THE INCORPORATION OF LINOLEIC ACID INTO THE TISSUES OF GROWING STEERS OFFERED A DIETARY SUPPLEMENT OF FORMALDEHYDE-TREATED CASEIN–SAFFLOWER OIL

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[Manuscript received 30 August 1971]

Abstract

A formaldehyde-treated casein–safflower oil supplement was included in the diet of nine Friesian steers and the incorporation of linoleic acid in their tissues, their liveweight gain, and their intake of dry matter and energy, were compared with those of nine steers given a conventional diet. Three steers from each group were slaughtered after 2, 4, and 8 weeks of feeding and the proportion of linoleic acid in samples of muscle and adipose tissue was measured. In addition, two steers were slaughtered prior to the start of the experiment to provide initial values for tissue composition. The steers were 9 months old when the experiment began.

Dietary linoleic acid, protected from hydrogenation in the rumen, was extensively incorporated into the body tissues studied; the progress of incorporation in each tissue was described by a curve of diminishing increments. The level of incorporation observed in deep body tissues was higher than in tissues located near the body surface. The supplemented steers ate less dry matter and energy intake was less than for the unsupplemented steers, the difference being most apparent at the beginning of the experiment. Although the supplemented steers tended to grow less rapidly than the unsupplemented steers, the difference was not significant.

I. Introduction

The lipids in the plant material eaten by ruminant animals generally contain high proportions of C_{18} polyunsaturated fatty acids (e.g. linoleic and linolenic). Microbes in the rumen hydrogenate these fatty acids, thus accounting for the low concentration of polyunsaturated fatty acids in ruminant tissues (Garton 1967). For this reason, the inclusion of polyunsaturated oils in ruminant diets does not significantly increase the proportion of these acids in the body tissues (Erwin, Sterner, and Marco 1963; Tove and Mochrie 1963).

A recent development (Scott *et al.* 1970; Scott, Cook, and Mills 1971) has made possible the production of oil supplements in which the constituent polyunsaturated fatty acids are protected from hydrogenation in the rumen. The polyunsaturated oils (e.g. safflower, sunflower, soybean, etc.) are embedded in a protein matrix and then treated with formaldehyde. When ruminant animals are given such supplements, marked changes occur in the fatty acid composition of milk and body fats (Cook *et al.* 1970; Scott *et al.* 1970; Scott, Cook, and Mills 1971).

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In a study of the alterations which occurred in the fatty acid composition of plasma and tissue lipids of growing steers given a supplement containing safflower oil (Cook *et al.* 1972), large increases were observed in the amount of linoleic acid in the tissue lipids. This paper describes the progress of incorporation of linoleic acid (18:2) into the tissue lipids during the period of supplementation. Data are also presented on food intakes and liveweight gain. A second group of steers, similar to and managed in the same way as those receiving the supplement, but maintained on a conventional diet, provided a basis for comparison.

II. Methods

(*a*) *Animals*

The 20 Friesian steers used were 9 months of age and had a mean liveweight of 185 (S.E. ±3) kg when the experiment started. They had previously been maintained at pasture. Two of the steers were slaughtered at the beginning of the experiment to provide control tissue samples. The 18 remaining steers were allocated at random to two experimental groups. They were confined in individual pens in a large open shed and weighed once weekly prior to feeding.

(*b*) *Diets*

Each animal was offered 8 kg of feed daily at 0900 hr; feed refused was removed at 0830 hr the following morning. The diet given to the supplemented animals consisted of a loose mixture of rolled barley (50%), chopped lucerne hay (30%), and formaldehyde-treated casein–safflower oil supplement (20%). The supplement was prepared by spray-drying an emulsion of equal parts by weight of safflower oil and casein as previously described (Scott, Cook, and Mills 1971). The safflower oil used contained 75 g 18:2 per 100 g fatty acids. The animals were gradually introduced to the supplement during the first 6 days of the experiment. The second group of steers was not given the untreated supplement because its inclusion in their diet was likely to cause refusal of feed (T. W. Scott and L. J. Cook, unpublished data). Thus, while providing comparative data for normally fed animals in the same physiological state as the supplemented steers, they are not, strictly speaking, a control group. They were given a diet which consisted of rolled barley (62·5%) and chopped lucerne hay (37·5%) for the first 2 weeks of the experiment. Thereafter the diet contained rolled barley (58·6%), chopped lucerne hay (35·2%), and peanut meal (6·2%). The chemical composition of the dietary components is tabulated below (DM, dry matter).

