The effect of free and protected oils on the digestion of dietary carbohydrates between the mouth and duodenum of sheep

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1. Sheep fitted with rumen and re-entrant duodenal cannulas were given diets of approximately 200 g hay and 400 g concentrate mixture alone, or supplemented daily with 40 g linseed or coconut oils free or protected with formaldehyde–casein in a 5×5 Latin-square arrangement. Chromic oxide paper was given as a marker at feeding time and passage to the duodenum of neutral-detergent fibre (NDF) and different sugars were estimated from the values for constituent: marker at the duodenum. Contributions of microbial carbohydrates to these flows were estimated from amounts of RNA present.

2. The carbohydrate composition of mixed rumen bacteria from sheep rumen digesta were similar regardless of diet. Of the sugars entering the duodenum all the rhamnose and ribose and 0·51, 0·24 and 0·35 of the mannose, galactose and starch–glucose respectively, were contributed by the microbes. Virtually all the arabinose, xylose and cellulose-glucose were contributed by the diet.

3. For sheep receiving the basal ration, coefficients of digestibility between mouth and duodenum, corrected where necessary for microbial contribution, were 0·95, 0·66, 0·67, 0·62, 0·45 and 0·51 for starch-glucose, mannose, arabinose, galactose, xylose and cellulose-glucose respectively. Corresponding values when free-oil-supplemented diets were given were 0·95, 0·55, 0·38, 0·55, 0·01 and −0·02 respectively. Values for diets supplemented with linseed oil or coconut oil did not differ significantly. Addition of protected oils to the basal feed also resulted in depressed digestibilities of dietary structural sugars but to a far lesser extent than those observed with the free oils.

4. Apparent digestibility of NDF was altered in the same direction as those of the main structural sugars, averaging 0·50, 0·17 and 0·29 in animals receiving the basal, free-oil-supplemented or protected-oil-supplemented diets respectively. The reasons for the difference between NDF and discrete carbohydrate analytical totals are discussed.

An extensive literature exists reporting the depressive effect of oils on the digestibility of crude fibre in the digestive tract of ruminants which has been well reviewed (Bull, 1971; Devendra & Lewis, 1974*a*). This depression of crude fibre digestion was found to occur in the rumen (Devendra & Lewis, 1974*b*).

The exact mechanism of the effect of fats on crude fibre digestion is not known. A number of theories have been put forward (Devendra & Lewis, 1974*a*). These included modification of the rumen microbial population or some of their activities concerned with fibre digestion (White *et al.* 1958; Ørskov *et al.* 1978) and coating of the fibrous feed material with lipid, thereby denying access to the microbes (Brooks *et al.* 1954; Ward *et al.* 1957). Much of the decrease in fibre digestibility has been shown frequently to be attributable to a decrease in cellulose digestibility, although this has been found to be highly variable and, in some cases, non-significant (Bull, 1971).

If the effect of oils in the rumen were on specific bacterial species it is possible that different structural carbohydrates of the fibrous feeds would not be affected in the same way whereas if coating of the fibrous materials by oils were responsible for the reduction in cellulose digestion, one would expect all structural sugars to be affected similarly. However, there appears to have been no study of the effect of oils on the digestibility of fibrous dietary carbohydrates other than cellulose.

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The present study, which formed part of a larger experiment (Sutton *et al.* 1983) was undertaken to examine the effects of free and protected oils on the digestion of the major dietary carbohydrates between the mouth and duodenum of sheep.

<div align="center">METHODS</div>

<div align="center">*Animals, diets and sampling of digesta*</div>

Five crossbred wether sheep, aged 2 years and weighing approximately 40 kg, were each fitted with a simple rumen cannula and a re-entrant duodenal cannula approximately 50 mm beyond the pylorus and before the point of entry of the bile and pancreatic ducts (Ash, 1962). The sheep were housed indoors in individual metabolism crates.

The basal diet consisted of 200 g chopped hay (100–150 mm) and 380 g concentrate mix (g/kg: 950 rolled barley, 50 decorticated groundnut meal, containing a mineral supplement; Sutton *et al.* 1983), to which were added 40 g free oil or the same amount of oil protected with formaldehyde-treated casein. An amount of protected casein (20 g/d) equal to that in the protected-oil supplements, was added to the basal and free-oil-supplemented diets. Protected-oil supplements were prepared as described by Sutton *et al.* (1983). The diets were given in two equal portions daily at 06.00 and 18.00 hours. Paper impregnated with chromic oxide supplying 1·76 g Cr_2O_3/d was introduced into the rumen at each feed.

