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Effects of clenbuterol on growth in underfed cattle

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Abstract. This study examined the effects of clenbuterol on the growth of young cattle (160 kg) that were fed a restricted quantity of a low-quality hay to simulate dry-season pasture conditions in the tropics. Twenty Brahman steers were used. Ten control animals lost an average of 0.24 kg/day in the first 17 days, then maintained their liveweight for the remaining 21 days of the experiment. By contrast, 10 clenbuterol-treated animals lost 0.3 kg/day for the first 17 days of the experiment, then continued to lose weight at a steady rate of 0.15 kg/day. In control steers, plasma concentrations of urea-nitrogen decreased over the course of the experiment, and this effect was accelerated by clenbuterol treatment ($P < 0.05$). There were no marked changes in plasma concentrations of glucose, potassium, or N^t-methylhistidine in response to clenbuterol treatment. Clenbuterol had no effect on β_2-adrenoceptor density in the longissimus muscle, but there was a marked increase in β_2-adrenoceptors in both groups of cattle over time. Despite their loss of liveweight, the carcasses of clenbuterol-treated cattle were not lighter than controls (74.3 *v*. 72 kg, respectively) and contained 10% more protein ($P < 0.05$). This was reflected by a trend towards increased weight of the biceps femoris muscle (9%; $P < 0.1$). These findings are consistent with clenbuterol causing a drive to deposit muscle protein at the expense of other tissues, even when dietary protein and energy are limited.

Additional keywords: β-adrenergic agonists, β-adrenergic receptors.

Introduction

Growth stasis and even liveweight loss is a seasonal problem with cattle that graze on tropical savannas, where low rainfall can lead to a shortage of good-quality feed. As a result, beef cattle in northern Australia can take up to 6 years to reach market weight. If the age of turnoff could be reduced through improved growth rates in the dry season, there would be benefits to the producer in terms of profitability, sustainability, and improved meat quality. Furthermore, maintaining muscle strength and accelerating the rate at which cattle adapt their metabolism to poor-quality feed may improve their survival during periods of severe drought.

Whilst there are a number of anabolic agents that can stimulate muscle protein synthesis, most of these compounds are only effective at increasing growth rates when dietary protein and energy are adequate (for review see Lindsay *et al.* 1993). In theory, however, compounds that decrease protein degradation should be able to enhance muscle growth with no additional input of protein or energy. Clenbuterol is a β_2-adrenergic agonist, one of the few classes of anabolic compounds that are reported to increase growth primarily through reducing muscle protein degradation (Reeds *et al.* 1986). The drug has been shown to increase muscle growth, weight gain, and feed efficiency in food-restricted rats (Choo *et al.* 1990; Cardoso and Stock 1996) and to decrease N loss in protein-deprived sheep (Hovell *et al.* 1989). We demonstrated that clenbuterol causes a marked reduction in plasma urea concentrations in underfed cattle, suggesting that this class of drug may be effective in decreasing dry-season weight loss (Sillence *et al.* 1993). However, our results were equivocal because no reduction in total urinary N output was seen.

In the present study we treated underfed cattle with clenbuterol again, but focussed our attention on growth and carcass characteristics.

Materials and methods

All animal procedures were approved by the Tropical Beef Centre Animal Care and Ethics Committee and were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

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Twenty Brahman steers were fasted for 24 h, weighed, then placed in individual pens in a covered barn. The cattle were allowed free access to water, but were fed a restricted quantity of a poor-quality roughage diet to simulate dry-season pasture conditions. The feed offered was 13 g Pangola grass hay (*Disitaria decumbens*)/kg fasted liveweight.day, divided equally between 2 rations given at 08 00 and 16 00 hours. While receiving this diet the animals were weighed twice weekly for 6 weeks. During the first 3 weeks there was a rapid loss of 20–25 kg liveweight, but from 3 to 6 weeks the liveweight of all cattle had stabilised. Before the first day of the experiment the cattle were subjected to a second 24-h fast to obtain an accurate liveweight, and adjustments were made to their ration so that the ratio 13 g feed/kg liveweight was maintained. Each steer was allocated to one of two groups of equal mean liveweight (160 ± 4 kg). Thereafter, one of the groups received treatment twice daily with 4 mg clenbuterol dissolved in 10 mL safflower oil and administered as a top dressing on the feed; the second group received 10 mL safflower oil alone, and served as controls. The clenbuterol was synthesised in our laboratories as described previously (Pegg *et al.* 1991).

