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The effects of soya-bean oil and type of forage in the diet on the plasma lipid composition of sheep

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1. Sheep were given four different diets which contained two types of forage and two types of concentrate. The forage portion of the diet consisted of either hay or silage, while the low-oil concentrate had high levels of starch and in the high-oil concentrate this starch was replaced by an isoenergetic amount of soya-bean oil.

2. The effects of these diets on various factors in the blood was measured.

3. The erythrocytes were more fragile in hypotonic solutions (6·0–8·5 g sodium chloride/l) of saline when they were eating a diet of hay and high-oil concentrates.

4. The levels of calcium, magnesium, phosphorus, glucose and β-hydroxybutyrate in the plasma were unaffected by dietary treatments.

5. Substitution of silage for hay in the diet increased the intake of all fatty acids, especially that of $C_{18:3}$.

6. When the sheep had low intakes of oil, the change from hay to silage led to increases in the amounts of triglyceride (TG) and total lipid (TL) in their plasma, whereas with high intakes of oil this change of forage increased the plasma TG content by an even greater amount but did not alter the concentration of TL.

7. Increasing the intake of dietary oil raised the levels of both TG and TL in the plasma, irrespective of the type of dietary forage.

8. On silage diets the sheep had much greater amounts of 18:0 and 18:1 fatty acids but smaller quantities of 18:2 fatty acid in their plasma than on hay diets.

9. Changing the dietary forage from hay to silage or the concentrate from low- to high-oil enhanced the levels of all the *trans* isomers of C_{18} fatty acids in the blood of the animals.

10. These results imply that unsaturated fatty acids in silage-based diets are hydrogenated to a greater extent in the rumen than those in hay diets which results in an elevation in blood TG levels and that this in turn could profoundly alter such functions as milk fat synthesis and fat deposition in ruminants.

Lipid nutrition in ruminants is now of major importance (Christie, 1979) both from the metabolic (Bell, 1979) and the production aspects in sheep (Steele, 1979) and cows (Steele *et al.* 1971*a*). Most of the experiments reported in the literature on this topic have been carried out with hay as the only forage in the ration and in the majority of cases the quantity of fat in the various dietary components has been determined with diethyl ether as the extracting solvent (Christie, 1973). Two important changes have occurred, however, which make further investigation of this subject necessary. In the first instance, there has been a change in the manner of grass conservation; the amount harvested for silage has doubled and that for hay has remained fairly constant (Wilkins, 1974). Also it is acknowledged that there are large inaccuracies associated with procedures in which the fat content of food is determined by diethyl ether extraction (Atkinson *et al.* 1972), whereas with more modern techniques not only can the amount of total lipid in the diet be more exactly quantified but it can also be easily and completely described in terms of individual fatty acids (Christie, 1973).

Therefore, an investigation has been undertaken in order to establish if the changes, which are produced in ruminants that have been given diets in which fat has been added to hay-based diets, differ from those which might be obtained when silage constitutes the only forage in the diet. Possible differences between these diets have been examined in sheep by monitoring certain factors in their blood, with special attention being given to the

Table 1. *Amounts (g fresh material/d) of each constituent of the low- and high-oil concentrates offered to sheep*

Constituent	Low-oil concentrate	High-oil concentrate
Ground barley	423	423
Soya-bean-oil meal	113	113
Starch	116	—
Molasses	26	26
Soya-bean oil	—	50
Minerals	14	14

triglyceride (TG) fraction because of the metabolic importance of this entity in the blood of ruminants (Moore & Christie, 1979).

EXPERIMENTAL

Animals and their diets

Four adult Finn × Dorset wethers with an average weight of 70 kg were housed in individual metabolism cages and used for the experiment.