The experiment was designed as a 5×5 Latin square. The five treatments were basal ration alone (B) or with free linseed oil (L), protected linseed oil (PL), free coconut oil (C) or protected coconut oil (PC). Each treatment period lasted 6 weeks and consisted of a 10 d period on the basal diet, 5 d changeover to the treatment, 14 d adaptation to the treatment and a 13 d sampling period.

Spot samples of duodenal digesta, approximately 150 ml each, were taken over a 5 d period (days 2–6 of the 13 d sampling period), to cover hourly intervals of the 12 h period from 12.00 to 24.00 hours. Equal portions were bulked, homogenized and stored for each sheep on each diet. On days 9–13 of the sampling period, approximately 350 ml rumen digesta were taken at 0, 3 and 5 h after the morning feed. Only one sample was taken daily. Mixed rumen bacteria were separated as described by Smith & McAllan (1974) and combined for each sheep providing one mixed bacterial sample/sheep per diet.

<div align="center">*Analytical*</div>

Samples were subjected to acid hydrolysis (McAllan & Smith, 1974) which released rhamnose, ribose, mannose, galactose, arabinose, xylose and starch-glucose. Amounts of these sugars were measured by ion-exchange chromatography of their borate complexes (McAllan & Smith, 1974). Cellulose-glucose was estimated after more stringent hydrolysis of the residue from the first hydrolysis by automated enzymic analysis (McAllan & Smith, 1974). Neutral-detergent fibre (NDF) was estimated by the method of Van Soest & Wine (1967) and Cr_2O_3 according to the procedure of Stevenson & de Langen (1960). RNA was determined by the method of McAllan & Smith (1969).

In the present work the use of RNA as an indicator has been applied to the estimation of the contribution that microbial carbohydrates make at the duodenum. Estimates of microbial RNA flow at the duodenum were corrected ($\times 0.85$) for possible dietary interference (Smith *et al.* 1978). By establishing the amounts of individual carbohydrates relative to RNA in samples of mixed rumen bacteria from each animal on each diet, and assuming those to be representative of the microbial population leaving the rumen, it was possible to estimate the amounts of microbial carbohydrates at the duodenum from the RNA concentrations at that site.

Table 1. *Carbohydrate and neutral-detergent fibre (NDF) content (g/kg dry matter) of feed components*

	Feed component	
	Hay	Concentrates
Rhamnose	2·3	0
Mannose	2·0	2·2
Arabinose	24·9	25·9
Galactose	13·5	16·4
Xylose	107·6	51·0
Starch-glucose	22·8	569·8
Cellulose-glucose	279·8	37·7
NDF	694·0	240·0

Digestibility of each carbohydrate between mouth and duodenum was calculated using measured carbohydrate intake (S_i); measured carbohydrate flow at the duodenum (S_d) and estimated bacterial carbohydrate flow at the duodenum (S_b) (all expressed in g/d):

$$\text{Apparent digestibility} = \frac{S_i - S_d}{S_i}$$

$$\text{True digestibility} = \frac{S_i - (S_d - S_b)}{S_i}$$

RESULTS

Carbohydrate composition of dietary components and mixed rumen bacteria

The carbohydrate and NDF contents of the major dietary components are presented in Table 1. The hemicelluloses of plants are a complex mixture of polymers with more than one type in each plant. Their chain lengths are much smaller than those of cellulose and can be linear or highly branched. In the present work, no attempt has been made to separate different hemicelluloses. However, it can be seen that xylose is by far the most abundant neutral sugar component of the hemicelluloses of the feeds used in this experiment, followed by arabinose and galactose, giving approximate values of xylose:arabinose and xylose:galactose in the diet of 4·5:1 and 5:1 respectively. Mannose was also consistently detected but in very much smaller amounts and was presumably present in traces of glucomannans or galactoglucomannans.

Carbohydrate contents of mixed rumen bacteria are shown in Table 2. The rhamnose contents of mixed bacteria separated from the rumen digesta from sheep receiving free linseed oil and protected coconut oil were significantly lower ($P < 0.05$ and $P < 0.01$ respectively) than those of bacteria from sheep receiving the basal diet. There were no significant differences for the other carbohydrate components of mixed bacteria between sheep receiving different diets.

