The Component Acids of Milk Fats of the Goat, Ewe and Mare

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(Received 1 September 1944)

In marked contrast to the fairly extensive range of published information on the fatty acid composition of cow-milk fats, there have appeared relatively few detailed analyses of the milk fats of other farm animals. Dhingra (1933) analyzed the milk fats of Indian goats and sheep by the ester fractionation procedure, using a Willstätter flask, and found them to be closely similar in composition. The two fats differed from cow-milk fat in the higher content of hexanoic, octanoic and decanoic acids and in the lower content of unsaturated C_{18} acids. Riemen-schneider & Ellis (1936) made an investigation of goat-milk fat and reported the presence of mono-ethenoid C_{10} , C_{14} and C_{18} acids. Investigation by Laxa (1927, 1931) of the milk fat of the mare and of the sow led to a deduction of very approximate proportions of the major constituent acids; and, very recently, de la Mare & Shorland (1944) have published a few more data of the major acids of sow-milk fat.

The present communication deals with fractionation analyses, using an efficient heated column, of the methyl esters of fats obtained from samples of milks of goat, ewe and mare.

The goat's milk was supplied by Mrs G. W. Howard, King's Lynn, Norfolk, from does on a daily feed of hay, kale, beet, 1-1½ lb. groundnut cake, and also 2 hr. pasture; the ewe's milk was sent by Prof. J. G. Wright of the School of Veterinary Science, University of Liverpool; and the mare's milk was obtained from a Shire mare, 8 years old, belonging to Mr H. C. Peers, Oakland Farm, Neston, Cheshire, which was fed on pasture in summer and on hay with a small amount of crushed oats in winter.

RESULTS

General characteristics. The general characteristics of the three fats are given in Table 1 (a), and in Table 1 (b) are added some values reported by other investigators.

The data confirm the evidence of earlier workers that goat- and sheep-milk fats have somewhat similar general characteristics; and that they have lower iodine values and higher Polenske values than cow-milk fats.

It would appear that whilst mare-milk fat is also characterized by a relatively high Polenske value, there is a notable difference between this fat on the

Table 1. *Characteristics of goat-, ewe- and mare-milk fats*

	Saponification equiv.	Iodine value	Reichert-Meissl value	Polenske value	Kirschner value
(a) Present series					
Goat	233.7	28.8	23.2	7.2	15.6
Ewe	244.5	36.7	26.2	3.6	17.6
Mare	253.1	84.3	6.2	5.9	2.6
(b) Data reported by other workers					
Goat (Dhingra, 1933)	238.1	28.8	31.7	8.2	19.9
Goat (Knowles & Urquhart, 1924)	—	24.7-36.9	24.5-27.8	4.9-8.7	16.8-19.0
Ewe (Dhingra, 1933)	232.4	32.1	33.9	8.0	22.8
Ewe (Faltin & Dedinszky, 1930)	—	—	22.5-39.4	2.2-6.9	15.8-21.0
Mare (Laxa, 1927)	246.3	61.0	—	6.1	—

METHODS

The component acids in the three milk fats were determined by separation and ester fractionation as described by Hilditch & Jasperson (1941). In the case of the mare-milk fat, a smaller column (12 in. by ½ in.) was used, instead of that described by Longenecker (1937), for distillation of the small amount of the steam volatile acids and of the esters of ethanol-insoluble lead soaps of the non-volatile acids.

one hand and goat- and sheep-milk fats on the other, in respect of the low Reichert-Meissl and Kirschner values and the high iodine value of the former.

Component fatty acids. The percentages (by wt. and mol.) of the component acids of the three fats are given in Table 2 and are arranged for comparison in Table 3.

