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The Component Acids of Cow Colostrum Fat

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Though much work has been done on the composition of milk fat, little attention has so far been paid to the fat secreted during the first few days after parturition. Baldwin & Longenecker (1944a) studied the fatty acid distribution in the fat of human milk during the first few days of lactation and found that it was different from that of mature milk fat. The results of these workers indicated that as the period of lactation progressed from the first day's colostrum to mature milk, the amount of the acids of greater molecular weight than C_{18} decreased and the amount of C_{12} and C_{14} acids increased. Later, Baldwin & Longenecker (1944b) investigated the colostrum fat of a single cow collected during the first 4 days after parturition and reported that its fatty acid composition was very similar to that of a typical butter fat. The results obtained were only from samples collected from a cow of unspecified breed and comparisons were made with the results of the analysis of typical butter fat by Hilditch & Longenecker (1938). It is possible that if a sample of butter fat from the same cow had been collected and analyzed at a later period some differences might have been observed. Apart from these investigations no work appears to have been carried out on this subject. In the present study attempts were made to collect colostrum fat from some of the common breeds of cattle in India and the results obtained are now presented.

METHODS

The colostrum from which the fat was extracted for the present studies was collected from four different breeds of cows, namely, Sindhi, Gir, Tharparkar and Sahiwal. Milking was done twice a day. In the case of each animal material collected during each 24 hr. after parturition was mixed together, diluted with water and the cream separated. Butter fat was prepared direct from the cream by the 'boiling off' method. The butter fat prepared from each 24 hr. collection from every animal was first analyzed for its general chemical characteristics. The collection of butter fat was continued during the first 15 days of lactation and samples from the first 5 days and the tenth day of lactation were used in the present studies.

During the dry period the cows were receiving 3 lb. of a concentrate mixture (wheat bran, ground-nut cake, grain husk and grain in the ratio of 4:2.5:2.0:1.5) in addition to $\frac{1}{2}$ lb. each of extra grain husk and ground-nut cake. Four weeks before calving pregnant animals were fed 3 lb. of the usual concentrate mixture mentioned above with 3 lb. of wheat bran. For the first 4 days after calving the animals received 8 lb. wheat bran, 1 lb. of *jaggery* (crude cane sugar), with a roughage consisting of 55 lb. green grass and 3 lb. ragi straw. From the fifth day of lactation onwards the animals were fed, together with the usual amount of roughage as mentioned above, different amounts of the common concentrate mixture according to milk yield.

Representative composite mixtures of the colostrum fat during the initial days of lactation were obtained by mixing equal quantities of the fat collected from the different cows

on the corresponding days of lactation. This mixed butter fat for each day of lactation was first analyzed for its physical and chemical characteristics and then subjected to detailed analysis by ester fractionation according to the method of Hilditch, as modified by Smith & Dastur (1938). The fat was converted into methyl esters directly and then the lower components fractionally separated from the whole bulk, the higher members being separated into liquid and solid acids by Twitchell's lead salt method, methylated and also fractionally distilled. The detailed results obtained for the composition of the three main fractions, the lower, solid and liquid esters of the colostrum fat on the first day are given by way of illustration in Tables 2 and 3, while the final figures are recorded in Table 4. Unfortunately the small amounts of material available for analysis prevented isolation of the lower unsaturated acids C_{10} , C_{12} and C_{14} in the present work. Their existence in butter fat has, however, been established beyond doubt by Bosworth & Brown (1933), Hilditch & Paul (1936) and other workers.

RESULTS

The chemical characteristics of the individual samples of colostrum fat and those of composite mixtures are shown in Tables 1 and 1*a*. Table 1 shows clearly that as time goes on there is a marked progressive change in the nature of the fat secreted by individual animals as judged by the butyro-refractometer reading, and the iodine, saponification and Reichert values. These changes were very similar for all the animals except cow 531, in which the transition was not so well marked. In every case the butyro-refractometer reading decreased from 45.4 to about 42.1 during the progress of lactation. The saponification value gradually increased from about 213 to 232. The Reichert value rapidly increased from about 13 on the first day to 20 on the second day and then gradually increased to about 30. The iodine value decreased from about 40 to 32. The results of analyses of the composite mixtures also reveal the same gradation in all their chemical constants (Table 1*a*). The most interesting observation concerns, perhaps, the physical state of the colostrum samples; the melting-point of the fat gradually decreases with the progress of lactation. This may be attributed to the presence of larger amounts of stearic acid in the colostrum fat relative to the normal butter fat collected later.