Component	DM (%)	Organic matter (% DM)	Nitrogen (% DM)	Lipid (% DM)	Gross calorific value (kcal/g DM)
Rolled barley	89·4	97·4	1·30	3·5	4·61
Lucerne chaff	91·7	89·9	3·14	2·5	4·50
Peanut meal	93·8	93·4	8·24	4·3	4·72
Casein–safflower oil	94·5	97·9	7·16	44·6	7·39

(*c*) *Sampling Procedures*

Three animals in each group were slaughtered 2, 4, and 8 weeks after being offered the maximal level of supplement. At slaughter, samples were taken from perirenal, omental, and subcutaneous fat depots and from two muscles, the M. semitendinosus and the M. psoas major. Approximately 2 g of each tissue was frozen on solid carbon dioxide and transferred into vials containing 10 ml of chloroform–methanol (2:1 v/v). Samples were stored under nitrogen at −15°C.

(*d*) *Chemical Analyses*

The dietary constituents and feed residues were analysed for nitrogen and organic matter by the methods of the Association of Official Agricultural Chemists (1960). The gross calorific values of the dietary components were determined by bomb calorimetry.

To determine the total lipid content of the dietary components and feed residues, samples were saponified with sodium hydroxide and ethanol, acidified with hydrochloric acid, and the free fatty acids and non-saponifiables extracted into hexane. The hexane extracts were evaporated and the lipid was determined gravimetrically. The proportion (g per 100 g fatty acids) of 18:2 in the tissue lipids was determined by gas–liquid chromatography (Scott, Setchell, and Bassett 1967).

The dietary components present in the feed residues were calculated by means of simultaneous equations based on the nitrogen, ash, and lipid contents of the residues and dietary components.

III. Results

(*a*) *Linoleic Acid (18:2) in Tissue Lipids*

The proportion of 18:2 in tissue lipids from the unsupplemented steers remained virtually constant throughout the experiment (Fig. 1). There was a dramatic increase

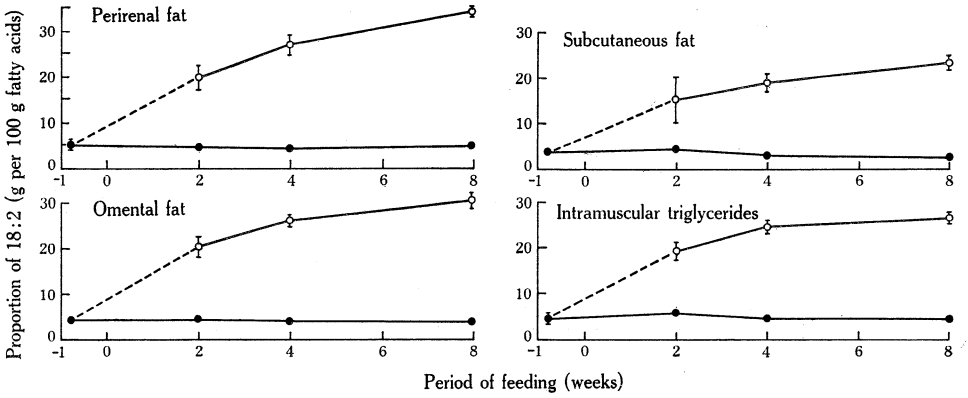


Fig. 1.—Effect of feeding a formaldehyde-treated casein–safflower oil supplement on the proportion of 18:2 in the tissue lipids of steers. ● Unsupplemented diet. ○ Supplemented diet.

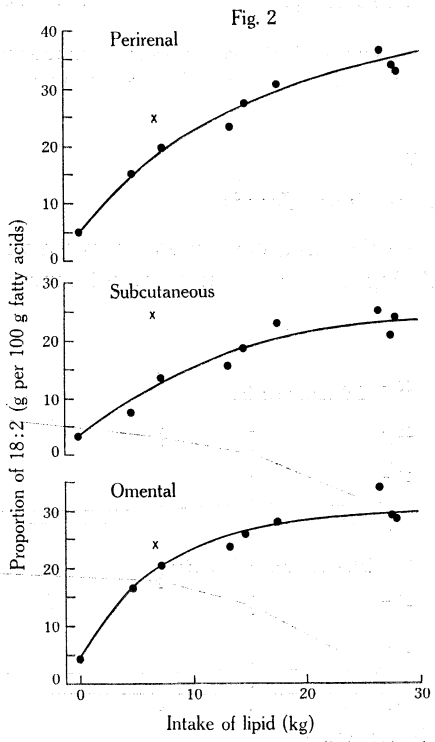
in the 18:2 content of these lipids when the supplement was given. After 2 weeks of feeding, intramuscular (M. psoas major) and subcutaneous triglycerides from the supplemented steers contained approximately three times as much 18:2 and perirenal and omental triglycerides contained approximately 4·5 times as much 18:2 as did the corresponding triglycerides from the unsupplemented steers. The proportion of 18:2 in these tissues increased with continued feeding but the rate of increase declined, suggesting that the response followed a curve of diminishing increments.

