Effect of clenbuterol on growth, nitrogen and energy balances and endocrine status in food-restricted sheep

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ABSTRACT

The aim of this study was to examine the effect of the β_2-adrenoceptor clenbuterol on food-restricted sheep. Clenbuterol was administered as a dietary admixture (4 mg/ kg diet) to a group of male Serra da Estrela sheep (n = 6).The animals were housed individually in metabolic cages and fed for 45 days at 65 % of estimated requirement for energy maintenance. An untreated group with the same energy intake level was included as a control. Changes in body mass, nitrogen and energy balances and insulin, insulin-like growth factor (IGF-1), and triiodothyronine (T_3) levels in the experimental animals were monitored. During the 4th week of the trial, clenbuterol-treated sheep showed increased mass gains, greater energy retention and serum IGF-1 levels and decreased T_3 serum concentrations. This study showed that clenbuterol may induce a protective effect in sheep subjected to periods of food deprivation, based on the body mass and digestible energy effects manifested by treated animals.

Key words: clenbuterol, food-restriction, sheep.

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INTRODUCTION

The anabolic effects of β_2-adrenoceptors on *ad libitum*-fed sheep have been reported before[2,9]. Studies of β_2-adrenergic agonists have focused on increasing animal production and carcass quality in situations in which food availability is not a limiting factor. However, about 70 % of the world's livestock is located in developing countries[15] where weather conditions often result in marked seasonal variations in the availability of food, severely affecting growth and causing 17–20 % body mass loss during drought periods[19]. The effects of clenbuterol on growth, body composition, endocrine status and nitrogen and energy balance in food-restricted rats showed that the β_2-adrenoceptor improved growth and offset the effects of food restriction on protein and energy metabolism[5,6]. Sheep are regularly subjected to drought conditions in tropical, subtropical and sub-arid regions. In the work reported here, the effect of clenbuterol on growth, nitrogen and energy balance and on several hormones in food-restricted sheep was examined.

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MATERIALS AND METHODS

Six-month-old (24.6 kg ± 0.44) male Serra da Estrela sheep were randomly divided into 2 groups (n = 6) and individually placed in metabolic cages in a room at 26 ± 1° C and fed daily with 135 g hay and 200 g concentrate (Table 1), with a metabolic energy (ME) density of 7.1 Mj/kg DM, crude protein of 12 %, and a q_m of 0.65, satisfying 65 % of estimated daily ME maintenance requirements[1] (Table 2). This ration was fed throughout the 45-day trial. One of the groups received clenbuterol (Sigma Aldriech Chemie Gmbh, Steinheim, Germany) incorporated into the diet at a concentration of 4 mg/kg DM. Food intake (corrected for spillage) and body mass were recorded daily and twice weekly, respectively. Urine and faeces were collected every 7 days from separate containers in the metabolic cages. Every 2 weeks, jugular vein blood samples were withdrawn for hormonal analysis. Urine and faecal nitrogen and gross energy contents were determined (duplicate samples) respectively by Kjeldahl digestion (Buchi 315, Schweiz, Germany) and Parr-1655 (Parr, Moline, IL) adiabatic calorimetric bomb, using benzoic acid as the thermodynamic standard.

Nitrogen balance was determined by subtracting urinary and faecal N from ingested N during the 24-h period prior to faecal and urinary sample collection. Digestible energy was determined by subtracting faecal N from ingested N during the 24-h period prior to faecal and urinary sample collection. Metabolic energy was calculated subtracting the urinary and methane energy values from digestible energy. Methane energy was calculated at 8 % of gross energy of the food intake[14]. Since clenbuterol-treated and control animals were on the same diet, it was assumed, as have other workers[11], that there were no significant changes in methane production.