The decrease in the butyro-refractometer reading and iodine value, and an increase in the saponification and Reichert values of the fat secreted by the animal as the colostrum fat changed to normal butter fat suggested a marked increase in the amount of fatty acids of low molecular weight and a slight decrease in the content of unsaturated acids. This was confirmed by detailed analysis based on fractional distillation methods. Tables 2 and 3 giving the detailed analytical results for fat in the first day of lactation, illustrate the methods. Table 4,

wherein the component fatty acids for all the samples by weight and molar percentages are presented in summarized form, shows clearly that there is a regular increase in the proportion of the low molecular weight fatty acids during the transition from the first day's colostrum fat to mature milk fat on the tenth day of lactation. The butyric acid content increased from 2.2 to 3.6 %. The amount of caproic acid in the fat was almost negligible on the first day. It gradually increased up to 1.5 % on the fourth day and then decreased to 0.6 %, at which level it remained constant. Caprylic, capric and lauric acids also increased till the fourth day after parturition and remained fairly steady. There appeared to be little change in the proportion of myristic acid, the slight increase in its content being counterbalanced by a slight decrease in stearic acid. The palmitic acid content of the secreted fat slightly increased on the second day after calving and then became constant. The amount of arachidic acid remained almost constant. The changes in the lower acids can be seen more clearly by reference to molar percentages. There is an increase of about 10 mol. % in the lower acids up to and including C_{14} . This is mainly accounted for by the fall of almost 6 mol. % in oleic acid. The fall in the percentage of stearic acid is also evident. In the unsaturated acids up to C_{14} there is virtually no change. The amount of hexadecenoic acid increased at first and then decreased to almost the original value. The chief change appeared to be in oleic acid, which decreased from 34.1 on the first day to 27.5 on the fourth day and then slightly increased to 29.3 on the tenth day of lactation. The amount of linoleic acid decreased gradually to almost one-quarter of its amount present in the first day's fat. There was no marked change in the C_{20-22} unsaturated acids. These changes, a decrease in the quantity of higher acid and an increase in the amount of lower acids, may well be associated with the functional development of the lactating mammary gland following parturition. A gradual decrease in the percentage of oleic acid and low amounts of volatile acids present in the colostrum fat are in close agreement with the findings that colostrum fat is richer in oleic acid and contains less volatile fatty acids than normal milk fat (Sommerfeld, 1909; Nilson, 1891). In comparison with the component fatty acids of human colostrum fat (Baldwin & Longenecker, 1944*a*) several differences in composition are apparent. The most interesting observation is the presence of larger amounts of low molecular weight fatty acids and smaller amounts of C_{20} and C_{22} acids in cow colostrum fat than in human colostrum fat. The first day's cow colostrum fat compares favourably with the mature human milk fat, the percentage of lower acids up to C_{14} being almost the same (16.4 and 17.8 respectively). The only point of