As there were differences between animals in intake of the supplement, the proportion of 18:2 in each of the tissues studied was plotted against the total intake of lipid from the supplement (Figs. 2 and 3). The values for all steers but one (shown

as × in Figs. 2 and 3) conformed to curves of diminishing increments. To describe the data for each tissue (excluding the values for the one exceptional steer), curves of the form

$$y = A - Be^{-kx},$$

where y = the proportion of 18:2 (% w/w) and x = total intake of lipid (kg), were fitted by an iterative procedure. The constant A represents the maximum or



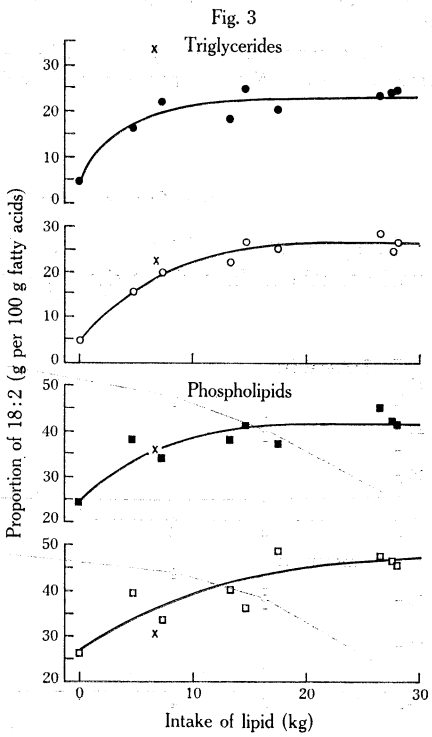


Fig. 2.—Relationship between total intake of safflower oil lipid and the proportion of 18:2 in perirenal, subcutaneous, and omental fat. Values for one steer (×) were omitted from curve-fitting calculations.

Fig. 3.—Relationship between total intake of safflower oil lipid and the proportion of 18:2 in intramuscular triglycerides (○, ●) and intramuscular phospholipids (□, ■). Open symbols, M. psoas major. Solid symbols, M. semitendinosus. Values for one steer (×) were omitted from curve-fitting calculations.

equilibrium value of x, B represents the maximum increment (so that $A-B$ represents the initial value of x), and k is a proportionality constant. The constants obtained for a solution of this equation for each tissue are shown in Table 1. The values obtained for the residual standard deviation and R^2 (coefficient of determination) show that the curves obtained accurately describe the data.

The maximum value calculated for the incorporation of 18:2 in perirenal fat (39%) was significantly higher ($P < 0{\cdot}01$) than those for subcutaneous (28%) and omental fat (31%). The difference between subcutaneous and omental fat was not

significant. The maximum values for intramuscular triglycerides were lower than for adipose tissue. The value calculated for the M. semitendinosus (23%) was significantly lower ($P<0\cdot01$) than that for the M. psoas major (27%). Intramuscular phospholipids normally contain more 18:2 than triglycerides (*c.* 25%, cf. 4%) and this class of lipids had the highest calculated maximum values. For the phospholipids of the M. semitendinosus and M. psoas major, these values were 42 and 49% respectively.

TABLE 1

CONSTANTS OBTAINED FOR SOLUTION OF THE EQUATION $y = A - Be^{-kx}$ FOR LIPIDS FROM ADIPOSE AND MUSCLE TISSUE

See text for definition of constants and variables

Tissue	A (± S.E.)*	B	$A-B$	k	S.D.†	R^2‡
	Adipose tissue triglycerides					
Perirenal	$38\cdot7_a \pm 2\cdot0$	34·2	4·5	0·074	1·24	0·991
Subcutaneous	$27\cdot8_b \pm 2\cdot9$	24·5	3·3	0·064	1·58	0·970
Omental	$31\cdot3_b \pm 1\cdot1$	27·1	4·2	0·119	1·37	0·988
	Intramuscular triglycerides					
M. semitendinosus	$23\cdot1_a \pm 0\cdot8$	18·7	4·4	0·237	1·63	0·971
M. psoas major	$27\cdot0_b \pm 0\cdot9$	22·3	4·7	0·148	1·42	0·982
	Intramuscular phospholipids					
M. semitendinosus	$41\cdot6_a \pm 1\cdot5$	17·6	24·0	0·170	2·21	0·936
M. psoas major	$48\cdot9_a \pm 5\cdot1$	22·4	26·5	0·081	3·79	0·848

* Within tissue lipid classes, values for A with same subscript do not differ at $P<0\cdot01$.

