British Journal of Nutrition (1987), **57**, 417–428

Effects of a β-agonist (clenbuterol) on growth, carcass composition, protein and energy metabolism of veal calves

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(*Received 5 August 1986 – Accepted 22 December 1986*)

1. Twenty-two British Friesian bull calves were used in a comparative slaughter experiment to determine the effects of a β-agonist (clenbuterol) on body composition and energy retention. Four calves were slaughtered at 18 d of age and constituted the initial slaughter group. Of the remaining calves, eight (group A, controls) were given milk replacer only, and ten calves (groups B and C, five calves per group) were given milk replacer plus clenbuterol (0·1 and 1·0 mg clenbuterol/kg milk replacer equivalent to approximately 2 and 20 μg/kg body-weight respectively over the 105 ± 3 d of the experimental period). Calves were slaughtered over the weight range 146–177 kg.

2. Clenbuterol had no significant effect on dry matter (DM) intake, daily live-weight gain or feed conversion ratio. DM digestibility of the milk replacer was not affected by treatment. Nitrogen balance was measured on three separate occasions starting when the calves weighed approximately 60, 110 and 130 kg. N retention was increased over the experimental period in clenbuterol-treated calves, although the effect only achieved significance in calves weighing approximately 110 kg live weight ($P < 0·05$).

3. Clenbuterol (20 μg/kg body-weight) increased estimated mean daily N retention in the carcass of the calves from 22 to 25 g whilst N retention in the non-carcass components decreased from 10 to 8 g/d. Effects of clenbuterol on N retention occurred mainly in skeletal muscle. Fat in both carcass and non-carcass components was reduced by treatment with clenbuterol. The total energy content of live-weight gain was reduced from 1077 to 897 MJ in clenbuterol-treated calves and mean daily heat production was estimated to increase from 23·1 in controls to 25·9 MJ/d in calves in group C.

4. In calves of mean live weight during balance of 120 and 136 kg, clenbuterol significantly increased daily urinary creatinine excretion and in 120 kg calves N^{τ}-methylhistidine was significantly decreased ($P < 0·05$). Based on estimates of muscle mass from urinary creatinine and protein degradation from N^{τ}-methylhistidine excretion, the fractional breakdown rate of muscle protein in clenbuterol-treated calves was only 0·66 of that in the controls when the calves weighed 120 kg.

Several reports (Baker *et al.* 1984; Dalrymple *et al.* 1984; Ricks *et al.* 1984; Jones *et al.* 1985; Moser *et al.* 1986) have identified that the β-adrenergic agonists clenbuterol (benzyl alcohol, 4-amino-*R*-(*t*-butyl-amino)methyl-3, 5-dichloro) and cimaterol (CL263, 780), when added to the diet of steers, lambs, poultry and pigs, produce an increase in muscle and a decrease in fat accretion which have been attributed to a shift in nutrient partitioning. From chemical analysis of the 9th to 11th rib sections it was estimated that carcass protein in clenbuterol-treated steers was 1·15 and carcass fat 0·75 of that in controls (Ricks *et al.* 1984). Estimation of body composition from rib analysis in animals treated with clenbuterol assumes that relations between rib composition and body composition which were obtained using untreated animals are the same for treated animals. These relations will only be the same if whole body composition is uniformly altered during repartitioning and this may not be the case. Furthermore, skeletal muscle accounts for only approximately 0·65 of total body protein, and components of the remaining 0·35 which are metabolically highly active, e.g. the hide and liver, may also be susceptible to the same metabolic change.

A comparative slaughter experiment was designed to examine the effects of the β-agonist clenbuterol on body composition, particularly with respect to the major sites of repartitioning of both fat and protein. Veal calves were chosen since their rate of weight gain is high with respect to body-weight (gaining approximately 10 g/kg body-weight per d); they

therefore may constitute a sensitive model for the study of agents affecting protein metabolism. Furthermore, during the later stages of growth of veal calves the proportion of fat in the gain is high, again making them potentially sensitive to the action of a repartitioning agent.

This paper reports the effects of clenbuterol on protein and fat accretion in growing veal calves. A clear response was obtained, the effects on body protein occurring primarily in skeletal muscle protein with histochemical evidence suggesting a reduction in muscle protein degradation.

MATERIALS AND METHODS

Animals. Twenty-two British Friesian bull calves were purchased locally at approximately 7 d of age (day 7). On day 17, eighteen of these were allocated into three treatment groups by a restricted randomization procedure such that the mean live weights of each group were similar (Table 3, p. 421); group A (eight calves; control group) and groups B and C (five calves per group; clenbuterol treated). The remaining four calves which were slaughtered on day 18 (mean weight 48·8 (SE 2·9) kg) constituted an initial slaughter group.

