The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep

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1. The effects of three levels of linseed oil (LSO) supplementation of a basal diet on rumen digestion and flow of nutrients to the proximal duodenum of three mature sheep provided with permanent rumen and duodenal re-entrant cannulas were studied.

2. A basal diet of 200 g hay and 400 g concentrates daily, providing approximately 7·0 MJ digestible energy and 13 g N/d, was given alone or with supplements of 13, 26 or 40 ml LSO/d in two equal portions at 06.00 and 18.00 hours. The flow of duodenal digesta was measured by spot-sampling using chromic oxide paper as the marker. Bacterial protein synthesis (BPS) was measured by the diaminopimelic acid technique.

3. Addition of LSO reduced the digestion of energy and organic matter, particularly acid-detergent fibre, in the stomach. Digestion in the intestines increased but at the higher levels of supplementation this failed to compensate completely for the reduction in rumen digestion. Total volatile fatty acid concentrations were not affected but molar proportions of acetate and butyrate were decreased by approximately 18 and 61% respectively while the molar proportion of propionate was increased twofold by the highest concentration of oil. The higher concentrations of LSO virtually eliminated protozoa from the rumen.

4. The second increment of LSO (26 ml/d) produced the highest duodenal flow of total N and bacterial N and the highest efficiency of BPS. The highest concentration of oil (40 ml/d) was without effect. Rumen and duodenal ammonia concentrations and plasma urea concentrations tended to be reduced by the higher concentrations of LSO.

5. It is argued that the results support suggestions made elsewhere that free oils reduce the efficiency of BPS but that they also reduce the numbers of protozoa which can cause an increase in the efficiency of BPS. The net effect of free oil supplementation on BPS is thus likely to be variable and difficult to predict.

Calculation of the feed requirements of ruminants for production is becoming increasingly dependent on the ability to predict the supply of nutrients resulting from the processes of digestion and synthesis in the digestive tract in general and the rumen in particular. The inclusion in diets of certain supplements known to alter rumen processes introduces further complications which make the prediction of nutrient supply more difficult.

The addition of free linseed oil (LSO) or coconut oil to diets of hay and concentrates given to sheep was found by Knight *et al.* (1978) to increase markedly the amount and efficiency of microbial protein synthesis (MPS). On the other hand, no change was found in earlier studies when cod liver oil was used (Sutton *et al.* 1975) and Czerkawski *et al.* (1975) concluded that LSO caused microbial synthesis to be reduced. A more consistent response to free oil supplementation has been a reduction in the apparent digestion of organic matter (OM) in the rumen ($ADOM_R$) due mainly to a reduction in fibre digestion (Devendra & Lewis, 1974; Kowalczyk *et al.* 1977; Knight *et al.* 1978). It seems probable that it is this reduction in digestion in the rumen that is the main factor limiting the amount of oil that can be added to ruminant diets. A further consequence is a reduction in the potential benefit, in terms of an increase in the absolute supply of microbial protein, resulting from any increase in the amount of microbial protein synthesised per unit $ADOM_R$.

The purpose of the present experiment was to see if, by varying the amount of LSO supplement, it might be possible to explain the discrepancies in the reported response in bacterial protein synthesis (BPS) to free oil supplementation and further, to see whether

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a level of supplementation could be identified that would enhance the efficiency of BPS without reducing $ADOM_R$.

A preliminary report of these results has been published elsewhere (Ikwuegbu & Sutton, 1981).

EXPERIMENTAL

Animals and management

Three Suffolk × Scottish half-bred wethers, aged approximately 10 months at the time of surgery and weighing between 35 and 45 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 but proximal to the point of entry of the common bile duct. Each sheep received each of the four experimental diets in a random order.

Housing

All the sheep were kept in individual metabolism crates in the sheep house throughout the experiment. The floor of the crate was made of expanded metal, the front half of which was covered with a rubber mat. The animals had considerable freedom of movement but could not turn around. No artificial light was provided except when collections were being made.