Table 1. *General analytical characteristics of colostrum and milk fats*

Day of lactation	Butyro-re-fractometer reading (at 40°)	Saponification value	Iodine value	Reichert value	Polenske value	Kirschner value
Cow 82. Gir (2nd lactation)						
1	45.2	213.7	38.60	13.16	0.95	12.31
2	44.2	220.4	35.20	18.99	1.40	17.31
3	43.0	224.2	33.40	22.22	1.61	20.75
4	42.1	224.8	29.70	23.39	2.20	21.62
5	41.6	226.2	30.00	29.21	2.30	25.86
10	41.0	228.2	29.45	32.82	1.27	30.54
15	41.0	229.3	33.95	30.71	1.99	29.17
Cow 251. Sindhi (11th lactation)						
1	45.3	215.4	38.30	14.75	1.10	12.02
2	44.0	223.0	35.30	21.32	1.25	19.87
3	42.9	224.4	32.80	24.35	1.40	22.80
4	41.3	226.0	31.00	30.23	1.25	26.81
5	42.0	231.2	30.60	31.30	2.10	28.26
10	42.5	230.1	34.20	31.40	2.10	27.80
15	42.0	231.4	32.10	31.77	1.75	27.14
Cow 257. Tharparkar (4th lactation)						
1	45.5	213.3	41.58	13.98	0.97	12.54
2	44.0	221.9	38.68	21.45	1.59	20.07
3	42.8	224.8	34.53	23.28	2.30	21.78
4	42.2	226.0	34.71	28.64	2.20	24.91
5	42.2	228.6	34.84	28.76	1.89	25.11
10	42.7	228.2	35.72	30.79	1.69	27.10
15	42.8	227.4	36.83	29.67	1.79	26.21
Cow 531. Ayrshire-Haryana (12th lactation)						
1	43.9	219.3	32.98	15.82	1.21	13.61
2	42.8	223.1	28.98	19.05	1.79	17.33
3	42.0	225.2	29.75	21.34	1.89	18.48
4	41.3	226.1	28.28	26.18	2.20	22.84
5	41.0	228.0	28.75	28.43	2.30	24.62
10	42.7	228.0	30.54	27.63	2.10	23.66
15	41.3	229.2	29.82	29.22	2.50	25.83
Cow 64. Sahiwal (3rd lactation)						
1	45.4	213.5	42.79	14.59	0.75	12.66
2	45.2	214.9	41.62	18.05	0.75	15.12
3	43.9	218.0	38.39	19.48	1.15	16.87
4	43.4	221.7	36.12	21.74	1.40	19.05
5	42.7	226.0	33.48	24.27	1.70	20.77
10	42.1	228.0	31.32	27.07	1.70	23.93
15	41.6	230.0	30.84	26.41	1.30	23.30
Cow 64. Gir (5th lactation)						
1	44.2	218.0	38.42	13.98	1.20	12.92
2	43.8	220.3	36.53	19.00	1.59	17.38
3	43.5	222.0	35.12	22.35	1.79	20.95
4	43.1	225.1	32.50	24.95	1.99	23.68
5	42.8	227.4	31.67	26.06	1.79	22.46
10	42.3	229.6	32.01	32.02	1.79	29.20
15	42.5	228.1	32.16	29.00	1.55	26.05

Table 1a. *Analytical characteristics of the composite mixtures of colostrum fat*

Day of lactation	Butyro-re-fractometer reading (at 40°)	Saponification value	Iodine value	Reichert value	Polenske value	Kirschner value	Melting-point
1	45.4	213.1	39.79	14.92	1.10	11.21	38.7
2	43.8	220.4	35.30	22.18	1.40	20.61	34.3
3	43.3	224.0	33.51	24.27	1.70	22.23	34.2
4	43.2	225.1	32.06	25.75	1.95	22.41	33.9
5	42.8	226.8	31.56	27.01	2.04	23.24	33.9
10	42.1	228.2	31.60	30.56	1.96	26.20	33.8
15	41.8	229.6	31.82	29.72	1.98	25.92	—

Table 2. *Fractionation of the methyl esters prepared from 300 g. of cow colostrum fat (first day of lactation)*

