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The action of the β-agonist clenbuterol on protein and energy metabolism in fattening wether lambs

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1. Five Greyface wethers (42–45 kg) fed on various fixed amounts of dried grass pellets (either approximately 1·3 times maintenance or 2 times maintenance) by means of belt-type continuous feeders were housed in open-circuit respiration chambers for periods of 45 d. Between days 15 and 35 they received daily oral doses of 1·5 mg of the β-adrenergic agonist clenbuterol (adsorbed on to the feed). Continuous energy and nitrogen balance measurements each of 5 d duration were conducted throughout the chamber confinement.

2. On six occasions (twice during the 15 d pre-clenbuterol period, on days +4, +11 and +18 of clenbuterol administration and once during the post-treatment period) animals were infused with [1-^{14}C]leucine to determine the rates of leucine oxidation and the amounts of leucine available for protein synthesis.

3. Clenbuterol administration caused a marked increase in N retention (2–3 g N/d; $P < 0.001$) throughout the 20 d treatment period. It also increased ($P < 0.001$) the energy expenditure of the animals (on average by 1·1 MJ/d over the first 5 d, compared with immediate pretreatment values, and 0·6 MJ/d over the 20 d period, compared with the mean of pre- and post-treatment control values). The effect of treatment was calculated to result, on average, in the daily retention of 19 (SE 1·5) g more protein and 30 (SE 5·5) g less fat.

4. During clenbuterol treatment leucine oxidation was reduced ($P < 0.01$). However, values for the amounts of leucine available for protein synthesis were equivocal, with an increase ($P < 0.001$) on day 11 of treatment, but no change on days 4 and 18.

5. Withdrawal of the clenbuterol resulted in rapid alterations of N and energy metabolism towards those expected of control animals of that weight.

The assertion that over-consumption of animal fat is detrimental to human health (National Advisory Committee on Nutrition Education, 1983; Department of Health and Social Security, 1984) has intensified the interest in methods which induce a leaner carcass conformation in growing animals. Some manipulation can be achieved by nutritional means (Turgeon *et al.* 1986) but economic considerations limit the extent of such applications. Within the genetic constraints of any particular breed, alternative ways of decreasing the ratio of fat gain:protein gain in growing and fattening animals usually involve the administration of exogenous compounds, e.g. ionophores, anabolic steroids, growth hormone, etc.

The phasing-out, within the European Economic Community at least, of the use of anabolic steroids has given added impetus to the search for alternative manipulators of the growth process. Recent interest has focussed on the range of β-adrenergic agonists, e.g. clenbuterol and cimaterol, which in growth trials involving poultry, pigs, sheep and cattle have been shown markedly to increase the areas of specific muscles, while concomitantly reducing body fat (Baker *et al.* 1984; Dalrymple *et al.* 1984*a*, *b*; Ricks *et al.* 1984; Beerman *et al.* 1986).

At present the majority of metabolic studies on the action of the β-agonists have been confined to rats where, for muscle protein metabolism at least, the evidence as to their mode of action is contradictory, with reports of both increases in protein synthesis (Emery *et al.* 1984) and decreases in protein degradation (Reeds *et al.* 1986). The present study represents the first stage in an examination of the action of clenbuterol on protein and energy metabolism in sheep. The protocol involved determination of the kinetics of energy

expenditure and whole-body leucine metabolism. A preliminary report of these findings has appeared previously (MacRae *et al.* 1986).

EXPERIMENTAL

Animals and diet

Five Greyface wethers (initial weight 42–45 kg) were accustomed to metabolism crates and to spending extended periods in an open-circuit respiration chamber. They were given rations of pelleted dried grass (23.5 g nitrogen/kg dry matter (DM), 9·9 MJ metabolizable energy (ME)/kg DM). Sheep nos. 92, 134 and 139 received a ration approximately equal to 2 times maintenance energy intake (1600–1800 g/d as fed (AF); maintenance assessed as 400 kJ/kg live body-weight (LBW)$^{0.75}$) while sheep nos. 180 and 181 received 1100 and 1300 g/d AF respectively. All rations were delivered by means of belt-type continuous feeders (Sutherland *et al.* 1964). The intake of each sheep was held constant for the duration of the experiment. Rations for individual sheep were prepared for the complete experimental period at a single weighing from a mixed batch of diet.

