

## **Long-term Effects of Feeding Protected Sunflower Seed Supplement on the Composition of Body Fat in Growing Sheep**

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### *Abstract*

Fifty-one Southdown crossbred and 37 Suffolk crossbred wether lambs were fed for 31 weeks a diet containing 6.6% by weight linoleic acid derived from a formaldehyde-treated casein-sunflower seed supplement. Serial biopsy samples of subcutaneous fat were obtained during this time and over the following 49 weeks when no supplement was given. Samples of omental, perirenal and subcutaneous fat were obtained from 16 sheep slaughtered 17 weeks after supplementation ceased and the proportion of linoleic acid determined in all samples. Total body fat was estimated from tritiated water space on five occasions during supplementation.

The proportion of linoleic acid reached a maximum value of 25% after 16 weeks and then remained constant while the supplement was being fed. Subsequent changes were related to changes in liveweight. Weight loss, either slow or rapid, resulted in only a slight decrease in linoleic acid while weight gain caused a rapid decrease. There was no difference between fat depots, nor was there any breed difference evident in the proportion of linoleic acid at any given time.

The Southdown crossbred sheep grew more slowly than the Suffolk crosses and when compared at the same liveweight had more total fat and linoleic acid.

### **Introduction**

As the result of microbial hydrogenation of dietary polyunsaturates in the rumen, adipose tissue of ruminant animals is characterized by a high proportion of saturated fatty acids in depot fats. The feeding of polyunsaturated lipid supplements protected from ruminal degradation (Scott *et al.* 1971; Cook *et al.* 1972*b*) results in ruminant adipose tissue and milk fat containing an increased proportion of polyunsaturated fatty acids particularly linoleic acid but little is known about the effects of prolonged supplementation on such changes or about changes after the supplement has been withdrawn. Accordingly, the present experiment was conducted to monitor changes in total body fat and in the proportion of linoleic acid during and after feeding a polyunsaturated supplement to sheep. Since breeds differ in body fatness (Searle and Griffiths 1976) comparisons were made between two sire genotypes.

### **Materials and Methods**

#### *Animals and Diet*

Eighty-eight wether lambs, the progeny of crossbred ewes mated to either Southdown or Suffolk rams, were removed from pasture at 7 months of age, shorn, treated orally to control intestinal parasites and allocated at random to groups of 11 which were kept in separate pens in an open shed. There were 51 Southdown and 37 Suffolk crossbreds and each group contained either six or seven Southdown and five or four Suffolk crossbred sheep respectively. They were fed a mixture of chopped

lucerne hay (50%), crushed barley (20%) and formaldehyde-treated casein-sunflower seed supplement (Scott *et al.* 1972) containing 22% linoleic acid (30%). This mixture subsequently referred to as the supplemented diet was fed for 31 weeks. Initially each group was given 11 kg daily and to allow for increasing liveweight this was increased to 12 and then 13 kg/group daily after weeks 9 and 17 respectively. The sheep were then fed restricted amounts of an unsupplemented diet comprising equal parts chopped lucerne hay and crushed oats for 17 weeks in order to bring about slow weight loss following which six Southdown and 10 Suffolk cross sheep were slaughtered and samples of omental, perirenal and subcutaneous adipose tissue obtained. The remaining sheep were put to pasture. In week 71 of the experiment (i.e. 40 weeks after the supplement had been withdrawn), three Suffolk-cross sheep were removed from pasture and each was fed 300 g unsupplemented diet daily for 8 weeks (which led to rapid weight loss). This was followed by near *ad libitum* feeding for 6 weeks. The rest of the sheep remained at pasture and the experiment concluded 49 weeks after supplementation ceased.