Diets and feeding procedure

The basal diet was similar to that used by Knight *et al.* (1978) and was composed of 200 g hay and 400 g concentrate mix. The concentrate consisted of (g/kg air-dry mix): rolled barley 884, soya-bean meal 46, protected sodium caseinate 46, copper-free mineral mix (Boots Farmsales Ltd, Witney, Oxon) 14, and sodium sulphate–ammonium molybdate 10. The sodium sulphate–ammonium molybdate was included to reduce the risk of Cu toxicity (Cammell, 1977). The sodium caseinate was protected by treatment with formaldehyde (MacCormac Products Ltd, Killeshandra, Ireland) and was included because it had also been a component of the diets used in the previous study by Knight *et al.* (1978). The composition of the hay and concentrate is shown in Table 1.

Raw LSO (Signpost Paints, Haverhill, Suffolk) was used as a supplement in three of the four diets. It was stabilized by an antioxidant, butylated hydroxy-toluene (2,6-ditert-butyl-*p*-cresol), added to give a concentration of 200 mg/l. LSO was added to the basal concentrate at 13, 26 and 40 ml/d to provide four experimental diets containing 0, 20, 39 and 60 g LSO/kg diet respectively.

The basal diet was fed in two equal portions at 06.00 and 18.00 hours. LSO was measured out by volume daily and well mixed by hand into the concentrate mix. Refusals were removed and weighed just before the afternoon feeding. Water was always available.

Experimental routine

Each period was divided into 42 d. The basal diet was given for the first 5 d and during this time an inoculum of approximately 100 ml rumen fluid from a sheep on the basal diet was given to all the sheep that had previously received an oil-supplemented diet to encourage re-establishment of protozoa. After this, LSO was introduced gradually over a period of 8 d. A period of 11 d was allowed for adaptation to treatment and 18 d for sample collection and measurements. In the sampling period, faeces and urine were collected for the first 8 d using a harness and bag for collection of the faeces. On day 4, 50 ml rumen fluid were taken every hour for 6 h and every 2 h for the next 6 h, using a stainless-steel strainer. The pH was determined immediately. A portion of the rumen fluid used for ammonia determination

Table 1. *The mean dry matter (DM) content and composition of the DM of the hay and the concentrate mix*

	Hay	Concentrates
DM (g/kg feed)	844	865
Composition of DM (g/kg DM)		
Organic matter	916	945
Acid-detergent fibre	368	63
Starch	14	495
Nitrogen	15·9	28·6
Gross energy (MJ)	17·80	17·96

was mixed with three drops of concentrated sulphuric acid and stored at −20°. The remainder was stored at −20°. A portion (10 ml) of a separate sample of rumen fluid used for protozoa counts was added to 10 ml of a mixture of glycerol–water–formalin (500 : 450 : 50, by vol.) and stored at room temperature. Blood samples were taken from the jugular vein every 3 h for 12 h on day 5. The flow of digesta at the duodenum was determined by taking spot samples of digesta, using chromic oxide paper (2·5 g paper containing 0·88 g Cr_2O_3 put into the rumen twice daily) as the marker. Approximately 150 ml duodenal digesta were taken from the proximal cannula on six occasions over 3 d starting on day 8. The collection of digesta was arranged such that a sample was taken every 2 h during the 12 h daytime feeding cycle. Six rumen samples of 100 ml each were taken for 2,4-diaminopimelic acid (DAPA) estimation over 12 h on day 11. Bacterial samples were isolated within 3 h of the last sample being taken using differential centrifugation as described by Smith & McAllan (1974). For measurement of rumen fluid kinetics, a single injection of a solution of approximately 100 μCi ^{15}Cr-EDTA solution was given at 07.00 hours on day 18 and the decline of radioactivity monitored in samples taken at hourly intervals for 11 h.

Chemical analyses

Feed samples were dried for 24 h at 105° and total duodenal digesta and faeces were freeze-dried for 3 d for dry matter (DM) content. They were analysed for OM by ashing at 550° for 4 h, for N by the micro-Kjeldahl technique, for α-linked glucose polymers by the method of MacRae & Armstrong (1968) and for acid-detergent fibre (ADF) by the method of Van Soest (1973). Determination of Cr_2O_3 was by the method of Stevenson & De Langen (1960). Duplicate 2 ml portions of rumen fluid and 0·5 ml ^{15}Cr-EDTA infusate were counted for 4 min using an Intertechnique CG4000 gamma spectrometer (Intertechnique Ltd, Uxbridge, Middlesex). Volatile fatty acids (VFA) in rumen samples were determined by gas–liquid chromatography (Sutton & Johnson, 1969), ammonia in acidified rumen samples by the method of Merry *et al.* (1982) and plasma urea-N by the method of Marsh *et al.* (1965). The DAPA content of bacteria and duodenal digesta was determined by ion-exchange chromatography using an Auto-Analyzer (Technicon Instruments Co, Basingstoke, Hants) by the method of Smith *et al.* (1978) which involves 22 h hydrolysis in 6 M-hydrochloric acid at 108°. The proportion of duodenal N present as bacterial N was calculated as mg DAPA/g N in duodenal digesta × g N/mg DAPA in bacterial samples.