Experimental design

Each animal was prepared with two indwelling silastic catheters (Dow Corning, Health Care Group, Reading), one in each jugular vein. One catheter (1·02 mm i.d., 2·16 mm o.d.) was inserted approximately 250 mm and used for sampling while the other (0·76 mm i.d., 1·65 mm o.d.) was placed with its tip in the anterior vena cava (position identified with the aid of a blood pressure monitor; approximate distance 350 mm) and used for infusion. Catheter patency was maintained for the total period of the experiment by continuous infusion of heparin solution (10 i.u. heparin/ml in 9 g sodium chloride/l; 6 ml/h).

Each animal was confined to an open-circuit respiration chamber for a total period of 45 d. Throughout that period they were harnessed to allow daily collection of faeces by chute (after Brockway, 1979) and urine by aspiration into 4 M-sulphuric acid. Excreta were pooled for analysis as 5 d batches. Heat production was estimated daily from gaseous exchange (Brouwer, 1965).

Measurements were made over three periods. The first was 15 d pretreatment. Next was a 20 d treatment period during which the sheep received daily 1·5 mg clenbuterol, administered by adsorbing a solution of clenbuterol dissolved in 0·1 M-hydrochloric acid (0·4 g/l) on to the grass pellets. Thereafter treatment with clenbuterol was discontinued and immediately a 10 d post-treatment period started.

Leucine kinetics

Leucine metabolism was measured on six separate occasions: twice in the pretreatment period, at days +4, +11 and +18 of clenbuterol administration, and finally once during the post-treatment period (7 d after drug withdrawal). The procedure was similar to that described previously (Lobley *et al.* 1985). Briefly, [1-^{14}C]leucine in sterile saline (9 g sodium chloride/l) was infused continuously (25 g/h, 0·2 μCi/g) for 8 h. For the last 3 h of infusion blood samples (20 ml) were withdrawn at 0·5 h intervals. The specific radioactivity and leucine concentration in blood were determined as described by Lobley *et al.* (1985). Expired $^{14}CO_2$ was determined directly by means of an on-line ionization chamber (volume 100 litres, constructed by J. Brockway, Rowett Research Institute) connected to the respiration chamber.

Calculation of leucine kinetics was as follows:

$$\text{irreversible loss rate (ILR; mmol/h)} = \frac{\text{infusion rate } (\mu\text{Ci/h})}{\text{SR blood free-leucine } (\mu\text{Ci/mmol})}, \quad (1)$$

where SR is specific radioactivity,

$$\text{fractional oxidation rate (FOR)} = \frac{\text{SR}^{14}\text{CO}_2\ (\mu\text{Ci/mmol}) \times \text{CO}_2\ \text{produced (mmol/h)}}{\text{infusion rate } (\mu\text{Ci/h})}, \quad (2)$$

$$\text{leucine oxidized (LO; mmol/h)} = \text{FOR} \times \text{ILR}, \quad (3)$$

$$\text{leucine for protein synthesis } (\text{ILR}_{\text{syn}}; \text{ mmol/h}) = (1 - \text{FOR}) \times \text{ILR}. \quad (4)$$

Leucine oxidation and ILR_{syn} can be converted from mmol/h to approximate equivalents of protein (g/d) by the factor × 51·5 (Lobley *et al.* 1980).

Statistical procedures

All values were analysed by two-way analysis of variance with orthogonal contrasts. The animal × day interaction cannot be used to provide an estimate of error as the animals will vary in the amount they change over the period. The interaction was therefore divided into three components: (1) (pretreatment control *v.* post-treatment control) × animal, (2) (treatment *v.* control) × animal, (3) other. Assuming natural changes to be largely linear the first component would be expected to be large. The second component, which provides a genuine estimate of error (but with only 4 df), was expected to be of the same order or slightly larger than the third. However, the analysis indicated that there was no difference between components (2) and (3), and the two components were combined to provide a measure of error. Unless otherwise stated all comparisons for treatment effects are against the combined values for pre- and post-treatment controls.

RESULTS

At the dosage of clenbuterol selected there were no feed refusals and no detectable change in the metabolizability of the ration.

