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*DEPARTMENT OF ANIMALS PHYSIOLOGY, VETERINARY FACULTY, AGRICULTURAL ACADEMY IN LUBLIN
**DEPARTMENT OF ANATOMY, BIOCHEMISTRY AND VETERINARY PHYSIOLOGY, UNIVERSITY OF PISA

Ryszard BOBOWIEC*, Franco MARTELLI**,
Krystyna RADYMSKA-WAWRZYNIAK*

Growth Efficiency, Liver Response, Acid-Base and Haematological Status of Chicken During Exposure to Anabolic Effects of β-Adrenergic Agonist Clenbuterol

Wpływ β-adrenergicznego agonisty clenbuterolu na masę ciała, wzrost, metabolizm wątroby oraz stan układu hematologicznego i równowagi kwasowo-zasadowej u kurcząt

A special interest of β-adrenergic agents responsible for repartitioning effect in metabolism was lain in reducing fat deposition. The exact mode of action of β agonists is continually under consideration. Some results (4, 5, 16, 17, 20) point to stimulation of lipolysis and termogenesis by products belonging to that group via augmentation of cyclic adenosine-3'5' monophosphate (c AMP) in adipocytes. Others underline rather anti-lipogenic activity of β agonistis and positive effect on protein metabolism (9, 11, 13, 15). Such alternations in metabolism, feed intake and feed efficiency are conceivably affected, but to date it has not yet been established unequivocally whether clenbuterol improves these parameters. Taking into account the second settlement according to which β agonist acts as a suppressor of creation of a new lipid we performed experiments on chickens to arrange impact of such an agent on bile salts production, fat deposition and growth performance. We hypothesised also that as a consequence of increased protein synthesis there have to be showed changes in acid-base balance and other blood constituencies.

MATERIAL AND METHOD

Chicken at the start of treatment with clenbuterol were 4 weeks old weighing 735-745 g and were fed *ad libitum* on a standard diet called mixture "Starter" until 1 month and "Finisher" up to bleeding at the 2nd month. The second mixture contained 22% crude protein and 12.2 MJ of ME/kg of DM. The animals were weighted daily, and at the beginning of experiment were separated into 3 groups according to dose of anabolic agent employed. The first group served as a control, the second one received clenbuterol *per os* (2.5 mg/kg b.w.) each day and the third, 5 mg/kg b.w. in the same manner. At the end of each treatment chicken were stunned and exsanguinated. Liver, alimentary tract and suet fat were dissected and weighed. Bile from gall bladder was collected volumetrically and used for quantification of bile salts according to the methods described by Levin et al. (12). Blood gas samples were collected into heparinized tubes placed on ice and analysed for PO_2, PCO_2 and pH within 0.5 h of sampling. Additional blood samples for haematological analysis were collected via plastic syringes and placed in heparinized tubes. All blood gas and pH samples were analysed on

a Blood Gas Analyser (Plastomed, Poland). Haematology values for hemoglobin (Hb, g dl^{-1}) hematocrit (%) red blood cells count (μl^{-1}) and white picture (%) were determined manually. Means and standard errors of means are presented; differences amount means were assessed by student t-test.

RESULTS

GROWTH PERFORMANCE

Both in the first and the second treated group blood weight gains were greater as compared to control birds, however the difference was statistically significant ($p < 0.05$) only for the second group (Tab. 1). In the treated chicken there was a demonstrable decline in feed intake, especially under the influence of a higher dose of clenbuterol. However, at each dose of clenbuterol feed efficiency (g gain/g food) was increasing significantly over a control group only for the first and last four days (Fig. 1). When the highest dose was used increases amounted to 134% of control group at the 10th day of experiment. Liver weight appeared to be enhanced in both groups as the difference approached significance at the 5% level. In relation to 100 g body weight liver weight was only increased in the first group. Clenbuterol increased individual body weight (1996 $\pm$224.87 g) (Tab. 1) compared with the control birds (1887.33 $\pm$221.66 g) at the 60th day. Clenbuterol had significant reduction effect on fat content despite the augmentation of body weight. Although only mesenteric fat was checked, probably entire fat content in the whole bird was reduced. In the first group the clenbuterol displayed a maximum reduction response. In this group both the greatest weight of alimentary tract and liver weight was observed. In both treated groups clenbuterol feeding had a significant effect on absolute weight of liver and alimentary tract but only significant effect on relative weight of liver and alimentary tract after treatment 2.5 mg/kg. Simultaneously the ratio (proportion of liver weight to body weight) liver: body weight was the biggest in the first group (2.75) in comparison to the control group (2.29).

