103. SHEEP BODY FATS

1. COMPONENT ACIDS OF FATS FROM ANIMALS FED ON HIGH AND LOW PLANES OF NUTRITION

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In 1938 an investigation on the effects of different planes of nutrition of ewes upon the birth weight and growth of lambs was undertaken at the Animal Nutrition Institute of the School of Agriculture, Cambridge, by Dr Verges, with the result that two groups of ewes respectively of about 70 and 150 lb. live weight were obtained from the restricted and full diets given. Five of the 70 lb. animals were then fed on a high plane of nutrition ('supermaintenance ration'), whilst five of the 150 lb. ewes were given a restricted diet ('submaintenance ration'), an animal from each group being killed at intervals until, finally, the first group attained 150 lb. live weight, whilst the second group had been brought down to about 70 lb. live weight. Dr J. Hammond suggested that the fat from these animals would repay study since throughout the experiment they had been on standard and known quantities of food. In this and the following communication some account is given of the component acids and glycerides in the perinephric and external tissue fats of some of the ewes. The opportunity to study sheep body fats from animals of known history was especially welcomed because all previous detailed information as to the acids and glycerides present referred to specimens of market origin (mutton tallow); the analyses now recorded afford data for sheep fats parallel with those already obtained for ox and pig depot fats from animals reared on specific diets.

Experimental

The 'supermaintenance rations' fed to the ewes of 70 lb. live weight consisted of lucerne hay of medium quality *ad lib.* together with 4–5 lb. daily of a concentrate mixture consisting of 1 part by weight each of locust bean meal and white fish meal, 2 parts by weight each of broad bran, flaked maize, linseed cake and split peas, and 3 parts by weight of crushed oats. The 'submaintenance ration' fed to the ewes of 150 lb. live weight consisted of wheat straw *ad lib.* until the live weight of the animal fell to 90 lb., and subsequently of $\frac{3}{4}$ lb. of wheat straw only each day.

Some general data for the animals at the time of slaughter are quoted in Table 1.

The component acids of the perinephric and external tissue fats of three ewes from each group (nos. 26, 12 and 4, and nos. 30, 9 and 21) have been examined in detail. (The quantity of perinephric fat available from the control animal no. 11 of 70 lb. live weight was too small to permit detailed analysis.) The yield of fat extracted by acetone from the tissues is shown in Table 2.

Table 1

Ewe no.	Days on special ration	Live weight lb.	Fatty tissues: Perinephric g.	Fatty tissues: External g.
	(*a*) Ewes on 'supermaintenance' ration			
11	0	70	27	223
26	22	91	138	671
20	38	105	253	1377
12	58	127	703	3939
4	81	151	2026	5580
	(*b*) Ewes on 'submaintenance' ration			
30	0	148	1019	7110
29	13	131	1441	5100
9	100	113	406	1218
10	147	91	465	1615
21	209	72	341	742

Table 2. *Composition of ewe fatty tissues*

Ewe no.	Live wt. at death lb.	Perinephric tissue: Fat %	Perinephric tissue: Fat I.V.	Perinephric tissue: Connective tissue %	Perinephric tissue: Water, etc. (by diff.) %	External tissue: Fat %	External tissue: Fat I.V.	External tissue: Connective tissue %	External tissue: Water, etc. (by diff.) %
		(*a*) Ewes on 'supermaintenance' rations							
26	91	90·3	48·2	6·0	3·7	44·6	52·0	18·8	36·6
12	127	96·7	43·4	2·7	0·6	80·8	49·1	8·2	11·0
4	151	97·5	42·7	1·6	0·9	83·6	50·2	8·0	8·4
		(*b*) Ewes on 'submaintenance' rations							
30	148	91·6	41·0	1·1	7·3	87·6	47·9	3·4	9·0
9	113	98·0	40·2	1·3	0·7	82·0	44·6	8·3	9·7
21	72	97·1	34·3	1·6	1·3	69·9	44·3	12·9	17·2

The proportion of fat in the external tissues of the ewe (no. 21) which had been reduced to 72 lb. live weight, although lower than in those of the fat animals, was considerably greater than that in the external tissues of the ewe (no. 26) of 91 lb. live weight (which had been brought up on a restricted diet until 22 days before it was killed). The general unsaturation of the fats, as indicated by their iodine values, declined somewhat in both the 'supermaintenance' and 'submaintenance' groups as the experiments proceeded.