† Residual standard deviation.

‡ Coefficient of determination, i.e. the proportion of the variance accounted for in fitting the curve.

(*b*) *Intake and Liveweight Gain*

The intakes of both DM and gross energy by the steers receiving the supplement were lower than those of the unsupplemented steers (Table 2). Non-orthogonal analysis of variance showed that these differences were significant ($P<0\cdot01$). The respective mean DM intakes of the supplemented and unsupplemented steers were 72 and 92% of the DM offered. The mean daily energy intakes (with their standard errors) of both groups are plotted in Figure 4. The energy intake of both groups was low in the first week of the experiment. The supplemented steers ate less energy than the unsupplemented steers and, although their intake improved as the experiment progressed, it remained lower than that of the unsupplemented steers. The amount of supplement offered was 1·51 kg DM per day and the mean intake was $1\cdot00 \pm 0\cdot06$ kg DM per day, i.e. 19·1% of DM eaten. Whereas feed was refused by all the supplemented steers throughout the experiment, several of the unsupplemented steers were eating all the feed offered them towards the end of the experiment. The concentration of supplement in the feed refused (24·2% of DM) was significantly higher ($P<0\cdot01$) than that in the diet offered (20·7% of DM), thus suggesting that the steers given the supplement discriminated against it to a small extent.

The steers in the unsupplemented group tended to grow faster than those receiving the supplement (Table 2), but the difference was not significant. Although the intake of dry matter per unit of gain tended to be lower for the supplemented steers, they consumed the same amount of energy per unit of gain as did the unsupplemented steers (Table 2).

TABLE 2

INTAKE OF DRY MATTER, ENERGY, AND CRUDE PROTEIN AND LIVEWEIGHT GAIN OF STEERS GIVEN A FORMALDEHYDE-TREATED CASEIN–SAFFLOWER OIL SUPPLEMENT

Parameter measured	Unsupplemented steers	Supplemented steers	S.E.
Dry matter offered (kg/day)	7·24	7·29	
Dry matter intake (kg/day)	6·68	5·24	±0·10**
Energy offered (Mcal/day)	33·1	37·5	
Energy intake (Mcal/day)	30·5	26·8	±0·5**
Crude protein offered (kg/day)†	1·01	1·41	
Crude protein intake (kg/day)†	0·91	0·95	±0·02 (n.s.)
Liveweight gain (kg/day)	1·25	1·09	±0·11 (n.s.)
Food conversion			
As kg dry matter/kg gain	5·34	4·79	
As Mcal intake/kg gain	24·4	25·0	

** Differences significant at $P < 0·01$. † Nitrogen content × 6·25.

IV. DISCUSSION

The results obtained in this experiment show that dietary 18:2, given as a formaldehyde-treated casein–safflower oil supplement, was rapidly incorporated into the body tissues. During the 8-week period of the experiment the level of incorporation increased continuously, but mathematical treatment of the data indicated that the values were approaching maxima which differed between tissues.

The proportion of 18:2 in the milk glycerides of lactating ruminants (Scott *et al.* 1970) and the depot fats of sheep (Scott, Cook, and Mills 1971) and lambs (Cook *et al.* 1970) increased when formaldehyde-treated casein–safflower oil supplements were included in the diet. Similar responses were obtained in the present experiment when this supplement was given to growing steers. The rate of liveweight gain of the steers implies that active lipogenesis was occurring. Thus the extensive incorporation of 18:2 into the tissues of the steers was consistent with their metabolic state. A comprehensive analysis of the fatty acid patterns and of the interrelationships between adipose tissue, muscle, liver, and plasma lipids has been given elsewhere (Cook *et al.* 1972).