The treatment period lasted for 38 days, during which we continued to weigh the cattle twice weekly. On Days 0, 7, and 38 of treatment, the animals were restrained, and blood samples obtained by jugular venipuncture were collected into heparinised tubes. The blood was centrifuged at 4°C to obtain plasma, which was then stored at –20°C until assayed. Concentrations of urea-N and N^t-methylhistidine (NMH) were determined by the methods of Technicon (1972) and Wassner *et al*. (1980), respectively. Plasma glucose concentrations were measured using a commercially available kit (Sigma Chemical, St Louis, MO), and K^+ was determined by flame photometry as described previously (Hoey *et al.* 1995).

A 2-g sample of longissimus muscle was also removed from each animal on Days 0 and 7 under sedation (Xylazine, 10 mg/animal, i.m.) and local anaesthesia (lignocaine, 100 mg/animal). The muscle samples were quickly placed into liquid N, then stored at –80°C until assayed for β-adrenoceptor density as described in detail by Sillence and Matthews (1994). A crude preparation of cell membrane fragments was prepared by homogenisation and centrifugation. β-Adrenoceptor density and affinity were measured by saturation binding assays with (–)[^{125}I]iodocyanopindolol (1–200 pM) as the radioligand and (–)-propranolol (1 μM) to determine non-specific binding. Ligand binding data were analysed with the LIGAND computer program (Munson and Rodbard 1980).

The animals were slaughtered on Day 38, and a third sample of longissimus muscle was obtained within 5 min of death. The carcasses were skinned and eviscerated, then weighed. The liver and heart were weighed also, as were the biceps femoris and semitendinosis muscles after removal from the left side of the carcass. The right side of each carcass was then placed in a freezer at –20°C. Once the sides were frozen, their composition was determined as described by Thomson *et al.* (1997). Briefly, the frozen sides were sliced transversely on a band-saw into 8-cm-wide strips, and the sawdust was collected from each carcass was stored at –20°C. An estimate of carcass water content was obtained by homogenising a subsample of sawdust in a known amount of distilled water, followed by freeze-drying to a constant weight. The freeze-dried homogenate was then subjected to Kjeldahl digestion, and its N content determined by the method of Williams and Twine (1967). Protein content was estimated by multiplying the N content by 6.25. To determine fat content, a subsample of the freeze-dried sawdust was sonicated in methanol, then subjected to complete lipid extraction using the method described by Christie (1973). Although the carcass sawdust method has some limitations, results for body composition in beef cattle correlate with those obtained through the more exhaustive technique of mincing the entire side (Williams *et al.* 1974) and with isotope dilution methods (Thomson *et al.* 1997). The results were analysed by Student's *t*-test. All data are presented as means ± s.e.m.

Results

Because the feed ration was adjusted when the experiment started, the control animals lost weight for the first 17 days of the experimental period (–4 ± 0.8 kg), then maintained their liveweight until Day 38 (Fig. 1). The clenbuterol-treated cattle lost a similar amount of liveweight over the first 17 days (–5.4 ± 0.9 kg), but in contrast to the controls they continued to lose weight steadily and had lost significantly more weight than controls by the end of the experiment (8.6 ± 1.6 kg; $P < 0.05$). Values for carcass weight and composition are shown in Table 1. Although clenbuterol-treated cattle lost more liveweight than controls, their carcasses were not lighter, and contained 10% more protein ($P < 0.05$). This was reflected by a similar trend towards increased weight of the biceps femoris muscle (9%; $P < 0.1$). Apparent differences in semitendinosis, liver, and heart weight, and carcass fat were small and did not reach statistical significance, but all trends were in the same direction observed in other published studies (for review see Sillence 1996).

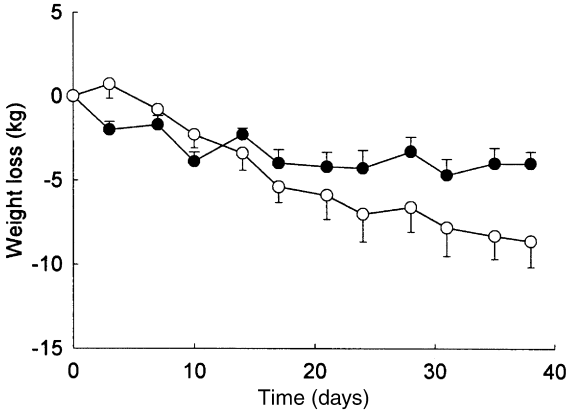

Fig. 1. Growth of control steers (●) and steers given 4 mg clenbuterol twice daily (○). All cattle were offered a restricted quantity of poor-quality hay. Initial liveweight 160 kg; $n = 10$.

