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# COMPOSITION OF MILK FAT IN NORMAL, KETOTIC AND FASTING COWS

By

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*Smith & Dastur* (1938) found that the composition of bovine milk fat changes markedly after starvation. After 11—12 days of starvation the percentage of the short-chain fatty acids ($C_4$-$C_{14}$) had greatly fallen and that of the long-chain fatty acids (particularly oleic acid, $C_{18}$:1) had increased. *Shaw et al.* (1942) reported corresponding changes, though less pronounced, in primary ketosis. *Luick & Smith* (1963) analysed the milk fat by gas chromatography, and the earlier findings were almost completely verified as regards clinically healthy, ketotic and fasting cows with hyperketonaemia. The experimental starvation in *Luick & Smith*'s studies lasted five days.

Through the use of radioactive isotopes it has been proved that milk fat synthesis from acetate is impaired during bovine ketosis (*Kronfeld et al.* 1959). Whether this has an aetiological significance has not been made clear, but it has been shown that the lipogenesis is impaired in essentially the same way in primary ketosis as after an experimentally induced condition of inanition accompanied by hyperketonaemia (*Simesen et al.* 1961; *Thin et al.* 1962). *Kronfeld et al.* launched the theory of a lipogenic lesion in the milk fat synthesis; according to *Thin et al.* this lesion exists somewhere in the addition of $C_2$ units between the $C_2$ and the $C_{12}$ fatty acids. The existence of a specific, probably enzymatic lesion is doubted by *Luick & Smith*, who consider that the failure to synthesize normal milk fat is due primarily to an inability to utilize the chief precursors, especially acetate and β-hydroxybutyrate, of the lower fatty acids, and that this in turn accounts for the relatively high levels of long-chain fatty acids found in the milk fat of fasting and ketotic cows.

A more detailed discussion of the possible aetiological significance of the impaired milk fat synthesis in primary ketosis will be found in the above-mentioned papers among others.

## MATERIAL AND METHODS

The material consisted of cows with the clinical diagnosis of primary ketosis (*ketotic cows*) and of clinically healthy cows in the same stage of lactation (*normal cows*). As regards the normal cows, a daily check was made of their appetite and of the presence of ketone bodies in urine or milk. They were grouped according to intensity of feeding, those receiving the maximal ration 5 or 10 days after calving forming one group and those receiving the same ration only 20 days after calving a second group. By maximal ration was meant a diet the energy content of which sufficed for the production of 27—28 kg of a fat-corrected milk (standard 0.4 f. u./kg fat-corrected milk*). Five high-lactating, clinically healthy cows were totally starved for 48 hours. All cows were of Swedish Red and White Breed.

Milk samples were collected in all cases from a morning and an evening milking. The two samples were mixed and kept at about — 20 °C until analysed. Samples from the ketotic cows were collected before treatment was commenced; from the cows subjected to different intensity of feeding, samples were collected on some occasion between the 10th and 20th day after calving; and from the fasting cows one sample was collected before the start and one after 48 hours of starvation.

After being thawed, the milk samples were homogenized by agitation. About 50 ml was centrifuged, the cream was skimmed off, shaken with ether, and the ether solution was dried with anhydrous calcium chloride. The ether was then evaporated.

Transesterification and preparation of the sample for gas chromatography were done by the method of *Smith* (1961) with the exception that methylene chloride was used as solvent. This means that one need not work at a temperature below room temperature and that one can use ampoules instead of test-tubes with airtight screw-on caps. On the other hand the shortest-chain fatty acids ($C_4$, $C_6$ and partly $C_8$) are lost both because their methyl esters evaporate with the methylene chloride and because the peaks of the methyl esters on the gas chromatogram coincide with the peak for residues of the methylene chloride.

*) f. u. = Scandinavian feed unit.

The gas chromatograph was a Perkin-Elmer Fractometer 116; an aluminium column was used, 2 m long and ¼″ wide, filled with butanediol succinate polyester (BDS), 20 %, on acid-washed Chromosorb W 60/80 mesh as carrier. The temperature was 190—200 °C and a flame ionization detector was used.

