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Effect of processing of dietary barley and of supplementary cobalt and cyanocobalamin on the fatty acid composition of lamb triglycerides, with special reference to branched-chain components

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1. The possibility that, when lambs are fed on barley-rich diets, vitamin B_{12} might limit hepatic utilization of propionate and result in the formation of branched-chain fatty acids was investigated with thirty-six animals allocated to twelve treatments according to a $3 \times 2 \times 2$ factorial design.

2. From 13 kg live weight to slaughter at 35 kg the lambs were given diets containing barley in one of three physical forms, and one of two levels of cobalt (0·1 and 0·9 mg/kg diet). In addition, half the lambs received twice-weekly injections of cyanocobalamin (40 μg/kg body-weight).

3. The fatty acid composition of triglycerides from subcutaneous and perinephric adipose tissue was determined at slaughter.

4. Neither additional dietary Co nor the administration of cyanocobalamin (alone or in combination with additional Co) influenced the proportions of branched-chain acids in the triglycerides. The possibility is discussed that the ability of the lamb to respond to the increased supply of propionate which is provided by a high-barley diet is not restricted by the availability of cyanocobalamin, but by hepatic capacity to utilize it for the production of methylmalonyl-CoA mutase (*EC* 5.4.99.2).

5. Regardless of diet, subcutaneous triglycerides contained greater proportions of propionate-derived fatty acids than did perinephric triglycerides which contained greater proportions of exogenously-derived C_{18} unsaturated acids. Lambs given whole barley in loose mix produced triglycerides containing relatively less odd-numbered *n*-acids and branched-chain acids than did lambs fed on either of two pelleted diets containing rolled or whole barley.

Previous investigations showed that, when lambs were reared on pelleted diets having a high content of rolled barley, they produced unusually soft subcutaneous adipose tissue which was due to the occurrence in the triglycerides of abnormal proportions of branched-chain fatty acids (Duncan, Ørskov & Garton, 1972). In addition to these acids, the triglycerides also contained unusually high proportions of odd-numbered *n*-fatty acids, and further studies (Garton, Hovell & Duncan, 1972*a, b*) indicated that the production of both types of fatty acid was probably associated with failure of the liver to metabolize excess propionate normally and hence to its direct incorporation into fatty acids and to the utilization of methylmalonate in fatty acid synthesis, giving rise to branched-chain components. In the normal course of gluconeogenesis, methylmalonate, derived by carboxylation of propionyl CoA (Flavin & Ochoa, 1957), is rapidly converted into succinyl CoA by the action of methylmalonyl-CoA racemase (*EC* 5.1.99.1) and methylmalonyl-CoA mutase (*EC* 5.4.99.2). The mutase contains 5′-deoxyadenosylcobalamin (Cannata, Focesi, Mazumder, Warner & Ochoa, 1965) and it has been found that methylmalonate accumulates in the liver and blood of vitamin B_{12}-deficient sheep (Smith, Osborne-White & Russell, 1969; Smith & Marston, 1971).

In our previous studies (Duncan *et al.* 1972; Garton *et al.* 1972*a*, *b*) the level of cobalt included in the diet (about 0·1 mg/kg diet) was in accord with the suggested allowance for sheep fed on conventional diets (Agricultural Research Council, 1965; Underwood, 1966; Sauchelli, 1969). Marston (1970) has, however, reported that sheep require 0·5–1·0 mg Co/d to maintain maximal vitamin B_{12} status, as indicated by serum and liver values. This observation, together with the findings of Elliot, Kay & Goodall (1971) and Sutton & Elliot (1972) that ruminal production and intestinal absorption of vitamin B_{12} are lower in sheep given high-grain diets than when they are given grass, suggested that in our previous studies the supply of dietary Co may have limited the amounts of vitamin B_{12} available for hepatic metabolism of methylmalonate. Further, it was possible that intestinal absorption of the vitamin might also have been limiting (cf. Marston, 1970) and thus precluded its utilization. These possibilities were investigated in an experiment in which the opportunity was taken simultaneously to investigate whether the physical state of the barley in the ration would influence the extent to which branched-chain fatty acids appeared in both subcutaneous and perinephric adipose tissue.

EXPERIMENTAL

Animals and their treatment

Thirty-six female lambs, Suffolk × (Finnish Landrace × Dorset Horn) crosses were randomly allocated, when each weighed about 13 kg, to different treatment groups based on a 3 × 2 × 2 factorial design.

These animals and their treatments were those described in their Expt 3 by Ørskov, Fraser & Gordon (1974) in which whole barley in loose mix, pelleted whole barley, and pelleted rolled barley constituted 900 g/kg diet. Cobalt sulphate heptahydrate was included in each diet at two levels, 0·43 mg/kg (basal) or 5·3 mg/kg; by spectrochemical analysis the Co contents of the low- and high-Co diets were 0·1 and 0·9 mg/kg respectively. In addition, three lambs in each dietary treatment group were each given twice-weekly intramuscular injections of 40 μg cyanocobalamin (Crookes Vitamins Ltd, Basingstoke, Hants) per kg body-weight. The animals were slaughtered when each weighed 35 kg.

Preparation of tissue samples

Samples, each of about 20 g, of subcutaneous (mid-back) and perinephric adipose tissue were taken from the dressed carcasses and stored at −20° for several weeks until analyses were made.

Analytical methods

Triglycerides were extracted from the adipose tissue and their component fatty acids determined as described by Garton *et al.* (1972*b*).