Table 1. Growth and body composition of underfed steers treated with clenbuterol

Item (kg)[A]	Control	Clenbuterol[B]	s.e.m.[C]
Initial liveweight	160.1	160.2	3.7
Final liveweight	156.1	151.6	3.3
Carcass weight	72	74.3	1.7
Carcass fat	7.14	6.11	0.56
Carcass water	47.6	49.5	1.1
Carcass protein	12.9	14.2	0.43*
Biceps femoris weight	1.27	1.39†	0.05†
Semitendinosis weight	0.47	0.50	0.02
Liver weight	1.71	1.63	0.09
Heart weight	0.64	0.59	0.02

† $P < 0.10$; * $P < 0.05$.
[A] Each value is the mean observation of 10 animals.
[B] 4 mg twice daily.
[C] Pooled standard error.

Plasma metabolite concentrations are shown in Fig. 2. There was no evidence that clenbuterol caused a marked change in plasma K^+, glucose concentration, or plasma NMH levels. The concentration of urea-N decreased in control animals over the course of the experiment, and this effect was accelerated by clenbuterol treatment. Thus, urea-N was 31% lower in clenbuterol-treated cattle than in controls on Day 7 ($P < 0.05$), but by Day 38, urea-N concentrations were similar in both treatment groups.

β_2-Adrenoceptor density was markedly higher in all animals on Day 38 than on Days 0 and 7, and was not altered by clenbuterol treatment (Fig. 3).

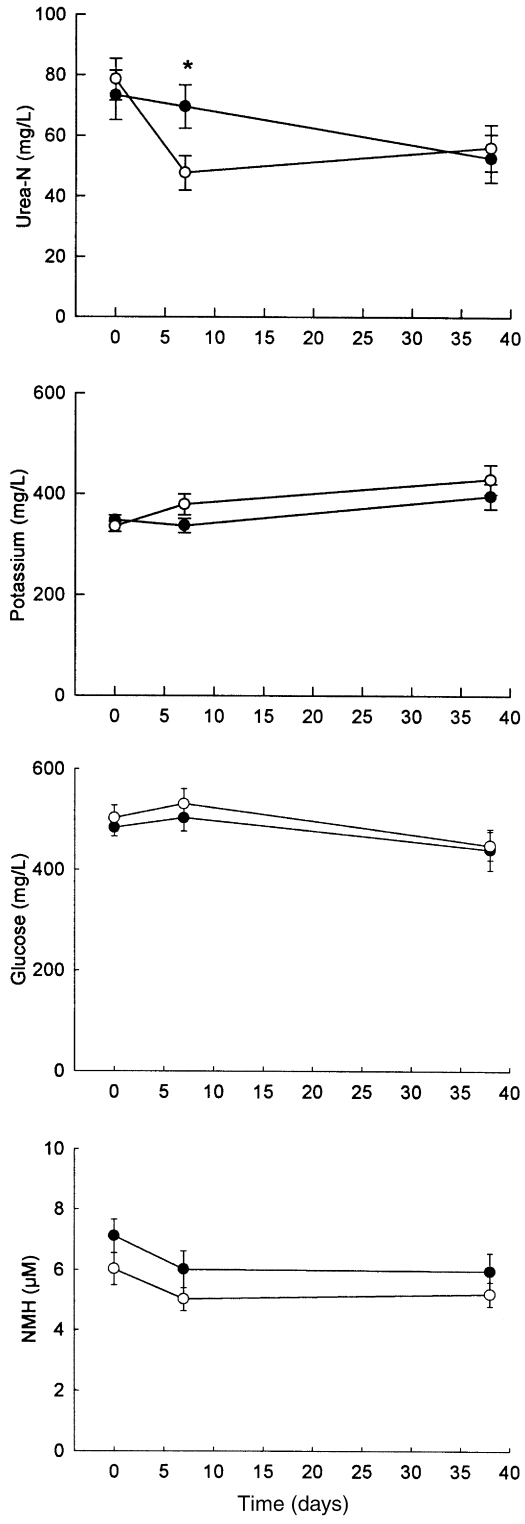

Fig. 2. Plasma concentration of metabolites (mean ± s.e.m.) in control steers (●) and in steers given 4 mg clenbuterol twice daily (○); $n = 10$; * $P < 0.05$.