Table 2. *Component acids of milk fats*

	Steam-volatile acids	Non-steam-volatile		Total	Fatty acids	
		'Solid' acids	'Liquid' acids		(% by wt.)	(% by mol.)
	(8.9%)	(43.4%)	(47.7%)			
Goat						
Butyric	2.98	—	—	2.98	3.0	7.5
Hexanoic	2.45	—	—	2.45	2.5	4.7
Octanoic	1.36	—	1.44	2.80	2.8	4.3
Decanoic	1.55	—	8.41	9.96	10.0	12.8
Lauric	0.38	1.13	4.46	5.97	6.0	6.6
Myristic	—	7.58	4.63	12.21	12.3	11.8
Palmitic	—	25.24	2.58	27.82	27.9	24.1
Stearic	—	5.97	—	5.97	6.0	4.7
As Arachidic	—	0.64	—	0.64	0.6	0.4
Decenoic	—	—	0.25	0.25	0.3	0.3
Dodecenoic	—	0.01	0.24	0.25	0.3	0.3
Tetradecenoic	—	0.07	0.76	0.83	0.8	0.8
Hexadecenoic	—	0.72	1.85	2.57	2.6	2.2
Oleic	0.18	2.01	18.86	21.05	21.1	16.5
Octadecadienoic	—	—	3.56	3.56	3.6	2.8
Unsaturated C ₂₀₋₂₂	—	—	0.25	0.25	0.2	0.2
Unsaponifiable	—	0.03	0.41	0.44	—	—
Ewe	(9.1%)	(45.4%)	(45.5%)			
Butyric	2.83	—	—	2.83	2.8	7.5
Hexanoic	2.57	—	—	2.57	2.6	5.3
Octanoic	2.16	—	—	2.16	2.2	3.5
Decanoic	0.77	—	3.98	4.75	4.8	6.4
Lauric	0.50	0.41	2.94	3.85	3.9	4.5
Myristic	—	5.79	3.88	9.67	9.7	9.9
Palmitic	—	20.45	3.39	23.84	23.9	21.6
Stearic	—	12.52	—	12.52	12.6	10.3
As Arachidic	—	1.10	—	1.10	1.1	0.8
Decenoic	—	—	0.13	0.13	0.1	0.2
Dodecenoic	—	0.01	0.12	0.13	0.1	0.2
Tetradecenoic	—	0.05	0.50	0.55	0.6	0.6
Hexadecenoic	—	0.55	1.69	2.24	2.2	2.0
Oleic	0.23	4.52	21.46	26.21	26.3	21.6
Octadecadienoic	—	—	5.21	5.21	5.2	4.3
Unsaturated C ₂₀₋₂₂	—	—	1.85	1.85	1.9	1.3
Unsaponifiable	0.04	—	0.35	0.39	—	—
Mare	(6.4%)	(18.1%)	(75.5%)			
Butyric	0.39	—	—	0.39	0.4	1.1
Hexanoic	0.88	—	—	0.88	0.9	1.9
Octanoic	2.45	—	0.13	2.58	2.6	4.4
Decanoic	1.39	—	4.08	5.47	5.5	7.9
Lauric	—	—	5.52	5.52	5.6	6.8
Myristic	—	3.42	3.43	6.85	7.0	7.4
Palmitic	—	10.46	5.44	15.90	16.1	15.4
Stearic	—	2.81	—	2.81	2.9	2.4
As Arachidic	—	0.26	—	0.26	0.3	0.2
Decenoic	—	—	0.87	0.87	0.9	1.3
Dodecenoic	—	—	0.99	0.99	1.0	1.2
Tetradecenoic	—	0.10	1.67	1.77	1.8	1.9
Hexadecenoic	—	0.39	6.98	7.37	7.5	7.2
Oleic	1.29	0.52	39.97 (-3.8H*)	41.78 (-3.7H*)	42.4†	36.9†
Octadecadienoic	—	—				
Linolenic	—	—				
Unsaturated C ₂₀₋₂₂	—	—	5.05	5.05	5.1	4.0
Unsaponifiable	—	0.14	1.37	1.51	—	—

* H = atoms of hydrogen (per molecule) short of saturation.

† The unsaturated C₁₈ acids have been shown, by application of the method of alkali-induced conjugation, to consist of 44% octadecenoic, 18% octadecadienoic and 38% octadecatrienoic acid (cf. p. 446).

