THE UTILIZATION BY GROWING LAMBS OF A CASEIN–SAFFLOWER OIL SUPPLEMENT TREATED WITH FORMALDEHYDE

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Abstract

A formaldehyde-treated casein–safflower oil supplement was given at levels of 0, 75, and 150 g/day to lambs receiving 600 g/day of a diet of equal parts chopped lucerne hay and crushed oats. The response of the lambs was studied in terms of food intake and liveweight gain, the chemical composition of the body, the incorporation of linoleic acid into body fat, the digestibility of the diets, and the levels of urea, α-amino nitrogen, and glucose in blood plasma.

The supplemented lambs appeared to discriminate against the supplement to a small extent. There was no detectable effect of the supplement on the gross chemical composition of the body but the linoleic acid in the supplement was extensively incorporated into body tissues; the progress of incorporation was described by a curve of diminishing increments. Approximately half the linoleic acid ingested was retained in the body. The supplement was readily digested and plasma levels of urea, α-amino nitrogen, and glucose were increased in the lambs given 150 g/day of the supplement.

I. INTRODUCTION

The ruminant mode of digestion provides a means of deriving metabolically useful energy from the structural carbohydrates of plants through the activities of symbiotic microorganisms. One of the consequences of this type of digestion is the marked sensitivity of ruminant animals to the inclusion of fat in the diet. Digestive disturbances and reductions in food intake, animal performance, and the digestibility of cellulose have been reported when the level of fat in the diet exceeded about 8% (Brooks *et al.* 1954; Ward *et al.* 1957; Davison and Woods 1960, 1963; Armstrong and Ross 1968; Johnson 1972; Johnson and McClure 1972). In addition, polyunsaturated fatty acids in the diet are substantially hydrogenated in the rumen and so do not appear in tissue and milk lipids (Reiser 1951; Tove and Mochrie 1963; Ward *et al.* 1964; Garton 1967).

It is now possible, by formaldehyde treatment of the material produced by embedding polyunsaturated oils in a matrix of protein (Scott *et al.* 1970, 1971), to produce a supplement in which the constituent polyunsaturated fatty acids are protected from hydrogenation in the rumen. When such supplements are given to ruminants, polyunsaturated fatty acids from the oil are incorporated into milk and body fats without modification (Scott *et al.* 1970; Cook *et al.* 1972). These supple-

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ments also provide a means of increasing the caloric density of the diet without adversely affecting digestion in the rumen (Faichney *et al.* 1972; Hogan *et al.* 1972). However, there is a possibility that an increase in the caloric density of the diet may increase the amount of fat laid down in the body and Lodge *et al.* (1972) have recently reported a tendency for this to occur in pigs. In the present experiment, the effect of a formaldehyde-treated casein–safflower oil supplement on the body composition of lambs was studied. In addition, digestibility was measured and observations made on the effects of the supplement on plasma levels of urea, α-amino nitrogen, and glucose and on the incorporation of linoleic acid (18:2) into body fat.

II. Methods

(*a*) *Animals*

The female Border Leicester × Merino lambs used were about 12 weeks old and their liveweights ranged from 22 to 28 kg when the experiment began. They were treated to control helminths, coccidia, and external parasites and were kept indoors in metabolism cages unless stated otherwise. They were weighed twice weekly prior to feeding.

(*b*) *Experimental*

Each lamb was offered its feed daily at 0900 hr; feed refused was removed at 0830 hr the following morning. The lambs began eating as soon as feed was presented and most of the ingestion occurred during the first 2 hr. Four lambs (group Ia) were given 600 g/day of the basal diet of equal parts chopped lucerne hay and crushed oats. Seven lambs were offered a formaldehyde-treated casein–safflower oil supplement mixed with their basal diet; three of these lambs (group II) were offered 75 g/day (first level) and four lambs (group III) were offered 150 g/day (second level) of the supplement. Two lambs (group Ib), kept in individual pens, were given sufficient of the basal diet to maintain a rate of liveweight gain similar to that achieved by the lambs in group III. The supplement was prepared by spray-drying an emulsion of equal parts by weight of safflower oil and casein as described previously (Scott *et al.* 1971). The safflower oil used contained 75 g 18:2 per 100 g fatty acids. The composition of the dietary components was as follows (DM, dry matter):