Carbohydrate and NDF entering the duodenum

Results are presented in Table 3 for the intakes, duodenal flows and mouth to duodenum digestibilities of the main neutral sugars released by acid-hydrolysis from natural carbohydrates. The proportions of mannose and galactose entering the duodenum, which were contributed by microbes, were 0·36–0·74 and 0·20–0·30 respectively and were not significantly affected by diet. The amounts of undigested feed galactose reaching the duodenum tended to be greater with free-oil-supplemented diets but the differences were not significant.

Table 2. *Carbohydrates in mixed bacteria from the rumen of sheep given a basal diet alone (B) or supplemented with free linseed (L) or coconut (C) oil or protected linseed (PL) or coconut (PC) oil*

(Mixed bacteria were bulked samples separated from rumen digesta taken at 0, 3 and 5 h post-feeding. Results (g/kg dry matter (DM)) are mean values for five animals on eact diet)

Diet... Sugar	B	L	PL	C	PC	SEM
Rhamnose	13·7	7·0*	11·1	10·8	7·4**	0·9
Ribose	10·4	9·8	11·8	8·3	9·8	1·0
Mannose	2·3	2·3	3·0	2·5	2·3	0·3
Arabinose	1·7	2·1	2·4	2·4	1·5	0·2
Galactose	6·0	5·3	7·1	7·9	6·0	0·5
Xylose	1·0	0·8	0·9	1·0	0·8	0·1
Starch-glucose	50·5	33·8	45·9	25·0	34·7	7·0

Cellulose-glucose was only analysed in a few samples and was found to be generally less than 0·5 g/km DM. Mean values were significantly different from those for diet B: * $P < 0.05$, ** $P < 0.01$.

Little or none of the arabinose, xylose or cellulose-glucose entering the duodenum was contributed by the microbes. The amounts of surviving feed arabinose were significantly increased ($P < 0.01$) in animals receiving either of the free-oil supplements compared with animals receiving the basal diet. This was reflected in significantly lower ($P < 0.01$) amounts disappearing between mouth and duodenum. Protection of the oils reduced but did not completely eliminate this effect. The pattern of effect of oil supplementation on cellulose-glucose passing to the duodenum was very similar to the effects on arabinose with the same levels of significance but the absolute effects were more severe. Free oils completely prevented the digestion of cellulose-glucose whilst the protected oils reduced digestion by about one-third. Results for xylose, whilst showing the same basic effects of oil supplementation as both arabinose and cellulose-glucose, were extremely variable both between and within animals as shown by the high standard error, and effects of free oils were thereby only significant at $P < 0.10$.

Amounts of rhamnose and ribose entering the duodenum were similar on all diets and averaged approximately 1·4 and 1·5 g/d respectively. These amounts were contributed almost entirely by the rumen microbes. Starch-glucose intake on all diets was approximately 190 g/d. Apparent coefficients of digestibility for starch-glucose were unaffected by the diet and averaged 0.91 ± 0.05. Microbial contribution to the total starch-glucose entering the duodenum ranged from 0·24 to 0·44 (i.e. 4 to 7 g bacterial starch/d) and was not significantly affected by the diet. The average amount of dietary starch-glucose truly digested between mouth and duodenum was 0.94 ± 0.06 g/g intake and amounts of surviving dietary starch-glucose entering the small intestine were 9–15 g/d.

The amounts of NDF consumed and passing the duodenum are shown in Table 4. Approximately 0·50 g NDF/g intake disappeared between mouth and duodenum of animals receiving the basal ration. This was significantly ($P < 0.001$) reduced when either of the free oils was added to the diet (0·19 and 0·12 for linseed and coconut oils respectively). Protection of the oils reduced the effect of the free oils (0·29 and 0·38 for linseed and coconut oils respectively), but these were still significantly lower ($P < 0.01$) than digestibilities recorded in animals receiving the basal ration.

