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Use of Terbutaline to Reduce Incidence of Ascites Syndrome in Native Tom Turkeys

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Abstract: In a completely randomized design experiment, 60 male turkeys of 8-weeks-old were randomly divided into different levels of terbutaline kg^{-1} of diet ($\text{TE}_1 = 0 \text{ mg kg}^{-1}$, $\text{TE}_2 = 7.5 \text{ mg kg}^{-1}$ and $\text{TE}_3 = 15 \text{ mg kg}^{-1}$). The period of experiment was 8 weeks. Birds and feed were weighed weekly. At 8th weeks, blood samples taken from four birds per pen for the determination of triiodothyronine (T_3), thyroxine (T_4) and hematocrit. Hearts were collected from all birds and scored for ascites. Weight and feed intake in birds received oral administration of terbutaline (TE_2 and TE_3) were significantly higher than control group ($p < 0.01$). Hematocrits were significantly higher in ascitic birds (TE_1) ($p < 0.05$). Adding terbutaline to the feed significantly reduced ascites mortality ($p < 0.01$). T_3 value was not affected by the dietary treatments, but terbutaline reduced significantly the plasma T_4 concentration ($p < 0.05$). Right/total ventricular weight ratio (RV/TV) in right ventricular failure turkeys (TE_1) was significantly ($p < 0.01$) higher compared with healthy birds (TE_2 and TE_3). This study showed that supplemented diet with terbutaline (7.5 and 15 mg kg^{-1}) may decrease heart failure syndrome in birds, of course adequate withdrawal times are ensured.

Key words: Ascites syndrome, terbutaline, intake, growth, Turkey

INTRODUCTION

Over the last 40 years, genetic selection for rapid growth and heavy body weight, which is particularly associated with a small skeletal frame, have been implicated in musculoskeletal and cardiovascular disease in meat-type in poultry. Ascites syndrome is a severe cause of loss in the broiler industry in many countries, not only due to high mortality, but also due to reduced weight gain and increased condemnations at slaughter (Hassanzadeh *et al.*, 2000). Fat storing in the body as triglycerides is hydrolyzed to free fatty acids and glycerol through the lipolysis process (Coppack *et al.*, 1994). Catecholamines are the most important stimulators of lipolysis working primarily through β_2 adrenergic receptors (β_2 ARs) expressed in adipocytes and in skeletal muscle (Coppack *et al.*, 1994; Lafontan and Berlan, 1993). Most β_2 agonists were originally developed for the treatment of bronchial diseases and as tolytic agents (Ricks *et al.*, 1984). Similar studies on poultry demonstrated that β_2 agonists has an analogous metabolic action in decreasing carcass fat and increasing protein deposition and carcass yield (Dalrymple *et al.*, 1984).

Rapid growth resulted from increased feed intake per unit of time and higher metabolic rate consequently leads to a higher demand for O_2 . Increased muscle mass (particularly breast muscle) has not been accompanied by a proportional increase in supply organs such as heart and lungs (Havenstein *et al.*, 2003).

It has been suggested that the ascites syndrome is a multifactorial disease with a common triggering factor to reduce cardiovascular reserve (Ocampo *et al.*, 1998).

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During the development of ascites syndrome in birds, classic hematological exhibit changes. Hematocrit, hemoglobin and Red Blood Cells count (RBC) all increase dramatically (Yersin *et al.*, 1992). It has been postulated that in hypoxic situations, lowered oxygen tensions reduce capillary blood flow moreover in combination with the polycythemia, ascites development is hastened (Olander *et al.*, 1967). Right Ventricle to Total Ventricle (RV/TV) ratio [wt.(g)/wt.(g)], hemoglobin, hematocrit, blood gases and specific clinical chemistries can be used to determine the ascites status in a bird before gross lesions become apparent. An RV/TV ratio of >0.29 is considered as an accurate measure of the onset of ascites (Peacock *et al.*, 1989). It has been shown that β -adrenergic agonists can increase cardiac performance and regulate the number and bioavailability of β receptors in target tissues (Ocampo *et al.*, 1998).