Feeding and management. The calves were housed in individual pens littered with straw. They were given milk replacer at levels adjusted weekly according to metabolic live weight ($LW^{0.75}$). It was given from a bucket twice daily, at 09.00 and 17.00 hours, up to 105 kg live weight and subsequently three times daily, at 07.00, 13.00 and 17.00 hours. The feed was reconstituted with warm water (38°) to a concentration of 125 g/l from 7 d of age to 90 kg live weight and to 215 g/l thereafter. Two formulations of a commercial milk replacer were used (Table 1). The change to the formulation with the high-oil content occurred when the calves reached 95 kg live weight. The calves were weighed weekly after the morning feed and the quantity of milk offered was adjusted according to the recorded live weight. Milk refusals were collected and recorded. The calves were allowed free access to drinking water.

Clenbuterol (as the hydrochloride) was added daily to each feed of the reconstituted milk replacer such that calves on treatments B and C received 0·1 and 1 mg clenbuterol/kg milk replacer dry matter (DM) respectively (equivalent to approximately 2 and 20 μg/kg body-weight over the experimental period).

Nitrogen balance and digestibility. Three digestibility and N balance trials were performed using four calves from each treatment, starting when the live weights of the calves were approximately 60, 110 and 135 kg and at approximately 28, 75 and 96 d of age respectively. The animals were placed in metabolism crates and allowed to adapt for 48 h before each 8-d collection. Urine was collected by gravity in plastic vessels containing 400 ml 4 M-H_2SO_4, weighed and sampled after the first 4 d of each balance trial and at the end. Faeces were collected daily and stored at 5° before chemical analysis.

Slaughter procedure. The calves in the initial slaughter treatment received their normal morning feed and were weighed at 09.00 hours. These animals were slaughtered by intravenous administration of sodium pentobarbitone followed by exsanguination; a standard procedure (Table 2) was adopted for separation and weight recording of body components. The remaining calves of the trial were slaughtered when they achieved a live weight of between 146 and 177 kg. Since in practice it has proved impossible, for comparative purposes, to achieve exactly the same mean slaughter weight, for different groups of animals, the range in weight at slaughter was imposed to allow adjustment by covariance to the same mean slaughter weight. On the morning of slaughter the normal 07.00 hours feed was withheld. The calves were weighed, and then transported to a commercial abattoir where they were stunned with a captive bolt and immediately exsanguinated.

Table 1. *Declared composition and chemical analysis of the milk replacers, and feeding programme of the calves*

	Low fat	High fat
Manufacturer's declared analysis		
Minimum crude protein (g/kg)	240	240
Oil (g/kg)	180	200
Maximum crude fibre (g/mg)	5	5
Vitamin A (I.U./kg)	45000	45000
Vitamin D (I.U./kg)	4500	4500
Vitamin E (I.U./kg)	20	20
Flavomycin (mg/kg)	16	16
Chemical composition		
Dry matter (DM) (g/kg)	966	964
Nitrogen (g/kg DM)	39·9	40·4
Ash (g/kg DM)	79·9	87·6
Fat (g/kg DM)	194	210
Gross energy (MJ/kg DM)	21·7	21·6

	Level of milk replacer given to calves from 30 kg live wt to slaughter wt				
Live wt (kg)	30–39	40–54	55–69	70–105	106–175
g dried milk/kg live $wt^{0·75}$ per d	40	45	55	60	65

Table 2. *Dissection procedure utilized during the experiment*

	Hot wt	Cold wt	Pre-mincing wt	Mincing	Sampling
Carcass both sides (excluding kidneys, perinephric and channel fat)	×	×	×	×	×
Blood	×		×	×	×
Hide	×		×	×	×
Head	×				
Feet	×		×	×	×
Tail	×				
Gut full, with fat	×				
Gut empty, with fat	×		×	×	×
Organs†	×		×	×	×
Perinephric channel fat	×		×	×	×

× Weight recorded.

† Liver, heart, lungs, spleen, pancreas, thymus, testis, kidneys and penis weighed separately and then the individual tissues were combined and composite samples taken.

Carcass separation. The procedure for separation, mincing and sampling of the carcasses was that shown in Table 2. Initially the kidneys and perinephric fat, plus the channel fat (fat depots in the pelvic channel) were removed and the two hot sides weighed. After storage for 24 h at 5°, the weights of the cold tissue were obtained. The 10th rib joint was then dissected out of the left side of the carcass, weighed in air and then in water (approximately 10°) and specific gravity calculated (Ledger *et al.* 1973). The joint was then dried and dissected into fat, muscle, bone and ligamentum nuchae. As a check on the components for mincing a pre-mincing weight was also taken and the components of the 10th rib were recombined with the left side. Components of the carcass were grouped as shown in Table 2 and minced in a Wolfking mincer to pass through a 14 mm screen and then an 8 mm screen. The minced material was mixed in a commerical planetary mixer and triplicate samples, each of approximately 200 g, were transferred to aluminium trays and frozen before freeze drying. The freeze-dried samples were ground and sub-sampled for chemical analysis.

Chemical analysis. The DM content of feeds and faeces was determined by oven drying at 105° for 48 h and DM of the carcass components by freeze drying. N was determined by the automated Kjeldahl method of Davidson *et al.* (1970). Organic matter was estimated by ashing at 580° for 2 h. Lipids were determined by a modification of the chloroform–methanol extraction, incorporating an acid hydrolysis stage (Atkinson *et al.* 1972). Urine was analysed for N^{τ}-methylhistidine (MH) by ion-exchange chromatography (Harris & Milne, 1981) and urinary creatinine by the automated technique of Technicon Instruments Co. Ltd (1965) which utilizes the Jaffe reaction (Hawk *et al.* 1947).