BILE ACID CONCENTRATION AND CONTENTS

We take into account that during fasting phase of bile secretion about 80% of the volume of bile is accumulated in gall bladder and about the same quantity of bile salts pool remains in this part of bile tree. The biggest dose of clenbuterol had little effect on bile salts content in gall bladder (47.22 and 45.65 μM respectively), although concentration of bile salts after treatment of these dose dropped significantly ($p < 0.05$). Quite the opposite effect clenbuterol exerts in the dose of 2.5 mg/kg. The concentration of bile salts pronouncedly decreased to the low level 28.07 μM/ml with simultaneous increase of bile salt content to the value of

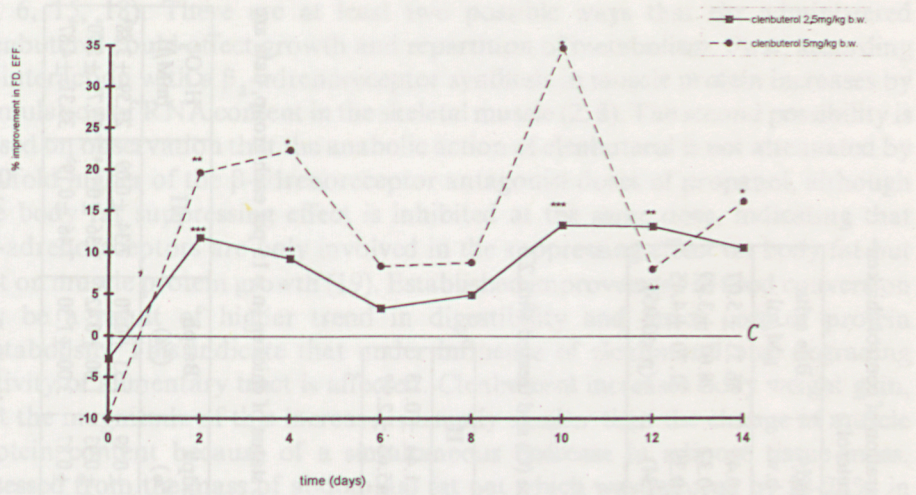

Fig. 1. Effect of daily clenbuterol feeding on feed efficiency. The percentage in improvement in average feed efficiency (FD EFF – gram gain/gram feed intake) compared to group C is plotted. N = 15. Significance: * $p<0.05$, ** $p<0.01$, *** $p<0.001$

53.61 μM which represents about 80% of bile salts pool. Treatment of chicken with clenbuterol caused bile volumes substantially increased in gall bladder which attained 1.91 ±0.19 ml in the second group versus 1.30 ±0.21 ml in the control group (Tab. 2).

HAEMATOLOGY

Dates collected from the control group are generally within the range normally expected for healthy chicken (Tab. 3). Clenbuterol-fed chicken had the same values of Hb (10.34 g%) as the control chicken. However the mean red blood cells (RBC) from group one was higher ($p<0.05$) than that of RBC from the control chicken. The percentage of hematocrit (Ht) with the value about 34% in the control group was similar in the clenbuterol-fed chicken and tended to be lowered in second group clenbuterol-fed chicken. Basic measurements of blood gas and pH are presented in Tab. 3. Standard bicarbonate levels and pH were all significantly higher in the second clenbuterol-fed group. Conversely, reductions in HCO_3 were seen for samples from the first group chicken. Values of pH in the first group were comparable to those seen in the control group.

DISCUSSION

The results of this experiment indicate that clenbuterol given orally is an effective anabolic agent in chicken. An increase of 13.4% in daily live weight gain in clenbuterol-fed birds over 30-day-period confirms the work by other authors

Tab. 1. Response of final body weight, tissue weights and bile acids concentration of control chicken (C) and treated with 2.5 mg (I) and 5 mg (II) of clenbuterol