Calculations

The values were analysed as a randomized block. Linear and quadratic trends were calculated for treatment effects. In the presentation of results the change in the amount or

Table 2. *The mean amounts of gross energy (MJ/d) consumed, passing through the duodenum and excreted in the faeces and its digestion in the stomach and total tract in three sheep given a basal diet alone or supplemented with 13, 26 or 40 ml linseed oil/d*

	Linseed oil (ml/d)					Effects	
	0	13	26	40	SEM	Linear	Quadratic
Flow (MJ/d)							
Food	9·19	9·73	10·15	10·48	0·080	***	NS
Duodenum	5·17	5·82	7·68	7·34	0·187	***	*
Faeces	2·15	2·24	2·64	2·77	0·098	**	NS
Digestion							
Stomach	0·44	0·40	0·24	0·31	0·017	***	*
Total	0·77	0·77	0·74	0·74	0·011	NS	NS
Stomach / Total	0·57	0·53	0·33	0·42	0·027	**	*

NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

Table 3. *The mean amounts of organic matter (g/d) consumed, passing through the duodenum and excreted in the faeces and its digestion in the stomach and total tract in three sheep given a basal diet alone or supplemented with 13, 26 or 40 ml linseed oil/d*

	Linseed oil (ml/d)					Effects	
	0	13	26	40	SEM	Linear	Quadratic
Flow (g/d)							
Food	479	497	508	521	4·7	***	NS
Duodenum	238	265	345	323	10·2	***	•
Faeces	101	105	124	129	4·6	**	NS
Digestion							
Stomach	0·50	0·47	0·32	0·38	0·019	***	*
Total	0·79	0·79	0·76	0·75	0·010	*	NS
Stomach / Total	0·64	0·59	0·42	0·51	0·028	**	NS

NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

proportion of nutrients apparently digested between the mouth and the proximal duodenum is referred to as digestion in the stomach. True digestion was calculated by adding bacterial flow to the amount apparently digested.

RESULTS

Energy digestion

The effects of LSO supplements on the digestion of energy (Table 2) and OM (Table 3) were very similar and will be considered together. The addition of LSO caused a highly significant ($P < 0·001$) linear reduction in digestibility in the stomach, though the reduction tended to be less with 40 ml than with 26 ml LSO leading to a quadratic ($P < 0·05$) effect also. There was a much smaller reduction in digestibility in the total tract with the result that the proportion of digestion occurring in the stomach also fell linearly ($P < 0·01$).

The amount of ADF flowing to the duodenum increased linearly ($P < 0·05$) with LSO

Table 4. *The mean amounts of acid-detergent fibre (g/d) consumed, passing through the proximal duodenum and excreted in the faeces and its digestion in the different sites of the digestive tract of three sheep given a basal diet alone or supplemented with 13, 26 or 40 ml linseed oil/d*

	Linseed oil (ml/d)					Effect†
	0	13	26	40	SEM	Linear
Flow (g/d)						
Food	83	84	84	84	1·2	NS
Duodenum	46	60	69	72	3·1	*
Faeces	37	40	46	49	2·3	*
Digestion						
Stomach	0·44	0·28	0·18	0·14	0·035	**
Total	0·55	0·52	0·46	0·41	0·031	*
$\frac{\text{Stomach}}{\text{Total}}$	0·82	0·55	0·40	0·35	0·087	*

NS, not significant.
* $P < 0·05$, ** $P < 0·01$.
† No quadratic effects were significant.

supplementation (Table 4). The increase with the two higher levels of LSO was over 50% and accounted for approximately one-quarter of the increased flow of OM on these diets. There was also a linear increase in faecal output but, as with the digestion of energy and OM, the reduction in total digestion was smaller than the reduction in digestion in the rumen with the result that the contribution of the stomach to over-all digestion fell linearly ($P < 0·05$) with increasing LSO. Thus a major consequence of the LSO supplementation was that 45–65% of total ADF digestion occurred in sites distal to the proximal duodenum when LSO was added compared to 18% on the basal diet.