Statistical analysis. The carcass characteristics and the 10th rib dissection values were adjusted by analysis of covariance using final live weight as a covariate. Differences due to treatment were examined using analysis of variance and significance levels are based on an F test.

Significant treatment effects were tested using Student's t test.

RESULTS

Feed intake, live-weight gain and feed conversion ratio. Addition of clenbuterol to the diet had no significant effect on DM intake, daily live-weight gain or feed conversion ratio ($P > 0{\cdot}05$) (Table 3). However, there was a tendency for the incidence of feed refusal to be greater in calves given clenbuterol.

Digestibility of milk replacer and N balance. For the three balance periods, DM digestibility of the milk replacer was similar for all three groups (0·91 (SE 0·003), n 35).

N retention. There were significant differences between the three balance periods in the amounts of N retained and the ratio of N retained:N intake ($P < 0{\cdot}01$), but there was no significant interaction between period and effect of clenbuterol treatment (Table 4). Only in period 1 was the N intake the same for each group of calves. In this period there was no significant effect of treatment on N retention, though a trend for greater retention was seen in the treated calves ($P < 0{\cdot}15$). In periods 2 and 3, N intakes varied between treatment groups. Calves treated with 20 μg clenbuterol/kg body-weight consumed less N but retained more N than the controls whilst the N retention of calves treated with 2 μg/kg was similar to that of the controls. In view of the differences in N intake between treatments, which however were small, the data were statistically compared on the basis of the ratio, N retained:N intake. Data from all three periods were pooled and the ratio of N retained:N intake corrected for differences in individual live weight of the calves at the start of each balance period. Treatment with 20 μg clenbuterol/kg significantly ($P < 0{\cdot}05$) raised the overall ratio of N retention:N intake (treatment A = 0·51; treatment B = 0·51; treatment

Table 3. *Effect of clenbuterol on the mean feed intake and daily live-wt gain of calves*

Clenbuterol (mg/kg milk replacer)...	0	0·1	1·0		Level of signif-
Clenbuterol (approximate concentration, μg/kg body-wt)...	0	2	20	SED	icance
No. animals	8	5	5		
Period on experiment (d)	102	108	106	7·23	NS
Initial wt (kg)	44·5	43·6	43·2		
Milk refusals (kg DM/d)	0·06	0·14	0·15	5·78	NS
Milk consumption (kg DM/d)	1·82	1·77	1·85	12·20	NS
Live-wt gain (kg/d)	1·10	1·08	1·07	0·058	NS
Feed conversion ratio (kg milk solids/kg live-wt gain)	1·67	1·65	1·74	0·091	NS

SED, standard error of difference, is the value of minimum treatment replication; DM, dry matter; NS, not significant.

Table 4. *Effects of clenbuterol on nitrogen retention, the ratio of N retained: N intake, creatinine and* N^{τ}*-methylhistidine (MH) excretion in calves*

Clenbuterol (mg/kg milk replacer)...	0	0·1	1·0		Level of signif-
Clenbuterol (approximate concentration, μg/kg body-wt)...	0	2	20	SED	icance
Period 1:					
No. of animals	4	3	4		
Mean live wt during balance (kg)	62	58	61		
N intake (g/d)	45·5	45·5	45·5		
N retained (g/d)	28·9	29·5	30·2	0·59	NS†
N retained: N intake	0·64	0·65	0·66	0·013	NS†
Creatinine (g/d)	1·96	2·04	2·05	0·144	NS
Creatinine (mg/kg live wt)	31·5	35·2	33·8	0·151	NS
MH (mmol/d)	0·249	0·255	0·262	0·026	NS
MH (μmmol/kg live wt)	4·0	4·4	4·3	0·22	NS
MH: Creatinine (mmol/(g × 10^{-1}))	1·27	1·25	1·28	0·218	NS
Period 2:					
No. of animals	4	4	4		
Mean live wt during balance (kg)	119	118	121		
N intake (g/d)	90·3	86·8	82·5		
N retained (g/d)	42·7	42·6	45·5		
N retained: N intake	0·47^{a}	0·49^{ab}	0·56^{b}	0·023	*
Creatinine (g/d)	4·30^{a}	4·82^{b}	5·12^{b}	0·229	*
Creatinine (mg/kg live wt)	36·2^{a}	40·9^{b}	42·4^{c}	0·098	**
MH (mmol/d)	0·800^{a}	0·625^{b}	0·618^{b}	0·080	*
MH (μmol/kg live wt)	6·7^{a}	5·2^{b}	5·1^{b}	0·40	*
MH: Creatinine (mmol/(g × 10^{-1}))	1·86^{a}	1·30^{b}	1·21^{b}	0·28	*
Period 3:					
No. of animals	4	4	4		
Mean live wt during balance (kg)	128	136	136		
N intake (g/d)	87·8	88·3	82·0		
N retained (g/d)	35·4	34·6	37·9		
N retained: N intake	0·41	0·39	0·46	0·041	NS
Creatinine (g/d)	4·9^{a}	6·49^{b}	6·34^{b}	0·436	*
Creatinine (mg/kg live wt)	38·5^{a}	47·7^{b}	46·6^{b}	3·06	*

SED, standard error of difference, is the value of minimum treatment replication; NS, not significant.
^{a,b,c}, Mean values followed by different superscript letters are significantly different: * $P < 0·05$, ** $P < 0·01$.
† $P < 0·15$.

