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# THE INFLUENCE OF CLENBUTEROL ON FAT DEPOSITION AND FATTY ACID COMPOSITION OF DIFFERENT ADIPOSE TISSUES IN CASTRATED AND UNCASTRATED HOGGETS

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## INTRODUCTION

The aim at obtaining leaner meat from both lambs and calves has induced numerous investigations into the use of ß-agonists. The effectiveness of these agents depends on finding the optimum dose amount and treatment duration. Different experimental design varying in the sex and age of animals used, the feeding systems employed and the mode of applying ß-agonists has made it difficult to arrive at a uniform standpoint on this question (Thornton *et al.*, 1985; Williams, 1987; Zimmerli and Blum, 1990).

On studying male lambs for fattening, it was established that the effect of the same high clenbuterol dose (10mg) depended on feeding conditions. A positive effect was observed only with high energy diets (Banskalieva *et al.*, 1991). Use of a tenfold lower dose applied over a long time (180 days) in both castrated and uncastrated hoggets also lead to a reduction in reserve lipids, fat thickness and adipocyte size (Banskalieva *et al.*, 1992). At both high and low doses, variations in the extent of changes in the composition of adipose tissue triacylglycerols are observed.

The objective of the present experiment was to study the effect of a high clenbuterol dose on some aspects of lipid metabolism in adult animals. Data obtained afford information about both the deposition and composition of reserve lipids as well as adipose tissue cellularity in uncastrated and castrated hoggets.

## MATERIAL AND METHODS

Two experiments were carried out on 18 months old uncastrated and castrated male hoggets. Animals in both experiments were divided into three groups: a control group and two experimental groups. During the experiment, which had a duration of 42 days, all groups received the same diet containing 4.1MJ energy and 130g of protein per kg. Animals in the two experimental groups also received 10mg of clenbuterol per kg/feed. The hoggets of both control and the first experimental groups were slaughtered at the same time. The second experimental group was slaughtered 14 days later, having received no clenbuterol for that time.

Fresh adipose tissue from the perirenal (PAT), subcutaneous (around the tail),intermuscular (around *m.semimembanosus*), breast (BAT-over 5-6$^{th}$ vertebra) regions and the caul were obtained at slaughtering. After 24 hours at 2°C, carcasses were divided into two parts by a transverse cut at 12$^{th}$ rib. Fat depths were measured at the base of the tail, at the 5-6$^{th}$ vertebra of breast bone and the second to last rib. The total subcutaneous fat was removed and was weighted, as well as the caul and perirenal adipose tissue.

Methyl esters of triacylglycerols (TG), isolated by preparative TLC, were prepared by transmethylation with 2% solution (v/v) of $H_2SO_4$ in dry methanol for 15 hours. The fatty acid composition was analyzed by gas-liquid chromatography, using a metal column (3mx2mm) packed with 3% SP 2330$_3$ on Supelcoport (100-200 mesh).

For determining the size of adipocytes, samples of 1cm were taken. Sections of adipocytes (16m thick) were strained with Sudan III, and their mean diameter was determined in 3-5 visual field on 100 cells.

The Student t-test was used as a criterion for statistical evaluation of results.

## RESULTS AND DISCUSSION

Clenbuterol treatment lead to reduced deposition of reserve fats and reduction of the fat layer in both uncastrated and castrated animals, but to a different extent (Table 1). Broadly speaking, the effect of clenbuterol was marked in castrated animals. After six weeks of treatment with clenbuterol, subcutaneous and caul fat was decreased by half in those animals. On the other hand, in uncastrated animals drastic reduction in the amount of perirenal adipose tissue (73%) was observed, surpassing the changes observed in castrated animals. A similar effect, which depended on the location of the fat depots, has been reported by previous investigations by our lab both with lambs and hoggets (Banskalieva *et al.*, 1991; 1992). The causes of this reduced lipid deposition in experimental animals are still open to discussion.

In the study of Miller *et al.*, (1988) clenbuterol decreased lipogenesis as estimated from lipogenic enzyme activities and $^{14}$C-acetate incorporation into triacylglycerol in subcutaneous adipose tissue but had no effect on intramuscular fat synthesis.

The effect of the higher dose of clenbuterol used in the present examination had a greater effect compared to the tenfold lower one used in other investigations on the same classes of animals (Banskalieva *et al.*, 1992). For example, on uncastrated animals, the changes in both perirenal (PAT) and subcutaneous (SAT) adipose tissue are 5.6 and 1.7 times higher respectively, compared with those observed with the lower dose. In castrated hoggets, perirenal adipose tissue appears to be more susceptible to the effect of the higher dose. Except for results obtained by us for both lambs and hoggets, no investigations have been carried out on caul fat. It appears to react specifically both to dose and to the duration of treatment and depends on the physiological condition of animals.

In investigations on lambs fed different energy and protein levels, clenbuterol treatment showed an effect in the group of animals on a high level of feed intake analagons to that established in the present experiment (Banskalieva *et al.*, 1991). The results give us reason to consider that administering clenbuterol according to the recommended scheme (10mg/kg feed for six weeks) has the desired effect only in animals which have forced reserve fat deposition, regardless of age or physiological condition. However, the mechanism regarding this process has not yet been clarified.

Reductions in the content of subcutaneous fat with clenbuterol treatment are accompanied by analagons changes in fat layer depth (Table 1). In castrated animals the fat thickness was decreased by half irrespective of the anatomical location. In uncastrated hoggets a similar change of layer depth is only observed in 5-6$^{th}$ vertebra with non-significant changes at the other two sites.