Component	DM (%)	Organic matter (% DM)	Acid-detergent fibre (% DM)	Nitrogen (% DM)	Lipid* (% DM)	Gross calorific value (kcal/g DM)
Lucerne chaff	85·8	88·9	28·7	3·49	1·7	4·50
Crushed oats	89·8	96·6	16·3	1·11	3·7	4·63
Casein–safflower oil	95·1	97·8	—	7·41	44·6	7·39

* Fatty acids plus non-saponifiables.

During the sixth week of the experiment, total faecal collections were made from the lambs in groups Ia, II, and III for the estimation of digestibility. Blood samples were taken by jugular venipuncture from all lambs prior to feeding and 2 hr and 6 hr after feed was offered on the last day of the collection period. At the end of the collection period one lamb in group Ia died (accidental strangulation) and the remainder of this group were offered their diet *ad libitum* until the end of the experiment. The 18:2 content of all lambs at the beginning of the experiment was estimated from the body fat content predicted from tritiated water space (Searle 1970) and the concentration of 18:2 in the total body fat of the lambs receiving the basal diet (groups Ia and Ib). Each lamb was slaughtered when it reached 36 kg liveweight.

(*c*) *Sampling Procedures*

A sample of the week's faeces from each lamb was taken for the determination of dry matter and another sample was macerated with water and stored at −10°C. Blood samples were drawn into heparinized syringes and promptly centrifuged; the plasma was stored at −10°C.

When they reached 36 kg, the lambs were shorn, fasted for 24 hr, and then killed and prepared for analysis as described by Searle (1970). In addition, representative samples were taken from perirenal, omental, and subcutaneous fat depots; approximately 2 g of each tissue was frozen on solid CO_2, transferred into vials containing 10 ml chloroform–methanol (2 : 1 v/v), and stored under nitrogen at −10°C.

TABLE 1

LIVEWEIGHT GAIN, FOOD INTAKE, AND FOOD CONVERSION OF LAMBS GIVEN A BASAL DIET WITH (GROUPS II AND III) AND WITHOUT (GROUP I) SUPPLEMENTS OF FORMALDEHYDE-TREATED CASEIN–SAFFLOWER OIL

Measurement	Group				
	Ia (restricted period)	Ia (*ad lib.* period)	Ib	II	III
Initial liveweight (kg)	24·4	26·4	23·7	26·4	25·9
Final liveweight (kg)	26·4	35·6	36·0	36·0	35·9
Liveweight gain (g/day)	47·6±11·0*	154·0±3·0	139·1±12·5	107·2±2·1	141·0±13·3
	108·7±7·6				
Dry matter intake (g/day):					
Lucerne chaff	257	394	395	250	247
Crushed oats	269	423	416	261	254
Casein–safflower oil	—	—	—	69	126
Total	526	817±29	811±17	580±7	627±4
	694±17				
Energy intake (kcal/day)	2404	3728	3698	2837	3215
	3167				
(percentage as casein–safflower oil)	—	—	—	17·9	29·0
Caloric density (kcal/g dry matter)		4·56	4·56	4·89	5·13
Food conversion (g dry matter/g gain)	11·1	5·3	5·8	5·4	4·4
	6·4				
(kcal intake/g gain)	50·5	24·2	26·6	26·5	22·8
	29·1				

* Standard error.

(*d*) *Chemical Analyses*

The chemical composition of the whole body of each lamb was determined using the methods described by Searle (1970). Acid-detergent fibre in feed, feed refusals, and macerated faeces was estimated as described by Van Soest (1963); acid-detergent fibre is reported as the loss after ignition at 600°C of the residue remaining after extraction and filtration. Other methods of analysis were those used previously (Faichney 1972; Faichney *et al.* 1972). The 18:2 content of the fat extracted from the whole body mince was determined on fresh frozen samples as it was found that losses of up to 66% occurred if the mince was freeze-dried.