Table 5. *Effect of clenbuterol on carcass characteristics and on the weight of liver, heart, and kidney and channel fat*

Clenbuterol (mg/kg milk replacer)...	0	0·1	1·0		Level of signif-
Clenbuterol (approximate concentration, μg/kg body-wt)...	0	2	20	SED	icance
No. of animals	8	5	5		
Mean final live wt (kg)	159·9	159·8	162·2	2·36	
Cold carcass wt (kg)	92·1	96·2	100·2	2·00	
Dressing† (%)	57·6[a]	60·3[b]	61·8[c]	0·64	*
Liver (kg)	3·12	2·84	2·56		
Liver‡ (kg)	3·14[a]	2·86[b]	2·51[c]	0·14	*
Heart (kg)	0·91	0·90	0·79	0·79	
Heart‡ (kg)	0·91	0·90	0·78	0·060	NS
Kidney and channel fat (kg)	1·71	1·41	1·11		
Kidney and channel fat‡ (kg)	1·74[a]	1·44[a]	1·07[b]	0·154	*

SED, standard error of difference, is the value of minimum treatment replication; NS, not significant.
[a,b,c], Mean values followed by different superscript letters are significantly different: * $P < 0·05$.

† Calculated as: $\frac{\text{cold carcass weight}}{\text{final live weight}} \times 100$.

‡ Weight of organs adjusted for differences in final live weight (where b is the regression coefficient and SE is the standard error): liver (kg), b 0·266, SE 0·00914, ($P < 0·01$); heart (kg), b 0·00321, SE 0·00287; kidney and channel fat (kg), b 0·0281, SE 0·0125 ($P < 0·01$).

C = 0·56; standard error of difference (SED) 0·015), but only during period 2 and in calves given 20 μg clenbuterol/kg was this ratio significantly higher than that in controls.

Creatinine and MH excretion. Urinary excretion of creatinine and MH is given in Table 4. There were no samples from period 3 available for MH analysis. During period 1 there was no significant effect of clenbuterol on creatinine or MH excretion although urinary creatinine tended to be higher in clenbuterol-treated calves. During periods 2 and 3, urinary creatinine was higher in clenbuterol-treated calves ($P < 0·05$). Furthermore, the difference between treated and untreated calves increased between the two periods. There was no significant effect of clenbuterol in period 1 on urinary excretion of MH or the urinary MH:creatinine ratio. In period 2, clenbuterol significantly reduced the absolute excretion of MH, MH per unit live weight and the ratio of MH:creatinine ($P < 0·05$).

Carcass characteristics and 10th rib dissection. Effects of treatment with clenbuterol on the weight of the cold carcass and weight of liver, heart and kidney and channel fat are shown in Table 5. Dressing percentage was increased and the weight of liver and perinephric plus channel fat significantly decreased by treatment with clenbuterol ($P < 0·05$).

The specific gravity of the 10th rib joint from calves treated with clenbuterol did not differ at the two levels of treatment but was significantly higher than that in untreated calves (1·091 *v.* 1·081; $P < 0·01$ (SED 0·0029)). Percentage dissectible lean and fat in the 10th rib joint showed significant differences between all three treatments (percentage lean 61·4, 65·5 and 71·2, $P < 0·05$, SED 1·70; percentage fat 20·6, 15·9 and 13·2, $P < 0·05$, SED 1·10; in untreated, 2 and 20 μg clenbuterol/kg treated calves respectively).

The results of the complete carcass separation and chemical analyses of the initial slaughter group, the controls and clenbuterol treated calves are given in Table 6. Total body N was increased and body DM and fat decreased by clenbuterol treatment. The increase in body N occurred in the carcass, in the non-carcass components the N content was actually lower in the clenbuterol-treated compared with the control calves. Although there was no difference in the daily N retention in the whole body between the controls and calves treated

Table 6. *Effects of treatment with clenbuterol (0·1 or 1·0 mg/kg milk replacer, approximately 2 or 20 μg/kg body-weight) on the body composition of calves*

(Values (in kg) adjusted by covariance for differences in final live weight to a mean overall live weight of 160·5 kg)

