Effect of Clenbuterol on Lipid Metabolism in Fattening Lambs

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SUMMARY: The effect of clenbuterol on both growth and fatty acid composition of different adipose tissues in fattening lambs was studied.

Administration of clenbuterol reduces the total body fat, especially the perirenal fat and of caul, as well as the thickness of different fat depots.

In all adipose tissues investigated clenbuterol reduces the relative amount of 16:0, but does influence differently on the other fatty acids. The unsaturation of both intermuscular and perirenal adipose tissues in treated animals did not change, however this of caul decreased and that of both subcutaneous and breast increased. No significant differences were established in treated animals after a week withdrawal period.

The results obtained show that clenbuterol exerts a different effect not only on the amount of depot lipids but also on their composition.

INTRODUCTION: Clenbuterol and cimaterol /became already "classical"/ are known to reduce deposition of reserve lipids in both monogastric and in ruminants. Mechanisms of that process are not yet clear, inducing numerous investigations. Results in some of them show that B-agonists depress lipogenesis and stimulate lipolysis in adipose tissue "BEERMAN" et al. (1985), BERSHAUER (1989), THORNTON et al. (1985)" and in other they are of contrary effect "MILLER et al. (1988), HU et al. (1988)" or exert no effect "COLEMAN et al. (1985), SCHIAVETTA et al. (1990)". Quantitative changes in reserve lipids occasionally are accompanied with a decreasing of fat layer thickness, with number and size of fat cells "BOHOROV" et al.(1987), COLEMAN et al. (1985), MILLER et al. (1988)".

Information about fatty acid profile of lipids from reserve depots after treating with B-agonists was obtained at investigations of HU et al.(1988), THORNTON et al. (1985)" in sheep. Treating, both with cimaterol and clenbuterol, although in different degree, leads to changes in relative fatty acid content of lipids from subcutaneous adipose tissue. In our previous investigations on lambs "BANSKALIEVA et al. (1989)" it was established that under the influence of feeding factor triacylglycerols change specifically their fatty acid composition depending on localisation of adipose tissues.

It is of interest to be studied to what extent quantitative changes of lipids from different adipose tissues are also accompanied to changes in their fatty acid composition after treating with B-agonists. It was the purpose of the present study on lambs where the effect of clenbuterol has been studied, applied in the stage of intensive fat deposition.

MATERIALS and METHODS: Experiment has been conducted on male lambs, semifine-fleeced. After weaning at an age of 45 days (average live weigth of 15.4 kg), animals received for 90 days a diet containing energy and protein - 6.0 MJ and 200 g/kg diet respectively. After reaching 26.5 kg of live weight lambs were divided into 3 groups (8 animals each). During 6 weeks (until the end of experiment) animals of both experimental groups received daily additionally 10 mg clenbuterol per kg of diet. Feed intake (ad libitum) was recorded daily, and live weight - every 14 days.

At the end of experiment 4 animals of each control and the first experimental groups have been slaughtered. Lambs of the second experimental group were slaughtered a week later, during that time they received no clenbiterol. Up till slaughter the animals were given free access to water and food.

Fresh perirenal, subcutaneous (around the tail), intermuscular (around m.Semimebranosus), breast (over 5-6th vertebra) adipose tissues and caul were obtained at slaughtering. After 24 h at 2 C carcasses were divided into two parts by a transverse cut at 12th rib. Fat depths were measered at tail-base, at 5-6th vertebra of breast bone and forelast rib. The total subcutaneous fat was removed from the left side of each carcass. The lipids of tissues were extracted thrice with CHCL /MEOH (1:1;v/v) and stored in a solution of 0.1% (w/v) of butylhydroxytoluen in chloroform at -30 C.

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 at 40 C for 15h. The fatty acid composition was analysed by gas-liquid chromatography, using a metal 2 4 column (3mx2mm) packed with 3% SP 2330 on Supelcoport (100-200 mesh). The Student test was used as a criterion for statistical evaluation of results.