The peaks of the curves were identified by means of the curves published by *Smith,* and the identification was checked and supplemented by studies of retention times. The quantitative determination was done by planimetry on the assumption that the area of a peak is proportional to the carbon content of the corresponding quantity of ester. That this assumption yields a satisfactory result has been shown by *Ackman & Sipos* (1964).

Statistical analyses were done by conventional methods.

Using this method, certain acids ($C_4$, $C_6$ and partly $C_8$) were lost. On this account all the percentages reported in this paper (Tables 1—3) are undoubtedly rather too high. The magnitude of the errors increases, of course, with the values of $C_4$-$C_8$. It has earlier been reported that the percentages of $C_4$-$C_8$ diminish as a result both of starvation and ketosis (*Smith & Dastur* 1938; *Luick & Smith* 1963). The values of $C_8$ in Tables 1 and 2 indicate corresponding reductions in our study. This means that the t-values recorded in Tables 1 and 2 are probably rather too low for acids the concentrations of which were numerically higher after fasting and in ketosis, and rather too high for those in numerically lower concentration in these conditions. As regards the latter acids, only for ($C_{14}$:1 + $C_{15}$) and $C_{18}$ both in ketotic and fasting cows (Tables 1 and 2) and for $C_{18}$:3 in fasting cows (Table 2) the differences are so small that the numerically lowered values recorded for these groups may in reality correspond to rises. For this reason no statistical comparisons have been made in Table 2 for ($C_{14}$:1 + $C_{15}$), $C_{18}$ and $C_{18}$:3, nor in Table 1 for ($C_{14}$:1 + $C_{15}$) and $C_{18}$.

## RESULTS

Tables 1 and 2 show the results of comparisons in respect of milk fat composition between ketotic and normal cows and between cows before and 48 hours after total starvation. All cows after 48 hours of fasting had more or less pronounced hyperketonaemia. As will be seen, the percentage of fatty acids with relatively few carbon atoms ($C_{10}$, $C_{12}$, $C_{14}$) was markedly lowered,

Table 1. Composition of milk fat (mol.%) of normal and ketotic cows.

| Fatty acid | Normal (n = 17) $\bar{x} \pm s$ | Ketotic (n = 12) $\bar{x} \pm s$ | t-value |
|---|---|---|---|
| $C_{10}$ | 4.09 ± 1.54 | 1.56 ± 0.73 | 5.28*** |
| $C_{12}$ | 5.04 ± 1.67 | 1.59 ± 0.64 | 6.79*** |
| $C_{14}$ | 12.50 ± 2.96 | 6.36 ± 2.09 | 6.17*** |
| $C_{14}$:1 + $C_{15}$ | 1.31 ± 0.45 | 1.29 ± 0.41 | |
| $C_{16}$ | 30.45 ± 1.68 | 32.68 ± 2.16 | 3.13** |
| $C_{16}$:1 | 0.98 ± 0.58 | 1.77 ± 0.38 | 4.10*** |
| $C_{17}$ | 0.02 ± 0.05 | 0.03 ± 0.09 | 0.40 |
| iso-$C_{18}$ | 0.11 ± 0.08 | 0.25 ± 0.09 | 4.43*** |
| $C_{18}$ | 13.15 ± 1.67 | 11.56 ± 2.36 | |
| $C_{18}$:1 | 29.84 ± 5.48 | 40.39 ± 2.13 | 6.31*** |
| $C_{18}$:2 | 0.96 ± 0.27 | 1.16 ± 0.22 | 2.15* |
| $C_{18}$:3 | 1.02 ± 0.55 | 1.11 ± 0.45 | 0.47 |
| $C_{10}$-$C_{14}$ | 21.62 ± 5.65 | 9.51 ± 3.24 | 6.66*** |
| $C_{16}$-$C_{18}$:3 | 76.53 ± 5.63 | 88.94 ± 3.40 | 6.79*** |
| $C_8$ | 0.55[a]) | 0.26[a]) | |

a) Probably too low.