	Whole body	Carcass	Non-carcass	Organs†	Gut	Kidney and channel fat	Hide
Initial slaughter group, four calves, mean live wt 48·8 kg:							
DM	11·96	7·27	4·69	0·65	0·51	—	1·34
N	1·46	0·84	0·62	0·08	0·07	—	0·23
Fat	1·07	0·63	0·44	0·06	0·08	—	0·028
Control, group A, eight calves, mean live wt 160kg:							
DM	52·7	33·7	19·1	2·68	4·3	1·5	4·75
N	4·73	3·07	1·66	0·27	0·17	0·008	0·68
Fat	17·04	9·78	7·26	0·79	3·14	1·46	0·77
Clenbuterol (0·1 mg/kg), group B, five calves, mean live wt 160 kg:							
DM	50·8	33·4	17·5	2·54	3·7	1·3	4·26
N	4·92	3·33	1·59	0·26	0·15	0·006	0·64
Fat	14·40	8·20	6·20	0·73	2·69	1·22	0·53
Clenbuterol (1·0 mg/kg), group C, five calves, mean live wt 162 kg:							
DM	48·3	32·5	15·8	2·21	3·4	0·9	3·88
N	5·04	3·52	1·51	0·24	0·16	0·005	0·60
Fat	11·30	6·18	5·12	0·57	2·32	0·83	0·42
SED							
DM	1·31	0·70	0·53	0·12	0·32	0·22	0·30
N	0·090	0·086	0·039	0·009	0·008	0·000 7	0·045
Fat	1·137	0·628	0·333	0·067	0·306	0·219	0·084
Significant differences (groups A, B and C):							
DM	**	NS	**	**	*	*	*
N	**	***	*	NS	NS	**	NS
Fat	***	***	**	*	*	*	***

DM, dry matter; SED, standard error of difference, is the value of minimum treatment replication; NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† Liver, heart, lungs, spleen, pancreas, thymus, testis, kidneys, penis.

with 2 μg clenbuterol/kg (3·206 and 3·203 g N retained/d respectively), clenbuterol at this level of administration was still affecting N metabolism since daily N retention in the carcass was increased and in the non-carcass decreased. Effects of clenbuterol on fat accretion occurred in both the carcass and non-carcass components with less fat deposited in both components at the two levels of clenbuterol treatment. The ratio of N:fat-free DM was lower in the total body (0·133 and 0·135 *v.* 0·136 for treatments B, C and A respectively) and in the carcass of clenbuterol-treated calves compared with the controls. The perinephric and channel fats were the only discrete portions of fat removed from the carcass which could be analysed and examined with respect to effect of treatment on a specific fatty tissue. The ratio of N:fat-free DM in perinephric and channel fats was considerably lower in the clenbuterol-treated compared with control calves (0·075 and 0·071 *v.* 0·20 for treatments B, C and A respectively).

There was no difference in the total ash content of the calves; total ash contents, adjusted by covariance for differences in final slaughter weight for treatments A, B and C calves, were 7·6, 7·4 and 7·4 kg (SED 0·27) respectively.

DISCUSSION

The total carcass separation and chemical analyses indicated that in calves, both muscle protein and fat depostion were affected at both levels of clenbuterol administration but that skeletal growth as indicated by total ash was unaffected. The proportion of lean and fat in the carcass was estimated from regression equations based both on sample joint specific gravity and also estimation of percentage fat in the side from percentage fatty tissue in the joint and lean in the side from lean in the sample joint (Ledger *et al.* 1973). From the specific gravity of the 10th rib joint the proportion of lean in the carcass was calculated to increase from 0·65 to 0·66 of the carcass and dissectible fat to decrease from 0·14 to 0·11. Similarly, from the 10th rib dissection the proportion of lean in the carcass was calculated to increase from 0·64 to 0·66 and 0·69 and fat to decrease from 0·18 to 0·14 and 0·13 in untreated, 2 and 20 μg clenbuterol/kg treated calves respectively. However, in the carcass, chemically determined lean (Table 6) as a proportion of that in the untreated calves increased by 0·15 whilst chemically determined fat decreased by 0·37 in calves treated with 20 μg clenbuterol/kg. These results demonstrate that specific gravity or dissection of the 10th rib joint considerably underestimated the changes in carcass composition in calves treated with clenbuterol. Results of carcass composition obtained from clenbuterol-treated steers using predictions based on specific gravity or 10th rib dissection should therefore be viewed with caution.

In spite of producing a significant decrease in gut-tissue DM and tending to reduce gut-tissue N, clenbuterol had no effect on the digestibility of the milk replacer. A lack of effect on digestive efficiency following a reduction in the N content of gut tissue is similar to the effect induced by growth-promoting antibacterials which produce a thinning of the gut epithelial lining and an N-sparing effect but whose effects on absorptive capacity are equivocal (Armstrong, 1985).