The component acids of the selected fats were determined by means of the procedure followed in recent similar work in this laboratory [Hilditch & Longenecker, 1937; Hilditch *et al.* 1939]: the mixed fatty acids from hydrolysis of 130–150 g. of each fat were separated into 'solid' and 'liquid' acids by the different solubilities of the lead salts in alcohol, and thereafter the methyl esters of each group of acids were fractionally distilled through an electrically heated and packed column at low pressure (0·1–0·2 mm.).

Considerations of space lead us to confine our data here to the final weight and molecular percentages observed for the acids in each of the fats (Table 3); but it should be mentioned that the unsaturation of the methyl esters of C_{18} acids from the 'solid' acids was uniformly large (I.V. from 13 to 25), presumably owing to the presence of small proportions of vaccenic acid [Bertram, 1928], a solid acid isomeric with oleic acid.

Table 3. *Component acids of perinephric and external tissue fats of ewes*

	'Supermaintenance' group			'Submaintenance' group			
Ewe no.	26	12	4	30	9	21	
Live wt. (lb.)	91	127	151	148	113	72	
Acid	(*a*) Weight % (excluding unsaponifiable matter)						Mean
	Perinephric fats						
Lauric	—	—	—	0·1	0·1	—	Trace
Myristic	2·9	2·7	2·3	1·7	3·0	2·8	2·6
Palmitic	24·0	24·7	26·2	26·8	23·6	23·0	24·7
Stearic	24·9	28·3	27·1	30·1	31·7	37·8	30·0
Tetradecenoic	0·7	0·3	0·3	0·2	0·2	0·2	0·3
Hexadecenoic	2·4	0·9	1·0	0·9	1·3	1·0	1·2
Oleic	39·2	36·8	38·7	34·8	35·4	32·1	36·2
Octadecadienoic	5·2	5·7	3·3	4·3	3·9	2·2	4·1
Unsaturated C_{20-22}	0·7	0·6	1·1	1·1	0·8	0·9	0·9
	External tissue fats						
Lauric	0·4	0·6	0·9	0·7	0·6	0·3	0·6
Myristic	3·4	3·0	3·1	1·9	2·2	3·7	2·9
Palmitic	27·8	28·0	28·3	33·9	30·5	24·3	28·8
Stearic	14·7	16·2	13·5	15·3	20·1	24·6	17·4
Tetradecenoic	0·4	0·3	0·2	0·3	0·3	0·3	0·3
Hexadecenoic	1·6	0·8	0·6	0·9	1·2	0·7	1·0
Oleic	46·3	46·6	50·8	41·2	41·4	44·1	45·0
Octadecadienoic	4·8	3·9	1·9	4·9	2·8	1·5	3·3
Unsaturated C_{20-22}	0·6	0·6	0·7	0·9	0·9	0·5	0·7
	(*b*) Molar % (excluding unsaponifiable matter)						
	Perinephric fats						
Lauric	—	—	—	0·2	0·2	—	0·1
Myristic	3·5	3·2	2·8	2·1	3·6	3·3	3·1
Palmitic	25·6	26·4	28·0	28·6	25·3	24·7	26·4
Stearic	23·9	27·3	26·1	29·0	30·5	36·6	28·9
Tetradecenoic	0·8	0·3	0·3	0·3	0·3	0·2	0·4
Hexadecenoic	2·6	1·0	1·1	1·0	1·4	1·1	1·4
Oleic	38·0	35·7	37·5	33·7	34·3	31·2	35·0
Octadecadienoic	5·0	5·6	3·2	4·2	3·8	2·1	4·0
Unsaturated C_{20-22}	0·6	0·5	1·0	0·9	0·6	0·8	0·7
	External tissue fats						
Lauric	0·5	0·8	1·2	1·0	0·8	0·4	0·8
Myristic	4·1	3·6	3·7	2·3	2·7	4·4	3·5
Palmitic	29·4	29·7	29·9	35·8	32·3	25·9	30·5
Stearic	14·1	15·6	12·9	14·5	19·2	23·6	16·6
Tetradecenoic	0·5	0·3	0·2	0·4	0·3	0·4	0·3
Hexadecenoic	1·7	0·8	0·7	1·0	1·3	0·8	1·1
Oleic	44·6	44·9	48·9	39·5	40·0	42·5	43·4
Octadecadienoic	4·6	3·8	1·9	4·7	2·7	1·5	3·2
Unsaturated C_{20-22}	0·5	0·5	0·6	0·8	0·7	0·5	0·6