#### *Measurement of Fatty Acid Composition of Adipose Tissue*

Samples of subcutaneous adipose tissue (50–100 mg) were obtained from all animals at the start of the experiment, on five occasions during supplementation and on eight subsequent occasions, the last sample being 49 weeks after the supplement had been withdrawn (see Fig. 1). In the three sheep subjected to rapid weight loss and regain, seven samples were taken at 2–3 week intervals (see Fig. 2). Samples were removed just anterior to the shoulder with the biopsy device of King (1976). Adipose tissue was dissected out from the biopsy sample and transferred to tubes containing 2 ml 0.5 M methanolic sodium hydroxide. Methyl esters of the fatty acids were prepared by the method of Metcalfe *et al.* (1966) and measured with a gas chromatograph (Hewlett Packard model 5700A) equipped with flame ionization detector and electronic integrator (3370B). A 2 m by 2 mm stainless steel column packed with 17% diethyleneglycol succinate on Chromosorb W (AW-DMCS) was used. Recoveries were determined from triacylglycerols (triglyceride) standards (99% purity—Applied Science Laboratories Inc. U.S.A.) using the same procedure.

Adipose tissue samples taken from the slaughtered sheep were similarly treated.

#### *Measurement of Whole Body Chemical Fat*

At the start of the experiment and on four subsequent occasions during supplementation tritiated water (TOH) space was determined and body fat estimated from TOH space and fleece-free liveweight (Searle 1970).

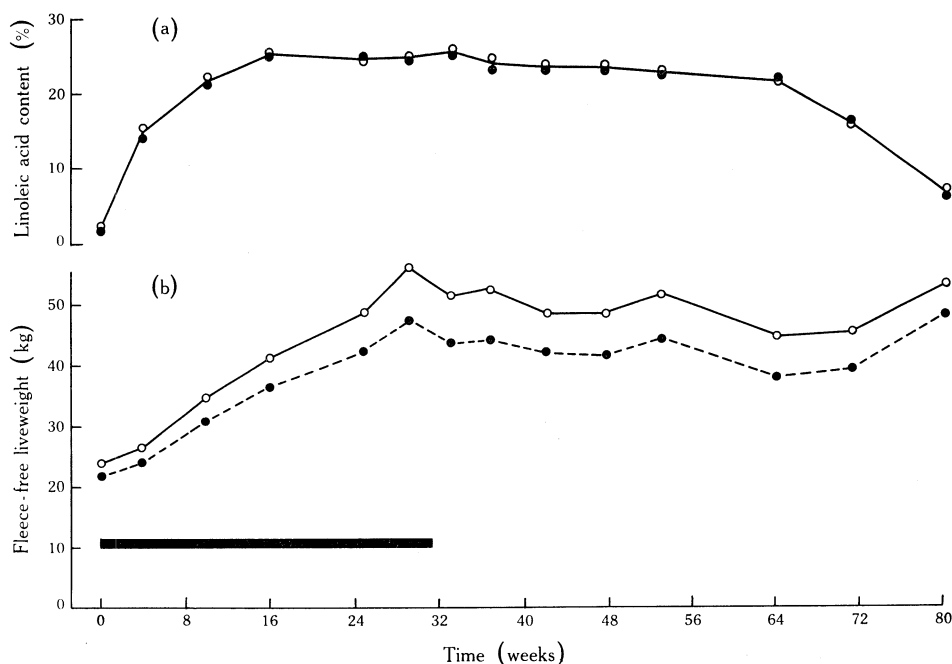
### **Results**

#### *Effect of Polyunsaturated Feed Supplement on the Percentage of Linoleic Acid in Body Fat*

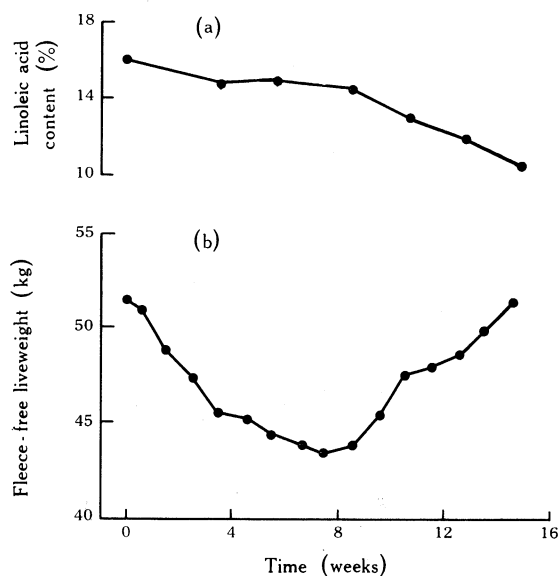
At the start of the experiment the Southdown and Suffolk crossbred sheep weighed  $21.5 \pm 0.6$  and  $23.8 \pm 0.6$  kg respectively (mean  $\pm$  s.e.) and  $47.6 \pm 1.0$  and  $53.6 \pm 0.9$  kg respectively at the end of supplementation (Fig. 1). The proportion of linoleic acid in subcutaneous adipose tissue rose from an initial value of 2.5% to a maximum of 25.0% after 16 weeks and then remained constant, no effect of breed being apparent.