Protein metabolism. Fig. 1 shows that incremental N retention in the whole body over the experimental period was 3·27, 3·46 and 3·58 kg, equivalent to 32·0, 32·0 and 33·7 g N retained/d in calves given 0, 2 and 20 μg clenbuterol/kg. Over the three balance periods calves treated with 20 μg clenbuterol/kg consistently retained more N than controls, whereas N retention was the same in controls and calves given 2 μg clenbuterol/kg. The carcass-separation results indicate, however, that although whole body N retention on a daily basis was not increased at the lower level of clenbuterol treatment compared with the

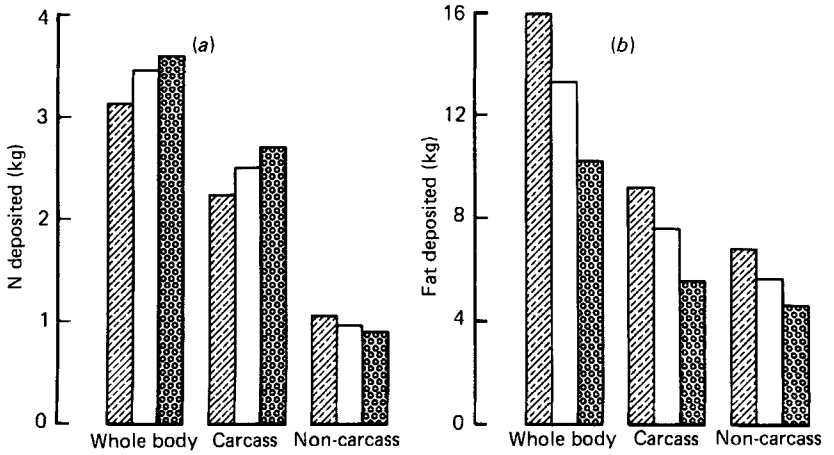

Fig. 1. Deposition of (*a*) nitrogen and (*b*) fat over the experimental period in the whole body, carcass and non-carcass components of calves untreated (▨) or given 0·1 (□) or 1·0 (▩) mg clenbuterol/kg milk replacer (approximately 2 or 20 μg/kg body-weight respectively).

controls, N retention in the carcass was significantly higher in both groups of clenbuterol-treated calves. Over the experimental period, N retained in the carcass increased from 2·23 kg (mean 22 g/d) in the controls, to 2·49 kg (mean 23 g/d) and 2·68 kg (mean 25 g/d) in calves treated with 2 and 20 μg clenbuterol/kg respectively. Increased N retention in the carcasses of calves treated with 2 μg clenbuterol/kg was compensated by a loss of N from the non-carcass components, whilst at 20 μg clenbuterol/kg this effect was combined with increased daily N retention. Since 0·73 of the total protein present in the carcass is present in skeletal muscle (Lobley *et al.* 1980) the results suggest that a major effect of clenbuterol was occurring in skeletal muscle protein, confirming results obtained by P. J. Reeds, S. M. Hay and R. M. Palmer (personal communication). Non-carcass components, the gut, hide and major organs, exhibited a decline in N retention from 1·04 kg (10 g/d) in untreated calves to 0·89 kg (mean 8 g/d) in calves receiving 20 μg clenbuterol/kg (Fig. 1), although the difference between N retention in the non-carcass components (0·15 kg) was insufficient to account for the increased retention in skeletal muscle (0·45 kg). Lobley *et al.* (1980) reported rates of total protein synthesis (g/d) in the gastrointestinal tract, liver and hide to be an average 2·4, 0·4 and 1·0 times the total synthetic rate obtained in muscle when the ratio of protein concentrations in the organs:muscle protein was 0·1, 0·3 and 0·3 respectively. As these organs have a considerable higher metabolic activity than skeletal muscle, small reductions in mass may yield considerable savings in N requirement for organ homeostasis. Whilst a primary effect of clenbuterol may be related to increased skeletal protein deposition, effects related to protein sparing from non-carcass components may be equally important.

Urinary excretion of creatinine, as a measure of total muscle mass (Graystone, 1968) increased with increasing live weight and was higher in each balance period in clenbuterol-treated calves compared with the controls. Furthermore, the magnitude of the differences between the creatinine excretion of controls and the mean of all treated calves increased up to and including period 3 (creatinine excretion, mean of all treated calves minus mean of controls, period 1 = +3·0; period 2 = +5·5; period 3 = +8·7 mg/kg live weight). Thus although daily N retention measured either by N balance or by whole body N retention

did not indicate whether clenbuterol had an effect at each of the balance periods, the cumulative effect on total muscle mass, estimated from creatinine excretion, strongly suggests that clenbuterol was affecting protein metabolism throughout the experimental period.

The use of MH elimination as an index of muscle protein degradation was criticized by Millward *et al.* (1980) but the criticism was challenged by Harris (1981). This challenge was supported by Afting *et al.* (1981) who concluded that in humans, skeletal muscle tissue is the major contributor of MH in urine, since it contributes as much as 75% to the urinary excretion. In the present experiment clenbuterol reduced the total protein content of the viscera. Although the effects of treatment on protein turnover in the viscera are unknown, and the contributing sources of MH are not quantified, the use of MH elimination as an index of muscle protein degradation may still have validity in qualitative terms. The rate of muscle protein degradation (g/d) was calculated by the equation

$$\text{muscle protein degradation} = \frac{\mu\text{mol MH excreted/d}}{\text{MH content of skeletal muscle protein.}} \quad (1)$$