Table 3. *Comparison of component acids of goat-, ewe- and mare-milk fats*

Acid	Goat	Ewe	Mare
	Percentages by weight		
Butyric	3.0	2.8	0.4
Hexanoic	2.5	2.6	0.9
Octanoic	2.8	2.2	2.6
Decanoic	10.0	4.8	5.5
Lauric	6.0	3.9	5.6
Myristic	12.3	9.7	7.0
Palmitic	27.9	23.9	16.1
Stearic	6.0	12.6	2.9
As Arachidic	0.6	1.1	0.3
Decenoic	0.3	0.1	0.9
Dodecenoic	0.3	0.1	1.0
Tetradecenoic	0.8	0.6	1.8
Hexadecenoic	2.6	2.2	7.5
Oleic	21.1	26.3	18.7
Octadecadienoic	3.6	5.2	7.6
Linolenic	—	—	16.1
Unsaturated C ₂₀₋₂₂	0.2	1.9	5.1
Molar percentages			
Butyric	7.5	7.5	1.1
Hexanoic	4.7	5.3	1.9
Octanoic	4.3	3.5	4.4
Decanoic	12.8	6.4	7.9
Lauric	6.6	4.5	6.8
Myristic	11.8	9.9	7.4
Palmitic	24.1	21.6	15.4
Stearic	4.7	10.3	2.4
As Arachidic	0.4	0.8	0.2
Decenoic	0.3	0.2	1.3
Dodecenoic	0.3	0.2	1.2
Tetradecenoic	0.8	0.6	1.9
Hexadecenoic	2.2	2.0	7.2
Oleic	16.5	21.6	16.3
Octadecadienoic	2.8	4.3	6.6
Linolenic	—	—	14.0
Unsaturated C ₂₀₋₂₂	0.2	1.3	4.0

DISCUSSION

The proportions of butyric acid show close parallelism with the Reichert-Meissl and Kirschner values given in Table 1. The percentages are similar in goat- and ewe-milk fats and somewhat lower than in typical milk fat from stall-fed cows. The low butyric acid content (1.1% by mol.) is an arresting feature of the short-chain acids of mare-milk fat, and corresponds to the low Kirschner value (2.6). It is generally accepted that comparative indications of the relative proportions of butyric acid are given by the Kirschner value, of butyric and hexanoic acids by the Reichert-Meissl value, and of octanoic and decanoic acids by the Polenske value.

The relatively high content of decanoic acid in goat- and sheep-milk fats, found by Dhingra (1933), has been generally confirmed in the present work, but a point of difference is seen from the fact that, whereas the present goat-milk fat contained 12.8% and the sheep-milk fat contained 6.4% decanoic acid, the decanoic acid contents of the Indian milk fats were very similar, namely 11.1 and 10.1% respectively.

The milk fat of the horse resembles those of the goat and sheep in being characterized by a relatively higher decanoic acid content than cow-butter fat: in fact, all three fats contain somewhat higher percentages of C₈, C₁₀ and C₁₂ acids than are found in the cow-milk fats recorded in the literature (cf. Hilditch, 1940).

With regard to the percentage of stearic acid in the three fats, it will be noted from the data in Table 3 that the ewe-milk fat (10.3% by mol. stearic acid) contains more than twice the percentage found in the goat-milk fat (4.7% by mol.) and four times that in the mare-milk fat (2.4% by mol.): in this respect the milk fat of the ewe resembles that of the cow.

Another interesting difference between the three fats is the relatively lower percentage (15.4) of palmitic acid in the mare-milk fat as compared with the more usual percentage (20–30) in goat-, sheep- and cow-milk fats. It is noteworthy, however, that mare-milk fat contains a corresponding relatively higher amount of hexadecenoic acid, namely 7.2% instead of about 2% in the other milk fats. Thus, the total C₁₆ acids approach near the average range of about 25–30% which apparently is characteristic of milk fats of most mammals so far studied.

The lower saturated acids from butyric to myristic as given in Tables 2 and 3 show goat-milk fat to contain 47.7%, ewe-milk fat 37.1% and mare-milk fat 29.5%. The high content in the goat-milk fat is caused mainly by the presence of higher proportions of decanoic and myristic acids; and the relatively lower figure for mare-milk fat results mainly from the small component proportions of butyric and hexanoic acids.

The unsaturated C₁₈ acids of mare-milk fat. The outstanding difference in the present series of analyses from those previously recorded for milk fats of herbivorous mammals emerged from a study of the component unsaturated C₁₈ acids, and in particular those of the mare-milk fat. Iodine value determinations of the methyl esters from the ethanol-soluble lead salts of the non-steam-volatile acids of this fat indicated that the mean unsaturation of the C₁₈ acids was considerably higher than of those of the goat-, ewe- or cow-milk fat, namely, i.v. 164.0 for mare-milk C₁₈ acids as compared with the more usual iodine values of 90–100.