Discussion

Only three detailed analyses of sheep depot fats (mutton tallows) have been recorded previously, and these do not take account of unsaturated minor component acids other than octadecadienoic; the earlier data are collected in Table 4.

These older figures fall for the most part within the range of those obtained in the course of our present work, and it would appear that, whilst the specimen examined by Collin *et al.* [1929] was wholly perinephric fat, those described by Armstrong & Allan [1924] may have been mixed body fats in which external tissue fat was also present.

Table 4. *Earlier analyses of sheep depot fats*

Acid (weight %)	Armstrong & Allan [1924] South American	Armstrong & Allan [1924] Australian	Collin *et al.* [1929] Australian
Myristic	1	2	4
Palmitic	21	25	25
Stearic	30	23	31
Oleic	43	47	36
Octadecadienoic	5	3	4

From the whole series of sheep fats which we have now studied, it appears that, in the perinephric fats, the molar content of palmitic (+myristic) acid is about $26{\cdot}5 \pm 2\%$ and is thus somewhat lower than the value ($30 \pm 3\%$) which Hilditch & Longenecker [1937] have shown to be characteristic for ox depot fats, and Hilditch *et al.* [1939] for pig depot fats. The molar palmitic acid content of the external tissue fats is distinctly higher (mean value $30{\cdot}5\%$) and is also more variable, values from $25{\cdot}9$ to $35{\cdot}8\%$ (mol.) being observed. It is notable that, in ox and pig depot fats, those from the external tissues contain somewhat less palmitic acid than the more saturated perinephric fats, but that in the sheep the external tissue fats, although as usual less saturated than the perinephric fats, nevertheless possess higher proportions of combined palmitic acid than the latter.

Of the minor component saturated acids, myristic acid is present to about the same extent as in ox depot fats, but arachidic acid was not definitely detected. In the unsaturated series, hexadecenoic acid (which is usually present to the extent of 2–3% in ox and pig depot fats) only forms about 1% of the total fatty acids, whilst the proportion of unsaturated C_{20-22} acids is similar and thus probably intermediate between that found in ox and in pig fats. Polyethenoid C_{18} acids approach in quantity those in pig depot fats and are definitely more prominent than in ox depot fats; the proportion, although somewhat variable, is usually of the order of $4 \pm 1\%$ (mol.) of the total fatty acids. Acids from ester-fractions consisting substantially of the unsaturated C_{18} group yielded, on addition of bromine, traces of ether-insoluble bromo-additive products which darkened at 165–170° without melting and of petrol-insoluble products which melted at 114°; oxidation of their potassium salts with cold dilute permanganate solution likewise yielded only minute amounts of tetrahydroxystearic acids, M.P. 172°, and M.P. 153–154° (accompanying large quantities of dihydroxystearic acid, M.P. 130°, from the oleic acid also present). Apart from possible traces of polyethenoid C_{18} acids, the octadecadienoic acid of the sheep fats is different from ordinary or seed-fat linoleic acid, although traces of the latter are also present. The sheep fats thus closely resemble ox depot and cow milk fats, and many animal liver fats, in containing small quantities of an octadecadienoic acid not identical with seed-fat linoleic acid.