In this equation, the denominator was taken to be 3·5106 μmol MH/g muscle protein (0·587 μmol/g wet muscle tissue) (Nishizawa *et al.* 1979). The fractional rate of muscle protein breakdown (%/d) was calculated by the equation:

$$\text{fractional breakdown rate} = \frac{\text{total muscle protein degradation (kg)} \times 100}{\text{total skeletal muscle (kg).}} \quad (2)$$

Muscle mass, the denominator in eqn 2, was calculated either from the mean creatinine excretion (Graystone, 1968) using a value of 17·9 kg muscle/g urinary creatinine or from an estimation of the muscle in the carcass of the calves using the values from chemical analysis. Muscle in the carcass was calculated using the values presented in Table 6 for N in the carcass of calves at 48·8 kg live weight (the initial slaughter group) and at 160·5 kg (final slaughter weight), with muscle mass at intermediate weights calculated by regression analysis assuming a linear increase in muscle growth over this weight range. The N content of muscle in the initial and final slaughter groups was taken as 33 g/kg from the comparative composition of longissimus dorsi muscle in calves and steers (Lawrie, 1985). Estimates of muscle mass, muscle protein degradation and fractional breakdown rates (FBR) are given in Table 7. These results suggest that in period 1, since N retention and creatinine excretion were higher but muscle degradation appeared unaffected in calves given 20 μg clenbuterol/kg compared with controls, the increased N retention may have been occurring as a result of increased protein synthesis. In period 2, in calves given 20 μg clenbuterol/kg, N retention was increased by 0·07 but FBR was reduced by 0·33 compared with the controls. This suggests that protein synthesis in treated calves was now depressed compared with the controls but that increased protein deposition was occurring as a result of the reduction in FBR. Emery *et al.* (1984) reported a 34% increase in fractional protein synthesis in clenbuterol-treated rats. Over the experimental period treatment with 20 μg clenbuterol/kg increased carcass N by 0·20; however, muscle FBR was apparently reduced by over 0·30 in calves weighing 110 kg suggesting that at times, the effects of clenbuterol were less than those recorded in the calves when they reached 110 kg live weight. It is interesting that the FBR increased in the control animals between 57 and 112 kg live weight. If indeed the major action of clenbuterol is mediated via a reduction in muscle degradation then the lack of a marked effect in period 1 may relate to the low FBR, and until FBR rises there may be less potential for the effect of the drug to be manifested.

Fat metabolism and energy balance. Total body fat was reduced in carcass and non-carcass

Table 7. *Estimates of muscle mass, muscle protein degradation and fractional breakdown rates of muscle protein in control or clenbuterol-treated calves*

Clenbuterol (mg/kg milk replacer)...	0	0·1	1·0
Clenbuterol (approximate concentration, μg/kg body-wt)...	0	2	20
Period 1			
Mean live wt (kg)	62	58	61
Muscle mass (i)	35·1	36·6	36·7
(ii)	34·6	32·7	35·3
Muscle degraded (g/d)	424	434	446
Fractional breakdown (%/d) (i)	1·2	1·2	1·2
(ii)	1·2	1·3	1·3
Period 2			
Mean live wt (kg)	119	118	121
Muscle mass (i)	76·9	86·2	91·6
(ii)	70·2	74·7	79·7
Muscle degraded (g/d)	1363	1065	1053
Fractional breakdown (%/d) (i)	1·8	1·2	1·1
(ii)	1·9	1·4	1·3

(i) Based on urinary excretion of creatinine; (ii) calculated from carcass nitrogen.

Table 8. *Effects of clenbuterol on energy balance of calves from 44 to 160 kg live weight*

Clenbuterol (mg/kg milk replacer)...	0	0·1	1·0
Clenbuterol (approximate concentration, μg/kg body-wt)...	0	2	20
Metabolizable energy intake* (MJ)	3434	3530	3637
Body energy gain† (MJ)	1077	1001	897
Total energy expenditure (MJ)	2357	2529	2740
Period on experiment (d)	102	108	106
Mean heat production (MJ/d)	23·1	23·4	25·9

* Based on a metabolizable energy value of the milk replacer of 18 MJ/kg dry matter.
† Based on energy values for fat and protein of 38·9 and 22·3 MJ/kg respectively (Blaxter, 1969).

components by treatment with clenbuterol, unlike the effects on protein metabolism. These results agree with the effects of clenbuterol in steers reported by Ricks *et al.* (1984) and cattle and sheep (Hanrahan *et al.* 1986) but are contrary to results published by Emery *et al.* (1984) which revealed that clenbuterol had not affected body fat content in rats. The energy balance results for the calves are shown in Table 8. Body energy gain was reduced and calculated energy expenditure was increased in the clenbuterol-treated animals. Thornton *et al.* (1984) reported that in isolated subcutaneous adipocytes, clenbuterol reduced lipogenesis rates down to basal levels and stimulated lipolysis rates more than fivefold, hence both processes may have been affected in the present study. Emery *et al.* (1984) reported that β_2-agonists raised energy expenditure and stimulated the proton-conductance pathway in brown adipose tissue. The presence of brown adipose tissue in calves was confirmed by Alexander *et al.* (1975) and Thompson & Bell (1976). Furthermore it was shown that early stimulation of brown adipose tissue prolonged the potency of the thermogenic response (Meuler *et al.* 1975). The exact role of clenbuterol in reducing the amount of fat in the carcass is difficult to define. Whether the effect was due to a direct action on the adipocyte with a subsequent liberation of free fatty acids and their utilization in either adipose tissue sites or in other