Table 3. *The daily intakes (g/d), duodenal flow (g/d) and rumen digestibilities (g/g intake)*
of dietary carbohydrates, together with absolute amounts of microbial carbohydrate at the
duodenum for sheep receiving a basal diet alone (B) or supplemented with free linseed (L) or
coconut (C) oils or with protected linseed (PL) or coconut (PC) oils

(Results are mean values for five sheep on each diet)

Diet...	B	L	PL	C	PC	SEM
Mannose						
Intake	1·07	1·06	1·07	1·07	1·07	0·001
Duodenal flow:						
Food residue	0·36	0·37	0·17	0·61*	0·36	0·052
Microbes	0·29	0·42	0·49	0·35	0·32	0·043
Digestibility in the rumen:						
Apparent	0·39	0·25	0·38	0·10*	0·36	0·118
True	0·66	0·65	0·84	0·43	0·66	0·151
Arabinose						
Intake	12·82	12·78	12·80	12·80	12·82	0·011
Duodenal flow:						
Food residue	4·17	7·78**	5·08*	8·30**	5·39*	0·550
Microbes		All values negligible ($< 0.5\%$ of total)				
Digestibility in the rumen:						
True	0·67	0·39**	0·60*	0·37**	0·58*	0·060
Galactose						
Intake	7·72	7·69	7·71	7·71	7·72	0·007
Duodenal flow:						
Food residue	2·90	3·47	2·68	3·46	2·98	0·355
Microbes	0·78	0·96	1·15	1·12	0·80	0·104
Digestibility in the rumen:						
Apparent	0·52	0·42	0·50	0·41	0·41	0·061
True	0·62	0·55	0·65	0·55	0·61	0·076
Xylose						
Intake	35·51	35·52	35·42	35·60	35·49	0·076
Duodenal flow:						
Food residue	19·58	36·36**	23·50†	33·62*	24·73†	3·350
Microbes		All values negligible ($< 1\%$ of total)				
Digestibility in the rumen:						
True	0·45	−0·02†	0·34	0·05†	0·30	0·074
Cellulose-glucose						
Intake	61·30	60·83	61·05	61·05	61·24	0·131
Duodenal flow:						
Food residue	30·16	63·54***	41·50*	61·18**	41·07**	2·020
Microbes		All values negligible ($< 2\%$ of total)				
Digestibility in the rumen:						
True	0·51	−0·04***	0·32*	0·00**	0·33*	0·080

Mean values were significantly different from those for diet B: † $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

DISCUSSION

The carbohydrate composition of mixed bacteria separated from sheep rumen digesta in the present work was similar to values reported for steers and sheep receiving roughage concentrate diets with adequate rumen-degradable N (McAllan & Smith, 1974, 1976). Corresponding information for protozoal carbohydrate composition is not available but it has been assumed in the present studies to be similar to the bacterial composition.

The use of the RNA:constituent ratio in rumen bacteria and the RNA contents of digesta

Table 4. *The amount of ash-free, neutral-detergent fibre (NDF) (g/d) consumed and passing through the proximal duodenum and the apparent digestion between mouth and duodenum of sheep given a basal diet alone (B) or supplemented with free linseed (L) or coconut (C) oils or with protected linseed (PL) or coconut (PC) oils*

(Results are mean values for five sheep)

Diet...	B	L	PL	C	PC	SEM
NDF intake	199	200	199	198	199	0·50
Flow at duodenum	99	160***	141**	173***	124*	7·6
Apparent NDF digestion in stomach	0·50	0·19***	0·29**	0·12***	0·38*	0·041

Mean values were significantly different from those for diet B: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

at the duodenum to assess the microbial contribution at that point has been applied previously (McAllan & Smith, 1976). Average values for the proportional contribution of bacterial rhamnose and ribose at the duodenum were 1·08 and 1·06 respectively. Of the main feed structural neutral sugars, only mannose and galactose included an appreciable bacterial contribution and, therefore, would be appreciably affected by any over-correction for microbial flow at the duodenum, but these sugars compromise only approximately 15% of the total ingested structural neutral sugars. Galactose and mannose were, however, the only structural sugars which were not apparently significantly affected by the addition of free oils to the diet. Whether the lack of effect of these supplements on their digestibility was because they were present as side chains on larger hemicellulose units or were more soluble as smaller galactomannan chains is unknown.