Each animal received in turn each of four diets which consisted of either hay or silage as the forage and either a low- or a high-oil content concentrate. The daily allocation to each animal of hay and silage was 600 and 2040 g fresh material respectively. The silage and hay were conserved from the same sward except that the grass which was used for making the hay was cut at the customary 20 d later than for the silage. Soya-bean oil replaced starch on an isoenergetic basis in the high-oil concentrate. The sheep were given their daily ration of food in two equal portions at 6.00 and 18.00 hours and water was available *ad lib*. The amounts of each of the constituents of the concentrates offered daily to each animal are given in Table 1. These amounts were calculated to be sufficient for maintenance plus a small daily live-weight gain of 25 g/d (Ministry of Agriculture, Fisheries and Food, 1975).

Experimental design and statistical analyses

Each sheep was randomly assigned to one treatment sequence of a 4 × 4 Latin Square which was balanced for 'carry-over' effects. Each period lasted for 28 d and change-overs between treatments were made abruptly.

All the results were analysed statistically by the methods outlined by Snedecor & Cochran (1967).

Collection of samples and methods of analyses

A representative sample of each dietary component was collected daily and bulked at the end of each period for chemical analyses.

Immediately before and at various times after feeding on the last 2 d of each experimental period, samples of blood were collected from the jugular vein.

Before the lipids were extracted from the various dietary components, each sample was first heated at 70° for 30 min with 5 M-hydrochloric acid since it has been found (W. Steele, unpublished observations) that this treatment enhances the quantity of fatty acids that are extracted by lipid solvents. This was especially true of the hay and the unsupplemented concentrate where the extraction rate without acid treatment was approximately 55 % of that with acid treatment. With the silage and the high-oil concentrate the improvement was of the order of 10 %. After this preliminary incubation the lipid was then extracted

Table 2. *Mean daily intakes (g) of individual and total fatty acids by sheep given diets containing hay or silage with either low- or high-oil concentrates*

Forage ...	Hay		Silage		
Concentrate ...	Low-oil	High-oil	Low-oil	High-oil	SED*
16:0	3·32	8·97	4·04	9·69	0·050
16:1	0·17	0·22	0·27	0·33	0·007
18:0	0·24	2·32	0·25	2·34	0·007
18:1	1·38	12·1	1·39	12·1	0·014
18:2	4·56	30·2	5·29	30·9	0·009
18:3	1·40	4·94	4·59	8·11	0·116
Total	11·2	59·2	15·9	63·9	0·129

* Standard error of difference between two treatments.

from the dietary constituents with chloroform–methanol according to the procedure of Bligh & Dyer (1959).

Plasma was separated from whole heparanized blood by centrifugation, the lipids extracted by the method of Bligh & Dyer (1959) and the TG fraction separated from the other lipid fractions by thin-layer chromatography (Mangold, 1962).

Methyl esters of the fatty acids contained in the TL and TG fractions of the blood were prepared and estimated by methods already described (Steele, 1979). The 18:1 and 18:2 fatty acids of the plasma TL and TG moieties were resolved into *cis* and *trans* isomers by argentation thin-layer chromatography (Kiuru *et al.* 1974). The various isomers were then extracted from the silica gel with chloroform–methanol–acetic acid–water (50:30:1:10, v/v) and determined by gas–liquid chromatography using an internal standard (Christie *et al.* 1970).

The concentrations of glucose in the blood and of β-hydroxybutyrate in the serum were determined by the procedures of Werner *et al.* (1970) and Williamson *et al.* (1962) respectively. The levels of calcium, magnesium and phosphorus in the blood serum were calculated according to the methods of Gindler & King (1972), Gindler & Heth (1971) and Chen *et al.* (1956) respectively.

The osmotic fragilities of the erythrocytes (RBC) were measured in various concentrations of hypotonic saline (0·0–8·5 g sodium chloride/l) according to the method of Parpart *et al.* (1947).

