Digestion and synthesis in the rumen of sheep given diets supplemented with free and protected oils

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1. Six wether sheep were each provided with a permanent cannula in the rumen and re-entrant cannulas in the proximal duodenum.

2. In a preliminary study, the sheep consumed 200 g hay and 400 g concentrates supplemented with up to 40 g linseed oil, coconut oil or cod-liver oil daily. Feed was refused at higher levels of supplementation.

3. Five of the sheep were used in a 5 × 5 Latin-square experiment. They were given 200 g hay and 400 g concentrates alone (B) or supplemented with 40 g linseed oil (L), coconut oil (C), protected linseed oil or protected coconut oil daily. The protected oils were prepared by emulsifying the free oils with formaldehyde-treated sodium caseinate. Formaldehyde-treated sodium caseinate was also included in the other three diets.

4. Digestion in the stomach was measured by spot sampling duodenal digesta, using chromic oxide-impregnated paper as the marker. Microbial flow at the duodenum was measured by use of both diaminopimelic acid (DAPA) and RNA as microbial markers.

5. Both the free oils had broadly similar effects despite their very different fatty acid compositions. Digestion in the stomach of organic matter (OM) was reduced from 0·48 (diet B) to 0·29 (diets L and C) and that of neutral-detergent fibre from 0·50 (diet B) to 0·19 (diet L) and 0·12 (diet C). The molar proportions of acetic acid and *n*-butyric acid were decreased and that of propionic acid was increased. Protozoal numbers were reduced by 78% (diet L) and 90% (diet C). The flow of total nitrogen and microbial N was increased by both oils and the efficiency of microbial protein synthesis (g N/kg OM apparently digested in the rumen) was increased from 30 (diet B) to 85 (diet L) and 74 (diet C) when based on DAPA and from 41 (diet B) to 94 (diet L) and 81 (diet C) when based on RNA. The efficiency when based on true digestion of OM (g N/kg OM truly digested in the rumen) was increased from 23 (diet B) to 46 (diet L) and 44 (diet C) when based on DAPA and from 29 (diet B) to 49 (diet L) and 46 (diet C) when based on RNA. The amounts of microbial OM (g/d) at the duodenum were increased from 68 (diet B) to 124 (diet L) and 106 (diet C) when based on DAPA and from 92 (diet B) to 136 (diet L) and 115 (diet C, non-significant) when based on RNA.

6. When the oils were given in the protected form, the effects on digestion in the stomach were reduced but not eliminated. No significant increases in the amount of total N or microbial N at the duodenum were established, though there was a tendency for an increase in the efficiency of microbial protein synthesis with protected linseed oil. The results suggested that the method of protection used reduced the effects of the oils on rumen digestion and synthesis but was only partially successful in preventing hydrogenation of the fatty acids.

7. It is concluded that free oils can markedly increase the efficiency of microbial protein synthesis, possibly by their defaunating effect, and that this may enhance the potential for using non-protein-N on oil-supplemented diets.

Dietary lipid supplements are known to cause extensive modification to digestion in the rumen. Reductions have been demonstrated in fibre and organic matter (OM) digestion, methanogenesis, ammonia concentrations and the acetate : propionate value (Devendra & Lewis, 1974*a*; Palmquist & Jenkins, 1980). More recently it has been suggested that free oils may also alter synthesis of protein and lipids by rumen microbes (Czerkawski *et al.* 1975; Sutton *et al.* 1975). The effects appear to be due partly to inhibitory activity by certain of the medium- and long-chain free fatty acids released by hydrolysis in the rumen, and partly to a physical coating of the microbes and feed particles. Methods developed for protecting lipids from rumen metabolism offer the possibility that lipids fed in such a form may interfere less with rumen metabolism, but indirect evidence from experiments with milking cows has suggested that the technique is only partly effective in this respect (Bines

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et al. 1978). No direct comparison of the effects of oils in the free and protected forms on rumen digestion appears to have been published.

The purpose of the present studies was to examine in greater detail the effects of free lipid supplements on the processes of digestion and synthesis in the rumen of sheep and to see whether the extent of these effects was reduced when the lipids were given in the protected form. A brief report of some of these results has been published previously (Knight *et al.* 1978).

EXPERIMENTAL

Animals and management. Six Suffolk × Scottish half-bred wether sheep, aged approximately 18 months at the beginning of the experiments and weighing approximately 40 kg, were used. They were each fitted with a permanent cannula in the rumen and a re-entrant cannula (Ash, 1962) in the duodenum, approximately 50 mm beyond the pylorus and proximal to the point of entry of the common bile duct.

All the sheep were kept in individual metabolism crates indoors throughout.

Feeds. The basal feeds were chopped hay (872 g dry matter (DM)/kg air-dry feed; 928 g organic matter (OM), 19·2 g nitrogen and 694 g neutral-detergent fibre (NDF)/kg DM and a concentrate mix (866 g DM/kg air-dry feed; 960 g OM, 21·5 g N and 240 g NDF/kg DM consisting of 936 kg rolled barley, 49 kg groundnut meal and 15 kg low-copper mineral supplement (Boots Farmsales Ltd, Witney, Oxon) per tonne. To reduce the possibility of Cu toxicity, each sheep was provided with 1·1 g anhydrous sodium sulphate and 0·02 g ammonium molybdate in the mix daily (Cammell, 1977).

