Afternoon session

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Dietary fat and milk fat secretion in the cow

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Although fat is normally a relatively minor component of the diet of the lactating cow, the daily intake of dietary fat by the grazing cow is of the same order as the daily output of fat in the milk. The possibility that the level of fat in the diet of the cow might exert some influence on milk fat production has been extensively investigated over the last 50 years but conflicting reports have appeared on almost every aspect of the subject. Rather than to review earlier feeding experiments in any detail, it is the purpose of the present communication to examine the effects of different dietary fats or oils on the composition and yields of the individual fatty acids in the milk fat of the cow. It is hoped that this approach may throw some light on the influence of dietary fat on the synthesis of milk fat in the mammary gland.

The inclusion of vegetable oils rich in 18:2 or 18:3 (e.g. groundnut oil or linseed oil) in the diet of the lactating cow increases the iodine value and saponification equivalent but decreases the Reichert-Meissl, Polenske and Kirschner values of the milk fat (Holland & Buckley, 1918, 1923; Sutton, Brown & Johnston, 1932; Hilditch & Thompson, 1936; Hill & Palmer, 1938; Williams, Cannon & Espe, 1939; Hilditch & Jasperson, 1943). Hilditch & Thompson (1936) and Hilditch & Jasperson (1943) showed that this increased iodine value of the milk fat was due to an increased percentage of oleic acid and decreased percentages of the saturated fatty acids containing from 4 to 14 carbon atoms. These earlier results, obtained by the classical techniques of ester fractionation, have been fully confirmed in more recent experiments in which the milk fatty acids have been analysed by gas–liquid chromatography (Brown, Stull & Stott, 1962; Tove & Mochrie, 1963; Parry, Sampugna & Jensen, 1964; Storry, Rook & Hall, 1967). Since diets supplemented with vegetable oils or diets low in roughage (King & Hemken, 1962) appeared to exert a similar effect on the fatty acid composition of cow's milk fat, it was of interest to determine whether the inclusion of vegetable oils in the diet of the cow altered the concentrations of the various volatile fatty acids in the rumen contents. An investigation along these lines was carried out by Steele & Moore (unpublished observations) who found that the inclusion of cottonseed oil at levels of 6 or 10% of the concentrate mixture given to cows did not alter milk yield but slightly depressed the yield and percentage of fat in the milk. When the concentrate mixtures containing either 6 or 10% cottonseed oil were given to the cows, the yields and percentages of 4:0, 6:0 and 8:0 in the milk fat were altered only slightly, but the yields and percentages of 10:0, 12:0, 14:0 and 16:0 were markedly decreased, whereas the yields and percentages of 18:0 and 18:1

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were markedly increased. When cottonseed oil constituted 10% of the concentrate mixture, *trans* 18:1 constituted 37% of the increase in the daily yield of total 18:1 in the milk fat. The inclusion of 6 or 10% of cottonseed oil in the concentrate mixture altered neither the total concentration of volatile fatty acids nor the proportions of the individual volatile acids in the rumen contents.

Hill & Palmer (1938) and Stull, Harland & Davis (1957) reported that the addition of tallow (rich in 16:0, 18:0 and 18:1) to the diet of the cow resulted in an increase in the iodine value of the milk fat. Steele & Moore (unpublished observations) found that dietary tallow, included at the level of 6% of the concentrate mixture given to cows, had little effect on the yield of milk or milk fat but it depressed the yields and percentages of 10:0, 12:0 and 14:0 and increased the yields and percentages of 18:0 and 18:1 in the milk fat. In spite of the fact that the tallow contained about 30% 16:0, neither the yield nor the percentage of 16:0 in the milk fat was altered by the addition of tallow to the diet. Similar observations were made by Storry *et al.* (1967) who studied the effect on milk fat composition of supplementing the diet of cows with red palm oil (containing about 40% 16:0). Steele & Moore (unpublished observations) investigated the effects on milk fat composition of including individual fatty acids in the diet of the cow. In one experiment, the inclusion of 18:0 (94% pure) or 16:0 (96% pure) at the level of 10% of the concentrate mixture given to cows did not affect milk yield but significantly increased the milk fat yield and percentage. The addition of 18:0 to the diet resulted in a reduction in the yields and percentages of 10:0, 12:0, 14:0 and 16:0 and marked increases in the yields and percentages of 18:0 and 18:1. When 18:0 was included in the diet, the increased yield of 18:1 in the milk fat was almost entirely due to the *cis* isomer. When cows were given 16:0 in the diet there were reductions in the yields and percentages of 10:0, 12:0, 14:0, 18:0 and 18:1, but there were pronounced increases in the yield and percentage of 16:0 in the milk fat. The addition of 16:0 or 18:0 to the diet did not alter the total concentration or molar proportions of the rumen volatile fatty acids. In a further experiment (Steele & Moore, unpublished) it was found that the inclusion of 18:1 (78% pure) at the level of 10% of the concentrate mixture given to cows did not affect milk yield but markedly reduced the milk fat percentage and the yield of milk fat. The yields and percentages of the fatty acids from 4:0 to 18:0 in the milk fat were decreased but the yield and percentage of 18:1 were increased. In this experiment the increased yield of total 18:1 in the milk fat was accounted for to a considerable extent by the *trans* isomer. The addition of 18:1 to the diet of the cows resulted in a decrease in the concentration of total volatile fatty acids in the rumen contents and a reduction in the proportion of acetic acid and an increase in the proportion of propionic acid.