RESULTS

Dietary intakes

There were no refusals of food during any of the experimental periods. The total dry matter (DM) intake was approximately 2% greater with the silage-based diets than with the hay-based diets since it was impossible to predict accurately the DM contents of the forages, especially that of the silage. The low-oil diets had the lowest energy density and in order to maintain an isoenergetic intake the animals' intake of DM was approximately 5% more during periods on low-oil than it was on high-oil diets.

The fatty acid composition of the grass before and after conservation was not significantly ($P > 0·05$) different. The total fatty acid contents (g/kg DM) of the hay, silage and low- and high-oil concentrates were 5·9, 14·8, 15·2 and 114 respectively. The intakes of the different fatty acids by the sheep on the four dietary treatments is given in Table 2. Substitution of silage for hay resulted in an increase of 2·5 times in the intake of total fatty acids from the forage portion of the diet.

It should also be noted that the fatty acid composition of the forage lipids was also

Table 3. *Concentrations (μg/ml) of the total fatty acids at various times after feeding, in the plasma total lipid (TL) and triglyceride (TG) fractions of sheep given diets containing either hay or silage with either a low- or a high-oil concentrate*

Plasma fraction	Period after feeding (h)	Forage ... Hay: Low-oil	Hay: High-oil		Silage: Low-oil	Silage: High-oil	SED
TL	0	613	1388		684	1441	
	3	654	1380		675	1285	
	6	589	1230		639	1261	71·6*
	9	581	1299		637	1268	
	12	613	1388		684	1441	
SED				45·9†			
	Mean	611	1323		664	1315	42·0
TG	0	47·5	109·8		65·8	184·3	
	3	54·0	151·3		68·0	184·3	
	6	55·3	169·0		74·8	178·8	12·6*
	9	56·5	145·7		68·8	177·8	
	12	47·5	109·8		65·8	184·3	
SED				10·9†			
	Mean	54·1	139·8		69·1	180·7	5·8

* Standard error of difference between treatments within a period.
† Standard error of difference between periods within a treatment.

very different; the major difference being that the silage contained more $C_{18:3}$. Apart from the obvious increase in the quantity of fatty acids that were ingested when the diets were changed from the low- to the high-oil concentrate, the qualitative composition of the dietary lipid also changed. The added soya-bean oil contained lower concentrations of C_{16} fatty acids and higher concentrations of all the C_{18} fatty acids than that found in the other dietary ingredients. Nevertheless, because of the higher lipid content of the high-oil concentrate, the intake of all fatty acids including that of 16:0 fatty acid increased when the diet contained the high-oil concentrate (Table 2). The animals' consumption of total fatty acids was 4·7 g more on silage-based diets and although this increase represented only 7 % on the high-oil treatments it accounted for a rise of 42 % on the low-oil diets. The major part of this increase was accounted for by the 18:3 fatty acid.

Blood lipids

Irrespective of the level of oil intake by the sheep, the concentrations of total and all except one of the major fatty acids in their plasma were greater when the forage in the diet was silage than when it was hay (Table 3, Fig. 1). The one exception was that the amount of 18:2 fatty acid in the plasma was greater during periods when the animals were given hay than when they were receiving silage. However, none of these differences were statistically significant ($P > 0·05$). There were large and highly significant increases ($P < 0·001$) in the amounts of all fatty acids in the plasma of the animals when their diet was changed from a low- to a high-oil one, the magnitude of this increase was much less for the 16:0 fatty acid than for the C_{18} fatty acids (Fig. 1, Table 3).