Insulin serum levels were determined with an RIA (Gamyt CR 10/20 counter, Diagnostic Products, Los Angeles, CA) with DPC-Coat-A-Count reagents (Diagnostic Products, New York). The insulin assay was conducted with intra- and inter-assay CVs of 7.5 and 8.3 %, respectively. Insulin-like growth factor 1 (IGF-1) levels were determined with DSL-9400 Free IGF-I IRMA Kits (Diagnostic Systems Laboratories, Webster, TX) as described elsewhere[20]. Insulin-like growth factor 1 intra- and inter-assay CVs were 8.2 and 9.7 %, respectively. Triiodothyronine (T_3) was determined with an enzymatic solid-phase immunoassay (DPC-Coat-A-Count, Diagnostic Products, New York) and a Labsystem FP-901 Chemistry Analyser spectrophotometer. This method was validated for sheep serum (DPC Application Sheet ML93) and had intra- and

Table 1: **Nutrient composition of ingredients in the diet** (% dry matter).

	Concentrate	Hay
Dry matter	88.4	92.3
Crude protein	15.26	10.9
Ether extract	2.3	1.1
Ash	7	9.8
Crude fibre	6.9	35
Nitrogen-free extract	56.9	35.5
Sunflower seed meal	13.1	–
Maize	43.4	–
Soyabean meal	11.2	–
Corn gluten	27	–
Ca carbonate	2.5	–
Na bicarbonate	0.5	–
Mg sulphate	0.5	–
Na chloride	1.17	–
Vitamins & mineral conc.	0.63	–

Table 2: **Live mass.**

		Day 0	Day 8	Day 15	Day 25	Day 37	Day 45	*F*-test Time	*F*-test Treat.	*F*-test Time × Treat.
Live mass	Treat.	$25.14^{ab\,A}$ (0.65)	$26.11^{ab\,A}$ (0.73)	$27.11^{a\,A}$ (0.87)	$25.66^{a\,A}$ (0.71)	$24.44^{b\,A}$ (0.90)	$24.27^{b\,A}$ (0.92)	3.05*	8.53*	0.73
	Contr.	$25.69^{a\,A}$ (0.96)	$24.19^{a\,A}$ (0.82)	$25.44^{a\,A}$ (0.86)	$22.99^{b\,B}$ (0.80)	$22.66^{b\,A}$ (0.78)	$22.71^{b\,A}$ (0.76)			
Mass change relative to day 0	Treat.	–	$0.97^{a\,A}$ (0.45)	$1.97^{a\,A}$ (0.17)	$0.52^{a\,A}$ (0.26)	$–0.7^{b\,A}$ (0.21)	$–0.87^{b\,A}$ (0.23)	9.14^{**}	50.7**	0.32
	Contr.	–	$–1.51^{a\,B}$ (0.42)	$–0.25^{a\,B}$ (0.59)	$–2.70^{b\,B}$ (0.54)	$–3.03^{b\,B}$ (0.70)	$–2.98^{b\,B}$ (0.71)			

Values are means (SEM), $n = 6$.

Different superscripts (^{a,b} and ^{ab}) in the same row indicate significant differences ($P < 0.05$) between days (day 0, day 8 etc.).

Different superscripts (^{A} and ^{B}) in the same column indicate differences between the control and treatment.

Significance of *F*-test results are indicated as follows: $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

Treat. = clenbuterol treated animals; Contr. = control animals; Time = duration of trial.

Table 3: **Energy balance.**

		Day 0	Day 8	Day 15	Day 25	Day 37	Day 45	*F*–test Time	*F*–test Treat	*F*–test Time × Treat.
Digest. E	Treat.	$876.3^{a\,A}$ (299.9)	$1153.4^{a\,A}$ (475.3)	$315.5^{\,b\,A}$ (125.5)	$952.6^{a\,A}$ (332.8)	$455.5^{b\,A}$ (85.7)	$702.1^{a\,A}$ (84.12)	3.49**	38.4***	2.7*
	Contr.	$850.04^{b\,A}$ (210.6)	$–364.8^{b\,B}$ (205.6)	$–908.5^{b\,B}$ (202.2)	$–12.25^{b\,B}$ (497.7)	$259.5^{\,a\,B}$ (96.3)	$209.3^{\,ab\,B}$ (142.1)			
Metab. E.	Treat.	$466.2^{a\,A}$ (325.8)	$770.9^{a\,A}$ (487.8)	$61.81^{ab\,A}$ (124.3)	$551.8^{a\,A}$ (121.7)	$94.81^{b\,A}$ (79.1)	$399.7^{a\,A}$ (88.45)	3.53**	36.9***	2.6*
	Contr.	$–276.9^{ab\,A}$ (209.8)	$–724.7^{ab\,B}$ (213.6)	$–1288.9^{b\,B}$ (190.67)	$–383.8^{ab\,B}$ (208.1)	$–98.4^{a\,B}$ (94.9)	$–159.7^{ab\,B}$ (143.9)			

Values are means (SEM), $n = 6$.