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*) *Liveweight Gain and Food Intake*

The mean values for the initial and final liveweights of the lambs, their food intakes, and food conversion ratios are shown in Table 1. Group II lambs, which received the first level of the supplement, grew more than twice as fast as the lambs given the basal diet (group Ia restricted). The lambs given the second level of the supplement (group III) grew somewhat faster than those given the first level ($P = 0{\cdot}06$). For the lambs in group Ia the rate of liveweight gain for the whole feeding period was the same as that for the group II lambs.

TABLE 2

MEAN PERCENTAGE DIGESTIBILITY OF THE DIETS AND OF THE CASEIN–SAFFLOWER OIL SUPPLEMENT

Diet	Group	Percentage digestibility of				
		Organic matter	Acid-detergent fibre	Nitrogen	Lipid	Energy
Basal	Ia	74·4±0·5*	52·6±1·0	74·2±0·8	57·8±2·0	72·4±0·4
Basal+first level of supplement	II	77·9±0·7	54·8±1·4	81·3±0·2	83·4±1·2	77·3±0·8
Basal+second level of supplement	III	77·1±0·5	50·2±1·7	80·3±0·9	85·7±1·9	77·0±0·6
Supplement†						
First level				98·0	95·3	100·3
Second level				87·5	92·2	88·1

* Standard error.

† Calculated by difference assuming that the digestibility of the basal diet remained constant.

The lambs given the supplement did not eat all the feed that was offered. The lambs in group II refused 4% of the supplement offered and group III lambs refused 11% of the supplement offered to them. The supplement comprised, respectively, 11·9 and 21·3% of the dry matter offered to groups II and III; it made up 11·8 and 20·1% of the dry matter eaten. The difference between the composition of the food offered and of that eaten was significant for group III ($P = 0{\cdot}04$), suggesting that these lambs discriminated against the supplement to a small extent. The supplement accounted for 37% of the dry matter refused by the group III lambs. The amount of dry matter and gross energy required per unit of gain was greatest for the unsupplemented lambs and decreased as the amount of supplement given was increased. The food conversion ratios of group Ib lambs, which were given the basal diet near *ad libitum* so as to gain at the same rate as group III, and of group Ia lambs eating to appetite, were similar to the values for group II lambs but were higher than those for group III lambs.

(*b*) *Digestibility*

The apparent digestibility coefficients are shown in Table 2. The overall digestibility of organic matter, nitrogen, lipid, and energy increased ($P < 0{\cdot}01$) when the supplement was given. The digestibility of acid-detergent fibre by the lambs given the basal diet (group Ia) did not differ significantly from its digestibility by the supplemented lambs; the difference between group II and group III lambs was significant ($P = 0{\cdot}02$). The additional nitrogen, lipid, and energy provided by the first level of the supplement was virtually completely digested. The values for the second level of the supplement were lower, indicating that, as the level of supplementation was increased, the apparent digestibility of the supplement declined.

(*c*) *Body Composition*

The mean values for the chemical composition of the body for each group of lambs are shown in Table 3. There were no statistically significant differences between the groups of lambs in any of the body components. The value for the fat content of the empty body of group I lambs was strongly influenced by one lamb. If the value for this lamb is omitted, the mean value increases from 25·9 to 27·1%. There was thus no statistically detectable effect of the supplement on body fatness.

TABLE 3

CHEMICAL COMPOSITION OF THE EMPTY BODY OF LAMBS GIVEN A BASAL DIET WITH (GROUPS II AND III) AND WITHOUT (GROUP I) SUPPLEMENTS OF FORMALDEHYDE-TREATED CASEIN–SAFFLOWER OIL

Values given are means±standard errors

Measurement	Group		
	Ia+Ib	II	III
Empty body weight (kg)	32·9±0·3	33·6±0·0	32·8±0·3
Water (%)	55·6±0·9	53·2±0·6	54·2±1·7
Fat (%)	25·9±1·3	29·2±0·7	28·1±2·2
Protein (%)	13·0±0·2	13·0±0·4	12·8±0·3
Ash (%)	3·2±0·1	3·0±0·1	3·0±0·1
Energy (Mcal/kg)	3·25±0·10	3·45±0·08	3·31±0·18