The appreciable reduction found in the digestion of fibre between mouth and duodenum when free oils were incorporated in the diet agrees with earlier work (Devendra & Lewis, 1974b). Some digestion of arabinose occurred even in the presence of free oils, the arguments for its availability to bacterial digestion being the same as those for mannose and galactose. There was, however, little or no observed digestion of cellulose-glucose or hemicellulose-xylose in the rumen of sheep receiving free oils, suggesting that certain major aspects of fibre digestion were completely inhibited. This was in contrast to the estimated loss of approximately 15% of NDF before the duodenum in the same animals receiving the same diets. This discrepancy may be a result of a number of causes. Estimation of NDF in feedstuffs containing a high proportion of starch without previous enzymic removal of the starch have been found to be in error (Mason & Kragelund, 1980), which could mean that our estimate of the intake of NDF could have included some starch and was too high and hence digestibility would have been over-estimated. On the other hand, recovery of individual carbohydrates after the hydrolysis procedures used in this work varies from 93 to 101% (McAllan & Smith, 1974) and some of the values obtained in the present work may therefore be under-estimates. Other workers have found incomplete agreement between discrete carbohydrate analytical totals and detergent-extraction values (Theander & Oman, 1977). Despite these discrepancies the effects of free oils on the mouth to duodenum digestion of NDF and individual structural carbohydrates were in good general agreement in the present studies.

The effects of fats on the total numbers or types of bacteria in the rumen are equivocal, being reported as increasing the numbers (Czerkawski, 1973; Czerkawski *et al.* 1975), having no effect (Henderson *et al.* 1977) or reducing the numbers (El-Hag & Miller, 1972). The reported increase in bacterial numbers by Czerkawski *et al.* (1975) was accompanied by an

apparent decrease in the diaminopimelic acid (DAP) content of mixed bacteria separated from rumen digesta, suggesting that a change in the numbers of some species of bacteria had occurred. Reduced DAP content of mixed rumen bacteria separated from digesta from sheep receiving free oils was also observed in the present work (Knight, 1980). Indeed, Henderson (1973) has shown a reduction in the numbers of cellulolytic bacteria in the presence of long-chain fatty acids. However, in the present work the results suggest that there would have to be an elimination of hemicellulolytic and cellulolytic species or a virtually complete inhibition of their glycan hydrolase activities.

One striking effect of oils on rumen microbiota has been the marked reduction in the numbers of protozoa present in the rumen of animals receiving free oils (Czerkawski, 1973; Czerkawski *et al.* 1975; Ikwuegbu & Sutton, 1982; Sutton *et al.* 1983) which could itself affect the numbers, types or metabolic activities of the bacteria. For example, it has been reported that in sheep the presence of protozoa results in an increase in the numbers of cellulolytic bacteria (Kurihara *et al.* 1978). The importance of the contribution of protozoa to the digestion of structural carbohydrates in the rumen is uncertain. Some genera ingest fibrous feed particles and can utilize all the ingested carbohydrates (Clarke, 1977; Delfosse-Debusscher *et al.* 1979). Large and small entodiniomorph protozoa have been shown to be cellulolytic (Bonhomme-Florentin, 1975; Coleman *et al.* 1976) and others are active in degrading hemicelluloses (Bailey *et al.* 1962; Abou Akkada, 1965). It has been calculated that the presence of protozoa in the rumen can account for approximately 0·3 of the total microbial fibre digestion (Demeyer, 1981). In the present experiment the reduction in rumen digestibility of the fibre fraction of the diet in the presence of free oils was considerably greater than this suggesting that the elimination of protozoa did not account for all the change but that it could have been a contributory factor.

We have shown in the present work that the effect of free oils on carbohydrate digestion applies equally to the hemicellulose and cellulose fractions of the fibre. However, it is obvious that not all the hemicellulose constituent carbohydrates are affected to the same extent as observed by the reduced response for mannose, galactose and, to a lesser extent, arabinose. Thus, if the reduction in fibre digestion was due to physical protection of the fibre by coating, it would appear to be either selective or incomplete. No such coating of the concentrate part of the ration appears to take place judging by the unaltered digestion of starch in the presence of free oils. It is not possible from the present work to distinguish unequivocally whether the reduction in fibre digestion was due to physical protection of the fibre or to changes in microbial composition or activities, although results tend to favour a change in the composition or activities of the microbes.

The intermediate effects observed when protein-protected oils were given are not surprising. It suggests either that protection was incomplete, a possibility supported by other evidence (Knight *et al.* 1977; Bines *et al.* 1978) or that even in their protected form, oils can reduce fibre digestion though they do not completely eliminate it.

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