Based on this hypothesis, it was considered useful to evaluate whether or not the feeding of poultry with terbutaline-medicated feed throughout the complete fattening cycle could reduce the incidence or severity of the ascites syndrome.

MATERIALS AND METHODS

At 8 weeks old (n = 60) male turkeys were randomly distributed in light-proof rooms containing 12 floor pens (5 bird per fresh wood shavings litter pen) in Tatar Animal Science Research Center from October 2007 to April 2008. The period of experiment was 8 weeks. The daily photoperiod was 23L:1D throughout the experiment. In this experiment we evaluated 3 levels of terbutaline with the following arrangement of treatments: feed with 0 mg kg⁻¹ terbutaline (TE₁, control), basal diet with 7.5 mg kg⁻¹ terbutaline (TE₂) and basal diet with 15 mg kg⁻¹ terbutaline (TE₃). The average of initial body weight of birds in TE₁, TE₂ and TE₃ groups respectively was 1.75, 1.81 and 1.82 kg. The diets were formulated to meet or exceeded National Research Council (1994) requirements. Composition and nutrient content of basal diets are shown in Table 1. Feed and water were provided *ad libitum*.

Table 1: Composition and nutrient content of basal rations (%)

Items	8-12 (week)	12-16 (week)
Ingredients (%)		
Corn	38.40	50.75
Wheat	20.00	15.50
Soy bean meal	34.20	26.50
Fish meal	1.00	1.00
Fatty acid	3.40	3.50
Salt	0.20	0.20
Di calcium phosphate	1.34	1.15
Min+vit.premix*	0.50	0.50
Dl-methionine	0.02	0.02
Lysine	0.10	-
Oyster shell	0.95	0.88
Total (kg)	100.11	100.00
Calculated analysis		
ME (kcal kg ⁻¹)	2900.00	2995.00
Cmde protein (%)	21.15	18.27
Lysine (%)	1.25	0.95
Methionine (%)	0.36	0.32
Methionine+cyste (%)	0.72	0.62
Ca (%)	0.82	0.72
P (%) (nonphytate)	0.40	0.36

*Premix for phases 8-12 weeks provided per kilogram of diet: vitamin A, 13,500 IU; vitamin D3, 4,500 IU; vitamin E, 100 mg; thiamine, 4 mg; riboflavin, 10 mg; pyridoxine, 8 mg; vitamin B12, 30 µg; choline chloride, 500 mg; nicotinic acid, 50 mg; pantothenic acid, 20 mg; folic acid, 1.8 mg; biotin, 220 µg; vitamin K3, 5 mg; iron, 40 mg; copper, 15 mg; manganese, 80 mg; zinc, 100 mg; iodine, 2 mg; cobalt, 0.2 mg and selenium, 0.25 mg. Premix for phases 12-16 weeks provided per kilogram of diet: vitamin A, 10,000 IU; vitamin D3, 3,000 IU; vitamin E, 60 mg; thiamine, 3 mg; riboflavin, 6 mg; pyridoxine, 5 mg; vitamin B12, 30 µg; choline chloride, 500 mg; nicotinic acid, 55 mg; pantothenic acid, 11 mg; folic acid, 1.5 mg; biotin, 150 µg; vitamin K3, 4 mg; iron, 25 mg; copper, 15 mg; manganese, 70 mg; zinc, 60 mg; iodine, 0.8 mg; cobalt, 0.4 mg; and selenium, 0.25 mg

BW and WG

Body weight was measured at least once a week and the mean daily Weight Gain (WG) in each age interval was calculated for each bird.

Hematocrit

In order to get hematocrit measurements blood sample was taken from a wing vein punctured into heparinized microcapillary tubes and centrifuged in a microliter centrifuge at 3000 rpm for 10 min (D-78532, Hettich Zentrifugen, Tuttlingen, Germany).

RV:TV

Hearts were collected from all birds, those that died during the trials and those killed at the end of the trial and the atria, major vessels and fat were trimmed off. The Right Ventricle (RV) was carefully separated from the left ventricle and septum. The right ventricle was weighed, the left ventricle and septum were added and the Total Ventricle (TV) was weighed. Those birds having a RV/TV ratio of over 0.299 were classified as suffering from right ventricular failure (Hassanzadeh *et al.*, 2001).