	Body weight (g)	Liver weight/ /100 g b.w.	Intestine/ /100 g b.w.	Fat pad/ /100 g b.w.	Bile salts μM/ml
C	1877.33 ±221.66	2.30 ±0.35	8.20 ±0.63	1.03 ±0.45	36.33 ±5.07
I	1953.33 ±264.75	2.76 ±0.26	10.20 ±1.05	0.79 ±0.32	28.07 ±3.99
II	1996.00 ±224.87	2.30 ±0.35	8.20 ±0.98	0.95 ±0.39	31.70 ±4.45
I–II	(p=0.638)	(p<0.001)	(p<0.001)	(p=0.34)	(p<0.05)

Tab. 2. Bile volumes and bile salts content of gall bladder of control chicken (C) and treated with 2.5 mg (I) and 5 mg (II) of clenbuterol

Bile parameters	C	I	II
Bile volumes (ml)	1.31 ±0.14	1.91 ±0.19	1.44 ±0.13
Bile salts (μM)	47.22 ±4.32	53.61 ±4.82	45.65 ±2.97

Tab. 3. The effect of clenbuterol at doses of 2.5 mg (I) and 5 mg (II) on hematology and acid-base status of chicken (n=15 per each group, data are means, ±SEM)

	Hb ($g\ dl^{-1}$)	Ht (%)	RBC ($mil\ \mu l^{-1}$)	Lymphc (%)	Monc (%)	Neutph (%)	Acidph (%)	Basph (%)	pH	HCO_3^- (mM/l)
C	10.64 ±1.04	34.00 ±2.62	2.434 ±0.304^a	66 ±10.53	1 ±0.01	31.06 ±10.34	2.20 ±0.39	1.33 ±0.70	7.34 ±0.09	27.59 ±3.88^a
I	10.34 ±1.19	33.00 ±3.13	2.648 ±0.462^b	67 ±5.70	1 ±0.41	29.00 ± 5.56	2.00 ±0.93	1.00 ±0.42	7.36 ±0.11^a	25.71 ±5.70^a
II	9.54 ±1.41	32.53 ±2.64	2.165 ±0.640^c	61 ±13.90	2 ±1.14	31.00 ±13.29	3.00 ±0.87	3.00 ±1.30	7.46 ±0.19^b	33.31 ±4.87^b

Means with dissimilar superscript in each column are significantly different (p<0.05).
Hb – hemoglobin, Ht – hematocrit, RBC – red blood cell, Lymphc – lymphocyte, Monc – monocyte, Acidph – acidophile, Basph – basophile.

(1, 6, 15, 18). There are at least two possible ways that the administered clenbuterol could affect growth and repartition of metabolism. First, according to interaction with a β_2-adrenoreceptor synthesis in muscle protein increases by stimulation of RNA content in the skeletal muscle (2, 8). The second possibility is based on observation that the anabolic action of clenbuterol is not attenuated by 100fold higher of the β-adrenoreceptor antagonist doses of propanol, although the body fat suppressing effect is inhibited at the same dose, indicating that β_2-adrenoreceptors are only involved in the suppressing effect on body fat but not on muscle protein growth (19). Established improvement in food conversion my be a result of higher trend in digestibility and lesser cost of protein metabolism. This indicate that under influence of clenbuterol also degrading activity of alimentary tract is affected. Clenbuterol increases body weight gain, but the magnitude of this increase is usually smaller than the change in muscle protein content because of a simultaneous decrease in adipose tissue mass, assessed from the mass of abdominal fat pat which was reduced by 18-23% in clenbuterol treated chicken. The results show that the clenbuterol increased growth more effectively in the second group of chicken which received clenbuterol in a dose 5 mg/kg b.w. To prove conclusively that clenbuterol exerts a proper anabolic response we tested some liver and hematologic features. We found that the growth improvement caused by feeding chicken with 2.5 mg/kg clenbuterol is associated with most clearly seen enlargement in liver mass up to 2.76 g/100 g b.w. and suppression in bile acid concentration in gall bladder bile to the level of 28.07 $\pm$μM/ml in the first group, tentatively due to as a result of stimulation of protein synthesis with simultaneous depression of fatty acids synthesis. The observed increase in bile acid pool size in the first group is solely a consequence of augmentation of the bile volume (3, 7, 10, 14) because the concentration of bile salts drops down. In addition, our experiments have shown that in the case of enlargement of bile volume in the first group the bile was more fluid and clarified. Therefore it contained less bile salts and other lipids. The reduction of fat content and increased bowels weight were common features. These facts demonstrate that lipids metabolism was reduced and there was a change towards protein synthesis. In this study we have given particular attention to the bile lipids content and liver parameters because of the close relationship between biliary lipid content and their metabolism in the liver (22).