Effects on energy and N retention

A noticeable feature of the experiments was that the same level of clenbuterol administration evoked different responses in energy expenditure between the individual animals (see Table 1). Increases in energy expenditure over the first 5 d of treatment, which were greater than over the subsequent 15 d in all animals, varied from 0·55 MJ/d in sheep no. 134 to 1·65 and 1·91 MJ/d in sheep nos. 92 and 181 respectively. On average the energy expenditure during the first 5 d of treatment was elevated by 1·3 MJ/d compared with the immediate pretreatment value (Table 1). Despite a decline in energy expenditure thereafter the average energy expenditure over the 3 weeks was 0·9 MJ/d greater ($P < 0·001$; Table 1) than that during the mean of pre- and post-treatment control periods. Post-treatment heat productions were greater than the pretreatment values. This may represent a carry-over effect of clenbuterol administration or, alternatively, may be a consequence of the increased weights of the animals at the latter part of the experiment (range of weight gain over the total period 3·0–11·5 kg).

There was an immediate and sustained increase in N retention ($P < 0·001$, Table 2) as a result of clenbuterol administration. The magnitude of the increase (2–3 g N/d) was similar for animals at low and high intakes. There were no apparent time-related changes in N retention during the 20 d treatment period. N retention declined when the drug was withdrawn.

Changes in protein and fat retention

From the combined N and energy balance values the retentions of protein and fat in individual animals can be calculated. Retained energy was assumed to be in the form of

Table 1. *Mean daily metabolizable energy (ME) intakes and energy expenditures (MJ/d) of sheep given dried grass pellets before, during and after treatment with 1·5 mg clenbuterol/d*
(Each value represents the mean of a 5 d measurement)

Sheep no.	ME intake (MJ/d)	Energy expenditure (MJ/d)								
		Pretreatment			For treatment days:				Post-treatment	
		C1	C2	C3	1–5	6–10	11–15	16–20	C4	C5
92	16·37	ND	ND	11·03	12·62	12·77	ND	11·19	ND	10·47
134	14·76	9·04	9·34	9·19	9·74	9·34	9·34	9·49	9·01	8·94
139	14·68	8·79	9·02	9·07	9·87	9·26	9·22	9·32	9·20	9·10
181	10·00	ND	9·04	9·57	11·22	10·81	10·92	10·90	10·62	10·73
180	9·20	7·33	7·41	7·95	8·73	8·80	9·05	9·19	8·98	9·12
Mean*†	13·00	8·96	9·12	9·36	10·41	10·25	10·25	10·11	9·65	9·67

C1, C2, C3, C4, C5 are each 5 d control periods before (C1–C3) and after (C4–C5) treatment; ND, not determined.

* Mean values include estimates for missing values.

† Mean increase in energy expenditure over treatment period compared with pre- and post-treatment controls 0·9 MJ/d ($P < 0·001$; standard error of difference 0·27, residual df 17).

Table 2. *Mean daily nitrogen intakes and N retentions (g N/d) of sheep given dried grass pellets before, during and after treatment with 1·5 mg clenbuterol/d*
(Each period represents mean of a 5 d measurement)

Sheep no.	N intake (g N/d)	N retention (g N/d)								
		Pretreatment			For treatment days:				Post-treatment	
		C1	C2	C3	1–5	6–10	11–15	16–20	C4	C5
92	39·1	ND	ND	11·0	11·9	11·2	ND	14·8	ND	7·8
134	39·9	7·2	5·9	6·1	ND	13·2	10·6	10·8	9·0	9·9
139	36·7	11·6	8·4	9·3	10·8	ND	11·8	10·5	7·4	4·7
181	25·5	ND	6·3	3·9	8·7	6·2	6·8	7·8	4·4	4·1
180	21·6	4·5	5·5	5·3	6·1	6·5	8·5	6·4	2·8	2·3
Mean*†	32·6	8·3	7·5	7·1	9·8	9·5	10·1	10·0	6·4	5·8

C1, C2, C3, C4, C5 are each 5 d control periods before (C1–C3) and after (C4–C5) treatment; ND, not determined.

* Mean values include estimates for missing values.

† Mean increase in N retention over treatment period compared with pre- and post-treatment controls 2·8 g N/d ($P < 0·001$; standard error of difference 0·79, residual df 17).

either protein (N × 6·25; assumed energy value 23·6 MJ/kg) or fat (assumed energy value 39·6 MJ/kg). Before treatment the animals given the twice maintenance level of intake (sheep nos. 92, 134 and 139) were retaining approximately 5 MJ energy/d in the proportions 60 g protein: 90 g fat; the other two animals fed on the lower intake were retaining 0·8 and 1·5 MJ/d, mainly as protein (35 g/d). Treatment with clenbuterol caused a marked alteration in the calculated ratio of protein:fat accreted. The calculated differences in individual sheep between pretreatment and treatment retention of protein