The equations describing the relationship between the intake of safflower oil lipid and the incorporation of 18:2 in the tissues indicate that, under the conditions of this experiment, the maximum levels of incorporation of 18:2 differed between tissues. Thus the deeper body fats, particularly perirenal, approached higher levels

of incorporation than external fat (subcutaneous). A similar pattern applied to the intramuscular lipids. The M. semitendinosus lies subcutaneously in the hind leg whereas the M. psoas major lies deep within the body, ventral to the transverse processes of the lumbar vertebrae. The internal depot fats of ruminants contain more stearic acid and less oleic and palmitoleic acid than those of external tissues (Duncan and Garton 1967) and this difference was apparent in the unsupplemented steers in the present experiment (Cook *et al.* 1972). Duncan and Garton (1967) have suggested that the internal tissues preferentially assimilate the fatty acids of chylomicron triglycerides which, in ruminants, normally have a high content of stearic acid. A large amount of the 18:2 absorbed from the small intestine of the supplemented steers would have been incorporated into the chylomicron triglycerides (Cook *et al.* 1972); thus preferential uptake of these triglycerides by internal tissues is consistent with the differences observed between tissues in the incorporation of 18:2.

Since only one level of supplementation was used in this experiment, the results obtained cannot be extrapolated to situations where different levels of the supplement are given. Comparisons might be further complicated by the nature and amount of the other dietary constituents.

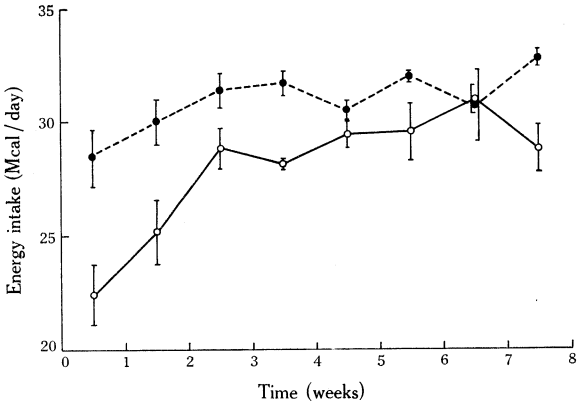


Fig. 4.—Mean daily energy intake (with S.E.) of unsupplemented (●) and supplemented (○) steers for each week of feeding plotted against time.

Despite the higher caloric density of their diet, the supplemented steers ate not only less dry matter but their gross energy intake was less than for the unsupplemented steers. The differences recorded must be regarded as minimum values because the unsupplemented steers were leaving no residues towards the end of the experiment. The lower intake of gross energy might be due, in part, to the tendency for the steers to discriminate against the supplement, perhaps because it was given as a fine powder. There is also the possibility that the release of large amounts of fat in the duodenum might inhibit food intake. In addition, since ruminants given diets containing high proportions of concentrates eat to satisfy their net energy needs (Montgomery and Baumgardt 1965; Baumgardt 1970), the difference in gross energy intake may be a reflection of the high net energy value of dietary fat (Hoffmann, Schiemann, and Nehring 1962). Thus the supplemented steers would need less gross energy per unit of gain than the unsupplemented steers. There was, however, no difference between the two groups in the overall mean

value for gross energy conversion, although the value for the supplemented steers may have been lower if their gross energy intake had not been so much lower than that of the unsupplemented steers in the first 2 weeks of the experiment (Fig. 4). The fact that the rate of liveweight gain of the supplemented steers tended to be lower than that of the unsupplemented steers suggests that their intake of energy may have been limited by some factor other than their net energy needs. It should be remembered, however, that any conclusions derived from the intake and liveweight data collected in this experiment remain tentative because the unsupplemented steers were not a control group and were not all eating to appetite; in addition short-term estimates of liveweight gain are unreliable.

The results of the present study, together with data reported earlier (Cook *et al.* 1970; Scott, Cook, and Mills 1971) show unequivocally that feeding formaldehyde-treated casein–polyunsaturated oil supplements to ruminants alters the fatty acid composition of body fats in a manner analogous to that observed when such oils are administered directly into the duodenum (Ogilvie, McClymont, and Shorland 1961) or when they are given to suckling ruminants (Stokes and Walker 1970). Because of their high caloric content, these supplements may also provide a means of increasing the caloric density of ruminant diets without affecting digestion in the rumen (Hungate 1966). Such an application would, however, be of limited use if the digestion of large quantities of lipid in the intestine inhibited food intake.

V. Acknowledgments

The authors' thanks are due to Mrs. N. B. Carter and Mr. P. N. Jones, Division of Mathematical Statistics, CSIRO, for computational assistance in curve fitting, to Mr. T. W. Searle for the determinations of gross calorific value, to Mrs. D. E. Margan, Miss E. Angus, Miss G. A. White, and Mr. V. H. Oddy for skilled technical assistance, and to Mr. C. E. McCulloch and Mr. K. Rixon for their care of the experimental animals.

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