Table 2. Composition of milk fat (mol.%) of high-lactating cows before and after 48 hours of complete fasting.

| Fatty acid | Before (n = 5) $\bar{x} \pm s$ | After (n = 5) $\bar{x} \pm s$ | t-value |
|---|---|---|---|
| $C_{10}$ | 3.66 ± 1.52 | 1.26 ± 0.41 | 3.41** |
| $C_{12}$ | 5.08 ± 1.50 | 1.78 ± 0.48 | 4.68** |
| $C_{14}$ | 13.32 ± 2.59 | 5.82 ± 1.06 | 6.00*** |
| $C_{14}$:1 + $C_{15}$ | 1.80 ± 0.52 | 1.20 ± 0.20 | |
| $C_{16}$ | 31.18 ± 1.78 | 31.54 ± 1.87 | 0.31 |
| $C_{16}$:1 | 1.10 ± 0.23 | 2.14 ± 0.58 | 3.71** |
| $C_{17}$ | trace | trace | |
| iso-$C_{18}$ | 0.10 ± 0.07 | 0.30 ± 0.07 | 4.47** |
| $C_{18}$ | 10.76 ± 2.49 | 9.66 ± 2.12 | |
| $C_{18}$:1 | 31.04 ± 2.65 | 44.48 ± 3.21 | 7.23*** |
| $C_{18}$:2 | 0.88 ± 0.19 | 0.98 ± 0.37 | 0.53 |
| $C_{18}$:3 | 0.88 ± 0.61 | 0.70 ± 0.27 | |
| $C_{10}$-$C_{14}$ | 22.06 ± 3.81 | 8.86 ± 1.72 | 7.06*** |
| $C_{16}$-$C_{18}$:3 | 75.94 ± 4.20 | 89.80 ± 1.70 | 6.84*** |
| $C_8$ | 0.20[a]) | 0.14[a]) | |

a) Probably too low.

Table 3. Composition of milk fat (mol.%) at varying feeding intensity after calving.

| Fatty acid | Intensity I[a] (n = 10) $\bar{x} \pm s$ | Intensity II[b] (n = 6) $\bar{x} \pm s$ | t-value |
|---|---|---|---|
| $C_{10}$-$C_{14}$ | 24.50 ± 4.86 | 17.27 ± 4.35 | 2.99** |
| $C_{16}$-$C_{18}$:3 | 73.61 ± 4.87 | 80.88 ± 4.11 | 3.05** |

a) Maximal ration 5 or 10 days after calving.
b) Maximal ration 20 days after calving.

and the sum of the percentages of acids with greater number of carbon atoms ($C_{16}$-$C_{18}$:3) was markedly increased. This applied very particularly to $C_{18}$:1 (oleic acid). As regards individual acids it appears to be of special interest that $C_{16}$ (palmitic acid) increased numerically both after fasting and in ketosis, in the latter case significantly. As regards ($C_{14}$:1 + $C_{15}$) and $C_{18}$ (stearic acid), Tables 1—2 show reductions which, however, on the afore-mentioned grounds may be suspected to be erroneous. *Smith & Dastur* (1938) and *Luick & Smith* (1963) found percentage increases of $C_{18}$ after fasting and in ketosis.

From Table 3 it is seen that, of clinically healthy cows with normal levels of blood acetone bodies, those which were offered a maximal ration only 20 days after calving had a significantly higher percentage of long-chain and lower of short-chain milk fatty acids than those which were given a higher energy diet.