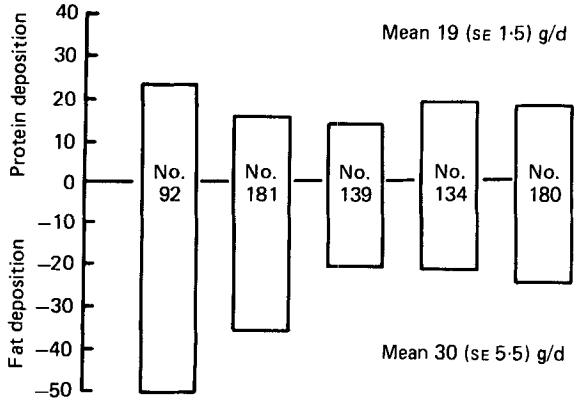

Fig. 1. Alterations in protein and fat deposition (g/d) for individual animals over the period during which they received 1·5 mg clenbuterol/d. Results are expressed against the mean pre- and post-treatment values for energy and nitrogen deposition (from Tables 1 and 2). Numbers within the bars refer to individual sheep identification. All animals showed a gain in protein and a decrease in fat deposition during treatment.

Table 3. *Rates of leucine oxidation (mmol/h) in sheep given dried grass pellets before, during and after treatment with 1·5 mg clenbuterol/d*

Sheep no.	Nitrogen intake (g N/d)	Leucine oxidation (mmol/h)					
		Pretreatment		For treatment days:			Post-treatment
		C1	C2	4	11	18	C3
92	39·1	ND	3·94	2·80	ND	2·45	2·97
134	39·9	3·25	3·54	3·89	2·37	2·96	2·67
139	36·7	2·55	2·70	2·51	2·65	2·17	3·49
181	25·5	ND	2·54	1·60	1·65	1·37	2·21
180	21·6	1·78	1·64	1·15	1·25	1·18	1·55
Mean*†	32·6	2·77	2·87	2·39	2·09	2·08	2·58

C1, C2, C3 each represent control measurements made either before (C1–C2) or after (C3) treatment; ND, not determined.

* Mean values involve estimates for missing values.

† Mean decrease in leucine oxidation over treatment period compared with pre- and post-treatment controls 0·6 mmol/h ($P < 0·001$; standard error of difference 0·24, residual df 13).

and fat, as a result of clenbuterol administration to individual animals, are shown in Fig. 1. The β-agonist increased the rate of protein deposition and reduced the rate of fat deposition, with an overall decrease in total energy retention. There was considerable between-animal variability particularly in the effect of fat deposition. Rates of protein deposition were, on average, increased by 19 (SE 1·5) g/d (by approximately 40 % of the rate of accretion in the control animals). Rates of fat deposition were reduced by 30 (SE 5·5) g/d; this represents a reduction of 30 % on the rate of fat gain for the high-intake animals, and an actual promotion of fat mobilization in the animals on the lower intake.

Protein turnover

The rates of leucine oxidation and of leucine available for protein synthesis calculated from leucine ILR and FOR are given in Tables 3 and 4. Clenbuterol caused a significant

Table 4. *Amounts of leucine available for protein synthesis (ILR_{syn}; mmol/h) in sheep given dried grass pellets before, during and after treatment with 1·5 mg clenbuterol/d*

Sheep no.	Nitrogen intake (g N/d)	ILR_{syn} (mmol/h) Pretreatment C1	Pretreatment C2	For treatment days: 4	11	18	Post-treatment C3
92	39·1	ND	10·2	9·9	ND	9·8	8·7
134	39·9	7·8	7·4	7·8	8·0	7·7	7·2
139	36·7	8·6	8·5	8·6	10·1	8·2	9·1
181	25·5	ND	8·4	8·5	10·1	7·4	8·4
180	21·5	6·3	6·5	7·4	8·0	7·7	7·2
Mean*†	32·6	8·3	8·2	8·4	9·4	8·1	8·1

C1, C2, C3 each represent control measurements made either before (C1–C2) or after (C3) treatment; ND, not determined.

* Mean values involve estimates for missing values.

† Mean increase in ILR_{syn} over treatment period compared with pre- and post-treatment controls 0·4 mmol/h ($P < 0·05$; standard error of difference 0·30, residual df 13).

reduction ($P < 0·001$) in LO throughout the treatment period. There was no significant increase in the flux of leucine to protein synthesis on days +4 and +18. At day +11 of treatment, however, this rate was elevated ($P < 0·001$).