areas, or whether indirectly with effects on fat metabolism occurring secondary to an increasing metabolic rate cannot be distinguished from the present results. Since the present study suggests that increased protein deposition occurred at least in part by reducing protein degradation it is unlikely that an increased energy requirement for protein synthesis was a primary reason for the reduction in fat deposition. Table 8 indicates, however, that heat production was increased by 0·3 and 2·8 MJ metabolizable energy/d in calves given 2 or 20 μg clenbuterol/kg, implying an increase in metabolic rate equivalent to 1 and 13% which was unrelated to actual costs of protein and fat accretion.

REFERENCES

Alexander, G., Bennett, F. W. & Gemmel, R. T. (1975). *Journal of Physiology* **244**, 223–234.

Afting, E.-G., Bernhardt, W., Janzen, R. W. C. & Rothig, H.-J. (1981) *Biochemical Journal* **200**, 449–452.

Armstrong, D. G. (1985). In *Control and Manipulation of Animal Growth*, Nottingham University Easter School, pp. 21–37 [P. J. Buttery, N. B. Haynes and D. B. Lindsay, editors]. London: Butterworths.

Atkinson, T., Fowler, V. R. F., Garton, G. A. & Lough, A. K. (1972). *Analyst, London* **97**, 562–568.

Baker, P. K., Dalrymple, R. H., Ingle, D. L. & Ricks, C. A. (1984). *Journal of Animal Science* **59**, 1256–1261.

Blaxter, K. L. (1969). *The Energy Metabolism of Ruminants*, p. 63. London: Hutchison and Co. Ltd.

Dalrymple, R. H., Baker, P. K., Gingher, P. E., Ingle, D. L., Pensack, J. M. & Ricks, C. A. (1984). *Poultry Science* **63**, 2376–2383.

Davidson, J., Mathieson, J. & Boyne, A. W. (1970). *Analyst, London* **95**, 181–193.

Emery, P. W., Rothwell, N. J., Stock, M. J. & Winter, P. D. (1984). *Bioscience Reports* **4**, 83–91.

Graystone, J. E. (1968). In *Human Growth*, p. 182 [D. B. Cheek, editor]. Philadelphia: Lea and Febiger.

Hanrahan, J. P., Quirke, J. F., Bomann, W., Allen, P., McEwan, J., Fitzsimons, J. & Kotzian, J. (1986). In Nottingham Feed Manufacturers Conference. London: Butterworths (In the Press).

Harris, C. I. (1981). *Biochemical Journal* **194**, 1011–1014.

Harris, C. I. & Milne, G. (1981). *British Journal of Nutrition* **45**, 411–422.

Hawk, P. B., Oser, B. L. & Summerson, W. H. (1947). In *Practical Physiological Chemistry*, p. 506. San Francisco: McGraw Hill Co.

Jones, R. W., Easter, R. A., McKeith, F. K., Dalrymple, R. H., Maddock, H. M. & Bechter, P. J. (1985). *Journal of Animal Science* **61**, 905–913.

Lawrie, R. A. (1985). *Meat Science* 4th ed, p. 61. Oxford: Pergamon International Library.

Ledger, H. P., Gilliver, B. & Robb, J. M. (1973). *Journal of Agricultural Science, Cambridge* **80**, 381–392.

Lobley, G. E., Milne, V., Lovie, J. M., Reeds, P. J. & Pennie, K. (1980). *British Journal of Nutrition* **43**, 491–502.

Meuler, V. Ter. & Molnar, S. (1975). *Zeitschrift fur Tierphysiologie, Tierernahrung und Futtermittelkunde* **35**, 243–256.

Millward, D. J., Bates, P. C., Grimble, G. K. & Brown, G. (1980). *Biochemical Journal* **190**, 225–228.

Moser, R. L., Dalrymple, R. H., Cornelius, S. G., Pettigrew, J. E. & Allen, C. E. (1986). *Journal of Animal Science* **62**, 21–26.

Nishizawa, N., Toyoda, Y., Noguchi, T., Hareyama, S., Itabashi, H. & Funabiki, R. (1979). *British Journal of Nutrition* **42**, 247–252.

Ricks, C. A., Dalrymple, R. H., Baker, P. K. & Ingle, D. L. (1984). *Journal of Animal Science* **59**, 1247–1255.

Technicon Instruments Co. Ltd (1965). *Technicon Methodology Sheet* N-11B. Basingstoke: Technicon Instruments Co. Ltd.

Thompson, G. E. & Bell, A. W. (1976). *Biology of the Neonate* **28**, 375–381.

Thornton, R. F., Tume, R. K., Larsen, T. W. & Johnson, G. W. (1984). *Proceedings of the Nutrition Society of Australia* **9**, 185.

Printed in Great Britain