(*d*) *Plasma Constituents*

The levels of urea, α-amino nitrogen, and glucose in the plasma of the lambs 0, 2, and 6 hr after feeding are shown in Figure 1. Two hours after feeding, the group III lambs, which were given the second level of the supplement, had higher plasma urea levels than the lambs in group Ia ($P = 0{\cdot}02$), group Ib ($P = 0{\cdot}09$), or group II ($P = 0{\cdot}01$). The group III lambs had lower plasma urea levels 6 hr after feeding than they did before feeding ($P = 0{\cdot}07$) or 2 hr after feeding ($P = 0{\cdot}02$). Plasma urea levels tended to fall from 2 hr after feeding in all except group II lambs. Analysis of variance showed that, taken across all groups, this effect was statistically significant ($P = 0{\cdot}04$).

At all sampling times, group III lambs had higher levels of α-amino nitrogen in their plasma than did the other lambs ($P < 0{\cdot}01$) but there were no significant differences between the other groups. The level of α-amino nitrogen in plasma did not change after feeding.

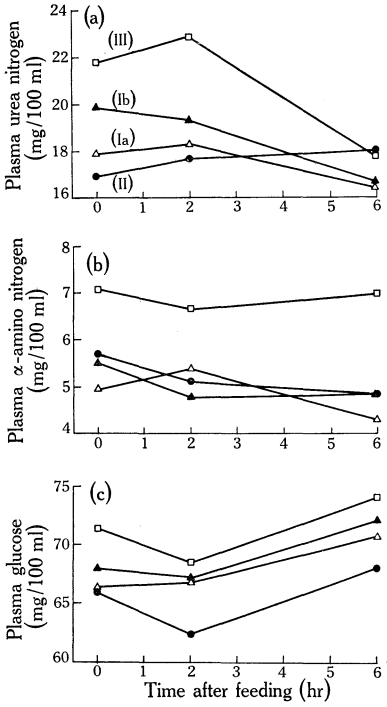

Fig. 1.—Changes in levels of urea nitrogen (*a*), α-amino nitrogen (*b*), and glucose (*c*) in the plasma of lambs after feeding.
△ Basal diet (group Ia).
▲ Basal ration *ad libitum* (group Ib).
● Basal diet + first level of supplement (group II).
□ Basal diet + second level of supplement (group III).

There were no significant differences between the different groups in plasma glucose levels at the different sampling times. However, analysis of the results pooled within groups showed that plasma glucose levels in group III lambs were higher than those in group II lambs ($P < 0{\cdot}01$) and a little higher than those in group Ia lambs ($P = 0{\cdot}08$); lambs in group Ib had levels a little higher than those in group II ($P = 0{\cdot}07$). The rise in plasma glucose levels which occurred between 2 and 6 hr after feeding was not statistically significant for any one group but was significant ($P < 0{\cdot}01$) across all groups.

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

For group III lambs at slaughter, the mean 18:2 content of perirenal fat (25·0%) was higher ($P < 0{\cdot}01$) than that of omental fat (20·8%), which was itself higher ($P = 0{\cdot}07$) than that of subcutaneous fat (19·3%). The differences between the values for group II lambs (20·1, 18·8, and 15·6% respectively) were not statistically significant ($P > 0{\cdot}26$). The mean values (±S.E.) for the whole body fat of group I, group II, and group III lambs were, respectively, 3·0±0·04%, 14·7±1·3%, and 18·4±0·6%; the differences were significant at $P < 0{\cdot}01$. The mean 18:2 contents of groups I, II, and III lambs at slaughter were 6·5, 35·7, and 42·6 g/kg

liveweight respectively, whereas the mean value for all lambs at the beginning of the experiment was 3·1 g/kg liveweight. It was calculated that group II lambs retained 53% of the 18:2 ingested and that group III lambs retained 45%.