Coconut oil, raw linseed oil and cod-liver oil (Super Solvitax; British Cod Liver Oils Ltd, Hull) were used. The linseed oil was stabilized by the addition of an antioxidant, butylated hydroxytoluene (2,6 ditert-butyl-*p*-cresol) to give a concentration of 0·2 g/l. Protected forms of linseed oil and coconut oil were prepared by a method similar to that described by Scott *et al.* (1971) in which an emulsion of 8 kg oil, 4 kg sodium caseinate and 550 ml formaldehyde solution (370–400 g/l) was prepared and spray-dried (Unigate Ltd, Bourton, Dorset). On analysis the protected products were found to contain 45 g N and 653 g total lipid/kg DM. Protected sodium caseinate was used in some treatments. It was prepared by dissolving 2 kg sodium caseinate in 10 l water at 70° and adding 275 ml formaldehyde solution. The resulting gel was dried at 80° on a hot-air-bed drier and milled.

Expt 1

The first experiment was a preliminary study to compare the acceptability and rumen fermentation characteristics in sheep given the basal diet supplemented with each of the three free oils. The three oils were allocated at random to the six sheep such that each oil was offered to two sheep. The basal ration of 200 g hay and 400 g concentrate mix/d was given in two equal portions at 06.00 and 16.30 hours. Uneaten food was removed and weighed at 16.00 hours. Drinking water was available at all times. The free oils were mixed daily by hand into the ration of concentrates. The amount of oil offered was increased by 10 g every 3 d until refusals of feed occurred. The sheep then continued on the basal diet supplemented with the highest level of oil that they would accept without refusals. After 5 weeks on the oil-supplemented diets rumen samples were taken for volatile fatty acid (VFA) analysis and protozoa counts on 3 d at 09.00 and 16.30 hours. Rumen samples continued to be taken on 3 d in each week for 6 weeks after withdrawal of the supplements.

Expt 2

Five of the six sheep in Expt 1 were used. They were given a basal diet alone or supplemented with 40 g daily of linseed oil or coconut oil, either in the free or protected

forms in a 5 × 5 Latin-square design. The basal diet consisted of (g/d) 200 hay, 380 concentrate mix and 20 protected casein. For the two free-oil treatments, the ration of oil was mixed into the basal concentrates and protected casein daily. For the protected-oil treatments, the protected casein was omitted and 60 g protected-oil supplement were added. The sheep were fed at 06.00 and 18.00 hours. For 2 weeks before and during collections of duodenal digesta, 2·5 g chromic oxide paper, providing 0·88 g Cr_2O_3, was added to the rumen at each feeding time.

Sampling. Each period consisted of 42 d. For the first 10 d, to reduce carry-over effects from the previous treatment, all the sheep were given the basal diet and, in addition, about 100 ml rumen fluid from a donor sheep receiving the basal diet were added to the rumen contents of each sheep to provide an inoculum of a mixed microbial population. A period of 19 d was allowed for introduction of the oils and adaptation to the diets; during this time oils were increased by 10 g/d every 2 d until an intake of 40 g/d was achieved. A period of 13 d was then allowed for sampling as follows: days1–9 faecal collection by harness and bag, days 2–6 spot sampling duodenal digesta and days 9–13 rumen samples for preparation of a bacterial fraction. Small samples of rumen fluid were also taken at 09.00 and 16.30 hours on 3 d each week; a portion was mixed with an equal volume of glycerol–water–formalin (500:450:50, by vol.) for protozoa counts and the remainder was stored at −20°. Faeces were bulked and stored at −20°. Duodenal digesta was collected by blocking the distal duodenal cannula and collecting about 150 ml digesta flowing from the proximal cannula. Twelve samples were taken from each sheep over 5 d to cover hourly intervals from 12.00 to 24.00 hours. The samples were bulked and stored at −20°. Samples of rumen fluid (300–500 ml) for bacterial preparations were taken over 5 d such that samples covered 0, 3 and 5 h after feeding. Bacterial samples were separated by differential centrifugation as described by Smith & McAllan (1974). Portions were removed and extracted for RNA analysis and the remainder was stored at −20° for other analyses.

Chemical analyses. The DM content of feeds was determined by drying at 100° for 2 d. The DM content of digesta, bacterial preparations and faeces was determined by freeze-drying. Samples of feed, digesta and faeces were analysed for OM by ashing at 550° for 4 h, N by the Kjeldahl technique, NDF (Van Soest & Wine, 1967) and gross energy by adiabatic bomb calorimetry. Ammonia in duodenal fluid was measured by the Conway (1957) method, rumen VFA by gas–liquid chromatography (Sutton & Johnson, 1969) and the Cr_2O_3 content of total duodenal digesta by the method of Stevenson & de Langen (1960). Duodenal digesta and rumen bacteria were analysed for RNA by the method of McAllan & Smith (1969) and for diaminopimelic acid (DAPA) by the method of Smith *et al.* (1978). Rumen bacteria samples were also analysed for OM and total N. Lipids were extracted in chloroform–methanol (2:1 v/v) and the fatty acids were analysed by gas–liquid chomatography of the methyl esters (Newport *et al.* 1979).

