal diet, and secondly that under these experimental conditions the addition of urea did not save dietary protein in barley from degradation in the rumen. These findings may not apply to younger ruminants, which have given definite responses in growth rate when a small amount of urea was added to a barley diet (Kay, MacLeod &

Macdearmid, 1969). The sheep in this experiment were over 1 year old and their nitrogen retention would be relatively very low. We have no direct evidence, but it may well be that in our animals there was much more recycling of nitrogen back to the rumen via the blood-stream than there would be with animals that were actively synthesizing body protein. Recent work reported by Sharma, Van't Klooster & Frens (1969) supports our observations. When these workers added urea to a semi-purified diet to supply about 1·1 g nitrogen/100 g dry matter, the amount of protein passing the duodenum did not increase when they added more urea.

The amount of protein disappearing in the large intestine did not vary significantly with the level of either fish meal or urea supplementation, the mean value for all treatments being 21 g/d. Most of this disappearance is likely to have been due to deamination, and the rate is likely to have been controlled largely by the amount of substrate fermented in the large intestine (Ørskov, Fraser, Mason & Mann, 1970).

It was shown by Ørskov, Fraser, Mason & Mann (1970) that starch infused via the terminal ileum into the caecum gave rise to much greater increases in faecal nitrogen than starch entering the rumen in the feed. It was further shown that this was due to the microbial growth when a substrate was provided in the large intestine. Since no digestion of microbes takes place beyond the large intestine, the influence of caecal fermentation on faecal nitrogen excretion would be greater than that of fermentation in the rumen.

To see how far these observations were confirmed by the present results, a study was made of the relationship between faecal nitrogen (*FN*, g/d) and the carbohydrate disappearance in the rumen (*CDR*, g/d) and in the large intestine (*CDL*, g/d). The regression coefficients were very similar for the urea and for the fish-meal diets and have been combined, giving the following equation

$$FN = 0{\cdot}0081CDR + 0{\cdot}0159CDL + \text{constant}; \text{ residual standard deviation} = 0{\cdot}97.$$

The standard errors of the regression coefficients were 0·0017 and 0·0042 for *CDR* and *CDL* respectively.

The constant took the value 0·17 for the urea and 0·96 for the fish-meal diets. Almost as good a fit could be achieved by an equation in which *CDR* and *CDL* were combined, with a single regression coefficient of $0{\cdot}0093 \pm 0{\cdot}0015$, so the results do not by themselves prove that more faecal nitrogen is derived per unit of carbohydrate disappearing in the large intestine than in the rumen. However, the difference in the regression coefficients is in the expected direction and almost reaches statistical significance ($P = 0{\cdot}05$) using a one-tailed test. The results may therefore be considered to lend some support to the observations of Ørskov, Fraser, Mason & Mann (1970).

The results here gave a faecal excretion of about 0·8 g nitrogen/100 g carbohydrate fermented in the rumen and about 1·6 g nitrogen/100 g carbohydrate fermented in the large intestine. It is possible that the variability which was not related to carbohydrate fermented was due to variation in the water-soluble fraction of the faeces which has not been found to have any meaningful relationship to intake (Mason, 1969 and personal communications).

The disappearances of starch, ether extractives, and ash were similar to what had been observed in previous work (Ørskov *et al.* 1971) except that the amount of ether

extractives apparently synthesized in the rumen was greater in this experiment (21 g/d).

The estimates of the rate of VFA production with the urea diets were not complicated by differences in protein disappearance. The estimate that total VFA production was equivalent to 59% of the digestible energy of the diets is in good agreement with other estimates obtained by isotope dilution procedure by Bergman, Reid, Murray, Brockway & Whitelaw (1965) of 62% of digestible energy and by Leng, Corbett & Brett (1968) of 53% of digestible energy.

The assumptions involved in the use of these calculations have been discussed (Ørskov *et al.* 1968) and will not be detailed here, but, in view of the similarity between the estimates obtained by this method and the isotope dilution procedure, a direct comparison between the two methods would be of value since the method used here is extremely simple.

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