The addition of coconut oil (rich in 12:0 and 14:0) to the diet of lactating cows reduces the iodine number of the milk fat (Holland & Buckley, 1918, 1923). When cows were given coconut oil (Hilditch & Sleightholme, 1930) or palm kernel oil (Hilditch & Jasperson, 1943), the concentrations of 12:0 and 14:0 in the milk fat were increased whereas the concentrations of all the other fatty acids (including 18:1) were decreased. Similar results were obtained by Mohammed, Brown, Riley

& Stull (1964) and Storry *et al.* (1967). Steele & Moore (unpublished observations) investigated the effect on milk fat composition of supplementing the diet of the cow with 14:0 (95% pure) or 12:0 (99% pure). In one experiment, the inclusion of 14:0 at the level of 10% of the concentrate mixture given to cows did not alter the yield of milk fat but decreased the yield of milk. The yield and percentage of 14:0 in the milk fat were markedly increased whereas the yields and percentages of all the other constituent fatty acids were decreased. In a further experiment, the inclusion of 12:0 at the level of 5% of the concentrate mixture given to cows markedly decreased the milk fat percentage and the yield of milk fat but did not alter milk yield. It was found that the milk fat produced by cows given the diet supplemented with 12:0 contained higher concentrations of both 12:0 and 14:0 but lower concentrations of the other constituent fatty acids. Analysis of rumen contents showed that dietary 12:0 or 14:0 reduced the concentration of total volatile fatty acids, slightly decreased the proportion of acetic acid and slightly increased the proportion of propionic acid in the total volatile fatty acids.

Hilditch & Williams (1964) reported that when cows were given 114 g cod-liver oil (rich in C_{20} and C_{22} unsaturated fatty acids) per day, the yield of milk fat was reduced from about 440 to 320 g per day. This reduction in milk yield was accompanied by increased percentages of 18:1 and the C_{20} and C_{22} unsaturated fatty acids and decreased percentages of all the other constituent fatty acids in the milk fat. Shaw & Ensor (1959) and Nottle & Rook (1963) have shown that the addition of cod-liver oil to the diet of the cow gives rise to a pronounced fall in the proportion of acetic acid and an increase in the proportion of propionic acid in the volatile fatty acid fraction of the rumen contents.

The level of fat production in the milk of the ruminant depends largely on the activities of two metabolic processes. In the first of these, acetate and β-hydroxybutyrate are taken up from the blood and are then utilized as precursors for the *de novo* synthesis in the mammary gland of the fatty acids from 4:0 to 16:0. These fatty acids are synthesized in mammary tissue by way of the malonyl CoA pathway and are then incorporated into triglycerides by the metabolic route that involves α-glycerophosphate, phosphatidic acid and α,β-diglyceride as intermediates (cf. Ganguly, 1960; Peeters & Lauryssens, 1964; Barry, 1964; Hibbitt, 1966; Annison, Linzell, Fazakerley & Nichols, 1967; Linzell, Annison, Fazakerley & Leng, 1967). In the second process, triglycerides circulating in the blood as chylomicra and low-density ($d < 1{\cdot}019$) lipoproteins are taken up by the mammary gland. At some stage in this process it appears that the triglycerides are partially or completely hydrolyzed and the resulting fatty acids are incorporated into new triglyceride molecules by the mammary gland (e.g. Barry, Bartley, Linzell & Robinson, 1963; Patton & McCarthy, 1963; Annison *et al.* 1967). Under normal dietary conditions, the major fatty acids present in the blood triglycerides of the cow are 16:0, 18:0 and 18:1 (Duncan & Garton, 1963) and these are the fatty acids that are normally taken up by the mammary gland for incorporation into milk triglycerides. However, it is clear that the fatty acid composition of the blood triglycerides is altered when the diet of the cow is supplemented with 12:0, 14:0, 16:0, 18:0 or 18:1 and increased amounts of these fatty acids are then taken up by the gland for milk fat synthesis. When the diet of the cow is supplemented with vegetable oils rich in 18:2 there is little or no increase in the yield or percentage of 18:2 in the milk fat. The conclusion of Tove & Mochrie (1963) that this 'is indicative of the efficiency and completeness of hydrogenation by the rumen microflora' is not acceptable since it may be calculated from the results of Duncan & Garton (1963) and Storry & Rook (1965) that 18:2 is, in fact, one of the main fatty acids circulating in the blood of the cow. However, this 18:2 occurs almost exclusively in the blood cholesterol esters and phospholipids, the fatty acids of which cannot be utilized for milk fat synthesis (e.g. Annison *et al.* 1967). When the 18:2 content of the blood triglycerides in the cow is increased by intravenous infusions of an emulsion of cottonseed oil then there is a pronounced increase in the concentration of 18:2 in the milk fat (Tove & Mochrie, 1963; Storry & Rook, 1965).