Calculations. Flow of digesta at the duodenum was calculated by reference to Cr_2O_3. The flow of microbial material at the duodenum was calculated from the concentration of RNA or DAPA in the separated bacterial fraction from rumen contents and in total duodenal digesta. As recent studies have established that a small proportion of the RNA at the duodenum may be of feed origin, the RNA content of duodenal digesta was multiplied by 0·85 (Smith *et al.* 1978). Although DAPA, unlike RNA, is generally considered to be a marker for bacteria only, for simplicity of presentation it will be described as a marker for microbial flow (i.e. bacteria and protozoa) except where a clear distinction between bacterial and protozoal contributions is discussed. In the present paper apparent digestion will be referred to as digestion whereas food minus duodenal flow plus microbial flow will be referred to as true digestion in the stomach.

RESULTS

Expt 1

All the free-oil supplements were accepted when provided at up to 40 g/d in the concentrate but some feed was uneaten when 50 g/d were offered. Supplementation was therefore reduced to 40 g/d.

The mean molar proportions of VFA in the rumen 5 weeks after the oils were first introduced were characterized by relatively low proportions of acetic acid and, to a lesser extent, butyric acid and high proportions of propionic acid (Table 1). The number of

Table 1. *Expt 1. Mean weekly values for the concentration of total volatile fatty acids (VFA), molar proportions of the major VFA and index of protozoa numbers in the rumen fluid of sheep receiving a basal diet supplemented with 40 g/d of one of three free oils*

(Each value is the mean for two sheep)

Time (weeks)	Total VFA (mmol/l)	Acetic acid	Propionic acid	Butyric acid	Protozoa nos. (scale 0–3)*
		(mol/mol total VFA)			
			Linseed oil		
After introduction					
5	71	0·58	0·30	0·07	0·5
After withdrawal					
1	78	0·59	0·26	0·09	1
2	66	0·63	0·18	0·14	2
3	74	0·64	0·19	0·12	1·5
			Coconut oil		
After introduction					
5	64	0·59	0·27	0·09	1
After withdrawal					
1	64	0·61	0·25	0·09	0
2	60	0·64	0·16	0·13	1
3	56	0·65	0·17	0·13	1
			Cod-liver oil		
After introduction					
5	72	0·54	0·32	0·10	1·5
After withdrawal					
1	64	0·58	0·25	0·11	1·5
2	61	0·63	0·20	0·12	2
3	62	0·63	0·19	0·13	2

* Scale corresponds approximately to 1×10^4 protozoa/ml at 0·5 to 5×10^5 protozoa/ml at 2·0.

protozoa was low compared to 2 and 3 weeks after withdrawal of the oils. Rumen VFA proportions returned to stable values within 2 weeks of withdrawal of the oils.

There were no clear differences among the oils in terms of their acceptability or effects on rumen fermentation. Two oils differing widely in their fatty acid compositions were required for Expt 2 and coconut oil and linseed oil were chosen.

Expt 2

Diets were normally eaten rapidly and no refusals of feed occurred during the sampling periods. Occasionally, sheep were reluctant to eat all the feed when the maximum intake of oil was first achieved, particularly in the case of coconut oil. However, full feed consumption resumed after 2–3 d.

Table 2. *Expt 2. Mean dry matter (DM) content and composition of the DM of the hay and of the basal concentrates supplemented with protected casein (B), protected casein plus linseed oil (L), protected linseed oil (PL), protected casein plus coconut oil (C) and protected coconut oil (PCO)*

Treatment ...	Hay	B	L*	PL*	C*	PC*
DM (g/kg feed)	872	873	884	884	884	884
Composition of DM (g/kg)						
Organic matter	928	962	966	966	966	966
Gross energy (MJ)	18·517	18·064	20·285	20·604	20·100	20·416
Neutral-detergent fibre	694	226	203	203	203	203
Nitrogen	19·2	28·3	25·4	25·1	25·4	25·1

* Calculated from the mean composition of the basal concentrates and supplements which were analysed separately.

Table 3. *Expt 2. The content (mg/g dry matter) of major fatty acids in the free and protected oil supplements*

Fatty acid	Linseed oil	Protected linseed oil	Coconut oil	Protected coconut oil
6:0	—	—	1·1	0·7
8:0	—	—	75·5	53·3
10:0	—	—	46·4	34·0
12:0	—	—	445·9	309·6
14:0	—	1·0	163·4	109·3
16:0	56·1	37·8	74·2	50·3
16:1	1·8	—	—	—
18:0	35·1	22·6	22·2	14·6
18:1	173·2	111·4	60·8	42·5
18:2	148·9	94·7	15·0	9·7
18:3	555·9	363·2	1·1	0·4
20:0	2·5	1·8	—	—
Others	10·1	6·7	14·1	5·2

The mean compositions of the hay and the various supplemented concentrates are shown in Table 2 and the fatty acid contents of the free- and protected-oil supplements are shown in Table 3.