Fraction	Boiling-point at 2 mm. up to	% of total esters	Mol. wt.	Iodine value	Fraction	Boiling-point at 2 mm. up to	% of total esters	Mol. wt.	Iodine value
Lower esters									
1	(Methyl butyrate)	2.62	—	—	7	127	0.60	211.9	6.42
2	54	0.34	156.7	3.08	8	137	0.97	229.6	7.18
3	80	0.59	163.6	3.93	9	140	1.02	239.2	7.56
4	92	0.50	181.4	5.46	10	145	1.13	244.0	7.52
5	103	0.61	190.8	7.87	11	150	0.86	247.9	8.04
6	115	0.42	200.9	7.08	12	153	1.03	255.2	8.78
Total							10.69		
Solid esters									
13	154	0.85	254.2	0.54	24	169	2.20	276.8	2.60
14	159	1.12	263.7	0.52	25	169	2.28	277.1	2.92
15	162	1.35	268.5	0.98	26	170	2.14	277.4	2.91
16	162	1.80	270.1	1.56	27	170	2.06	280.9	3.06
17	164	2.13	271.9	1.56	28	174	2.58	281.3	3.53
18	165	2.04	272.4	1.84	29	174	2.24	282.8	4.06
19	165	2.18	273.0	1.93	30	176	2.28	286.4	4.51
20	164	2.58	273.3	1.93	31	180	2.18	289.4	6.63
21	167	2.45	273.3	2.04	32	185	1.29	293.8	5.88
22	168	2.12	273.5	2.27	—	Residue	1.44	309.1	8.28
23	169	2.43	275.8	2.66	Total		41.74		
Liquid esters									
33	115	0.36	238.2	19.28	44	172	2.00	289.8	69.48
34	142	0.71	244.5	19.62	45	172	2.42	291.2	71.06
35	144	1.02	246.8	19.54	46	173	2.64	292.7	76.92
36	149	1.36	250.7	19.58	47	174	2.50	293.5	80.72
37	155	1.24	257.2	19.63	48	175	3.22	295.3	82.66
38	164	1.35	264.0	30.77	49	174	3.62	296.1	87.52
39	167	1.63	271.6	51.16	50	174	3.30	296.8	91.18
40	170	1.98	277.1	54.06	51	174	2.89	297.0	92.30
41	170	2.20	282.1	57.61	52	174	3.06	297.4	93.87
42	171	2.29	284.5	64.36	53	175	3.81	297.6	96.03
43	171	2.36	288.7	67.79	—	Residue	0.61	309.4	92.47
Total							47.57		

difference is in the very high content of palmitic acid (about 6% more) and low amount of linoleic acid (6.3% less) in cow colostrum fat. Further, both colostrum and mature human milk fat contain C_{22} and C_{24} unsaturated acids which are not found in either milk fat or colostrum fat secreted by the cow.

DISCUSSION

The analytical results indicate definite trends in the change in composition of the fat secreted by the cow as the period of lactation progresses. The change in composition of the fat secreted from the first day of lactation onwards is a gradual one. The component fatty acids are different from those of mature milk fat. In this respect the results confirm the findings of Baldwin & Longenecker (1944a) that there is difference in the composition of mature and colostrum fat obtained from human milk. But the same authors (1944b) later reported that the fatty acid of cow colostrum fat is similar in composition

to the typical butter fat as given by Hilditch & Longenecker (1938). Our findings do not agree with the observation made by these workers. The cow colostrum fat analyzed by them was obtained from a single animal, collected moreover during the first 4 days after parturition. We have analyzed composite samples of fat collected from six cows during the progress of lactation, and it may well be that if they had analyzed mature milk fat from the same cow the difference in composition might have been apparent. This change in composition of colostrum fat observed by us cannot be attributed to dietary effects since all the animals received the same diet during the period under observation.

At the start of lactation the cow probably uses her reserves for milk production, but whether these reserves are used specifically to synthesize fat or to furnish energy is unknown. Peterson, Palmer & Eckles (1929) indicate that in the process of milk secretion a fat intermediate in character is first deposited in the glandular tissue and then modified to form milk fat itself. The short chain acids in

Table 3. *The component fatty acids and esters of cow colostrum fat (first day of lactation)*