RESULTS and DISCUSSION: The control and treated animals had equal live weights (about

39 kg) after 42 d on trial (table 1). However clenbuterol treated lambs had heavier deboned carcass, probably as a result of increasing weight of single muscles "SHINDARSKA etal. (1991)", as well as of decreased lipid content in carcass (table 1). The ratio between slaughter carcass weight and deposed fat is 1:12 and 1:22 for control and experimental group respectively. Results obtained differ from those of SCHIAVETTA et al. (1990) in calves also treated in the stage of intensive deposition of reserve fats, where clenbuterol does not change quantitative characteristics of lipid depots. Total subcutaneous fat (table 1) also decrease significantly (by about 27 %), this being analogically to data of THORNTON et al. (1985). It shoud be noted that the effect of clenbuterol on lipid content in the carcass was discernable, even at a visible comparison of both control and experimental animals. Drastic decreases are observed in the quantities of caul (by 52%) and perirenal adipose tissue (by 39%), as well as of lipids in these two depots. Results are similar to the communication of BOHOROV et al.(1987), while COLEMAN et al.(1985) find out no changes.

Incorporation of clenbuterol also leads to significant changes in the fat layer thickness - a finess is observed by about 50 % at 5-6 th rib, less at the tail layer and that at breast bone (table 1). Changes obsrved in fat content of different depots follow the changes in total lipid content of carcass "SHINDARSKA" et al. (1991).

Decreasing of fats of clenbuterol-treated animals is accompanied with specifical changes in fatty-acid composition of TG of each investigated tissue (table 2). A common for all depots is decreasing of relative part of 16:0 and increasing of 18:2, while the effect on the other fatty acids is not synonymous. Stearic acid content does not change at breast and subcutaneous adipose tissue and it increases for other three ones. Contrary, the level of oleic acid is constant in intermuscular fat, significantly decreases in caul and perirenal adipose tissues. Its elevated content in TG of both breast and subcutaneous adipose tissue also conditions higher total unsaturation of lipids from these both depots. Changes are interesting in breast adipose tissue, being differently conservative to changes "BANSKALIEVA et al. (1989)". Changes in 16:0 and18:1 levels are analogical to the communication of HU et al. (1988), THORNTON et al.(1985) for subcutaneous adipose tissue in sheep.

In more species as well as in sheep, the rate of fatty acid synthesis of adipocytes is known to be directly proportional to the cell size "HOOD et al. (1982)". Relative palmitic acid content is known to be an indicator for the degree of biosynthesis of fatty acids de novo in different adipose tissues "INGLE et al. (1972)". Decreased level of that acid (table 2), changes in the actuvity of some lipogenic enzymes, paralelly to smaller sizes of fat cells in some depots "MILLER et al. (1988)", support the affirmation for depressing the lipogenesis after treating with B-agonists. According to the results of THORNTON et al.(1985), BERSHAUER (1989), reduction of reserve lipids is due to decreased lipogenesis. Sharply thining out of fat layer (table 1) is probably as a result of that process. MILLER et al. (1988), SCHIAVETTA et al. (1990) however established no changes in lipogenesis of both intermuscular and subcutaneous adipose tissue. The effect of B-agonists on lipogenesis has not been studied in the other adipose tissues.

Regardeless of the similarities of changes in fatty acid composition after treating with B-agonists, results of HU et al. (1988) for increasing lipogenesis in subcutaneous adipose tissue in sheep are opposite to those of THORNTON et al. (1985).

In high-concentrated feeding of lambs, incresed insulin secretion "DIMOV et al. (1989)" stimulate lipid synthesis and deposition of more reserve lipids "BANSKALIEVA et al. (1989)". Treating with clenbuterol, however, does not change the content of that hormone (unpublished data). Probably in this case the inhibiting effect of the compound on the fat deposition is exerted by a reduced lipogenesis, presumamly caused by decreased insulin sensitivity

A week after terminating the treating with clenbuterol, no significant changes are observed in traits studied (table 1). In fatty acid composition a certain trend exists toward reaching the values of control group (table 2), being different for different fatty acid and for each adipose tiessue. From these data results a question to what extent occured changes are reversible after terminating the treating.

The results obtained show that clenbuterol exerts a different effect not only on the amount of depot lipids but also on their fatty acid composition. They are an evidence for

complex and tissue specific interactions of B-agonists with different metabolite processes in the organism.