A portion (2.11 g.) of the mare-milk fat C₁₈ esters was hydrolyzed and the resulting acids were brominated in ether at 0° to give 0.78 g. insoluble bromides, m.p. 178–180° (recrystallized from benzene, m.p. 181°; mixed m.p. 181° with authentic specimen of hexabromostearic acid). This yield corresponded to 13.6% 'α'-linolenic acid and would be indicative of about 40% linolenic acid in the C₁₈ unsaturated acids if the hexabromide ratios of Shinowara & Brown (1938) were applied. Another

portion (2.05 g.) was brominated in light petroleum (40–60°) at 0° to yield 0.87 g. insoluble bromides melting at 166–170°. Pure hexabromostearic acid, m.p. 181°, was readily isolated from this mixture but no tetrabromostearic acid, m.p. 114°, was obtained.

The proportion of octadecadienoic and octadecatrienoic acids present in methyl ester fractions which consisted wholly of esters of unsaturated C_{18} acids has been determined by a modification of the method of Mitchell, Kraybill & Zscheille (1943) which will be described elsewhere by one of us. Briefly, the basis of the method is isomerization by alkali under standardized conditions of the mixed acids, which results in the production of definite proportions of conjugated diethenoid acids from linoleic acid, and of conjugated di- and tri-ethenoid acids from linolenic acid or from their geometric isomers. The conjugated acids give characteristic bands in the ultra-violet spectrum respectively at 234 and 268 μ . From the extinction coefficients of these bands the amounts of the two acids may be calculated. Application of this method in the present instance indicated that the unsaturated C_{18} acids in the 'liquid' acids of mare-milk fat consisted of 38% octadecatrienoic, 18% octadecadienoic and 44% octadecenoic acid.

Thiocyanometric analyses suggested the presence of about 44.5% of triethenoid acid, with consequent absence of diethenoid acid. So far as our experience goes at the moment, it is believed that the new spectroscopic method is the most reliable at present available for the analysis of mixtures of oleic, linoleic and linolenic acids or esters, or their geometric isomers. We consider that the proportions of the three acids indicated by the spectroscopic analysis are probably close to the truth, and it will be noted that the amount of octadecatrienoic acid (38%) accords with the figure of about 40% linolenic acid deduced above from the crystalline hexabromostearic acid obtained on bromination.

The presence of linolenic acid in mare-milk fat is of considerable interest and shows a notable difference from the composition of cow-milk fat under similar conditions of feeding. The mare in question was on a pasture diet during the summer months when it was being foaled, and hence the feeding conditions were somewhat analogous to those of cows on summer pasture recorded in the investigations of Hilditch & Sleightholme (1930), Hilditch & Dean (1933) and Hilditch & Jasperson (1941). Although the lipids of pasture probably contain about 40% linolenic acid (Smith & Chibnall, 1932; Shorland, 1944), none of this acid was found in the milk fat of the cows, and furthermore, none has been isolated from ox depot fats (Hilditch & Longenecker, 1937). Perhaps similar factors which lead to the presence of a small proportion (2%) of linolenic acid in the body fat of the horse (Heiduschka

& Steinrock, 1921) also influence the appearance of this acid in the milk fat.

The goat- and sheep-milk fat unsaturated C_{18} acids appear to resemble in nature those of the cow-milk fat: the point of difference lies in the lesser content (by about 10% by mol.) of oleic acid. The mixed unsaturated C_{18} acids of the goat-milk fat were subjected to low temperature crystallization as described previously for the cow-milk fat C_{18} acids (Hilditch & Jasperson, 1941). The resulting polyethenoid concentrate, i.v. 123, was brominated at 0° in ether and in light petroleum (40–60°). No evidence for the presence of ' α '-linoleic acid was obtained, and it was concluded that the main polyethenoid constituent is the octadecadienoic acid of uncertain constitution also found in the cow-milk fat.

The above data have been ascertained from the milk fats of animals which may be considered to be influenced by the same factors as shown by Hilditch and co-workers to operate in the case of the cow, for instance, diet, state of lactation, age of the animal, degree of exercise and seasonal temperatures. In view of the fact that the mare-milk fat has not been hitherto analyzed in any detail, the results recorded for this fat in the present paper should be considered as tentative until definite conclusions can be drawn when an opportunity has occurred to study further specimens.