In the current experiment average values of pH and HCO_3 increased expecially in the second treated group. According to Henderson – Hasselbach equation, blood pH, of which the HCO_3 system is the principal buffer, is determined by the ratio of base (HCO_3) to acid (pCO_2) (21). As acidogenic factors of blood drop down, HCO_3 increases. This causes suppression of the acid load and elevated pH. However, as discussed by Schaffer et al. for any given HCO_3 in metabolic alkalosis there is a wide range of pH (hydrogen ion concentration). Without additional data on urine electrolytes and blood

concentrations electrolytes (NH_2, lactate) it is difficult to make a definite diagnosis. Ammonia is very soluble in water (520 g L^{-1}) and reacts with water to form NH_4 and OH^-. This suggest that large protein synthesis is associated with a large transport of NH_2 group (exert alkalogenic effect) with resetting of acid-base status. At physiological pH (7.4) about 99% of the NH_3 is actually NH_4 and only 1% is NH_3. Birds are principally uricotelic, but significant levels of other N waste are present in their excreta, including ammonia (2-30% N) and urea (2-10% N). For the blood parameters measured the clenbuterol-fed birds tended to have lower values of Hb content (9.54 g%) and Ht (32.53%). The increase in the number of RBC in the first treated group may account for the better supply of oxygen to the tissue. Taking into account lack of significant changes in total WBC and their differential counts forms, we can assume that under the influence of clenbuterol the treatment of immunological systems of treated birds is effective and stable.

REFERENCES

1. Bobowiec R., Radymska-Wawrzyniak K.: Effect of beta-2-adrenergic agonist clenbuterol on plasma lipoprotein fractions, fatty acid content and muscle fiber types in chickens. Medycyna Wet. **50**, 9, 1994.
2. Bohorov By. O., Buttery P. J., Correia J. H. R. D., Soar J. B.: The effect of the beta-2-adrenergic agonist clenbuterol or inplanation with oestradiol plus trenbolone acetate on protein metabolism in wether lambs. Br. J. Nutr. **57**, 99, 1987.
3. Caple I. W., Halpin C. G., Heath T.: Bile and pancreatic secretion in chicken: the effects of bile salts, feeding and acid. Comp. Biochem. Physiol. **61A**, 653, 1978.
4. Choo J. J., Horan M. A., Little R. A., Rothwell N. J.: Anabolic effects of clenbuterol on skeletal muscle are mediated by β_2-adrenoreceptor activation. Am. J. Physiol. **263**, E50, 1992.
5. Dalrymple R. H., Ricks C. A., Baker P. K., Pensack J. M., Gingher P. E., Ingle D. L.: Use of the agonist clenbuterol to alter carcass composition in poultry. Poultry Sci. **63**, 2376, 1984.
6. Dalrymple R. H., Baker P. K., Gingher P. E., Ingle D. L., Pensack J. M., Ricks C. A.: A repartitioning agent to improve performance and carcass composition of broilers. Poultry Sci. **63**, 2376, 1984.
7. Eyssen H., Vandeputte M., Evrard E.: Effect of various dietary bile acids on nutrient absorption and on liver size in chicks. Arch. int. Pharmacodyn. **158**, 292, 1965.
8. Hu C. Y., Suryawan A., Forsberg N. E., Dalrymple R., H., Ricks C. A.: Effect of cimaterol on sheep adipose tissue lipid metabolism. J. Anim. Sci. **66**, 1393, 1988.
9. Jones R. W., Easter R. A., McKeith F. K., Dalrymple R. H., Maddock H. M., Bechtel P. J.: Effect of the adrenergic agonist cimaterol (CL 263, 780) on the growth and carcass characteristics of finishing swine. J. Anim. Sci. **61**, 905, 1985.
10. Uste C., Demarne Y., Corring T.: Response of bile flow, biliary lipids and bile acid pool in the pig to quantitative variations in dietary fat. J. Nutr. **113**, 1691, 1983.
11. Lafontan M., Berlan M., Prudhon M.: Les agonistes beta-adrenergiques. Mecanismes d'action: lipomobilisation et anabolisme. Reprod. Nutr. Develop. **28**, 61, 1988.
12. Levin S. J., Johnson C. G., Boyle A. J.: Spectrophotometric determination of several bile acids as conjugates. Anal. Chemistry **33**, 1407, 1961.

