QUANTITY AND FATTY ACID COMPOSITION OF VARIOUS FAT DEPOTS IN CASTRATED AND NONCASTRATED HOGGETS FED CLENBUTEROL

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SUMMARY:

A trial has been conducted on male castrated and noncastrated hoggets fed diets containing energy and protein - 4.1 MJ and 130 g per kg of feed, respectively.

The effect of long-term treating (6 months) with clenbuterol (1 (1 mg/kg diet) on both quantity and fatty acid composition of lipids from different adipose tissues (perirenal, tail, breast, intermuscular and caul) has been studied.

A decrease of lipid amount was established in adipose tissues in treated (castrated and noncastrated) animals compared to control groups.

Clenbuterol leads to some changes in the size of adipocytes and fatty acid composition of adipose tissue triacylglycerols. Changes observed are associated with physiological state of animals and localisation of fat depots.

INTRODUCTION

Species B-agonists are established to decrease lipid deposition, regardless of animal changes (WILLIAMS, 1987).Lipid content decrease, however, correlates not always to the B-agonists of fat layer thickness. Contradictory also are the results about the effect of does not correspond to the constancy in quantity of subcutaneous adipose tissue, established in cattle (SCHIAVETTA et al., 1990). Changes in adipose cell size in lambs (COLEMAN et al., 1985, HU et al, 1988) or cattle (MILLER et al., 1988) are different in various fat depots. Studies of HU et al., (1988) and THORNTON et al., (1985) show that clenbuterol and cimaterol increase unsaturation of lipid from subcutaneous adipose tissue in sheep. It is also established that the effect of clenbuterol on fatty acid composition also depends on the type of fat depots (BANSKALIEVA et al., 1991).

It is unknown, however, to what extent fatty acid profile corresponds to quantitative changes of lipids in depots, or of adipose cells, which also determined the trend of our studies. In this connection, the effect of long-term treating with clenbuterol has been studied in both castrated and noncastrated hoggets on both the content, adipocyte size and fatty acid composition of lipids from fat depots of different anatomical localisation.

MATERIAL AND METHODS

Two experiments on male noncastrated and castrated hoggets have been conducted. At starting the experiment, animals were 10 months old, 42 - 46 kg of live weight, divided in a control and two experimental groups for each experiment. Duration of experimental period was 6 months, animals being under the same rearing condition and received diet, containing 4.1 MJ energy and 130 g protein per 1 kg of diet. Experimental animals also received daily 1 mg of clenbuterol per kg of mixture. At the end of the experiment, 4 animals of each group were slaughtered. Hoggets of the second experimental group were slaughtered one week later, receiving no clenbuterol in this time.

Fresh perirenal, subcutaneous (around the tail), intermuscular (under m.Semimembranosus), breast (over 5-6th verteba) adipose tissues and caul were obtained at slaughtering. After 24h at 2°C, carcasses were divided into two parts by a transverse cut at 12 rib. Fat depths were measured at tail-base, at 5-6th vertebre of breast bone and forelast rib. The total subcutaneous fat was removed and was weighted, as well as the caul and perirenal adipose tissue.

The lipids of tissues were extracted thrice with CHCL3/MEOH (1:1;v/v). Fatty acid composition of triacylglycerols was analysed by gas chromatography, using a glass column (3mx2mm) packed with 3% SP 2330 on Supelcoport (100-200 mesh).

For determining the size of adipocytes, samples of 1 cm3 were taken, of which transverse cuts of 16 mm thickness were prepared. Coloration of adipocytes was made with Sudan III, and their mean diameter was determined in 3-5 visual field on 100 cells.

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

RESULTS AND DISCUSSION

Results presented in table 1 show that in castrated animals deposed subcuteneous fat and that in caul is by 70% more, compared to noncastrated. No differences exist between both types of animals, concerning the thickness of subcutaneous adipose tissue, measured in various topographic areas. Only in the area around the tail in noncastrated animals higher value in fat layer thickness was established, showing that in these animals accumulation of subcutaneous fat is more irregular.

To the increased subcuttaneous fat content in castrated animals, also correspond relatively more cells of greater diameter (figure 1). In intermuscular adipose tissue, maximum of curve in castrated animals is also removed to righter, while in perirenal adipose tissue, differences between castrated and uncastrated animals are minimum.

According to saturation extent TG of different fat depots (both in noncastrated and castrated animals) the following rank exists: breast adipose tissue, subcutaneous

adipose tissue, intermucular adipose tissue, perirenal adipose tissue and caul. Analogic result has also been established in lambs (BANSKALIEVA et al., 1991). No differences ha been observed between castrated and noncastrated animals.