There was no significant effect of supplementation on digestion of starch (α-linked glucose polymers). Of the average daily intake of 174 g, only 13 g (7%) entered the duodenum and virtually none was excreted in the faeces.

The effect of LSO supplements on rumen VFA are presented in Table 5. Mean total VFA concentrations were not affected by the addition of LSO but there were marked changes in the molar proportions of the major VFA. There were significant ($P < 0·05$) linear decreases in the molar proportions of acetate and butyrate and a linear increase in the proportion of propionate.

Rumen fluid and protozoa numbers

These values are presented in Table 5. Rumen fluid volume increased significantly ($P < 0·05$) as the concentration of LSO in the basal diet was increased, though only with the two higher concentrations of LSO were the increases, of approximately 26%, significant (26 ml/d, $P < 0·01$; 40 ml/d, $P < 0·05$). Rumen fluid clearance rate was not significantly affected by LSO supplements although it tended to be lower at the higher levels of supplementation. An opposite effect was observed with rumen outflow rate which tended to increase slightly with LSO supplementation.

Protozoa numbers were severely reduced when the concentration of LSO in the basal diet was increased. There were significantly linear ($P < 0·001$) and quadratic trends ($P < 0·05$). The two higher concentrations of LSO almost completely eliminated protozoa from the rumen ($P < 0·001$). Rumen pH was slightly lower on the LSO-supplemented diets but the effects were not significant.

Table 5. *The mean concentration of total volatile fatty acids (VFA) (mmol/l) and molar proportions (mmol/mol total VFA) of the major VFA, the volume (l), clearance rate/h, outflow rate (l/d), pH and protozoa numbers ($\times 10^{-4}$/ml) in the rumen fluid of three sheep given a basal diet alone or supplemented with 13, 26 or 40 ml linseed oil/d*

	Linseed oil (ml/d)					Effects	
	0	13	26	40	SEM	Linear	Quadratic
Total VFA	64·5	62·7	66·8	65·3	5·25	NS	NS
VFA proportions							
Acetate	640	608	536	528	21·3	*	NS
Propionate	182	261	330	367	45·4	*	NS
n-Butyrate	122	76	57	47	12·1	*	NS
Volume	4·3	4·6	5·5	5·4	0·02	*	NS
Clearance rate	0·062	0·063	0·055	0·053	0·0106	NS	NS
Outflow rate	6·4	7·0	7·3	6·9	1·00	NS	NS
pH	6·62	6·35	6·41	6·48	0·123	NS	NS
Protozoa	372	211	9	1	33·2	**	*

NS, not significant.
* $P < 0·05$, ** $P < 0·01$.

Table 6. *The mean amounts of nitrogen (g/d) consumed, passing through the duodenum, excreted and retained and the apparent digestion of nitrogen in three sheep given a basal diet alone or supplemented with 13, 26 or 40 ml linseed oil/d*

	Linseed oil (ml/d)					Effects†
	0	13	26	40	SEM	Linear
Food	13·3	12·3	12·6	11·9	1·08	NS
Duodenum	11·0	11·8	17·0	14·2	1·04	*
Faeces	3·0	3·2	3·6	3·8	0·17	*
Urine	7·2	6·8	6·1	4·8	0·60	*
Retained	3·0	2·3	2·9	3·3	1·21	NS
Apparent digestion						
Stomach	0·16	0·03	−0·38	−0·19	0·127	*
Total	0·77	0·73	0·71	0·68	0.033	NS

NS, not significant.
* $P < 0·05$.
† No quadratic effects were significant.