DISCUSSION

The actions of clenbuterol and certain other β-adrenergic compounds can be conveniently divided into two parts: the effects on energy expenditure and on protein gain. In experiments where intake has been controlled, or the administration of the drug has not stimulated voluntary food intake, there has been a reduction in either total body fat (calves, Williams *et al.* 1987; rats, Reeds *et al.* 1988), carcass fat (sheep, cattle and pigs; Ricks *et al.* 1984) or the estimated rate of fat deposition (sheep, present study). The reduction in lipid retention in the present study resulted partly as a consequence of an increased proportion of retained energy being as protein and partly because the rates of energy expenditure were increased. From consideration of ME intake and total energy deposition Williams *et al.* (1987) estimated that mean energy expenditure was increased, compared with controls, by 0·12 (2·8 MJ/d) over a 100 d treatment period in calves. This value is somewhat greater than the increase in heat production measured directly for sheep in the present study (mean +0·6 MJ/d; 0·07 increase). The calorimetric determinations also highlight the initial marked increase in energy expenditure during the first 24–96 h, which is probably associated with other β_2-adrenergic effects, such as tachycardia. After this period the elevation in heat production was less but energy expenditure was still greater ($P < 0·001$) than that in control periods. Increased energy expenditures were also reported for the rat (Rothwell *et al.* 1983; Emery *et al.* 1984) but since in those experiments the clenbuterol-treated animals ate more than controls (+0·13 and +0·22 respectively), at least a portion of the increased heat production could have been the result of the greater intake.

The increased N retention observed in the present study represented an average daily improvement of 3 g, or a proportional increase of 0·46. The absolute increase is similar to that calculated from slaughter values on clenbuterol-treated calves, in which the

proportional increase in rate of gain was only 0·08 (Williams *et al.* 1987). In rats, Emery *et al.* (1984) observed improvements in body protein gain of +0·22 over a 16 d treatment period, while for specific muscles Reeds *et al.* (1986) noted a doubling in the rate of protein retention in the initial phase of the action of the drug. The proportional improvements are to some extent misleading, dependent as they are on the initial gain in the control animals; nonetheless the absolute improvements appear to represent a persistent protein anabolic response, which can be observed for entire males as well as for castrates and females.

The improvement in protein deposition appears to be confined to skeletal and cardiac muscle, for in both rats (Reeds *et al.* 1986) and calves (Williams *et al.* 1987) no extra N gain could be detected for the other tissues. Indeed, in both species, a part of the improved carcass (muscle) retention appeared to be accomplished at the expense of the other tissues. Thus in the calf study carcass N gain was 0·45 kg, whereas total N retention was only enhanced by 0·31 kg. Similarly, in the rat study, by 21 d of clenbuterol treatment, the weights of liver and kidney in treated animals were lower ($P < 0·05$) than in untreated animals.

Based on intermediate N balance studies the rate of protein deposition in clenbuterol-treated calves was similar throughout the 100 d of treatment (Williams *et al.* 1987) and, in the present study, there were no obvious signs of the protein anabolic effect showing temporal changes. In young rats, however, the action of the drug is fairly short-lived, probably no longer than 14 d (Reeds *et al.* 1986). The reasons for this are not clear, although some restriction may be imposed by the ability of the skeleton to support the extra muscle mass. Increased weight, and protein content, for the muscles from the lower leg of the rat were enhanced by 0·23–0·33 above the approximate tripling already achieved by normal growth for the controls. Clearly, in the larger and more slowly growing commercial species, such rapid fractional increases in growth do not occur, but if all the extra N retention observed in the present study were as muscle protein, this would also represent an increase in muscle:bone value of approximately 0·2.