Clenbuterol treatment has confirmed the effect of B-agonists (became alread classic) on deposition of reserve lipids. Regardless of the type of animals (castrated not) a decrease was established both in lipid content of various fat depots and in lay thicknoss of subcutaneous adipose tissue (table 1). This result differs from data SCHIAVETTA et al.,(1990) for cattle. The effect of clenbuterol has been esteblished depend both on anatomical localisation of depots and on physiological condition of anim (table 1). In noncastrated animals caul weight decreases drasticaly (over 50%), compared other depots. In castrated animals - contrariwise: such an effect is observed subcutaneous adipose tissue. Changes in perirenal adipose tissue are between 25-20 % for and II experiments, respectively. Similar are data of SCHIAVETTA et al., (199 BANSKALIEVA et al., (1991), while COLEMAN et al., (1985) find no effect of clenbuterol perirenal adipose tissue. Clenbuterol leads to decreasing the thickness of subcutaneo adipose tissue, but at significantly greater extent in noncastrated animals.

In general, it is to notice that reduced lipid content in fat depots in experimental animals is accompanied by decreasing of diameter in adipocytes and increasi the relative part of adipose cells of less dismeter (figure 1). Analogical results ha also been obtained in other investigation on sheep and cattle (MILLER et al., 1988, HU al., 1988, SCHIAVETTA et al., 1990). Decreasing the fat or thickness of adipose layer most likely conditioned by changes in the size of adipose cells. Similar to changes reserve lipids (table 1) the effect of clenbuterol on the size of adipose cell depend physiological condition and is different for various fat depots. To the sharp decrease subcutaneous adipose tissue in castrated hoggets (compared to noncastrated) corresponds a a more significant increase of relative part of cells of less diameter. Analogical is the result for intermuscular adipose tissue. On the contrary, in perirenal adipose tis (where changes in fats are less) the effect of clenbuterol is more clearly marked noncastrated animals. Data of SCHIAVETTA et al., (1990) and MILLER et al., (1988) point out a dependence between the effect of clenbuterol and anatomical localisation adipose depots. Results about fatty acid composition (table 2) show that different adipo tissues react in different way to clenbuterol treatment. With the exception of results our previous study in lambs (BANSKALIEVA et al., 1991) other investigations about B-agonists effect on lipid composition of various fat depots have not been made.

Clenbuterol treatment leads to no changes in fatty acid composition triacylglycerols of both breast and perirenal adipose tissue in experimental animals of both trials (table 2). An increase of total unsaturation of subcutaneous adipose tissue intermuscular adipose tissue and caul was established in noncastrated animals, condition by increasing of oleic acid and decrease of stearic one. Increasing unsaturation of lipids from subcutaneous adipose tissue was also established in investigations on both lambs and sheep (BANSKALIEVA et al., 1991, HU et al., 1988, THORNTON et al., 1985). In present experiments no significant changes have been observed in relative amount of 16:0, regarding to the of localisation of depots, not yet of physiological condition of hoggets. According to this result, data obtained differ from established ones in lambs fed high-concentrated die (BANSKALIEVA et al., 1991) as well as data of THORNTON et al ., (1985), HU et al., (1985). Palmitic acid content is in a certain extent indicator for fatty acid synthesis de novo. Results obtained suppose clenbuterol - used in recommended dose and duration - exerts effect in that process. In investigations of sheep COLEMAN et al., (1985) also find effect of clenbuterol on lipogenesis adipose tissue.

B-agonists probably do not change fatty acid synthesis de novo (EADARA et al., 19 EISEMAN et al., 1988) and rather exert influence on acylation of fatty acids in adipose tissue triacylglycerols and deposition of more reserve fats, respectively. Our data sh that reduced lipid content in studied lipid depots is not accompanied by respective chang in fatty acid composition. The cause of this effect is not yet known. In general, results about fatty acid composition (table 2), as well as those of plasma free fatty acids (unpublished data) do not suppose stimulation of lipolysis in adipose tissue after use B-agonists. No correspondance was observed between changes in the size of adipocytes in different fat depots and their fatty acid composition (figure 1, table 2). Increasing relative part of cells of less diameter in subcutaneous adipose tissue and intermuscular adipose tissue for castrated animals is not accompanied by changes in fatty acid composition. Results obtained show that between both quantity and thickness of adipose layer, on the one hand, and mean diameter of adipocytes on the other hand, no relationship exists (table 1, figure 1).

At a week withdrawal period clenbuterol effect decreases in different extent on different parameters studied. Animals of experimental groups begin to depose fats, reaching in uncastrated animals and even surpassing the quantity of subcutaneous reserve fats of control animals. A similar trend is also observed in caul, changes in thickness of adipose layer, however, were not observed (table 1). In general, a decrease of relative part of cells of less diameter is observed, regardless of depot and state of animals (figure 1). Changes in fatty acid composition of some cases are directed to control animals, in other such ones were observed (table 2).

Results obtained show that clenbuterol exerts in different way effect on different processes in organism of animals,the exact mechanism of their influence being not yet kno

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Table 1.