13. Ligget S. B., Shah S. D., Cryer P. E.: Characterization of beta-adrenergic receptors of human skeletal muscle obtained by needle biopsy. Am. J. Physiol. **254**, E795, 1988.
14. Lisbona F., Jimenez R., Esteller A.: Secretion biliar en el pollo: efectos de la secretina, colecistoquinina – pancreozimina y del extracto de mucosa duedenal. Rev. Esp. Fisiol. **42**, 199, 1986.
15. MacLennan P. A., Edwards R. H. T.: Effects of clenbuterol and propranolol on muscle mass: evidence that clenbuterol stimulates muscle beta-adrenoreceptors to induce hypertrophy. Biochem. J. **264**, 573, 1989.
16. Maltin C. A., Hay S. M., Delday M. I., Smith F. G., Lobley G. E., Reeds P. J.: The action of the β-agonist clenbuterol on protein metabolism in innervated and denervated phasis muscle. Biochem. J. **261**, 965, 1989.
17. McElligot M. A., Barreto A., Chaung L. Y.: Effect of continuous and intermittent clenbuterol feeding on rat growth rate and muscle. Comp. Biochem. Physiol. **92C**, 135, 1989.
18. Muir L. A., Wein S., Duquette P. F., Olson G.: Effect of the β-adrenergic agonist L-640,033 on lipid metabolism, growth and carcass characteristics of female broiler chicks. J. Anim. Sci. **61**, 138, 1985.
19. O'Lamhna M. P., Roche J. F.: Effect of long or short acting anabolic agents, given singly or repeated, on growth rate and carcase weight of steers. Wet. Rec. **114**, 182, 1984.
20. Pringle T. D., Lonergan S. M., Calkins C. R., Jones S. J., Miller P. S., Koohmaraie M.: Temporal response of rabbits to adrenergic agonist feeding: tissue weight, calpains and calpastatin activities and nucleic acid and protein concentrations. J. Anim. Sci. **72**, 68, 1984.
21. Schneider P. L., Beede D. K., Wileox C. J.: Effects of supplemental potassium and sodium chloride salts on ruminal turnover rates, acid-base and mineral status of lactating dairy cows during heat stress. J. Anim. Sci. **66**, 126, 1988.
22. Vestergaard M., Henckel P., Oksbjerg N., Sejrsen K.: The effect of cimaterol on muscle fiber characteristics, capillary supply, and metabolic potentials of longissimus and semitendinosus muscles from young friesian bulls. J. Anim. Sci. **72**, 2298, 1994.

STRESZCZENIE

W przeprowadzonych badaniach na kurczętach postanowiono określić parametry hematologiczne, reakcje wątroby i efekty produkcyjne u kurcząt pod wpływem β-adrenergicznego stymulatora wzrostu (clenbuterolu). Kurczęta w wieku 1 miesiąca podzielono na trzy grupy: kontrolną i dwie doświadczalne, którym podawano codziennie *per os* clenbuterol w dawkach 2,5 i 5 mg/kg m.c. Clenbuterol powodował statystycznie istotny wzrost masy ciała (do 1996 ±224 g) w porównaniu do grupy kontrolnej (1887 ±221 g). Nie zaobserwowano istotnych różnic w masie ciała ptaków w zależności od dawki preparatu. U badanych kurcząt stwierdzono efektywniejsze o 15% wykorzystanie paszy mierzone stosunkiem zużycia paszy do przyrostu masy ciała. Pod wpływem clenbuterolu dochodziło do obniżenia zawartości depo tłuszczowego (0,79/100 g m.c.) z równoczesnym zmniejszeniem stężenia soli żółciowych z 36,33 do 28,07 μM/ml. Stosowany preparat nie zmieniał w istotny sposób badanych parametrów hematologicznych, obserwowano jednak przesunięcia w równowadze kwasowo-zasadowej. Mniejsza dawka clenbuterolu powodowała obniżenie zawartości jonów HCO_3^-, natomiast większa zwiększała zawartość jonów HCO_3^-. Otrzymane wyniki wykazały, iż anaboliczny efekt clenbuterolu u kurcząt połączony jest z przesunięciem metabolizmu w kierunku zwiększonej syntezy białka i równoczesnej supresji odkładania się tłuszczu. Desynchronizacja metabolizmu przejawia się także odchyleniami w stanie równowagi kwasowo-zasadowej.