Table 1. *Effect of cobalt and cyanocobalamin on the major component fatty acids in the subcutaneous and perinephric triglycerides of lambs fed on barley-rich diets*

(Mean values for nine animals/treatment expressed as percentages by weight of total fatty acids)

Treatment*	16:0	18:0	18:1	18:2+18:3	Odd-numbered *n*-acids†	Branched-chain acids‡
			Subcutaneous			
Basal diets	23·8	10·1	44·9	5·0	6·2	4·7
Diets + additional Co	23·9	10·6	45·1	4·8	6·1	4·7
Diets + cyanocobalamin	24·2	10·1	44·8	5·5	5·5	4·6
Diets + additional Co + cyanocobalamin	24·2	9·5	44·9	4·6	6·0	5·4
SE of differences between treatment means	0·69	0·53	0·95	0·40	0·40	0·60
			Perinephric			
Basal diets	20·0	23·1	39·7	5·8	4·2	2·3
Diets + additional Co	19·2	23·4	39·8	6·3	4·2	2·5
Diets + cyanocobalamin	20·2	24·5	38·3	6·6	3·4	2·1
Diets + additional Co + cyanocobalamin	20·6	22·7	39·6	5·6	4·1	2·4
SE of differences between treatment means	0·84	0·97	1·14	0·46	0·34	0·22

* See p. 72 for details of Co in diets and dosage of cyanocobalamin.
† Mostly fatty acids 15:0, 17:0 and 17:1.
‡ Mostly monomethyl-substituted fatty acids 14:0, 15:0, 16:0 and 17:0.

Other observations

After slaughter, the degree of firmness of the subcutaneous adipose tissue of the dressed carcasses was assessed manually by Ørskov *et al.* (1974) in the context of wider studies with these and other lambs.

RESULTS AND DISCUSSION

Neither additional Co nor supplementary cyanocobalamin (alone or in combination with additional Co) had any effect on the proportions of fatty acids derived from propionate (i.e. odd-numbered *n*-acids and branched-chain acids) or on the proportions of any other acids (Table 1). These observations suggest that the ability of the liver to respond to an increased supply of propionate is not restricted by the availability of vitamin B_{12} but possibly by its capacity to store or utilize the vitamin for the production of methylmalonyl-CoA mutase. Similarly, from experiments in which sheep were fed on wheaten hay chaff and wheat gluten and given Co orally or cyanocobalamin parenterally, Marston (1970) concluded that the factor which limited hepatic concentration of vitamin B_{12} was neither its production in the rumen nor its absorption from the intestine, but rather the inherent capacity of the liver to store the vitamin.

As Table 2 shows, the physical form of the dietary barley did, however, have a marked influence on the fatty acid composition of the subcutaneous and perinephric

Table 2. *Influence of the physical form of barley-rich diets on the fatty acid composition of the subcutaneous and perinephric triglycerides of lambs*

(Mean values for twelve animals/diet expressed as percentages by weight of total fatty acids)

Nature of barley and form of diet	16:0	18:0	18:1	18:2+ 18:3	Odd-numbered *n*-acids	Branched-chain acids
			Subcutaneous			
Rolled barley (pelleted)	23·1	9·2	45·7	4·5	6·6	5·8
Whole barley (pelleted)	24·6	8·8	45·0	5·3	6·2	4·9
Whole barley (loose mix)	24·3	12·3	44·1	5·1	4·9	3·9
Difference in mean values (pelleted – loose mix)	−0·44	−3·29	1·25	−0·20	1·46	1·50
SE of difference	0·52	0·40	0·72	0·30	0·30	0·45
Significance of difference	NS	***	NS	NS	***	**
			Perinephric			
Rolled barley (pelleted)	20·0	21·7	40·9	5·6	4·5	2·5
Whole barley (pelleted)	20·2	22·8	39·5	6·3	4·0	2·4
Whole barley (loose mix)	19·8	25·7	37·7	6·2	3·5	2·1
Difference in mean values (pelleted – loose mix)	0·29	−3·43	2·46	−0·26	0·74	0·42
SE of difference	0·63	0·72	0·85	0·34	0·26	0·16
Significance of difference	NS	***	**	NS	**	*

NS, not significant.
* $P < 0·05$, ** $P < 0·01$, ** $P < 0·001$.

triglycerides of the lambs. The animals which were given the pelleted diets produced triglycerides of similar composition, whereas those fed on the diets which included whole barley as part of a loose mixture produced triglycerides which contained significantly less odd-numbered *n*-acids and branched-chain acids and correspondingly more stearic acid (18:0). These observations reflected the degree of firmness of the corresponding subcutaneous adipose tissue as assessed manually by Ørskov *et al.* (1974) and are associated with their finding that propionic acid makes a greater proportional contribution to the total volatile fatty acids in the rumen contents of lambs given pelleted barley diets as compared with its contribution when whole barley, as such, forms the bulk of the diet.

Regardless of dietary treatment, and in agreement with previous observations (Garton *et al.* 1972*b*), the perinephric triglycerides contained smaller proportions of propionate-derived fatty acids and somewhat greater proportions of polyunsaturated C_{18} acids (18:2+18:3) than did the corresponding subcutaneous triglycerides. As noted by Duncan & Garton (1967) in sheep and by Faichney, Davies, Scott & Cook (1972) in cattle, it seems that exogenously-supplied polyunsaturated C_{18} fatty acids are preferentially deposited in the internal tissues of ruminants. As with the subcutaneous triglycerides, the perinephric triglycerides of the lambs given the pelleted diets were similar in composition, whereas those of the lambs given barley in loose mix contained significantly less propionate-derived fatty acids and octadecenoic acid (18:1), together with a significantly increased proportion of 18:0.

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