SUMMARY

1. Detailed analyses of the fatty acid composition have been determined of the milk fats of the goat, ewe and mare.
2. The general similarity between goat- and ewe-milk fats has been confirmed; and differences have been indicated by the relatively higher content of decanoic and a relatively lower content of stearic acid in goat-milk fat than in ewe-milk fat.
3. Mare-milk fat, which has not been analyzed in detail hitherto, has been shown to comprise the following acids (% by mol.): butyric 1.1, hexanoic 1.9, octanoic 4.4, decanoic 7.9, lauric 6.8; myristic 7.4, palmitic 15.4, stearic 2.4, as arachidic 0.2, decenoic 1.3, dodecenoic 1.2, tetradecenoic 1.9, hexadecenoic 7.2, oleic 16.3, octadecadienoic 6.6, linolenic 14.0, unsaturated C_{20-22} 4.0. The most arresting features are the low butyric and hexanoic acid contents and the presence of substantial proportions of linolenic acid amongst the C_{18} unsaturated acids.

We are indebted to Prof. J. G. Wright, Mrs R. G. Howard, Mr H. C. Peers and Miss E. Thomas for placing the experimental material at our disposal; to Mr F. Burke and Miss E. H. Harrop for valuable assistance in the ester fractionation analyses; to Mr A. McKerrigan for determination of the general analytical characteristics of the fats; and to the Directors of Messrs J. Bibby and Sons, Ltd., for permission to publish the results of the investigation.

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The Stabilization and Estimation of Pyruvic Acid in Blood Samples

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(Received 21 September 1944)

Two methods are available for assessing the aneurin (vitamin B₁) nutritional level of the body. The first of these involves the determination of aneurin itself in blood or urine, especially after test doses, and has met with only partial success. (For a review of the literature, see Bicknell & Prescott, 1942.) The other procedure depends upon the fact that pyruvic acid, an intermediary in carbohydrate metabolism, is utilized by the tissues at a rate which is largely determined by their co-carboxylase (aneurin pyrophosphoric ester) content; thus when dietary aneurin is inadequate and the tissues contain sub-optimal amounts of co-carboxylase, pyruvate is only slowly metabolized and its concentration in the blood tends to increase.

While pyruvate is readily estimated in protein-free filtrates (Long, 1942), the concentration of pyruvic acid present *in vivo* in the blood is not easily determined since its value falls during the period following the drawing of the blood sample. Lu (1939) and Friedemann & Haugen (1942) overcame this difficulty by deproteinizing the blood specimen as quickly as possible after withdrawal from the vein. Bueding & Wortis (1940), however, did not find this method satisfactory, but claimed to have stabilized the level of blood pyruvate for a few minutes by means of iodoacetate. More recently, Golberg & Gillman (1943) have shown that in the presence of both iodoacetate and fluoride the pyruvate level remains constant for 30 min.

The aim of the present investigation has been to find some method of stabilizing, for a longer period,

the pyruvate level of a blood sample at its initial value. This would allow the postponement of an estimation until a sufficient number of samples had accumulated; specimens might also be sent by post. This aim has been so successfully achieved that blood samples may now be kept at room temperature for at least 3 weeks without variation in the pyruvate level. The method of stabilization requires haemolysis at a slightly acid reaction in the presence of fluoride.

FACTORS CONTROLLING CHANGES IN THE PYRUVIC ACID LEVEL OF SHED BLOOD

Materials

Na pyruvate. Prepared according to Peters (1938). A solution containing a known amount (about 5 µg.) in 2 ml. 10% trichloroacetic acid was used as a standard.

Na iodoacetate. Iodoacetic acid was neutralized with 40% NaOH to pH 7.5. Final concentration in blood, 0.2–2.0%.

Citrate buffers of suitable pH values. Citric acid monohydrate (mol. wt. 210) (0.840 g.), dissolved in about 1.5 ml. water, was treated with 40% NaOH till the required pH was attained. One-fifth of the total volume was then pipetted into a tube and evaporated to dryness (see Methods (a)); this amount was used for each 10 ml. blood sample, giving a final concentration of 0.08 M.

Haemolytic agents. 'White' saponin (F. E. Becker and Co.) and thymol were used at final concentration 0.5 and 0.25% respectively. Cetyltrimethylammonium bromide ('Cetavlon') in solid form (75%) and as a 7.5% solution was obtained from Imperial Chemical (Pharmaceuticals) Ltd. Final concentration 0.3%.