Irrespective of the time after feeding, the level of TG in the plasma was greater on silage- than on hay-based diets (Table 3). Although all these differences were statistically significant for the high-oil treatments, only the mean values on the low-oil treatments reached a statistical level of significance ($P < 0·05$). All the individual fatty acids contributed to the difference in the plasma TG levels between diets at feeding time, whereas in the

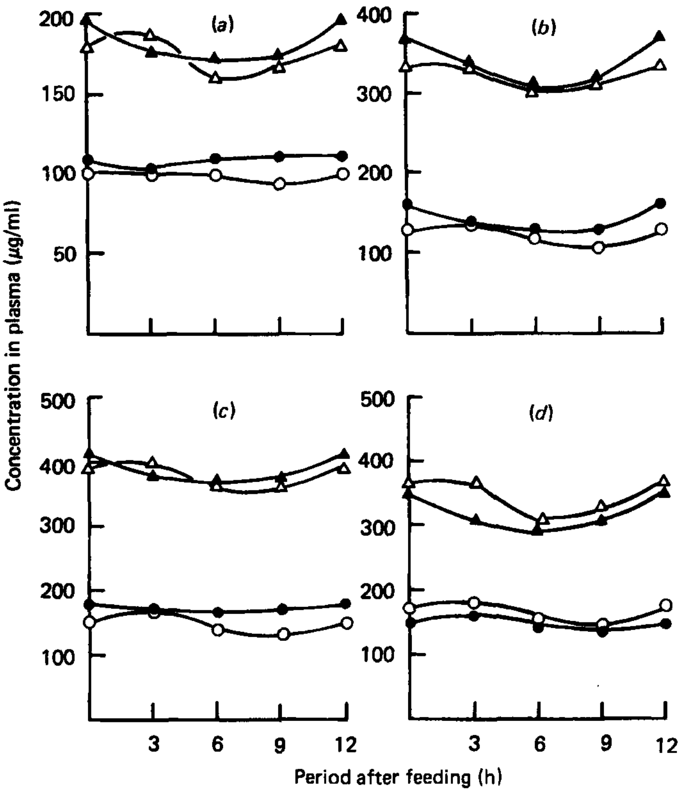

Fig. 1. Concentrations (μg/ml) of the major fatty acids: (*a*) 16:0, (*b*) 18:0, (*c*) 18:1, (*d*) 18:2, in the plasma of sheep at intervals after being given diets containing either hay (○ △) or silage (● ▲) with either low- (○ ●) or high-oil (△ ▲) concentrate.

intervening period between meals these differences were maintained mainly by that of $C_{18:1}$ (Fig. 2).

The concentration of TL in the plasma reached its maximum value at feeding time and its minimum in the intervening period, while that of TG increased in the interval between meals and declined to its lowest at meal time. The amounts of the *trans* isomers of both 18:1 and 18:2 fatty acids in the plasma TL and TG fractions were always greater on silage than on hay-based diets and also greater on high-oil than on low-oil diets (Table 4). The value for *trans*:*cis* isomers in both the 18:1 and 18:2 fatty acids was always larger in the TG than it was in the TL component of the plasma, irrespective of the dietary treatment.

Blood glucose and β-hydroxybutyrate

Immediately before meal time, the plasma glucose concentration was unaffected by diet (Table 5). Whereas, 4 h later its concentration was increased by the high-oil with silage diet in comparison to the other three diets and also compared to that in the pre-feeding sample ($P < 0.05$).

The lowest concentration of β-hydroxybutyrate in the plasma at 06.00 hours ($P < 0.05$) was recorded when the animals were receiving the diet of high-oil concentrate and silage (Table 5). While at 10.00 hours the level of this metabolite in the blood was increased both by over-all ($P < 0.001$) and by individual treatments ($P < 0.05$), none of the inter-treatment difference were significant ($P > 0.05$).