Acids	% as methyl esters				Fatty acids (excluding unsaponi- fiable)	
	Lower	Solid	Liquid	Total	% (wt.)	% (molar)
Saturated						
C ₄	2.38	—	—	2.38	2.2	6.3
C ₆	0.02	—	—	0.02	Trace	Trace
C ₈	0.87	—	—	0.87	0.9	1.5
C ₁₀	1.24	—	—	1.24	1.2	1.8
C ₁₂	1.38	—	0.04	1.42	1.4	1.8
C ₁₄	3.52	0.86	5.06	9.44	9.5	10.4
C ₁₆	0.75	27.89	1.79	30.43	29.7	29.0
C ₁₈	—	10.90	2.57	13.47	13.7	12.1
C ₂₀	—	0.61	—	0.61	0.6	0.5
Total	10.16	40.26	9.46	59.88	59.2	63.4
Unsaturated						
C ₁₀	0.08	—	—	0.08	0.1	0.1
C ₁₂	0.06	—	—	0.06	0.1	0.1
C ₁₄	0.29	—	0.54	0.83	0.8	0.9
C ₁₆	0.10	—	3.01	3.11	3.0	3.0
C ₁₈	—	1.48	31.98	33.46	34.1	30.3
C ₁₈	—	—	1.48	1.48	1.5	1.3
C ₂₀₋₂₂	—	—	1.10	1.10	1.2	0.9
Total	0.53	1.48	38.11	40.12	40.8	36.6
Sum of saturated and unsaturated acids	10.69	41.74	47.57	100.00	100.0	100.0

milk fat are probably derived, as Hilditch first suggested, from the breakdown of some of the long chain acids in the blood fat. It is not clear whether the same process occurs at the initial stages of lactation. It may be that the lower acids are used in the synthesis of oleic acid, but during the first few days of lactation, when the gland is producing much less than the usual amount of fat, the smaller amount of synthesis taking place is able to proceed to its final stages, so that lower acids are almost wholly converted into oleic acid. It is in any case evident that, whatever the mechanism, the mammary gland takes some days to synthesize normal butter fat with the result that the first day's colostrum fat possesses a composition intermediate in character between body fat and milk fat.

SUMMARY

1. Samples of colostrum fat collected from six cows during the progress of lactation have been analyzed for chemical characteristics and the composite mixtures were subjected to fractionation in order to study the fatty acid composition.

2. The results indicate that there is a gradual decrease in the oleic acid, and an increase in the fatty acids of low molecular weight in the colostrum fat as lactation advances.

3. The colostrum fat differs in composition from normal butter fat.

Table 4. *Summary of the component fatty acids of cow colostrum fat (expressed in weight and molar percentages)*

Acids	Weight percentages Days in lactation						Molar percentage Days in lactation					
	1	2	3	4	5	10	1	2	3	4	5	10
Butyric	2.2	2.4	2.6	2.8	3.6	3.6	6.3	6.7	7.1	7.6	9.6	9.9
Caproic	—	0.5	0.8	1.5	0.6	0.6	—	1.0	1.6	3.0	1.2	1.3
Caprylic	0.9	1.2	1.8	2.0	1.8	1.4	1.5	2.5	3.0	3.2	3.0	2.3
Capric	1.2	1.8	2.6	2.3	2.0	1.9	1.8	2.6	3.7	3.2	2.7	2.5
Lauric	1.4	1.8	2.0	2.7	2.6	2.6	1.8	2.2	2.2	3.2	3.1	3.1
Myristic	9.5	9.5	10.3	10.7	11.1	10.8	10.4	10.2	10.9	11.2	11.6	11.3
Palmitic	29.7	32.1	30.5	29.0	30.3	29.4	29.0	30.6	28.6	26.8	28.1	27.3
Stearic	13.7	12.5	12.0	12.6	11.6	12.9	12.1	10.8	10.2	10.5	9.7	10.8
Arachidic	0.6	0.7	0.9	0.8	0.7	1.2	0.5	0.5	0.7	0.6	0.5	0.9
Total	59.2	62.5	63.5	64.4	64.3	64.4	63.4	67.1	68.0	69.3	69.5	69.4
Decenoic	0.1	0.1	0.2	0.2	0.2	0.2	0.1	0.2	0.3	0.3	0.3	0.2
Do-decenoic	0.1	0.1	0.1	0.2	0.3	0.4	0.1	0.2	0.2	0.3	0.3	0.5
Tetra-decenoic	0.8	0.8	0.9	1.1	1.2	0.8	0.9	0.8	0.9	1.1	1.3	0.8
Hexa-decenoic	3.0	3.0	4.7	4.7	3.3	3.5	3.0	2.8	4.4	4.4	3.0	3.3
Oleic	34.1	31.0	28.2	27.5	28.8	29.3	30.3	26.8	24.0	23.1	24.2	24.7
Linoleic	1.5	1.7	1.6	0.8	0.5	0.4	1.3	1.5	1.5	0.7	0.4	0.3
C ₂₀₋₂₂ unsat.	1.2	0.8	0.8	1.1	1.4	1.0	0.9	0.6	0.7	0.8	1.0	0.8
Total	40.8	37.5	36.5	35.6	35.7	35.6	36.6	32.9	32.0	30.7	30.5	30.6
Sum of acids up to C ₁₄	16.2	18.2	21.3	23.5	23.4	22.3	22.9	26.4	29.9	33.1	33.1	31.9