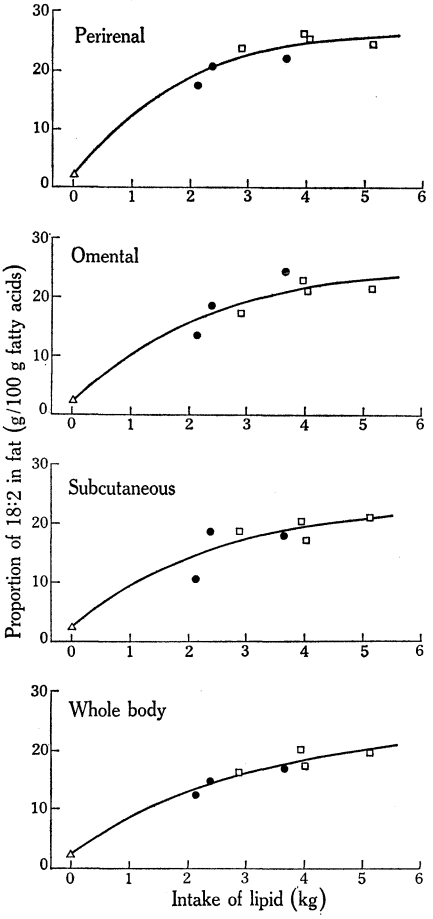

Fig. 2.—Relationship between total intake of safflower oil lipid and the proportion of linoleic acid (18:2) in the perirenal, omental, subcutaneous, and total body fat.
△ Mean of groups Ia and Ib.
● Group II.
□ Group III.

Although the lambs in group III ate approximately twice as much of the supplement as the lambs in group II, there was no difference between the groups in the progress of incorporation when the 18:2 content of their tissue lipids was plotted against the total intake of lipid from the supplement (Fig. 2). The values obtained for the adipose tissues sampled and for the whole body extract conformed to curves of diminishing increments. To describe each set of data, curves of the form

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

where y = the proportion of 18:2 in the fat (% w/w) and x = total intake of lipid (kg), were fitted by an iterative procedure. The constant A represents the maximum or equilibrium value of y, B represents the maximum increment (so that $A-B$ represents the initial value of y), and k is a proportionality constant. The constants

obtained for a solution of this equation for each tissue are shown in Table 4. The values obtained for the residual standard deviation and R^2 (coefficient of determination) show that the curves obtained provide an accurate description of the data. These results indicate that the variation which occurred in the length of time the lambs were given the supplement (group II, 71–117 days; group III, 48–96 days) affected the degree of incorporation only to the extent that it affected intake of the supplement. Although the maximum values calculated for the incorporation of 18:2 into perirenal and omental fat were higher than those for subcutaneous fat, the differences were not statistically significant.

TABLE 4

CONSTANTS OBTAINED FOR SOLUTION OF THE EQUATION $y = A - B\,e^{-kx}$ FOR LIPIDS FROM ADIPOSE TISSUES AND FOR WHOLE BODY LIPIDS

See text for definition of constants and variables

Tissue	A	B	$A-B$	k	S.D.*	R^2†
Perirenal	27·6±2·1‡	24·8	2·8	0·516±0·120	1·43	0·995
Omental	27·3±4·4	24·5	2·8	0·368±0·137	1·83	0·990
Subcutaneous	24·0±5·2	20·9	3·1	0·366±0·188	2·07	0·983
Whole body	24·6±3·0	21·7	2·9	0·308±0·079	0·91	0·996

* Residual standard deviation.

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

‡ Standard error.

IV. DISCUSSION

Although the supplement was readily digested and its constituent 18:2 extensively incorporated into tissue lipids there was no detectable effect on the body fat content of the lambs. This suggests that tissue fatty acid biosynthesis was inhibited by the supply of exogenous fatty acids for lipogenesis as is the case for non-ruminant species (Leveille 1970). Thus the tendency for lambs to lay down fat and protein in proportions determined primarily by stage of growth (Searle *et al.* 1972) was not affected by the differences between groups in the supply of nutrients at the tissue level brought about by the supplement (Hogan *et al.* 1972).