#### *Changes in the Percentage of Linoleic Acid in Subcutaneous Fat After Supplementation Ceased*

After 17 weeks of unsupplemented feeding during which time a mean weight loss of 6–7 kg occurred in all lambs, linoleic acid represented 23.4% of the total fatty acids, and it was still 22.0% 33 weeks after supplementation ceased with a total weight loss of c. 10 kg. This represented a decline of  $0.016 \pm 0.002\%$  per day in



**Fig. 1.** (a) Proportion of linoleic acid in samples of subcutaneous adipose tissue from Southdown (●) and Suffolk (○) crossbred sheep when fed protected sunflower seed supplement (solid bar) or unsupplemented. (b) Mean fleece-free liveweight at sampling for Southdown (●) and Suffolk (○) crossbred sheep.



**Fig. 2.** (a) Proportion of linoleic acid in samples of subcutaneous adipose tissue from Suffolk cross sheep previously fed a protected sunflower seed supplement and subsequently subjected to rapid weight loss and regain 40 weeks after supplement had been withdrawn. (b) Mean fleece-free liveweight at sampling.

linoleic acid. It was only during the final 16 weeks when liveweight increased by c. 10 kg in response to improved pasture growth that the percentage declined at the significantly faster rate of  $0.134 \pm 0.006$  per day.

*Effect of Rapid Weight Loss and Regain on the Percentage of Linoleic Acid in Body Fat*

When three sheep underwent rapid weight loss equivalent to c. 1 kg/week (Fig. 2) the percentage of linoleic acid in subcutaneous fat declined slowly by  $0.025 \pm 0.008\%$  per day but with improved feeding, when a weight gain of 1 kg per week occurred, declined rapidly ( $0.093 \pm 0.003\%$  per day). These slopes differed significantly ( $P < 0.05$ ).

*Linoleic Acid Content of Fat from Different Depots*

The linoleic acid content of perirenal, subcutaneous and omental fat depots of sheep slaughtered 17 weeks after the removal of the polyunsaturated supplement is shown in Table 1. Although the values for the subcutaneous depot were marginally lower than those for the other depots, there was no statistically significant difference between depots or breeds.

**Table 1.** Linoleic acid content of adipose tissue of sheep 17 weeks after the cessation of feeding a polyunsaturated supplement

Values are for mean linoleic acid content expressed as percentage by weight of all fatty acids present

Sire genotype	No. of sheep		Perirenal	Subcutaneous	Omental
Southdown	6	Mean	24.53	23.18	24.25
		s.e.	1.07	0.77	0.84
Suffolk	10	Mean	25.11	24.30	25.47
		s.e.	0.58	0.72	0.64
All sheep	16	Mean	24.89	23.88	25.01
		s.e.	0.52	0.54	0.51

*Total Body Fat*

Inspection of graphs of body fat content against fleece-free liveweight for individual sheep suggested a linear relationship. This was confirmed by regression analysis and standard tests revealed no evidence that within sire breeds the relationships for individual sheep were divergent in slope or intercept.

The estimates of the regression coefficients for fat on liveweight (Table 2) differed between breeds ( $P < 0.001$ ), Southdown crossbreds containing more fat than the Suffolk crossbreds at any given liveweight.