It is interesting to compare (Table 5) the total molar proportions of C_{16} and C_{18} acids in the sheep fats (for this purpose including minor component C_{14} and C_{12} acids with the C_{16} group, and the minor proportions of C_{20} and C_{22} acids with the C_{18} acids).

The approximate constancy of the total C_{16} and C_{18} groups of acids within each series of depot fats is as well-marked as in ox or pig depot fats previously studied in this laboratory [Banks & Hilditch, 1931; 1932; Dean & Hilditch, 1933; Hilditch & Longenecker, 1937]. In the six perinephric fats, the C_{18} (stearic, oleic, octadecadienoic) acids amount to 68–70 mols. per 100 mols. of

Table 5. *Molar percentages of C_{16} and C_{18} groups of acids*

	'Supermaintenance' group			'Submaintenance' group			
Ewe no. ...	26	12	4	30	9	21	
Live wt. (lb.) ...	91	127	151	148	113	72	
	Perinephric fats						Mean
C_{16} (+C_{14}, C_{12}) acids	32·5	30·9	32·2	32·2	30·8	29·3	31·4
C_{18} (+C_{20-22}) acids	67·5	69·1	67·8	67·8	69·2	70·7	68·6
	External tissue fats						
C_{16} (+C_{14}, C_{12}) acids	36·2	35·2	35·7	40·5	37·4	31·9	36·2
C_{18} (+C_{20-22}) acids	63·8	64·8	64·3	59·5	62·6	68·1	63·8

total fatty acids, although the stearic acid content, for example, varies from 23·9 to 36·6% (mol.); in the external tissue fats which, with one exception, contain more C_{16} acids than the perinephric fats, the total C_{18} acids form from 59·5 to 64·8% (mol.) of the total fatty acids, whilst the stearic acid content varies from 12·9 to 19·2% (mol.). These relationships are exactly similar to those observed in depot fats of oxen or pigs and, as will be seen from the communication which follows [Hilditch & Zaky, 1941], are consequent upon the characteristic glyceride structure which, as depot fats rich in stearic acid, sheep fats share with ox, etc. depot fats.

Up to this point, the twelve fats under investigation have been discussed as a whole, and as typical of depot fats produced in lean or fat sheep which have received diets of normal, though differing, character. Some interesting features are revealed, however, by consideration of the separate dietary experiments in which on the one hand, some of the animals were given a fattening ration, whilst on the other hand others were depleted in fat by feeding on a low plane of nutrition.

Fat deposited by ewes fed on 'supermaintenance' rations

From the weight of fat in the depots of each animal and the weight percentages of the component fatty acids (Table 3) it is possible to obtain a rough estimate of the weight of each fatty acid present in the depots of the control animal (91 lb. live weight) and of the animals killed respectively after 58 and 81 days' intensive feeding at respective live weights of 127 and 151 lb. (Table 6).

Table 6. *Total amounts of fatty acids in sheep depot fats* (*g.*)

	Perinephric fats			External tissue fats		
Ewe no. ...	26	12	4	26	12	4
Fatty tissue (g.)	138	703	2026	671	3939	5580
% fat	90·3	96·7	97·5	44·6	80·8	83·6
Fat (g.)	125	680	1975	299	3183	4664
Fatty acids (g.)	119	650	1887	286	3041	4456
Lauric	—	—	—	1	18	40
Myristic	3	17	43	10	91	138
Palmitic	29	161	494	79	852	1261
Stearic	30	184	512	42	493	601
Tetradecenoic	1	2	6	1	9	9
Hexadecenoic	3	6	19	5	24	27
Oleic	46	239	730	132	1417	2264
Octadecadienoic	6	37	62	14	119	85
Unsaturated C_{20-22}	1	4	21	2	18	31