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The Capsular Substance of *Bacillus anthracis*

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Ivánovics and his colleagues (Ivánovics & Erdős, 1937; Brückner & Ivánovics, 1937; Ivánovics & Brückner, 1937, 1938), following up the earlier work of others (Kramar, 1922; Tomcsik & Bodon, 1934), isolated the capsular substance from capsulated strains of *Bacillus anthracis* and some related organisms in a state approaching purity; the material was shown to have the properties of a hapten, giving precipitates with anti-sera to capsulated strains of *B. anthracis* but failing to induce antibody formation or to confer protection against infection with *B. anthracis* when injected into animals. On hydrolysis, the capsular substance gave good yields of *d*-(-)-glutamic acid, enantiomorphous with the *l*-(+)-acid normally encountered in proteins, and Ivánovics & Brückner (1937, 1938) concluded that it was a polypeptide built up solely of *d*-(-)-glutamic acid residues. The work described in the present paper had as its immediate object the elucidation of the detailed chemical structure of this material.

Our work has mostly been carried out with two capsulated strains of *B. anthracis*, viz. 'Vollum', a highly virulent strain originally obtained from Dr R. L. Vollum of the Sir William Dunn Institute of Pathology, Oxford, and 'HM', a non-sporing variant of low virulence obtained by Dr Bruce White, F.R.S., of the National Institute for Medical Research, Hampstead, by growing 'Vollum' in Selavo serum (see Appendix).

Isolation of crude capsular material

After some preliminary trials the two following methods were found convenient for the isolation of the crude capsular substance:

(a) *Cultures grown on agar* are reaped, suspended in water and killed by autoclaving (45 min. at 115°).

The autoclaved suspension is strongly acidified (HCl) and treated with 3 vol. of ethanol; after removing bacterial debris by filtration or centrifugation, the crude capsular substance is precipitated from the filtrate by bringing to pH 8–9 with NaOH. (This procedure is a development of a method, fully described in the Appendix, originally devised by Dr Bruce White.)

(b) *Cultures grown in liquid media* are autoclaved and centrifuged or filtered. The filtrate is treated with 10% CuSO₄ solution and the precipitated copper salt collected by centrifugation; the washed salt is dissolved in acid and decomposed with H₂S. The filtrate from the CuS is adjusted to pH 8–9 and precipitated with ethanol (3 vol.).

Crude materials so prepared from the Vollum and HM strains (and also from another strain, '99', of American origin) were serologically active, giving precipitates with the appropriate anti-serum at dilutions down to 1 in 10⁷. These crude preparations give extremely viscous solutions in water, and the best of them probably approximate very closely to the capsular substance as it occurs attached to the organism.

Further purification of capsular substance

Further purification of the capsular substance without extensive degradation is not easy. Dialysis in acid solution is not wholly satisfactory since, the molecular weight, calculated from the amino-nitrogen content on the assumption that each molecule contains only one free amino-group, decreases considerably during the process, e.g. the amino-nitrogen content of a crude preparation was doubled by keeping at 25° for 48 hr. in solution at pH 2. The most satisfactory method of purification is by way of the silver salt, which is precipitated