The intake and digestion of OM are given in Table 4. Digestion in the total tract was reduced by both free oils but was unaffected by the protected oils. The amount of OM entering the duodenum was increased by all the supplements but the increase was greater for the oils in the free form than in the protected form. This increase partly reflected the higher intake on the supplemented diets but a bigger contributor, particularly with the free oils, was a large depression in digestion in the stomach. Digestion was reduced by 40% of the free oils, but the reduction was only 21% with the protected linseed oil and it did not attain significance for protected coconut oil. Associated with the reduced digestion in the stomach was a reduction in the contribution of digestion in the stomach to over-all digestion of 37% for the free oils but rather less for the protected oils. Thus, whereas only 37% of OM digestion occurred in the intestines on the basal diet, this was increased to 60% with the free oils.

Table 4. *Expt 2. The mean amounts (g/d) of organic matter (OM) consumed, passing to the duodenum and excreted in the faeces and its apparent digestion in the stomach and total tract of five sheep given a basal diet alone (B) or supplemented with 40 g/d linseed oil or coconut oil in the free (L, C) or protected (PL, PC) forms*

(Each value is the mean for five sheep)

Treatment ...	B	L	PL	C	PC	SEM
OM (g/d)						
Food	490	532	537	529	531	2·3
Duodenum	255	378***	333***	377***	299**	8·4
Faeces	116	142***	128	152***	126	4·2
Digestion						
Stomach	0·48	0·29***	0·38***	0·29***	0·44	0·016
Total tract	0·76	0·73*	0·76	0·71***	0·76	0·008
$\frac{\text{Stomach}}{\text{Total tract}}$	0·63	0·40***	0·50**	0·40***	0·57	0·021

Differences between the supplemented diets and the basal diet were significant: $*P < 0·05$, $**P < 0·01$, $***P < 0·001$.

Table 5. *Expt 2. The mean amounts (MJ/d) of gross energy passing to the duodenum and excreted in the faeces and its apparent digestion in the stomach and total tract of five sheep given a basal diet alone (B) or supplemented with 40 g/d linseed oil or coconut oil in the free (L, C) or protected (PL, PC) forms*

(Each value is the mean for five sheep)

Treatment ...	B	L	PL	C	PC	SEM
Gross energy (MJ/d)						
Food	9·457	10·979	11·250	10·967	11·105	0·0422
Duodenum	5·288	8·466***	7·477**	8·008***	6·501***	0·1884
Faeces	2·431	3·083***	2·806*	3·212***	2·701	0·0951
Digestion						
Stomach	0·44	0·23***	0·34**	0·27***	0·41	0·019
Total tract	0·74	0·72	0·75	0·71*	0·76	0·009
$\frac{\text{Stomach}}{\text{Total tract}}$	0·59	0·32***	0·45**	0·38***	0·55	0·025

Differences between the supplemented diets and the basal diet were significant: $*P < 0·05$, $**P < 0·01$, $***P < 0·001$.

The intake and digestion of energy are shown in Table 5. The results were closely similar to those for OM.

All the supplements caused large depressions in the digestion of NDF in the stomach (Table 6). The depression was particularly large with the free oils, amounting to 62% for linseed oil and 76% for coconut oil, but even in the protected form, linseed oil and coconut oils reduced digestion by 42% and 24% respectively. The oils also caused large reductions in digestion throughout the digestive tract, the effect of the free oils again being greater than the effect of the protected oils, but the depression was smaller for digestion in the total tract than for digestion in the stomach. In consequence, although only 24% of NDF digestion occurred post-ruminally on the basal diet, the percentage increased to 35–49% with the protected oils and 63–74% with the free oils. Thus increased post-ruminal digestion compensated to a considerable extent for the severe depression in rumen digestion caused by the oils.

Table 6. *Expt 2. The mean amounts (g/d) of neutral-detergent fibre (NDF) passing to the duodenum and excreted in the faeces and its apparent digestion in the stomach and total tract of five sheep given a basal diet alone (B) or supplemented with 40 g/d linseed oil or coconut oil in the free (L, C) or protected (PL, PC) forms*

(Each value is the mean for five sheep)

Treatment ...	B	L	PL	C	PC	SEM
NDF (g/d)						
Food	199	200	199	198	199	0·5
Duodenum	99	160***	141**	173***	124*	7·6
Faeces	68	94***	83**	106***	84**	2·9
Digestion						
Stomach	0·50	0·19***	0·29**	0·12***	0·38*	0·041
Total tract	0·66	0·53***	0·59**	0·46***	0·58**	0·014
$\frac{\text{Stomach}}{\text{Total tract}}$	0·76	0·37**	0·51*	0·26***	0·65	0·078

Differences between the supplemented diets and the basal diet were significant; $*P < 0·05$, $**P < 0·01$, $***P < 0·001$.