Different superscripts (^{a,b} and ^{ab}) in the same row indicate significant differences ($P < 0.05$) between days (day 0,day 8 etc.).

Different superscripts (^{A} and^{B}) in the same column indicate differences between the control and treatment.

Significance of *F*-test results are indicated as follows: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Digest E = digestable energy; Metab E = metabolised energy; Treat. = clenbuterol-treated animals; Contr. = control animals; Time = duration of trial.

inter-assay CVs of 8 and 9.5 %, respectively.

All data are presented as mean ± SEM, and were subjected to ANOVA to determine the effect of clenbuterol treatment, duration of the trial and the interaction. *Post hoc* significant differences between treated and control groups were identified by least significant difference (LSD) tests ($P < 0.05$). All probabilities are 2-tailed.

RESULTS

Changes in live mass

Table 2 shows the changes in live mass during the trial. By the 4th week the clenbuterol-treated animals were significantly heavier ($P < 0.05$) than the control group due to a significant decrease of 2.7 kg in the mass of control animals by day 25 compared to day 0. Mass changes from day 0 onwards indicate that there were significant differences between treated and control animals throughout the trial, with control sheep consistently having lost mass. Although the treated animals showed negative growth from day 37 onwards, the losses in mass were significantly smaller than those observed in the control sheep.

Nitrogen metabolism

No significant changes occurred in N digestibility and balance during the experimental period (data not shown).

Energy metabolism

Clenbuterol treatment induced significant increases in energy intestinal absorption and metabolised energy during the trial (Table 3). These changes began at the end of the 1st week and were consistent during the remainder of the trial. Treated animals presented positive ME values whereas in the control sheep the ME levels were always negative. The treated and control animals showed no differences in urinary energy (data not shown)

Hormone profiles

Insulin concentrations did not change with clenbuterol treatment during the trial (Table 4). Insulin-like growth factor 1 serum concentrations were increased in the β-adrenoceptor agonist-treated animals from the 2nd to the 4th week of the trial (respectively 3.4 % and 9 % compared to the control), with a tendency to invert this relationship during the last week. Triiodothyronine serum concentrations in treated sheep were lower than in the control animals, up to 82 % during the 2nd week. Throughout the trial the control animals tended to show an increase in serum T_3 compared with the treated ones.

DISCUSSION

Clenbuterol-treated animals presented a significant increase in mass by the 4th week compared with the controls, and thereafter the tendency for the treated sheep to maintain a higher mass was

Table 4: **Hormone profile.**

						F-test		
		Day 0	Day 15	Day 30	Day 45	Time	Treat	Time × Treat.
Insulin (μIU/mℓ)	Treat.	7.31$^{a\,A}$ (0.36)	6.06$^{a\,A}$ (0.92)	6.39$^{a\,A}$ (1.66)	5.52$^{a\,A}$ (2.03)	1.45	1.39	0.55
	Contr.	6.54$^{a\,A}$ (0.35)	5.82$^{a\,A}$ (0.63)	4.69$^{a\,A}$ (1.30)	5.58$^{a\,A}$ (1.44)			
IGF1 (ng/mℓ)	Treat.	299.4$^{a\,A}$ (7.7)	317.3$^{ab\,A}$ (2.4)	283.6$^{b\,A}$ (3.8)	299.7$^{ab\,A}$ (11.8)	13.82*	0.86*	2.7
	Contr.	302.6$^{a\,A}$ (0.6)	306.6$^{a\,B}$ (3.1)	260.2$^{b\,B}$ (7.1)	312.8$^{a\,A}$ (5.36)			
T_3 (ng/dℓ)	Treated	119.56$^{a\,A}$ (9.57)	83.21$^{b\,A}$ (8.16)	84.17$^{b\,A}$ (4.92)	72.05$^{b\,A}$ (11.08)	13.81***	19.60***	0.16
	Contr.	125.04$^{a\,A}$ (7.56)	111.25$^{b\,B}$ (5.16)	103.54$^{b\,A}$ (11.72)	94.32$^{b\,A}$ (21.31)			

Values are in means (SEM), $n = 6$.