## DISCUSSION

With insignificant exceptions, the results presented in Tables 1 and 2 confirm the earlier reported changes in composition of the milk fat in primary ketosis and after inanition accompanied by hyperketonaemia, namely a reduced percentage of short-chain and increased percentage of long-chain fatty acids, and that the chief increase is in oleic acid ($C_{18}$:1). They also show that no essential difference existed between the changes in cases of clinical ketosis and after fasting. The resemblances in this respect between cows with clinical ketosis and with fasting ketosis have been reported earlier by *Luick & Smith* (1963). As appears from Table 3, a corresponding change existed between the short-chain and long-chain fatty acids in cows which received a low-energy diet during the first weeks of lactation without incurring ketosis either in the clinical or *blood chemistry* sense. This indicates

that the changes in composition of the milk fat are intimately bound up with the energy supply as such and not with the occurrence of ketosis. The experimental results thus raise the question whether the impaired lipogenesis found in bovine ketosis (*Kronfeld et al.* 1959) is not a physiological response to the relative starvation which is both one of the symptoms of primary ketosis and very often precedes it on account of insufficient energy supply in the diet (*Pehrson* 1966). In such case impaired lipogenesis would lack aetiological significance for primary ketosis.

In some details the results presented in Tables 1—2 differ from those reported earlier. *Smith & Dastur* (1938) and *Luick & Smith,* for example, found that the percentage of $C_{16}$ (palmitic acid) fell after starvation. In the present study $C_{16}$ rose both after starvation and in ketosis. The explanation may lie in differences of breed or possibly in the duration of the complete fasting.

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## SUMMARY

By means of gas chromatography the authors have found changes in the composition of the milk fat in ketosis and after fasting, the concentration of short-chain fatty acids falling and of long-chain rising. This is interpreted as indicating that no essential difference in

this respect exists between ketotic cows and cows after a period of fasting associated with hyperketonaemia. Since clinically healthy cows without hyperketonaemia likewise had a lowered concentration of short-chain and elevated concentration of long-chain fatty acids in the milk fat if they received a low-energy diet during the first weeks after calving, the authors question whether the impaired lipogenesis reported by others in primary ketosis is not a physiological consequence of fasting and therefore lacks aetiological significance for the primary ketosis.


## ZUSAMMENFASSUNG

*Die Zusammensetzung des Milchfettes bei klinisch gesunden Kühen, bei primärer Acetonämie und nach Hunger.*

Mit Hilfe der Gaschromatographie haben die Verfasser Veränderungen in der Zusammensetzung des Milchfettes bei Acetonämie und nach Hunger gefunden, so dass die kurzkettigen Fettsäuren in verminderter und die langkettigen in vermehrter Konzentration vorliegen. Die Resultate werden so gedeutet, dass keine grundsätzlichen Unterschieden in dieser Hinsicht bei Acetonämie und nach einem mit Hyperketonämie verbundenen Hungerzustand vorliegen. Klinisch gesunde Kühe ohne Hyperketonämie hatten gleichfalls verminderten Gehalt von kurzkettigen und vermehrten Gehalt von langkettigen Fettsäuren im Milchfett, wenn sie aus Energiegesichtspunkt in den ersten Wochen nach Kalben wenig ausgefüttert wurden. Die Verfasser ziehen daher in Erwägung, wenn nicht die gestörte Lipogenese, die andere Verfasser bei primärer Acetonämie festgestellt haben, eine physiologische Folge eines Hungerzustandes ist, und deswegen ohne ätiologische Bedeutung für die primäre Acetonämie ist.

## SAMMANFATTNING

*Mjölkfettets sammansättning hos normalkor, kor med primär acetonemi och svultna kor.*

Med hjälp av gaskromatografi har författarna funnit att mjölkfettets sammansättning vid acetonemi och efter svält ändras så att de kortkedjiga fettsyrorna föreligger i minskad och de långkedjiga i ökad koncentration. Resultaten tolkas så att inga principiella skillnader i detta avseende föreligger vid acetonemi och efter ett svälttillstånd förbundet med hyperketonemi. Kliniskt friska kor utan hyperketonemi hade likaledes sänkt halt kortkedjiga och förhöjd halt långkedjiga fettsyror i mjölkfettet om de blev ur energisynpunkt svagt utfodrade under de första veckorna efter kalvningen. På grund därav ifrågasätter författarna om inte den ändring i lipogenesen som andra författare har konstaterat föreligga vid primär acetonemi är en fysiologisk följd av ett svälttillstånd och följaktligen saknar etiologisk betydelse för den primära acetonemien.


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