N metabolism

The over-all N transactions in the whole animal are shown in Table 6. There was no significant effect on N retention in these mature sheep. Excretion of N in the faeces increased significantly ($P < 0·05$) but the resulting fall in digestibility did not attain significance. There was a linear ($P < 0·05$) fall in N excretion in urine and the highest concentration of LSO was associated with a reduction of approximately 33% in urinary N. Flow of N at the duodenum was increased by over 50% ($P < 0·05$) when 26 ml LSO were given. At the highest level of supplementation the increase was smaller and non-significant but over-all a linear response was established ($P < 0·05$). Ammonia-N flow averaged 0·9 g/d and was unaffected by diet. The pattern of flow of non-ammonia-N (NAN) for the four diets was

Table 7. *Flow of bacterial organic matter (OM) and nitrogen at the duodenum, the true and apparent digestion of OM in the stomach, and the efficiency of bacterial protein synthesis in three sheep given a basal diet alone or supplemented with 13, 26 or 40 ml linseed oil/d*

	Linseed oil (ml/d)					Effects	
	0	13	26	40	SEM	Linear	Quadratic
Bacterial flow (g/d)							
OM	90	96	115	99	6·7	NS	NS
N	7·5	8·1	9·7	6·7	0·56	NS	NS
OM digested in stomach (g/d)							
Apparent ($ADOM_R$)	240	232	163	198	9·5	**	*
True ($TDOM_R$)	330	328	277	297	14·5	NS	NS
True digestion of OM in stomach	0·69	0·66	0·55	0·57	0·027	*	NS
Efficiency of bacterial protein synthesis							
g N/kg $ADOM_R$	32	35	59	34	2·9	NS	***
g N/kg $TDOM_R$	23	25	35	22	1·3	NS	**

NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

Table 8. *The mean concentration (mmol/l) of ammonia in rumen and duodenal fluid and of urea in blood plasma in three sheep given a basal diet alone or supplemented with 13, 26 or 40 ml linseed oil/d*

	Linseed oil (ml/d)					Effects†
	0	13	26	40	SEM	Linear
Rumen ammonia	16·7	9·3	7·1	6·2	1·53	**
Duodenal ammonia	8·2	5·9	4·8	5·1	0·95	NS
Plasma urea	5·5	4·5	4·6	3·2	0·93	NS

NS, not significant.
** $P < 0·01$.
† No quadratic effects were significant.

therefore similar to that of total N and averaged 10·0, 10·9, 15·9 and 13·4 g/d for the four diets respectively.

Aspects of the effects of the LSO on bacterial synthesis are shown in Table 7. Estimates of bacterial synthesis were based on the use of DAPA as the marker and average values for mg DAPA/g bacterial N from rumen samples varied between 40 and 43, well within the published range. The only level of LSO supplementation to affect bacterial OM or N flow significantly ($P < 0·05$) was 26 ml/d which resulted in increases of slightly less than 30%. The effects of LSO on the amount of OM truly digested in the rumen ($TDOM_R$) were similar to the effects on apparent digestion and a significant ($P < 0·05$) linear decrease in true digestibility was established. Supplementation with LSO resulted in large increases in the efficiency of BPS (85% when based on $ADOM_R$ and 52% when based on $TDOM_R$) when 26 ml/d were used but no significant increases at the other levels and this is reflected by the highly significant ($P < 0·001$ and $P < 0·01$) quadratic relationships.

The concentrations of ammonia in rumen and duodenal fluid and of urea in blood plasma fell with increasing LSO, though only in the instance of rumen ammonia was a significant ($P < 0·01$) linear trend established (Table 8).

DISCUSSION

The method of measuring digesta flow at the duodenum in this experiment was spot sampling from re-entrant cannulas with Cr_2O_3 as the marker. The case for using 'dual-phase' markers with spot sampling was discussed at length by Faichney (1975) but this was for use in conjunction with simple cannulas which are likely to yield samples that are not representative of the digesta flowing. When re-entrant cannulas are used, as in the present study, none of the digesta can by-pass the sampling site, so it is reasonable to conclude that Cr_2O_3, when administered in a form that allows its relatively steady release from the rumen, will be an adequate if not perfect marker. Corse & Sutton (1971) found no difference in estimates of DM flow between spot samples and samples obtained by total collections from re-entrant cannulas in sheep when Cr_2O_3 was the marker. The close similarity of the values on the basal diet in the present experiment to the mean values recently published by the Agricultural Research Council (1980) for $ADOM_R : ADOM_{Total\ tract}$ (0·64 this paper *v.* 0·65 ARC) and for the efficiency of protein synthesis (g bacterial or microbial N/kg $ADOM_R$) (32 this paper *v.* 30 ARC) is strong evidence that the technique yielded reasonable estimates of the flow of the major constituents of digesta reported here.