Table 7. *Expt 2. The mean concentration of total volatile fatty acids (VFA) (mmol/l), molar proportions of the individual VFA and the protozoa numbers ($\times 10^{-4}$/ml) in the rumen fluid of five sheep given a basal diet alone (B) or supplemented with 40 g/d linseed oil or coconut oil in the free (L, C) or protected (PL, PC) forms*

(Each value is the mean for five sheep)

Treatment ...	B	L	PL	C	PC	SEM
Total VFA (mmol/l)	59·3	58·2	57·8	63·4	62·4	2·24
Molar proportions (mol/mol total VFA):						
Acetic acid	0·651	0·542***	0·615**	0·521***	0·642	0·0077
Propionic acid	0·179	0·333***	0·237**	0·374***	0·211	0·0093
Isobutyric acid	0·011	0·010	0·014**	0·005***	0·010	0·0006
n-Butyric acid	0·127	0·082***	0·093**	0·076***	0·105**	0·0057
Isovaleric acid	0·017	0·019	0·024**	0·006***	0·016	0·0013
n-Valeric acid	0·013	0·013	0·016	0·018*	0·015	0·0014
n-Caproic acid	0·002	0·000**	0·002	0·001	0·003	0·0004
Protozoa numbers (10^{-4}/ml)	85·1	18·8***	45·9**	8·5***	63·4	7·26

Differences between the supplemented diets and the basal diet were significant: $*P < 0·05$, $**P < 0·01$, $***P < 0·001$.

Oil supplementation of the diet had no effect on the concentration of total VFA but marked changes in the molar proportions of individual VFA were observed (Table 7). Both free oils caused large reductions in the molar proportions of acetic acid and *n*-butyric acid and approximately doubled the proportion of propionic acid. In addition, coconut oil caused large reductions in the proportions of isobutyric acid and isovaleric acid, whereas linseed oil was without effect. The effects of linseed oil in the protected form were similar to those of the free oil but considerably less marked except in the case of isobutyric and isovaleric acids, both of which were increased. Protected coconut oil had only very small and generally non-significant effects on rumen VFA.

The numbers of protozoa were greatly reduced by the free oils but, as with changes in VFA, the effects were less marked with the protected oils. Protected linseed oil almost halved the numbers of protozoa but protected coconut oil was without significant effect.

In addition to the rumen samples taken during the sampling period, samples were also taken throughout the experiment. The results indicated that, following withdrawal of the oils, proportions of rumen VFA and protozoa numbers returned to values found on the basal diet by the end of the 10 d recovery period at the beginning of each experiment period and, further, that the main effects of the oils on rumen fermentation were achieved within 5 d of the full treatments being applied.

Table 8. *Expt 2. The mean amounts (g/d) of total nitrogen consumed, passing to the duodenum and excreted in the faeces and the flow (gN/d) of some nitrogenous constituents in duodenal digesta in five sheep given a basal diet alone (B) or supplemented with 40 g/d linseed oil or coconut oil in the free (L, C) or protected (PL, PC) forms*

(Each value is the mean for five sheep)

Treatments ...	B	L	PL	C	PC	SEM
Total N						
Food	12·87	12·70	12·97	12·78	12·91	0·087
Duodenum	15·41	18·81*	16·50	19·90**	14·68	0·865
Faeces	3·61	3·66	3·23	3·60	3·14*	0·128
N flow in the duodenal digesta						
Ammonia-N	1·17	1·14	1·32	1·15	1·19	0·085
Non-ammonia-N	14·24	17·67*	15·18	18·75**	13·21	0·827
Microbial N†						
(*a*) DAPA	7·07	12·75***	9·20	10·18**	8·42	0·718
(*b*) RNA	9·54	13·90*	13·00	12·44	10·28	1·230

Differences between the supplemented diets and the basal diet were significant: $*P < 0·05$, $**P < 0·01$, $***P < 0·001$.

† Based on the use of (*a*) diaminopimelic acid (DAPA) or (*b*) RNA as the microbial marker.

The amounts of total N in the food, duodenal digesta and faeces and various components of total N in duodenal digesta are show in Table 8. With all the diets, the amount of N passing to the duodenum exceeded the amount consumed. On the basal diet the increase ws 2·5 g/d and broadly similar increases were obtained with the protected oils, but with free-oil supplementation it increased to 6·1 (linseed oil) and 7·1 (coconut oil) g/d. The amount of total N entering the duodenum from the stomach was approximately 20% and 30% greater with linseed oil and coconut oil respectively than with the basal diet, whereas with the protected oils the flow of total N was similar to that on the basal diet. No differences were detected among the treatments in the amount of ammonia-N so effects of the treatments on the amounts of non-ammonia-N closely reflected the effects on total N. Estimates of microbial N based on DAPA as the marker indicated that the increase in total N was primarily due to large increases in microbial protein synthesis. When RNA was the marker, all estimates of microbial N flow were higher and, although the free oils were again found to cause increases in the amount of microbial N, the increases were smaller and were only significnt in the case of free linseed oil. The protected oils generally had only small and non-significant effects on N components of duodenal digesta though there was a tendency for microbial N to increase, particularly with protected linseed oil. Faecal N was unaffected by the free oils but it was reduced by both the protected oils, significantly ($P < 0·05$) so in the case of protected coconut oil. This suggests that the extent of protection of the casein may have been less in the commerically-prepared products than in the protected casein prepared in the laboratory. However, in vitro incubation of the three protected products in rumen contents before the start of the experiment had shown ammonia production from all three products to be almost completely inhibited.