Different superscripts (^{a,b} and ^{ab}) in the same row indicate significant differences ($P < 0.05$) between days (day 0,day 8 etc.).

Different superscripts (^{A} and ^{B}) in the same column indicate differences between control and treatment.

Significance of *F*-test results are indicated as follows: *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

IGF1 = insulin-like growth factor 1; T_3 = triiodotyroxine; Treat. = clenbuterol-treated animals; Contr. = control animals; Time = duration of treatment.

persistent for the duration of the trial. The effect of clenbuterol on the maintenance of mass is also evident in comparisons of mass differences from day 0 onwards. However, after the 4th week the anabolic effect of clenbuterol was not in evidence anymore, probably due to down-regulation of the β-adrenoceptor agonist receptors. A decrease in β-adrenoceptor effects in *ad libitum*-fed lambs by the 33rd day of treatment has been reported in the literature[7]. These results are consistent with studies on rats, in which most of the anabolic effects of clenbuterol reached a maximum after about 8–10 days of treatment, whereafter treated and control animals grew at similar rates[10,16,17]. However, clenbuterol's anabolic effects have been shown to be more marked in rats fed restrictively than in those given free access to food[5,6].

From the 2nd week until the end of the trial, treated animals showed a significant increase, compared to the controls, in digestible energy. This increase in energy uptake by the digestive tract is related to changes in the intestinal epithelium structure induced by the β-adrenoceptor agonist. In clenbuterol-treated, food-restricted rats, there is, compared to the controls, an increase in protein content in gastrointestinal tract tissue ($P < 0.001$), as well as an enhancement of anatomical and functional features of the intestinal (duodenal) epithelium (Cardoso and Ferreira, unpubl. data). As a consequence of this enhancement in energy digestibility, the treated sheep also showed a parallel increase in metabolic energy, as urinary energy did not differ between treatments.

The lower levels of serum T_3 in the treated sheep do not appear to be responsible for the higher energy savings shown by these animals. The results are dependent on the increase in energy digestibility in clenbuterol-treated animals, as no changes due to clenbuterol were seen in metabolised energy. In *ad libitum*-fed rats, clenbuterol is known to reduce serum T_3[6].

Clenbuterol induced higher IGF-1 serum levels from the 2nd to the 4th week, with a consistent tendency to maintain this increase thoughout the trial. Significant differences in IGF-1 concentrations coincided with the period during which the growth of treated animals was significantly greater than in the controls. Similar results have been reported for underfed rats[6]. It is also known[13] that clenbuterol and growth hormone (GH) have an additive effect on growth in cattle. However, the independent anabolic action of these agents has been emphasised[3] although an increase in β-adrenergic receptor numbers with GH concentration has been demonstrated[18]. According to other studies[12], the re-feeding of rats restored the liver GH binding sites and serum IGF-1 levels influenced by a restricted-feeding regime. Our results show that clenbuterol enhanced energy digestibility. This could have induced the observed increase in serum IGF-1 and eventually in the GH binding sites in the treated animals, as shown by Bates and Pell[3]. Thus, IGF-I can be related to the clenbuterol growth effect in the treated sheep. The decrease of IGF-1 serum levels after day 15 of the trial is consistent with the results of other authors[8].

Insulin levels were not altered by clenbuterol treatment in the present trial, and similar results have also been reported for rats[6]. In lambs treated with the β_2-adrenoceptor agonist cimaterol, a reduction of 55 % in insulin serum levels resulted[4], indicating that the anabolic effects of insulin in protein metabolism may not be present in β_2-adrenoceptor agonist-treated sheep.

Our results show that clenbuterol may induce a protective effect in sheep that are deprived of food, evidenced by the mass and digestible energy effects shown by treated animals.

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