The amount of OM truly digested in the rumen ($TDOM_R$) was estimated by adding to the amount of OM apparently digested ($ADOM_R$) the amount of bacterial OM as calculated from the DAPA : OM concentration in bacterial samples from the rumen and using DAPA as the bacterial marker at the duodenum. This under-estimates $TDOM_R$ by the amount of protozoal OM but if, as seems probable, protozoal OM constitutes only 25% or less of microbial OM at the duodenum on conventional diets (Weller & Pilgrim, 1974; Harrison *et al.* 1979) then the under-estimate of $TDOM_R$ would be only about 10% on the basal diet and far less on the diets supplemented with LSO, particularly at the two higher levels when protozoa were almost eliminated. There are other sources of error in estimating $TDOM_R$ which have been extensively discussed by Czerkawski (1978) but the method used in the present study probably yields a reasonable approximation to the true value.

Changes in energy digestion

The principal aim of this work was to examine the effects on OM digestion and BPS in the rumen of increasing levels of LSO added to the diet with a view to establishing an optimal level of supplementation for maximizing the supply of bacterial protein to the intestines.

The results confirmed those of previous experiments (Devendra & Lewis, 1974; Knight *et al.* 1978) which showed that the inclusion of free oils in the diet of sheep reduced the digestion of energy and OM in the rumen. The maximum depression, of approximately 34%, in the proportion of digestible OM apparently digested in the stomach in the present studies was very similar to that reported by Knight *et al.* (1978) with 67 g free LSO or coconut oil/kg diet but considerably greater than the 4–9% depression found by Devendra & Lewis (1974) when two diets were supplemented with 80 g maize oil or tallow/kg diet. In the present experiment, increasing the concentration of LSO in the diet did not have a consistent effect on the reduction in OM digestion in the stomach. The greatest depression occurred with 39 g LSO/kg diet (26 ml LSO/d) whereas the effect with the highest level of oil (67 g LSO/kg diet (40 ml LSO/d)) was smaller, though not significantly so, and it was also smaller than the depression found by Knight *et al.* (1978) at a similar level of inclusion.

The reduction in OM digestibility was associated with a marked depression in ADF digestion in the stomach. This is consistent with the reduced digestibility of cellulose or crude fibre reported by many workers (Brooks *et al.* 1954; Czerkawski *et al.* 1966; Devendra & Lewis, 1974; Kowalczyk *et al.* 1977) when free oils were added to various diets. A major effect of the oil was to shift the site of digestion of digestible ADF to the intestines, presumably the caecum, with the result that the reduction in over-all digestion was small.

The absence of any charge in the concentration of VFA in the rumen is surprising in view of the large reduction in OM digestion and the 25% increase in rumen fluid volume, but it is in agreement with the results of Knight (1980). However, in other experiments (Czerkawski *et al.* 1975; Devendra, 1975; Sutton *et al.* 1975) a variety of free oils reduced VFA concentrations. In view of the large reduction in $ADOM_R$ there is little doubt that the production of VFA was reduced by the LSO in the present studies even though their concentration remained unchanged. The changes in molar proportions of VFA in response to the free oils were similar to those reported by many other workers and their extent was closely related to the amount of oil fed.

Protozoa were almost completely eliminated from the rumen at the two higher levels of LSO supplementation. This defaunating effect of oils has been reported previously (Purser & Moir, 1966; Czerkawski *et al.* 1975; Knight *et al.* 1978) and is probably due to the fatty acids rather than the oil itself (Czerkawski, 1973; Van Nevel & Demeyer, 1981). The consequences of defaunation are still a subject for much debate but, in the context of the present study, it is probable that it contributed to the reduced fibre digestion and, as discussed later, the changes in the efficiency of bacterial protein synthesis (see Demeyer, 1981).