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Table 1. C a r c a s s v a r i a b l e s

Variable	G R O U P S		
	control	experimental 1	experimental 2
Live weight wt,kg	38.5 + 1.5	39.0 + 1.0	39.0 + 1.0
Deboned carcass wt, kg	14.6 + 0.5	19.3 + 0.5	19.0 + 0.4
Fat thickness, cm:			
tail-base	1.48 + 0.15	1.08 + 0.19	1.08 + 0.15
forelast rib of			
breast bone	0.50 + 0.11	0.26 + 0.02	0.28 + 0.07
5-6th vertebra	1.41 + 0.20	1.10 + 0.10	1.10 + 0.10
Total subcutaneius			
adipose tissue wt, kg	1.20 + 0.25	0.88 + 0.04	0.88 + 0.05
Perirenal adipose			
tissue wt, kg (PAT)	0.26 + 0.05	0.13 + 0.01	0.13 + 0.01
	1	3	2
Caul wt, kg	0.52 + 0.06	0.33 + 0.02	0.38 + 0.02
	1	1,2	3
PAT fat (%)	80.4 + 3.0	79.2 + 3.6	71.7 + 2.2
Caul fat (%)	78.2 + 4.1	70.7 + 6.2	69.3 + 5.1

If the smallist possible difference between the superscrips (D) is:
D = 1, p < 0.05, D = 2, p < 0.01

TABLE 2. Fatty acid composition of different adipose tissues from both control and clenbuterol-fed lambs

Fatty acids	G R O U P S					
	control	exp. 1	exp. 2	control	exp. 1	exp. 2
	Breast adipose tssue			Subcutaneous adipose tissue		
14:0	6.9 + 0.9	5.6 + 0.8	6.0 + 0.4	4.4 + 0.5	3.9 + 0.5	4.2 + 0.7
				1	2	2
16:0	29.7 + 1.8	23.9 + 2.6	25.5 + 1.1	26.6 + 0.6	22.7 + 1.2	23.1 + 1.0
16:1	3.4 + 0.5	3.7 + 0.6	3.1 + 0.1	2.0 + 0.1	2.0 + 0.1	2.4 + 0.5
18:0	8.6 + 0.5	8.6 + 1.6	10.5 + 1.1	16.4 + 1.0	16.8 + 1.2	15.1 + 2.7
18:1	47.5 + 2.5	54.2 + 3.9	49.0 + 1.7	45.9 + 1.6	49.2 + 0.6	49.4 + 0.7
18:2	3.9 + 0.3	4.1 + 0.3	5.9 + 1.3	4.7 + 0.3	5.2 + 0.7	6.1 + 1.0
	Intermuscular adipose tissue			Perirenal adipose tissue		
14:0	5.0 + 0.6	5.2 + 0.2	6.1 + 0.3	4.6 + 0.5	3.8 + 0.6	3.9 + 0.6
	1	2	1,2	1	2	2
16:0	26.0 + 0.9	22.2 + 0.9	25.0 + 1.0	25.4 + 1.2	20.6 + 1.2	20.5 + 1.0
16:1	2.2 + 0.2	2.9 + 0.3	2.4 + 0.2	2.1 + 0.4	2.1 + 0.1	1.6 + 0.3
	1	3	1	1	2	2
18:0	16.6 + 0.9	20.8 + 0.3	16.6 + 1.2	20.4 + 0.8	26.5 + 2.3	26.1 + 2.1
				1	1,2	2
18:1	44.6 + 0.7	44.2 + 1.2	43.6 + 1.3	42.4 + 0.6	40.8 + 0.8	40.7 + 0.3
18:2	4.6 + 1.2	4.8 + 0.4	6.3 + 1.3	5.0 + 0.9	6.2 + 1.3	6.7 + 1.4
	C a u l					
14:0	5.3 + 1.2	5.0 + 1.0	5.4 + 0.9			
	1	1,2	2			
16:0	28.3 + 0.9	24.7 + 1.8	24.6 + 1.0			
16:1	1.8 + 0.1	1.8 + 0.1	1.7 + 0.1			
18:0	20.8 + 0.7	26.5 + 2.9	21.9 + 1.5			
	1	3	1			
18:1	39.2 + 0.3	36.2 + 0.7	39.0 + 0.2			

Jf the smallest possible aifference between the superscrups (D) is: D = 1, p < 0.05; D = 2 p < 0.01