Table 9. *Expt 2. The mean amount of microbial organic matter (OM) at the duodenum, the mean amount of OM digested in the stomach and the efficiency of microbial protein synthesis in the stomach of five sheep given a basal diet alone (B) or supplemented with 40 g/d linseed oil or coconut oil in the free (L, C) or protected (PL, PC) forms*

(Each value is the mean for five sheep)

Treatment ...	B	L	PL	C	PC	SEM
Microbial OM (g/d)†						
(*a*) DAPA	68	124***	89	106**	75	8·1
(*b*) RNA	92	136*	127	115	92	12·7
OM digested in stomach (g/d)						
Apparent ($ADOM_R$)	235	154***	204*	152***	232	8·4
True ($TDOM_R$)†						
(*a*) DAPA	303	278	293	258**	307	9·1
(*b*) RNA	327	290*	331	267**	324	11·2
Efficiency of microbial protein synthesis†						
g N/kg $ADOM_R$						
(*a*) DAPA	30	85***	46	74***	37	6·4
(*b*) RNA	41	94**	64	81**	44	8·9
g N/kg $TDOM_R$						
(*a*) DAPA	23	46***	31	44***	27	5·5
(*b*) RNA	29	49***	39*	46**	31	6·4

Differences between the supplemented diets and the basal diet were significant: $*P < 0·05$, $**P < 0·01$, $***P < 0·001$.

† Based on the use of (*a*) diaminopimelic acid (DAPA) and (*b*) RNA as the microbial marker.

Further details of microbial synthesis in the rumen are given in Table 9. The effect of treatments on the amount of microbial OM at the duodenum was very similar to the effect on the amount of microbial N. A consequence of the increased flow of microbial OM with the free oils was that, although the oils depressed the amount of OM apparently digested in the rumen by 35% ($P < 0·01$) their effect on the amount of OM truly digested was much less, being approximately 15–20% with coconut oil ($P < 0·01$) and approximately 10%, and significant ($P < 0·05$) only with RNA as the marker, with linseed oil. There were no significant effects of the protected oils on the amount of OM truly digested in the stomach.

The efficiency of microbial protein synthesis in relation to apparent or true OM digestion was always less when based on DAPA than when based on RNA. However, the free oils clearly caused large increases whichever of the four methods of calculating efficiency was used. There were no significant differences between the oils, so the mean effect in relation to apparent digestion of OM was an increase of 165% based on DAPA or 113% based on RNA. When related to truly-digested OM, the increases were 96 and 64% for the two markers respectively. Small increases were observed with the protected oils, particularly protected linseed oil, but only in one instance did the increase achieve significance ($P < 0·05$).

DISCUSSION

Free oils and digestion in the rumen

The considerable depression caused by the free oils in the digestion of OM in the rumen is in agreement with the observations of Devendra & Lewis (1974*b*) who gave 80 g maize oil or tallow/kg diet to sheep receiving 1100 g/d of two basal diets containing either 100 or 700 g hay/kg diet. However, the depression they found averaged only 10% for the

low-roughage diet and 15% for the high-roughage diet compared with the value of 40% found in the present studies with diets supplemented with 67 g oil/kg diet. In contrast, Sutton *et al.* (1975) found no significant depression in OM digestion in the rumen of sheep when a diet of hay and concentrates was supplemented with 33 g cod-liver oil/kg. This difference may be explained by the recent experiment of Ikwuegbu & Sutton (1982) who confirmed the large depression in OM digestion in the rumen caused by linseed oil, but who also found that the size of the depression was related to the amount of free oil included in the diet. In their experiments, Devendra & Lewis (1974*b*) found that the depression caused by maize oil was greater than that caused by tallow. It seems probable that the addition to diets of free oils will frequently reduce OM digestion in the rumen but that the size of the depression will depend on the amount and nature of oil supplement and the type of basal diet.

The major cause of the depression in rumen digestion was the severe reduction in the digestion of NDF. Equally large depressions in the digestion of crude fibre due to maize oil or tallow supplementation were reported by Devendra & Lewis (1974*b*) but no effect on the digestion of acid-detergent fibre due to cod-liver-oil supplementation was found by Sutton *et al.* (1975). The causes of the depression and more detailed analysis of individual carbohydrates are considered elsewhere (McAllan *et al.* 1983). Whereas 100 g NDF/d were digested in the rumen on the basal diet, this was reduced to 40 g/d with linseed oil and only 25 g/d with coconut oil. This reduction of 60 and 75 g NDF/d exceeds the depression of 25 and 45 g OM/d truly digested in the stomach for the two oils respectively. It is unlikely that the digestion of any major dietary constituent was increased by the oil supplements so the discrepancy probably reflects the difficulties in accurately defining the various constituents of duodenal digesta (Sutton, 1979). Despite these difficulties it is apparent that the reduction in fibre digestion is the main cause of the reduced digestion of OM and energy in the rumen.

The amount of NDF digested in the intestines was more than doubled from 31 g/d on the basal diet to over 65 g/d with the free oils. The ability of the hind-gut to compensate for reduced fibre digestion in the rumen was reported earlier by Thomson *et al.* (1972), who found that the reduced digestion of cellulose and hemicellulose in the rumen due to grinding and pelleting lucerne (*Medicago sativa*) was associated with increases of up to 50% in post-ruminal digestion. However, the amount of compensation in the present studies, though incomplete, was even greater than this and emphasized the considerable digestive capacity of the hind-gut fermentation of sheep.