The addition to the diet of the cow of vegetable oils rich in 18:2 results in marked increases in the yield and percentage of 18:1 in the milk fat. This could be due to the complete hydrogenation of 18:2 by rumen micro-organisms, the absorption of the resulting 18:0 and its incorporation into blood triglycerides which would then be taken up by the mammary gland where *cis* 18:1 is synthesized by the desaturation of 18:0 (e.g. Annison *et al.* 1967; Linzell *et al.* 1967). However, over one-third of the increase in the yield of 18:1 in the milk fat under these dietary conditions is accounted for by the *trans* isomer. Ward, Scott & Dawson (1964) and J. W. Czerkawski (unpublished observations) have shown that *trans* 18:1 is an intermediate in the hydrogenation of the C_{18} polyunsaturated fatty acids by rumen micro-organisms. Thus it would appear that the hydrogenation of 18:2 in the rumen is far from complete and that some of the *trans* isomer of 18:1 is absorbed and incorporated into blood and milk triglycerides. The observation (Wilde & Dawson, 1966; J. W. Czerkawski, unpublished) that rumen micro-organisms also catalyse the isomerization of *cis* 18:1 to *trans* 18:1 would account for the increased secretion of *trans* 18:1 in the milk fat of cows given pure oleic acid (*cis* 18:1) in the diet.

Although the decreased secretion of the shorter-chain fatty acids in the milk fat of cows given cod-liver oil could be due, at least in part, to the reduction in the proportion of acetic acid and the increase in the proportion of propionic acid in the volatile fatty acids of the rumen contents, the decreased secretion of the shorter-chain fatty acids (up to 14:0) in the milk fat of cows given cottonseed oil or tallow in the diet cannot be accounted for by alterations in the production of the various volatile fatty acids in the rumen. The question now arises whether the increased uptake of 16:0, 18:0 or 18:1 from the blood triglycerides inhibits the *de novo* synthesis in the mammary gland of the shorter-chain fatty acids from acetate or β-hydroxybutyrate. This seems distinctly possible for it has been established that the addition of fat to the diet of the rat decreases the rate of fatty acid synthesis in the liver (cf. Hill, Linazasoro, Chevalier & Chaikoff, 1958; Hill, Webster, Linazasoro & Chaikoff, 1960). According to Bortz, Abraham, & Chaikoff (1963), the impairment of fatty acid synthesis in the liver of rats given diets containing fat is due to the inhibition of acetyl CoA carboxylase which catalyses the carboxylation of acetyl CoA, the rate-limiting step in fatty acid synthesis in rat liver (Numa, Matsuhashi & Lynen, 1961) and also in the bovine mammary gland (Ganguly, 1960). It has been shown that fatty acids or fatty acyl CoA derivatives inhibit acetyl CoA carboxylase in rat liver (Bortz & Lynen, 1963) and rat mammary gland (Howanitz & Levy, 1965) and that the rates of fatty acid synthesis from acetate by particle-free supernatant fractions of rat (Smith & Dils, 1966) or cow (Hibbitt, 1966) mammary gland are reduced by the addition of fatty acids to the incubation medium. Tubbs & Garland (1964) have shown that the concentrations of long-chain fatty acyl CoA derivatives in rat liver were increased when the animals were given diets containing fat. It seems reasonable to suppose, therefore, that an increased uptake by the mammary gland of triglycerides from the blood stream would result in higher concentrations of long-chain fatty acids in fatty acyl CoA derivatives in the mammary gland. The acetyl CoA carboxylase in the mammary gland would thus be inhibited and there would be a reduction in the rate of the *de novo* synthesis from acetate of fatty acids up to and including 16:0.

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