The mechanism(s) by which the increased protein retention is achieved is controversial. In rats Emery *et al.* (1984) reported a stimulation of muscle protein synthesis at high daily doses of clenbuterol (2 × 1 mg/kg body-weight; 1200–1400 μg/kg $LBW^{0·75}$) but under these conditions food intake was also increased, a situation well-known to elevate protein synthesis (Reeds *et al.* 1980). In contrast Reeds *et al.* (1986), using daily dosages of clenbuterol more comparable (200 μg/kg body-weight; 110 μg/kg $LBW^{0·75}$) with those used both in the present study (28–35 μg/kg body-weight; 75–90 μg/kg $LBW^{0·75}$) and other growth trials with farm species (e.g. calves 20 μg/kg body-weight; 70 μg/kg $LBW^{0·75}$; Williams *et al.* 1987), found no stimulation of fractional synthesis rate in either m. gastrocnemius or m. soleus. Indeed, they reported a significant decline in protein synthesis after the protein anabolic action of the drug had apparently ceased, and concluded that the primary increase in muscle protein content was due to a decrease in fractional breakdown rate. The situation may be more complex, however, since at days +4 and +11 of treatment, i.e. while anabolism was still actively proceeding, absolute synthesis (the product of fractional synthesis rate × protein mass) in the rat muscles did increase, despite no increase in food intake. Thus, while the initial action of the drug does appear to involve a decrease in protein degradation, later maintenance of the anabolism may require elevation of muscle total protein synthesis. Similar observations with sheep were reported by Bohorov *et al.* (1987), where improvements in the rate of protein gain in both m. longissimus dorsi and m. vastus lateralis were 0·33 greater in animals which received clenbuterol (approximately 400 μg/kg body-weight/d; 1060 μg/kg $LBW^{0·75}$). After 37 d of treatment muscle fractional protein synthesis rates were indistinguishable between control and treated lambs but total synthesis was elevated by 0·33–0·66. The authors were also in

general agreement with the conclusion that a decrease in muscle protein degradation was involved in the primary action of clenbuterol. Additional evidence which supports a partial role, at least, for a decline in muscle protein degradation comes from the reduced N^{τ}-methylhistidine elimination observed for treated as compared with control calves (Williams *et al.* 1987).

The measurement of leucine ILR through the blood pool allows a comparative estimate of whole-body kinetics, provided the drug treatment does not alter the relation between the SR of the leucyl-tRNAs and blood free leucine. In whole-animal terms, leucine oxidation (which can be equated to overall amino acid catabolism) declined as urinary N elimination decreased and N retention increased. The flux of leucine for protein synthesis was unaltered from control values at day +4, was elevated at day +11 and declined again at day +18. Only at day +11 were these changes significant ($P < 0{\cdot}001$). The reasons for these trends are unclear, although the values at day +18 are similar to those reported by Bohorov *et al.* (1987) for plasma tyrosine flux in lambs compared, after 37 d of treatment with clenbuterol, with controls. In that report (Bohorov *et al.* 1987), tyrosine flux (uncorrected for oxidation) was slightly lower, but not significantly so, in the treated animals; this was despite an increase in absolute protein synthesis for certain muscles (see p. 463). The pattern in whole-body protein synthesis observed in the current study ($ILR_{syn} \times 51{\cdot}5$; from Table 4) is, however, similar to that observed for muscle total protein synthesis in rats treated with clenbuterol, i.e. no increase, increase and then decline (from Reeds *et al.* 1986). Muscle protein synthesis, however, makes a minor contribution to whole-body protein synthesis (0·15–0·25; Lobley *et al.* 1980) and, at day +18, N retention is still augmented in sheep, whereas in rats the decline in absolute muscle synthesis appears to accompany the loss of the protein anabolic effect: thus such comparisons must be considered with caution. An additional complication involves the changes in carcass composition, as observed for example in calves (Williams *et al.* 1987), where there is actually a reduction in protein mass of the viscera compared with control animals. Tissues such as the gastrointestinal tract, liver, skin, etc. make important contributions to body protein turnover and changes in the metabolic activity of these tissues may produce profound effects on the kinetics of amino acid metabolism disproportionate to net changes in protein gain. The importance of these non-muscle tissues to the viability of the animal may require that 'resistance' to extended depletion through the action of an exogenous agent, be induced. Therefore, to compensate, the rates of synthesis in these tissues may be elevated until a new balance between the action of the drug and normal endogenous mechanisms is established. This may account for the increased rate of synthesis observed at day +11, but obviously such speculations need to be tested by experiment.

Clearly, the β-agonist clenbuterol is a highly effective means of altering the relative rates of protein and fat deposition and, therefore, of changing the carcass conformation of the animal. Unfortunately, some problems are encountered with the use of clenbuterol, e.g. tachycardia, reduced blood pressure and, at dose rates above 1·5 mg/d, inappetence in sheep (Brockway *et al.* 1987). Reeds *et al.* (1988) have shown that the effects of clenbuterol on energy expenditure and protein anabolism can be dissociated, at least in the rat: the use of selective β-antagonists prevented the elevated energy expenditure but maintained the protein anabolic response. This raises the possibility that suitable compounds could be synthesized which would retain the potential to improve protein gain without associated deleterious actions, making them more suitable for commercial application.

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