Bacterial synthesis

As in the studies of Knight *et al.* (1978), the efficiency of BPS was increased by addition of LSO to the diet. However, in the present experiment the increase was approximately 80% and only occurred with 39 g LSO/kg diet whereas with 60 g LSO/kg diet, which Knight *et al.* (1978) found to cause an increase of 180%, no increase was detected. Czerkawski *et al.* (1975) calculated that even though there was an increase in bacterial numbers associated with a decrease in protozoal numbers as the concentration of LSO in the diet of sheep was increased to 100 g/kg diet, there was a fall in the amount of BPS as calculated from DAPA concentrations and outflow rates of rumen fluid. Sutton *et al.* (1975) found no change in MPS when sheep were given a diet containing 33 g cod liver oil/kg diet.

Thus although the experiment did succeed in defining a level of LSO inclusion that maximized the supply of bacterial protein, it is quite apparent that the response to free oil supplementation is very variable. A probable explanation for the variability of response is provided by a recent investigation by Van Nevel & Demeyer (1981). From in vitro studies of the effect of methane inhibitors on the metabolism of rumen microbes, they concluded that LSO hydrolysate could either increase or decrease MPS depending on the net effect of two opposing factors. They argued that its direct effect on bacteria was to depress protein synthesis but that this effect might be masked because it also removed protozoa which, as they had shown earlier (Demeyer & Van Nevel, 1979) would result in an enhancement of both total and net synthesis. Such a theory fits the results of the present study well. The lowest level of LSO that virtually eliminated protozoa from the rumen (26 ml/d) coincided with the greatest efficiency of BPS. At still higher additions of LSO (40 ml/d) BPS was reduced, presumably because no further appreciable reduction in protozoal numbers was possible and the inhibitory effect of the LSO on bacterial metabolism was the dominant response. On the basis of this theory, the net effect of such agents as free oils on MPS is likely to remain highly unpredictable, depending as it does on the net effect of opposing responses.

An additional complication is that lipid supplements are normally provided in the form of the oil or fat, as in the present study, yet it is the free fatty acid that appears to be the toxic agent (Henderson, 1973; Maczulac *et al.* 1981). Thus the effect of dietary lipids on microbial metabolism probably depends to a considerable degree on the rate of hydrolysis of the supplement.

Many other factors have been implicated in observed variations in the efficiency of MPS.

Ishaque *et al.* (1971) correlated a high efficiency of MPS with high molar proportions of propionate when sheep were given a high-concentrate diet of hay, barley and maize. Harrison *et al.* (1976) found that high fluid clearance rates brought about by intra-ruminal infusion of artificial saliva were associated with increased efficiency of MPS but also with high molar proportions of acetate and low proportions of propionate. In the present experiment the highest efficiency of BPS, found with 26 ml LSO/d, was associated with an increase in propionate and a decrease (non-significant) of approximately 11% in rumen clearance rate, yet at still higher proportions of propionate the efficiency of BPS fell. Kennedy *et al.* (1976) suggested that a high rumen clearance rate was more important in increasing MPS than was manipulating an increase in the molar proportion of propionate. It is clear that the relationships between the efficiency of MPS, rumen VFA proportions and fluid clearance rates are complex and it seems probable that on these diets, the major factor influencing BPS was the LSO itself.

The effect of the two higher levels of LSO on the flow of NAN at the duodenum was greater than the effect on bacterial N flow. With 26 and 40 ml LSO/d, the flow of NAN exceeded the flow of bacterial N by 6·2 and 6·7 g/d respectively, whereas with 0 and 13 ml LSO/d the difference was only 2·5 and 2·8 g/d respectively. This suggests that LSO reduced feed protein degradability by approximately 4 g N/d, though it is also possible that part of the increase reflected a greater endogenous secretion of N due to the higher flow of undigested fibre from the rumen.

It must be concluded that addition of free LSO to a mixed diet is likely to reduce the digestion of OM, particularly fibre, the numbers of protozoa, the concentration of ammonia, the ratio of acetic acid to propionic acid and possibly protein degradability in the rumen and that the response is broadly dose-related. Unfortunately, the potentially important effect on MPS is much less predictable and does not bear a simple relationship to the level of inclusion of oil. It must further be borne in mind that in the present experiment and that of Knight *et al.* (1978), free oil was stirred by hand into the loose concentrate mix daily. Responses may well be different if the oil is incorporated into a pellet as in the studies of Czerkawski *et al.* (1975) and more normal commercial practice.

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