Although the pattern of rumen VFA was markedly altered by the oil supplements, as has been found in many other studies, no change in the concentration of total VFA occurred. The amount of OM apparently digested in the rumen, which is an approximate measure of the amount of fermentation and hence VFA production in the rumen (Sutton, 1979), was reduced by 35% and an approximately equivalent reduction in VFA production would be expected. Similar results have recently been reported for diets supplemented with 20, 39 or 60 g linseed oil/kg (Ikwuegbu & Sutton, 1982). Hence it must be concluded that the linear relationship of the concentration of VFA in the rumen to their rate of production for certain diets (Leng, 1970) does not apply when free oils are added to the diet.

Free oils and microbial protein synthesis

The very large increase in the efficiency of microbial protein synthesis when the diets were supplemented with the free oils was unexpected. Because of the accompanying depression in OM digestion, the increase in the amount of microbial protein synthesized was considerably less than the increase in efficiency of synthesis. The increase in the daily flow of total N in duodenal digesta due to oil supplementation was similar in size to the increase

in microbial N, and examination of the results of Devendra & Lewis (1974*b*) shows that in that experiment also, duodenal N was increased by 25–100% by oil supplementation. On the other hand, Sutton *et al.* (1975) found no effect of supplementation with cod-liver oil on the amounts of total N or microbial N at the duodenum. Czerkawski *et al.* (1975) concluded that supplementation of a dried-grass diet with up to 100 g linseed oil/kg diet actually decreased the synthesis of microbial matter though it appeared to increase the rate of VFA production. These conclusions were based on concentrations and outflow rates in the rumen rather than measurements of digesta flow at the duodenum, but if they are correct, the efficiency of microbial protein synthesis was reduced in the work of Czerkawski *et al.* (1975) in comparison with the two- to threefold increase in our experiment.

Many methods exist for measuring the flow of digesta at the duodenum and measuring microbial protein. The methods used in the present experiment were similar to those by Ikwuegbu & Sutton (1982). The use of Cr_2O_3 with spot sampling has been criticized (Faichney, 1975) but mainly in relation to spot sampling from simple cannulas. However, Corse & Sutton (1971) found no differences in estimates of DM flow in the duodenum of sheep betwen total collections and spot collections of digesta from re-entrant cannulas when Cr_2O_3 was the marker. The choice of microbial marker presents even greater room for disagreement. Large but inconsistent differences have been shown by direct comparisons among different techniques (see Harrison & McAllan, 1980). In the present studies RNA, which measures protozoa as well as bacteria, gave consistently higher estimates of flow than DAPA which measures bacteria only. This difference would appear reasonable for the basal diet and suggests that approximately 25% of microbial N entering the duodenum was of protozoal origin, a value similar to that reported by Harrison *et al.* (1979). However, on the oil-supplemented diets the difference, though smaller, was not reduced to the same extent as the reduction in protozoal numbers.

No satisfactory means exist at present for establishing the absolute accuracy of estimates of microbial protein synthesis in the complex situation of the normally-fed ruminant. The estimate for the basal ration in the present study that 30 g microbial N were synthesized/kg OM apparently digested in the rumen when DAPA was the marker is identical to the mean value adopted by the Agricultural Research Council (1980). Furthermore, the proportion of OM digestion apparently occurring in the rumen on the basal diet, 0·63, was also very close to the mean value of 0·65 adopted by the Agricultural Research Council (1980). These results show that, for the basal diet, the techniques used in the present study yielded values very similar to those published elsewhere. It is recognized that this does not provide direct evidence for the accuracy of the results obtained on the oil-supplemented diets and it would obviously be important to obtain confirmation of the results by other techniques.

The calculated efficiences of microbial protein synthesis on the two oil-supplemented diets were two to three times greater than those on the basal diet and considerably greater than even the highest previously-published values from in vivo experiments. The supplements used in the present experiment had a high energy concentration but this is unlikely to explain the high efficiency of microbial synthesis. Dietary oils are hydrolysed in the rumen and the fatty acids are extensively hydrogenated, but there is no evidence that they are catabolized. They could not, therefore, have contributed directly to the energy supply of the microbes and explanations must be sought in indirect effects of the oils.

Many factors have been shown to alter the efficiency of microbial protein synthesis (see Harrison & McAllan, 1980). High propionate fermentations have been associated with increased ATP production and hence greater microbial yields, but this association has not been observed in all instances (Bergen & Yokoyama, 1977) and may not apply in the present situation in which the increased proportion of propionic acid was due to the oil supplements rather than an alteration of the type of dietary carbohydrate as is the more common