There was no detectable difference between the two levels of supplementation in the relation between lipid intake and the incorporation of 18:2 into tissue lipids. Thus the lambs given the higher level of the supplement were approaching the maximum degree of incorporation earlier in time and so retained a lower proportion of the 18:2 ingested than did the lambs given the lower level of supplement. The tendency for the deeper body fats to reach higher levels of incorporation of 18:2 than external fat is consistent with previous results (Faichney *et al.* 1972).

The lambs given the supplement did not eat all the food offered them and appeared to discriminate against the supplement to a small extent, as did Friesian steers given this supplement (Faichney *et al.* 1972). The energy intake of group III lambs relative to their liveweight was 245 kcal per day per $kg^{\frac{3}{4}}$ and that of group Ia lambs when eating to appetite was 284 kcal per day per $kg^{\frac{3}{4}}$. These values represent similar amounts of net energy but are less than the potential for such lambs since

Searle and Graham (1972) reported a value of 385 kcal per day per $kg^{\frac{3}{4}}$ for crossbred lambs offered a pelleted diet containing approximately equal parts lucerne, oats, and linseed meal. In addition Weston (1971) reported growth rates of 267 g/day for crossbred lambs offered a pelleted diet containing approximately equal parts lucerne and concentrates, a value considerably higher than those reported here. Thus, although the higher net energy value of the dietary fat (Hoffman *et al.* 1962) resulted in improvements in food and energy conversion ratios, the lambs in group III were unable to approach their growth potential. The relatively high digestibility of the basal diet suggests that physical factors should not have limited energy intake (Baumgardt 1970), yet the lambs in group Ia did not achieve their growth potential. The failure of the lambs in this experiment to achieve expected levels of food intake cannot be explained.

As the lipid in the supplement was not released in the rumen, the absence of any effect on the digestibility of acid-detergent fibre is consistent with the view that unprotected lipid supplements affect the digestibility of other nutrients through an effect on the type and activity of rumen microorganisms (Brooks *et al.* 1954; Nieman 1954). The apparent digestibilities of the other components of the diet were higher when the supplement was present, and calculations of the digestibility of the supplement by difference show it to be highly digestible, a finding in agreement with the results of Hogan *et al.* (1972). The reason for the decline in the digestibility of the supplement as the level of supplementation increased is not readily apparent but stimulation of endogenous secretion rates following the release in the duodenum of the protein and oil from the supplement may be involved.

Plasma urea levels normally increase with increasing protein intake (e.g. Faichney and Davies 1972) and the present results tend to follow the same pattern. The fall in plasma urea levels after feeding is consistent with the results of Thornton (1970) and Faichney (1971). The plasma α-amino nitrogen levels found in the lambs given the higher level of supplement (group III) were much higher than those in the other lambs and suggest that the supply of amino acids in relation to energy at the tissue level was in excess of these lambs' requirements. The tendency for the plasma glucose levels to fall after feeding and then rise above the prefeeding level is consistent with the findings of Bassett (1972). The higher levels of glucose in the plasma of group III lambs might be the result of a reduction in the requirement for reduced NADP due to inhibition of endogenous fatty acid biosynthesis by dietary fat. However, this explanation cannot account for the fact that group II lambs had the lowest plasma glucose levels.

The present results confirm that dietary polyunsaturated fatty acids can be protected from hydrogenation in the rumen and efficiently incorporated into tissue lipids as previously reported (Cook *et al.* 1970, 1972; Faichney *et al.* 1972). It is now possible to produce foods with a markedly increased content of polyunsaturated fatty acids from ruminant animals and it has been suggested that these may be of use in the management of cardiovascular disease in man (Nestel *et al.* 1973). The apparent limitation of voluntary food consumption associated with the supplement here and in previous work (Faichney *et al.* 1972) must be overcome if protected lipid supplements are to be used successfully to increase the caloric density of diets for ruminants.

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