It is permissible to take the data for ewe no. 26 as an approximate measure of the quantities of fatty acids already present in the depots of the other animals

at the outset of the experiment, although there are indications that the ewes varied somewhat in total fat content at corresponding stages of the experiment. Nevertheless, the quantities of each acid at the commencement are small in comparison with those added during fattening, and any error thus introduced will not be serious. We thus arrive at an estimate of the percentage (weight) composition of the fatty acids deposited as depot fats in ewes nos. 12 and 4 (Table 7).

Table 7. *Estimated proportions (weight %) of fatty acids deposited by ewes nos. 12 and 4*

	Perinephric fats		External tissue fats	
Ewe no.	12	4	12	4
Approx. fat deposited (g.) ...	555	1850	2884	4365
Fatty acids (wt. %):				
Lauric	—	—	0·6	0·9
Myristic	2·6	2·3	2·9	3·1
Palmitic	24·9	26·3	28·1	28·4
Stearic	29·0	27·3	16·4	13·4
Tetradecenoic	0·2	0·3	0·3	0·2
Hexadecenoic	0·6	0·9	0·7	0·5
Oleic	36·3	38·6	46·6	51·1
Octadecadienoic	5·8	3·2	3·8	1·7
Unsaturated C_{20-22}	0·6	1·1	0·6	0·7

The figures in Table 7 are of interest from several points of view. They suggest that the close constancy in the contents of the C_{16} and C_{18} groups of acids is maintained throughout the whole period of fattening, with the characteristically slight differences maintained in each group as between the perinephric and the external tissue fats. There is also definite indication that in the earlier stages of fattening somewhat more stearic and octadecadienoic acids (and correspondingly less oleic acid) are produced than in the later stages; indeed, the figures suggest the possibility that in the final stages some of the octadecadienoic acid previously deposited in the external tissues has disappeared either by mobilization or by conversion into oleic glycerides. At the same time the close constancy of the combined proportions of oleic, stearic and octadecadienoic acids may be remarked (71·1 and 69·1 % in the added perinephric and 66·8 and 66·2 % in the added external tissue fats). It may reasonably be concluded that the proportions of component acids shown in Table 7 are specific for fat deposition by sheep on a normal fattening diet.

Fat lost by ewes fed on 'submaintenance' rations

Table 8 shows the approximate weight of each fatty acid in the depots of the control animal (148 lb. live weight) and of the ewes killed respectively after 100 and 209 days' restricted feeding when the respective live weights were 113 and 72 lb.

Taking the data for ewe no. 30 as an approximate measure of the amounts of each fatty acid present in the depots of nos. 9 and 21 at the commencement of restricted diet (as in the case of the intensive feeding experiment above), an estimate can be made of the percentage (weight) proportions of each fatty acid removed from the fat depots in ewes nos. 9 and 21 during their maintenance on the restricted diet (Table 9).

Table 8. *Total amounts of fatty acids in sheep depot fats (g.)*

	Perinephric fats			External tissue fats		
Ewe no. ...	30	9	21	30	9	21
Fatty tissue (g.)	1019	406	341	7110	1218	742
% fat	91·6	98·0	97·1	87·6	82·0	69·9
Fat (g.)	932	398	331	6230	999	519
Fatty acids (g.)	891	380	316	5950	954	496
Lauric	1	Trace	—	42	6	2
Myristic	15	11	9	113	21	18
Palmitic	239	90	73	2017	291	121
Stearic	268	121	119	911	192	122
Tetradecenoic	2	1	1	18	3	2
Hexadecenoic	8	5	3	54	11	3
Oleic	310	134	101	2450	395	219
Octadecenoic	38	15	7	291	27	7
Unsaturated C_{20-22}	10	3	3	54	8	2