A significant decrease in mean adipocyte diameter was observed at the base of the tail in castrated animals, but not in the intact animals (Table 1). Clenbuterol reduced adipocyte size in perirenal adipose tissue in both noncastrated and castrated animals, but had the opposite effect in intramuscular adipose tissue (MAT). Miller *et al.*, (1988) also pointed out that clenbuterol has a differential effect on both the size and number of fat cells depending on their anatomical location. Either a change in the number of fat cells or a reduction in their diameter may contribute to the thinning of fat layer (Hu *et al.*, 1988; Miller *et al.*, 1988; Schiavetta *et al.*, 1990). It has not yet been elucidated, however, to what extent those processes depend on the type, dose and the duration of treatment with ß-agonists.

Results from the fatty acid analysis of TG from all five fat depots are presented in Table 2 according to the extent of their total unsaturation. The higher fat content in the SAT and caul of castrated animals was accompanied by changes in the TG fatty acid composition.

With clenbuterol treatment, a trend towards increased unsaturation of TG from BAT, MAT, PAT and caul was observed in uncastrated animals, and was mediated through changes in relative content of 18:0 and 18:1. Increasing of unsaturation in lipids from subcutaneous adipose tissue has been reported for both lambs and sheep (Banskalieva *et al.*, 1991; Hu *et al.*, 1988; Thornton *et al.*, 1985). For castrated animals, a similar trend occurred in both PAT and caul,

while changes in fatty acid profile of TG in BAT and MAM are in a reverse order. TG of SAT do not change in castrated hoggets and uncastrated ones they become more saturated. According to Eadara *et al.* (1989) and Eiseman *et al.* (1988) ß-agonists are not likely to change de novo fatty acid synthesis; rather, it is likely that they influence acylation of individual fatty acids in adipose tissue TG.

After a 14 day withdrawal period, the animals began to exhibit increased fat deposition which was between one and 22% than that in the first experimental group. However, the adipose tissue weights in the second experimental group were still far below those of the control group. Changes in fat layer thickness were insignificant. The fatty acid composition of TG in some depots remains uncharted, and in other ones, it trends to reach the values of control groups.

Although ß-agonists are metabolize very quickly (MacRay *et al.*, 1986; Hovell *et al.*, 1987), from humanitarian and economic points of view, it is important to find an optimum withdrawal period. An optimum withdrawal period would maintain the repartitioning effects of the ß-agonists while ensuring a complete absence of residues. It should be noted that compared to the seven day period tested in both lambs and hoggets (Banskalieva *et al.*, 1991; 1992), a two week pause after clenbuterol treatment slowly reduced the effect of that substance on the traits studied (Tables 1 and 2). Use of a higher dose for a shorter period in adult animals followed by two week pause will ensure maintenance of the observed results. On the other hand, a more complete metabolism of residual clenbuterol may also be acceptable.

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Table 1. Carcass variables.

| Variable | Castrate | | |
|---|---|---|---|
| | C | E1 | E2 |
| Total subcut-aneous adipose tissue wt, g | 653 ± 24 | 440 ± 69 | 450 ± 19 |
| Caul wt, g | 580 ± 34 | 340 ± 17 | 330 ± 17 |
| Perirenal adipose tissue wt, g | 150 ± 16 | 40 ± 9 | 70 ± 10 |
| Fat thickness, cm: | | | |
| tail-base forelast rib, | 0.40 ± 0.12 | 0.28 ± 0.04 | 0.30 ± 0.05 |
| breast bone | 0.16 ± 0.05 | 0.12 ± 0.01 | 0.15 ± 0.03 |
| 5-6$^{th}$ vertabra | 0.41 ± 0.13 | 0.18 ± 0.05 | 0.21 ± 0.11 |
| Adipocyte diameter, m: | | | |
| perirenal adipose tissue | 44.6 ± 5.6 | 39.8 ± 1.1 | 41.2 ± 1.5 |
| intermuscular adipose tissue | 34.1 ± 0.3 | 39.4 ± 3.0 | 37.4 ± 2.9 |
| subcutaneous adipose tissue | 47.2 ± 5.4 | 39.0 ± 1.1 | 45.1 ± 2.7 |

Table 1 (cont). Carcass variables.

| Variable | Noncastrated C | E1 | E2 |
|---|---|---|---|
| Total subcut-aneous adipose tissue wt, g | 1027 ± 64 | 567 ± 14 | 610 ± 90 |
| Caul wt, g | 850 ± 63 | 370 ± 25 | 375 ± 15 |
| Perirenal adipose tissue wt, g | 180 ± 8 | 115 ± 39 | 140 ± 15 |
| Fat thickness, cm: | | | |
| tail-base forelast rib, | 0.44 ± 0.32 | 0.24 ± 0.04 | 0.25 ± 0.13 |
| breast bone | 0.20 ± 0.01 | 0.11 ± 0.01 | |
| 5-6$^{th}$ vertabra | 0.59 ± 0.35 | 0.31 ± 0.10 | 0.15 ± 0.05 |
| | | | 0.35 ± 0.09 |
| Adipocyte diameter, m: | | | |
| perirenal adipose tissue | 46.1 ± 2.9 | 40.4 ± 0.4 | 42.9 ± 1.9 |
| intermuscular adipose tissue | 40.7 ± 2.2 | 39.2 ± 0.9 | 40.4 ± 0.9 |
| subcutaneous adipose tissue | 43.5 ± 3.3 | 42.8 ± 3.4 | 45.3 ± 4.9 |

C = control; E1, E2 = experimental groups.
* = significant difference between control and experimental group ($P<0.05$).

Table 2. Fatty Acid Composition of Triacylglycerols of Various Adipose Tissues of Hoggets.

damaged