situation. A more likely explanation is the reduction in protozoal numbers due to supplementation with free oils. Such reductions due to supplementation with linseed oil or its hydrolysate have been found by others (Czerkawski *et al.* 1975; Van Nevel & Demeyer, 1981; Ikwuegbu & Sutton, 1982). Demeyer & Van Nevel (1979) found in in vitro studies that defaunation resulted in the energetic efficiency of the net synthesis of microbial protein being approximately doubled from 13 to 30 g microbial N/kg OM fermented. This expression of efficiency is similar to the term g microbial N/kg OM truly digested in the rumen used in the present paper and the size of the increase was similar to that caused by the oil supplements in the present study. However, in subsequent in vitro experiments, while confirming this response, Van Nevel & Demeyer (1981) also concluded that defaunating agents such as linseed oil hydrolysate can reduce protein synthesis by bacteria by a direct toxic effect, and that the net effect on protein synthesis by the complete microbial population depends, therefore, on a balance between an increase in synthesis due to defaunation and a decrease due to the direct toxic effect of the agent on bacterial metabolism. In vivo studies by Ikwuegbu & Sutton (1982), in which sheep were given diets containing different amounts of linseed oil, appeared to confirm this conclusion. On the other hand, efficiencies were well within the nomal range in young calves in which only very small numbers of protozoa had become established (Smith *et al.* 1978), suggesting that low numbers of protozoa *per se*, even without use of a defaunating agent, do not automatically result in high efficiencies. Clearly, more detailed studies of microbial metabolism and the role of protozoa will be required to resolve these problems satisfactorily.

Protected lipids

In almost all aspects of digestion and synthesis examined in this experiment, the effects of the protected lipids were intermediate between those of the basal diet and the two free lipids and further, the effects of protected coconut oil were generally less than those of protected linseed oil and only rarely differed significantly from the responses on the basal diet. These results show that the method of protection used greatly reduced the effects of the oils on fibre digestion, rumen VFA and microbial synthesis. The reason for the difference in response to the two protected oils is unclear. When given in the free form, the two oils had similar effects and both protected oils were prepared in exactly the same way.

Although these results indicate that the method of protection was successful in reducing the effects of dietary lipids on rumen metabolism, examination of fatty acids entering the duodenum in the digesta showed that the technique was far less effective in preventing metabolism of fatty acids in the protected lipids themselves (Knight *et al.* 1977).

Hogan *et al.* (1972) supplemented a basal diet of lucerne hay with sunflower oil protected by a similar method to that used in the present studies. They found a reduction in OM digestion in the rumen on the supplemented diet similar to that reported here. They also concluded that the formaldehyde treatment effectively protected the casein and part of the basal lucerne hay from rumen digestion but, as in the present study, it was only partially effective in protecting the fatty acids from hydrogenation. Hogan *et al.* (1972) suggested that these apparent discrepancies may be because the oil droplets are close to the surface of the particles and hence some hydrogenation could occur even if the casein remained protected. It is also possible that the extent of protection resulted in a release of oil or fatty acids into the rumen fluid at a slow rate which allowed hydrogenation of the fatty acids to occur but which prevented a high concentration of oil developing in the rumen and hence greatly reduced the effects of the oil on digestion of OM and fibre. These results demonstrate the importance of distinguishing between protecting the rumen contents from the effects of the added lipid and protecting the lipid from metabolism by the rumen digesta.

CONCLUSIONS

The free lipids clearly had major effects on rumen metabolism. The potential importance of these effects are both beneficial, in improving the efficiency of microbial protein synthesis, and deleterious, in reducing fibre digestion. The possibility of reducing the effects on fibre digestion while retaining the improved efficiency of synthesis clearly merits further study, but recent attempts to achieve these effects by reducing the amount of linseed oil were unsuccessful (Ikwuegbu & Sutton, 1982). It was noteworthy that both the oils had essentially the same effect on rumen processes despite their very different fatty acid compositions. The linseed oil was rich in C_{18} unsaturated fatty acids, containing (mg/g) 18:1 173, 18:2 149 and 18:3 556, whereas the coconut oil contained 12:0 446 and 14:0 163 and a total of only 77 mg C_{18} unsaturated fatty acids/g. All these fatty acids have been shown to be inhibitory to certain rumen bacteria, particularly the cellulolytic species, in vitro (Henderson, 1973) so it is not possible to tell from the results how far the effects on fibre digestion represented specific effects of fatty acids on bacterial metabolism and how far they were due to more generalized coating effects of either the microbes or the feed particles. However, the stimulatory effect of both oils on microbial protein synthesis clearly showed that at least part of the action of the oils was a direct effect on the microbes.

Even when associated with a reduction in OM digestion in the rumen, the increased efficiency of microbial synthesis resulted in an increased total flow of non-ammonia-N and microbial N to the duodenum. To achieve this, between 6·1 and 7·1 g N/d were added to digesta between the mouth and proximal duodenum. Assuming that 0·03 g N/d per kg body-weight (Harrop, 1974) were secreted in the abomasum, then between approximately 5 and 6 g N/d were re-cycled to the rumen. The ability to re-cycle large amounts of N to the rumen is well-established for wether sheep given low-N diets at maintenance but it is arguable that a high-producing animal, such as a fast-growing calf or a high-yielding dairy cow, would be far less capable of re-cycling N because of the large demands for tissue N. In such animals there would appear to be considerable potential for adding non-protein-N to oil-supplemented diets to fulfil the increased N demands of the rumen microbes caused by the oils. The interaction between urea and lipid supplements was examined by Ørskov *et al.* (1978) in lambs given dried grass as a basal diet. They found slightly greater responses to urea supplementation, in terms of feed intake and acid-detergent fibre digestion, when dried grass was given with tallow than when it was given alone, but this effect of urea was primarily an alleviation of the depression in intake and digestibility caused by the tallow rather than a positive stimulation. No measurements of N metabolism were reported.

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