For ruminant adipose tissue it was calculated (on a molecular basis) that fatty acids constitute 94–96% of the weight of sheep body fat, and hence the weight of linoleic acid present in the body at any sampling period can be calculated by multiplying 95% of total body fat by the fraction of linoleic acid in total fatty acids. The relationship between the weight of linoleic acid and liveweight is given in Table 2. The Southdown cross sheep had significantly more linoleic acid than the Suffolk cross at any given liveweight ( $P < 0.001$ ).

During the first 16 weeks of supplementation the weight of linoleic acid stored was 31% of linoleic acid given in the diet. This proportion declined to 25% subsequently.

**Table 2.** Regression equations of the weight of fat (kg) and linoleic acid (kg) on fleece-free live-weight (kg) for Southdown and Suffolk crossbred sheep fed a polyunsaturated supplement for 31 weeks ( $n = 51$  and 37 respectively)

	Sire genotype	Intercept ( $\pm$ s.e.)	Slope ( $\pm$ s.e.)	R.s.d. <sup>A</sup>	$r^2$
Fat	Southdown	-8.06( $\pm$ 0.54)	0.49( $\pm$ 0.02)	1.71	0.86
	Suffolk	-7.51( $\pm$ 0.48)	0.42( $\pm$ 0.01)	1.65	0.87
Linoleic acid	Southdown	-2.43( $\pm$ 0.13)	0.13( $\pm$ 0.004)	0.45	0.88
	Suffolk	-2.31( $\pm$ 0.09)	0.11( $\pm$ 0.002)	0.36	0.92

<sup>A</sup> Residual standard deviation.

## Discussion

In a feeding trial with crossbred sheep given a protected lipid supplement for 96 days, Garrett *et al.* (1976) reported a progressive increase in the proportion of linoleic acid in subcutaneous adipose tissue with a maximum of 20% after 69 days. A similar rate of increase was found in the current experiment but the slightly higher maximum proportion of 25% was not achieved until after 112 days of supplementation. The proportion of dietary linoleic acid that was stored (31% decreasing to 25%) was similar to that found by Garrett *et al.* (1976) and by Hogan and Hogan (1976) but much less than reported by Faichney *et al.* (1973) where 45–53% of dietary lipid was stored.

In the current experiment no difference was found between subcutaneous, perirenal and omental adipose tissue in the proportion of linoleic acid. This result is similar to that of Garrett *et al.* (1976) who fed crossbred lambs on a protected lipid supplement for 14 weeks; however, Faichney *et al.* (1973) and Hogan and Hogan (1976) (with lambs) and Faichney *et al.* (1972) and Hood and Thornton (1976) (with steers) found that the deeper body fat, particularly the perirenal, contained a higher proportion of linoleic acid than the subcutaneous after supplementation for 4–8 weeks. Thus it appears that, in the short term, the internal tissues preferentially assimilate the linoleic acid absorbed from the gut but with a longer period of supplementation equilibrium is reached in all body tissues.

After supplementation ceased there was only a slight decrease in the proportion of linoleic acid in subcutaneous adipose tissue irrespective of whether weight loss was slow or rapid (Figs 1 and 2 respectively). Therefore linoleic acid was probably catabolized to the same degree as other fatty acids in the adipose tissue pool and not removed preferentially. The rapid decrease in the proportion of linoleic acid during weight gain (Figs 1 and 2) can be interpreted as a dilution effect resulting from increased deposition of triacylglycerols containing fatty acids other than linoleic acid.

The difference in fatness between Suffolk cross and Southdown crossbred sheep at the same liveweight in the present experiment is consistent with Reid (1972) who found pure-bred Southdowns had considerably more fat than Suffolks. Differences in fatness between other breeds and crosses have been shown by Searle and Graham (1972) and Searle and Griffiths (1976) and these authors have suggested that an

animal of small mature size has a higher proportion of its body as fat at any given weight than an animal of large mature size.

Thus the present results show that once high levels of polyunsaturation have been achieved in the adipose tissue of ruminants, they can be maintained without additional feeding of expensive supplements provided the animal does not continue to gain weight. They also demonstrate the influence of sire breed type on body fatness.

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