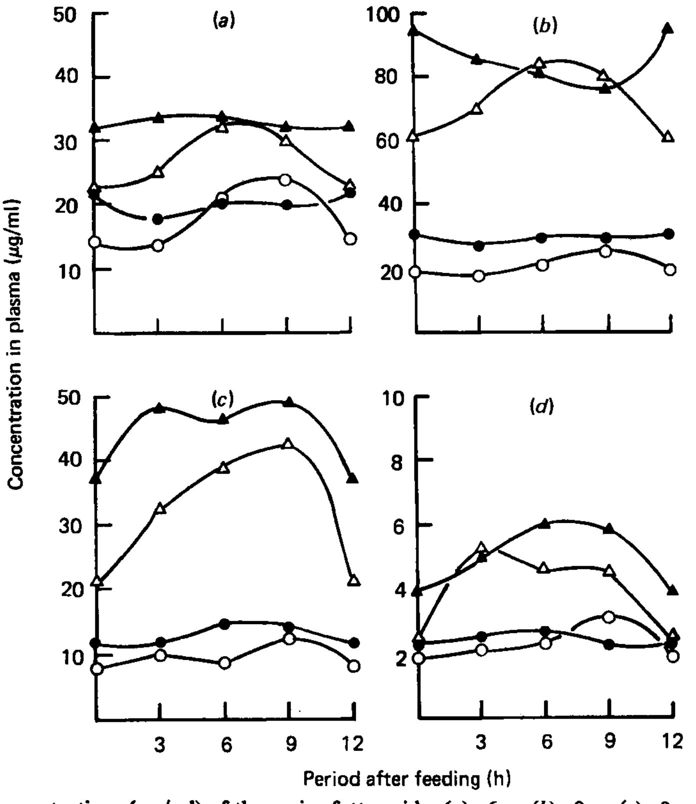

Fig. 2. Concentrations (µg/ml) of the major fatty acids: (*a*) 16:0, (*b*) 18:0, (*c*) 18:1, (*d*) 18:2, in the plasma triglycerides of sheep at intervals after being given diets containing either hay (○ △) or silage (● ▲) with either a low- (○ ●) or high-oil (△ ▲) concentrate.

Table 4. *Concentrations* (*µg/ml*) *of* cis (c) cis-cis (cc) *and* trans (t) *isomers of* 18:1 *and* 18:2 *fatty acids in the plasma total lipid (TL) and triglyceride (TG) fractions of the plasma of sheep given diets containing either hay or silage with either a low- or a high-oil concentrate*

Forage ...		Hay		Silage		
Concentrate ...		Low-oil	High-oil	Low-oil	High-oil	SED†
Plasma fraction	Fatty acid					
TL	*c* 18:1	136	340	158	323	44·0
	t 18:1	10·2	34·9	13·4	60·3	6·25
	cc 18:2	156	336	145	309	43·4
	*t** 18:2	2·2	2·2	2·3	3·6	0·73
TG	*c* 18:1	8·75	21·8	10·5	24·5	3·48
	t 18:1	1·35	13·1	2·64	21·3	2·96
	cc 18:2	2·23	3·96	2·14	5·45	0·90
	*t** 18:2	0·22	0·19	0·28	0·42	0·12

* Includes *cis-trans* and *trans-trans* isomers of 18:2.
† Standard error of difference between two treatments.

Table 5. *Concentrations (mmol/l) of glucose in the blood and of β-hydroxybutyrate (β-OHC$_4$) in the serum of sheep immediately before and at 4 h after feeding on a diet containing either hay or silage with either a low- or a high-oil concentrate*

Blood constituent	Time of day (hours)	Forage ... Hay			Silage			
		Low-oil	High-oil		Low-oil	High-oil	SED	Over-all mean
Glucose	06.00	2·80	2·77		2·76	2·75	0·091*	2·77
	10.00	2·90	2·93		3·10	3·15		3·00
								0·064
SED				0·128†				0·12
β-OHC$_4$	06.00	0·14	0·14		0·13	0·08	0·056*	0·28
	10.00	0·27	0·24		0·40	0·22		
SED				0·037*				0·030

* Standard error of difference between treatments within a period.
† Standard error of difference between periods within a treatment.

Blood Ca, Mg and P

The concentrations of Ca, Mg and P in the blood of the animals were not changed ($P > 0·05$) by the various dietary treatments. The only significant difference was an increase in the mean over-all concentration of Mg from 0·79 mM immediately before feeding to 0·89mM at 4 h later.