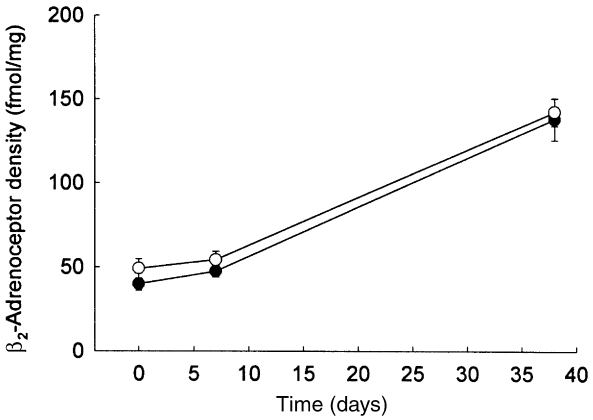

Fig. 3. Density of β_2-adrenoceptors in the longissimus muscle of control steers (●) and in steers fed 4 mg clenbuterol twice daily (○). Samples taken on Days 0 and 7 were through biopsy with sedation and local anaesthesia; those taken on Day 38 were obtained through necropsy; $n = 10$.

Discussion

The continued loss of liveweight in clenbuterol-treated cattle is in contrast to the positive effect of clenbuterol on weight gain in energy-restricted rats (Cardoso and Stock 1996). This loss of liveweight was not reflected in the carcass of treated animals, however. Although the difference in mean liveweight between the control and clenbuterol-treated cattle increased daily, this had not reached statistical significance when the animals were killed on Day 38. Nevertheless, on the assumption that differences in liveweight and carcass weight were real, it can be calculated that 4.5 kg of liveweight was lost in clenbuterol-treated cattle relative to controls, despite a 3.2 kg increase in muscle mass (protein + water). This loss of liveweight could be accounted for partly because of a reduction in carcass fat (–1.03 kg), but mainly because of a reduction in the mass of viscera and/or hide (–6.8 kg estimated by difference).

The repartitioning of protein and energy is consistent with many previous reports on the effects of β-agonists (Moloney *et al.* 1990; Chickhou *et al.* 1993). However, when cattle are provided with an adequate diet the increase in muscle mass usually equals or outweighs the loss of

viscera and fat, so that liveweight is either maintained or increased (Vestergaard *et al.* 1994). The present results show that there is a relentless drive to deposit muscle protein in β-agonist-treated cattle, because it is apparent even in underfed animals that are losing liveweight. At first this effect may seem lethal, but it is not necessarily so. In fact, a marked loss of protein from the tissues such as the liver is a normal physiological response of ruminants to underfeeding, and this organ is given priority for protein repletion once an adequate feed supply becomes available again (Wester *et al.* 1995). This may offer some energetic advantage to the animal, as the liver and gut account for a disproportionate amount of whole-body energy consumption (52%) compared with their relative mass (Burrin *et al.* 1989). The preservation of skeletal muscle may also be of advantage; death from starvation can occur long before the body is depleted of its protein and energy stores because of the impairment of muscles that allow the animal to breathe (Anon. 1989). Muscle strength is also important for mobility during a severe drought, allowing cattle to continue to forage for food.

Although a certain amount of repartitioning to muscle may benefit underfed cattle, it is also important to conserve other body protein for the activity of enzymes, and maintenance of the immune system. Thus, it is desirable to increase the overall efficiency with which dietary protein and energy are used, reducing their total loss from the animal. It is clear from the present results that the action of clenbuterol is not solely one of reducing the rate of muscle protein degradation. Protein turnover must have altered in tissues other than muscle, although our results give no indication of whether changes occurred through altered rates of synthesis or degradation. Nevertheless, the loss 6.8 kg of non-carcass components would be expected to release about 1.4 kg protein, assuming a 20% protein content. As carcass protein was increased by 1.3 kg in clenbuterol-treated cattle, this represents a relatively efficient repartitioning of dietary and/or body protein to muscle, with little or no impact on overall N balance. With regard to energy use, clenbuterol usually causes a small and transient increase in metabolic rate in well-fed animals (Lindsay *et al.* 1993), but Sainz *et al.* (1990) reported values for heat production in cimaterol-treated sheep ranging from +10% to –13% of control values, depending on the amount and quality of the feed offered.