Carcass Variables

Animals

Variable / Groups	Noncastrated Hoggets C	Noncastrated Hoggets E1	Noncastrated Hoggets E2	Castrated Hoggets C	Castrated Hoggets E1	Castrated Hoggets E2
Total subcutaneous adipose tissue wt, g						
Caul wt, g	503 ± 13 a	430 ± 42	540 ± 23	858 ± 14 b,1,2	473 ± 39 4,2	673 ± 11 4
Perirenal adipose tissue wt, g (PAT)	274 ± 56 a,1	118 ± 20 2	158 ± 15 1,2	477 ± 7 b,1,7	396 ± 8 4,7	255 ± 21 4
Fat thickness, cm:	119 ± 16	103 ± 14	90 ± 8	141 ± 15	115 ± 26	142 ± 27
tail-base	0.54 ± 0.08 a,1	0.31 ± 0.06 1,2	0.23 ± 0.05 2	0.38 ± 0.03 b	0.32 ± 0.08	0.17 ± 0.06
forelast rib of	0.23 ± 0.05 1	0.12 ± 0.07 4	0.19 ± 0.01 2,4	0.21 ± 0.05	0.19 ± 0.03	0.15 ± 0.03
breast bone	0.52 ± 0.02 1	0.28 ± 0.01 4	0.26 ± 0.09 2,4	0.51 ± 0.01 1	0.32 ± 0.02 4	0.63 ± 0.06 1,2
5-6th vertebra						
Caul:						
moisture (%)	8.1 ± 1.5 1	20.7 ± 7.1 4	29.5 ± 1.5 4	6.1 ± 1.9 1	13.7 ± 1.6 2,3	17.3 ± 1.9 3
fat (%)	81.9 ± 3.4 1	73.9 ± 8.2 1	72.6 ± 0.9 1,2	84.2 ± 2.6	81.6 ± 5.6	76.5 ± 2.1
PAT:						
moisture (%)	8.2 ± 0.1 1,2	23.2 ± 1.2 4,2	18.1 ± 0.6 4	6.6 ± 0.3 1	17.1 ± 1.4 4	18.8 ± 1.1 4
fat (%)	80.8 ± 1.1 1	69.8 ± 0.3 4	73.3 ± 1.1 3	82.1 ± 2.2 1	73.2 ± 1.7 2,3	71.9 ± 0.8 3

C-control group; E1, E2 - experimental groups; If the smallest possible difference between superscribs (D) is:D=1, P<0.05, D=2, P<0.01, D=3, P<0.01; a,b-significant differences between control groups.

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

Adipose Tissues	Breast			Subcutaneous			Intermuscular			Perirenal			Caul	
Groups	C	E1	E2	C	E1	E2	C	E1	E2	C	E1	E2	C	E1
Fatty acids														
							N O N C A S T R A T E D							
16:0	25.2	25.8	24.5	24.3	24.2	25.2	23.4	24.9 ^{2,3}	23.0 ^{1}	23.3	21.2	22.9	24.4	23.3 ^{1}
16:1	6.1	6.8	5.9	2.8	3.3	2.8	2.1	2.8	3.2	2.2	2.4	2.4	2.7	2.7
18:0	12.0	11.1	11.4	24.8	20.2 ^{1}	22.6 ^{2}	30.1 ^{1,2}	24.0 ^{1}	21.7 ^{2}	32.7	35.9	31.0	31.1	30.4 ^{1}
18:1	50.4	50.1 ^{1,2}	51.0 ^{1}	42.2	47.3 ^{2}	42.6	38.5	42.6	44.8 ^{2}	36.1	35.6	38.5	35.5	39.6
18:2	6.2	6.0	7.4	5.8	4.9	7.1	5.8	5.7	7.2	5.6	4.8	5.2	6.1	4.3
TUFA:	63.6	62.9	64.3	50.8	55.5	50.5	46.4	51.1	55.2	43.9	44.6	46.1	44.3	46.6
							C A S T R A T E D							
16:0	24.5	24.9 ^{1}	24.6 ^{2}	26.0 ^{1,2}	23.7	25.1	25.1	25.4 ^{1,2}	24.7 ^{1}	22.5	22.1	23.8	25.5	24.2
16:1	7.2	4.6	5.8	3.1	3.0	3.2	2.4	2.7	2.3 ^{2}	2.4	2.7	2.6	2.8	2.7 ^{1}
18:0	10.1	14.4	10.8	24.2	27.8	23.8	26.4	24.4	28.0	32.6	33.3	35.2	31.2	31.9 ^{1}
18:1	52.9	51.1 ^{1,2}	52.6 ^{1}	42.9 ^{2}	40.5 ^{1}	41.7 ^{2}	41.7 ^{4}	43.1 ^{1}	39.0 ^{1}	38.0	36.6	36.0	35.2	36.7 ^{1,2}
18:2	5.2	4.9	6.2	3.7	4.7	6.2	4.4	4.3	5.9 ^{3}	4.5	5.3	5.9	5.3	4.5
TUFA:	65.3	60.6	64.6	49.7	48.2	51.1	48.5	50.1	47.2	44.9	44.6	44.5	43.3	43.9

C - control group; E1,E2 - experimental groups; TUFA - total unsaturated fatty acids; The smallest possible difference between superscribs (D) is: D = 1, $P < 0.05$; D = 2, $P < 0.01$.

Fig.1. Adipocyte diameter distribution of subcutaneous (I), perirenal (II) and intermuscular (III) adipose tissues.