Fragility of RBC

Within the low-oil treatments the RBC of the sheep were more fragile when the basal ration was silage; none of these differences, however, were significant ($P > 0·05$). Although the RBC were more fragile during periods of high-oil intake, the only differences which reached a statistical level of significance ($P < 0·01$) were when the two hay diets were compared and even then only at saline concentrations between 6 and 8·5 g/l.

DISCUSSION

In the present investigation the level of $C_{18:3}$ in the grass before and after ensiling was approximately the same thus differing from the results of Lough & Anderson (1973) who observed a large reduction in the concentration of this acid after ensiling; they ascribed this change to microbial action. The most probable cause of the difference between these two experiments is that Lough & Anderson (1973) examined a silage which had been made without additives whereas the material in the present experiment was preserved with the aid of formic acid which would inhibit microbial activity.

As grass matures the quantity of total lipid as well as the concentration of 18:3 fatty acid in it declines (Moore *et al.* 1968), which is probably a reflection of an increase in the value of structural:leafy tissue (Gray *et al.* 1967). Thus in the experiment now reported the difference between the hay and silage in their contents of total lipid and 18:3 fatty acids probably resulted from the fact that the grass was conserved for hay at a more mature stage of growth than for silage.

Neither the type of forage nor the substitution of oil for starch in the diet caused the blood levels of glucose or β-hydroxybutyrate (Table 5) to fall outside the normal range (Doxey, 1977). Even the highest level of β-hydroxybutyrate recorded was only approximately 20 % of the maximum level which sheep are capable of utilizing without ill-effect (Bergman & Kon, 1964).

Table 6. *Mean fatty acid compositions (g/100g) of the triglycerides (TG) and lipid fractions other than TG in the plasma of sheep*

	Lipid fraction of plasma	
Fatty acid	TG	Other lipids
16:0	22·5	13·6
18:0	47·4	20·5
cis-18:1	14·2	26·3
trans-18:1	8·4	2·3
cis-cis 18:2	3·0	27·0
trans isomers of 18:2	0·27	0·23

It was surprising to find that a change of diet from hay to silage, which resulted in an increase in the daily intake of fatty acids of only 4·7 g, should increase plasma levels of TG by such a large amount and even more surprising that the magnitude of this increase should be greater when the two high-oil diets were compared (Table 3). One possible explanation for this may be that the extent to which unsaturated fatty acids are hydrogenated in the rumen depends on the nature of the basal forage. Consequently this affects the amount of each fatty acid which enters the blood stream. Thus when the diet was silage-based the amounts of 16:0, 18:0 and *trans* 18:1 fatty acids in the plasma of the sheep exceeded that when their diet was hay-based (Fig. 1, Table 4). These three acids are preferentially used for the synthesis of TG compared to other lipid tissues in the plasma (Table 6).

The very large increase in the level of plasma TG which resulted from the high-oil diets (Table 3) has not previously been reported in sheep. The only values in the literature which can be used for comparison have been obtained with cows. However the comparison may not be valid because in the one case with cows in which there was no response in plasma TG levels to added fat the experimenters had used a different sampling site for the blood from that used in the trial now reported (Moore *et al.* 1969; Steele *et al.* 1971 *b*) and in the other case where the plasma TG levels of the cows was increased by dietary fat the workers had supplemented the diet with fat in the protected form (Macleod *et al.* 1977; Yang *et al.* 1978).

The results of the present investigation demonstrate that not only is the type of forage, *per se*, in the diet responsible for the lipid composition of the blood plasma of sheep but it also affects the metabolism of added dietary oils. It is concluded from these results that the addition of oil to the diet of sheep is more conducive to increasing the concentrations of TG in the plasma when the basal forage is silage compared to when it is hay. This implies that because of the increases in plasma TG brought about by these dietary regimens there could be profound changes in such functions as milk fat synthesis (Annison *et al.* 1967) and fat deposition in ruminants (Christie, 1979).

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