The only metabolite that showed a significant response to clenbuterol treatment was urea. Because urea is the principal form of N excretion in most circumstances, a reduction in plasma urea-N is sometimes taken as an indicator of improved N balance. Furthermore, the short-term, but marked, effects of clenbuterol on this variable are consistent with our previous observations in underfed heifers (Sillence *et al.* 1993) and appear to support the theory that clenbuterol accelerates the improvement in N balance that occurs as a natural adaptation to underfeeding. However, these results should be treated with caution, because in contrast to the well-fed animal in which urea-N accounts for the up to 70% of urinary N excretion, as little as 8% of total N may be excreted in this form in underfed ruminants (McIntyre 1969; Sillence *et al.* 1993). In fact, clenbuterol caused a marked reduction in urinary urea-N output in our previous experiment, without affecting total N output (Sillence *et al.* 1993). In the present study, the repartitioning of protein from non-carcass components to muscle suggests that there was no net N loss, consistent with our results for plasma NMH and urea-N. This finding is in contrast to the impaired N balance and increased urinary N excretion seen in clenbuterol-treated rats when their dietary protein is severely restricted (Perézs-Llamas *et al.* 1992). Nevertheless, it can be concluded that in cattle, clenbuterol accelerates a metabolic change that occurs naturally in underfeeding, but this is not necessarily associated with an altered N balance.

Consistent with the short-lived effect of clenbuterol on plasma urea-N concentration, no further increase in muscle mass occurs in ruminants beyond 2 or 3 weeks of treatment with β-agonists (O'Connor *et al.* 1991; Pringle *et al.* 1993). A rapid attenuation of the growth response also occurs in rats, and this has been correlated to a reduction in β-adrenoceptor density (downregulation) in skeletal muscle, which can be as great as 50% (Rothwell *et al.* 1987; Sillence *et al.* 1991; Kim *et al.* 1992). However, β-adrenoceptors were not downregulated by clenbuterol in the present study (Fig. 3), and we have repeated this observation in studies of well-fed cattle where all samples were taken by biopsy (M. N. Sillence and M. L. Matthews, unpubl.). Our observation also supports a similar finding made in pigs, where the β-agonist ractopamine failed to downregulate β-adrenoceptors in skeletal muscle, even though they were downregulated in adipose tissue (Spurlock *et al.* 1994). Because ractopamine increases porcine muscle growth but has little effect on fat deposition, these data were interpreted as showing that receptor downregulation does limit the response to β-agonists in livestock. Our conclusions are opposite, however. We suggest that attenuation of the muscle growth response in ruminants results from factors other than downregulation, perhaps involving changes in post-receptor events such as G-protein coupling and adenylyl cyclase activation. Because their effects are only short-term, the use of β-agonists is likely to be constrained to a finishing strategy in feedlots. If similar technologies are to benefit grazing animals, then the mechanism through which cattle rapidly become insensitive to the effects of β-agonists needs further investigation.

Finally, our data show that β-adrenoceptor density was higher in all animals on Day 38 than on Day 0. It is tempting to speculate that the upregulation of β-adrenoceptors is a physiological response to underfeeding, providing a natural way to enhance nutrient repartitioning through increased sensitivity to endogenous catecholamines. A second possibility though, is that the β-adrenoceptor density measured in

the first 2 samples was comparatively low because of the use of a sedative and/or local anaesthetic. Further studies are required to resolve this question.

Conclusions

In summary, clenbuterol causes a drive to deposit muscle protein even in cattle that are underfed and losing liveweight. By reducing plasma urea and the mass of viscera, its effects appear to accelerate, and to some extent mimic, those which occur naturally as an adaptation to underfeeding. The greater preservation of muscle, more effective mobilisation of body fat stores, and loss of energy-consuming viscera may all enhance the survival of a drought-stricken animal. On the other hand, β-agonists do not reduce total N loss in underfed cattle. In addition, cattle rapidly become insensitive to the effects of these drugs, and the reason for this remains to be discovered.

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