Table 9. *Estimated proportions (weight %) of fatty acids mobilized from depots of ewes nos. 9 and 21*

	Perinephric fats		External tissue fats	
Ewe no. ...	9	21	9	21
Approx. fat lost (g.)	534	601	5231	5711
Fatty acids (wt. %):				
Lauric	0·2	0·2	0·7	0·7
Myristic	0·8	1·0	1·8	1·8
Palmitic	29·1	28·9	34·5	34·8
Stearic	28·8	25·9	14·4	14·5
Tetradecenoic	0·2	0·2	0·3	0·3
Hexadecenoic	0·6	0·9	0·9	0·9
Oleic	34·4	36·3	41·2	40·9
Octadecadienoic	4·5	5·4	5·3	5·2
Unsaturated C_{20-22}	1·4	1·2	0·9	0·9

The figures in Table 9 should be compared with the corresponding data in Table 7 (referring to the component acids of fat which is being added to the depots). In the perinephric fats of the group on restricted diet, the proportions of the fatty acids in the mobilized fat are on the whole similar to those added by the animals which were intensively fed. Some slight differences are however observable, and these are much more accentuated in the external tissue fats. The proportion of palmitic acid in the mobilized fat is slightly higher than in the deposited fats (Table 7), whilst that of oleic and stearic acids is correspondingly somewhat lower. Thus palmitic glycerides have been, to a slight degree, preferentially mobilized as compared with oleo- or stearo-glycerides.

In the external tissue fats this tendency is more marked. It has already been noted (p. 935) that these possess higher proportions of combined palmitic acid than the sheep perinephric fats, and the proportion of palmitic acid deposited in the depot fats during intensive feeding (Table 7) is also somewhat greater in the external tissues than in the perinephric fats. Similarly, during maintenance of restricted diet, the fat withdrawn from the external tissues is definitely richer in combined palmitic acid, and poorer in oleic and (to a less degree) stearic acid, than that deposited during intensive feeding.

This general tendency towards preferential mobilization of palmito-glycerides in both groups of sheep fats may be further illustrated by noting the united proportions of C_{18} (oleic, stearic and octadecadienoic) acids removed from the depots: 67·7 and 67·6 % in the cases of the perinephric fats, and 60·9 and 60·6 %

in the external tissue fats. The proportions in the perinephric fats are only slightly lower than those of the added C_{18} acids in the 'supermaintenance' experiments (71·1 and 69·1 %), but those in the fats removed from the external tissues are considerably lower than the C_{18} acids deposited during full feeding (66·8 and 66·2 %). These results seem to differ materially from those obtained from the fats of pigs which had been fasted for prolonged periods [Hilditch & Pedelty, 1940]. In the pig fats, although no wide degree of selectivity in the mobilization of any one fatty acid component of the depot glycerides was detected, it was clear that oleic glycerides were removed to a greater extent than those of the saturated acids; whereas in the sheep fats palmitic acid has consistently been shown to be somewhat more freely mobilized than the other major components. Again, there is definite reluctance in the pig to mobilize the minor proportions of octadecadienoic and unsaturated C_{20-22} glycerides present, but in the sheep the glycerides of these acids appear to be removed at least as readily as the oleic glycerides. The conditions in the two series of experiments were, however, not identical: the pigs were fasted and only received water during the trials, whilst the sheep were given a restricted amount of wheat straw. It is therefore not certain whether the observed difference in the nature of the fat withdrawn is specific for the animals concerned, or whether it may have been influenced by the different dietary conditions.

Attention may again be directed to the circumstance that practically the whole of these depot fats consists of mixed glycerides (in which palmitodioleins and oleopalmitostearins are most abundant) and that therefore any tendency towards withdrawal of a particular fatty acid is partly obscured by the fact that other acids present in the mixed glycerides are inevitably concurrently involved.

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