Thyroid Hormones

Serum samples were collected from four birds per pen at the end of experiment, for the determination of total triiodothyronine (T₃) and thyroxine (T₄) levels by radioimmunoassay, using standard commercial kits (Kavoshyar kit) according to the procedure of Kloss *et al.* (1994) (Gama manicl, Contron, Italy, with Automatic Gama Counter).

Statistical Analysis

All percentage data were subjected to arc sine transformation. The data were analyzed by general linear model procedure of SAS software (SAS, 1998). When necessary mean separation was accomplished by using Duncan's multiple-range test (Duncan, 1955) a probability value of less than 0.01 and 0.05 was considered significant, unless otherwise noted. The experimental design was a completely randomized design.

RESULTS AND DISCUSSION

From days 56 to 84, no significant differences in weight gain and feed intake between treatments were shown (Table 2). Birds receiving the diet supplemented with terbutaline had significantly higher

Table 2: Feed intake, BWG¹, FCR² and mortality of male turkeys due to ascites syndrome in control and terbutaline-treated group

Items	Treatments				Sig. ⁴
	TE ₁	TE ₂	TE ₃	SEM ³	
Feed intake (kg bird⁻¹)					
56-84 (day)	10.26	9.85	9.79	1.045	ns ⁵
56-112 (day)	18.32 ^b	19.02 ^a	19.33 ^a	1.020	**
BWG (kg bird⁻¹)					
56-84 (day)	3.95	4.12	4.18	0.079	ns
56-112 (day)	6.62 ^b	7.13 ^a	7.22 ^a	0.022	**
FCR (feed intake/body weight gain)					
56-84 (day)	2.60 ^a	2.39 ^b	2.34 ^b	0.005	**
56-112 (day)	2.76 ^a	2.66 ^b	2.67 ^b	0.002	**
Total mortality (%)	4.20 ^a	3.10 ^b	3.40 ^b	0.004	**

¹BWG: Body Weight Gain ²FCR: Feed Conversion Ratio ³SEM: Standard Error of Mean ⁴Sig.: Significance, ⁵ns: Not significant, Within rows, values with same superscripts are not significantly different ******(p<0.01), TE₁: Control (zero terbutaline), TE₂: 7.5 mg kg⁻¹ terbutaline, TE₃: 15 mg kg⁻¹ terbutaline

Table 3: Relative organs weight and serum hormones level of male turkeys due to ascites syndrome in control and terbutaline-treated group

Treatments	Relative ventricle weight (g) and blood hormones						
	Right ventricle	Left ventricle	Total ventricle	Ventricle index	Serum T ₃ (µg dL ⁻¹)	Serum T ₄ (µg dL ⁻¹)	Hematocrit (%)
TE ₁	4.780 ^a	10.930	15.710 ^a	0.300 ^a	3.25	11.850 ^a	41.30 ^a
TE ₂	2.890 ^b	10.970	13.860 ^b	0.210 ^b	3.12	7.010 ^b	36.20 ^b
TE ₃	2.780 ^b	10.680	13.460 ^b	0.200 ^b	3.18	6.960 ^b	37.40 ^b
SEM ¹	0.003	0.012	0.014	0.002	0.02	0.012	0.07
Sig. ²	*	ns ³	*	**	ns	*	*

¹SEM: Standard Error of Mean, ²Sig.: Significancy, ³ns: Not significant; Within columns, values with same superscripts are not significantly different **($p < 0.01$), *($p < 0.05$); TE₁: Control (zero terbutaline), TE₂: 7.5 mg kg⁻¹ terbutaline, TE₃: 15 mg kg⁻¹ terbutaline

weights from days 56 to 112 ($p < 0.01$). Feed intake in compare with TE₂ and TE₃ birds was lower in control birds and this was significant during the 56-112 days of ages ($p < 0.01$; Table 2). Generally, TE₂ and TE₃ supplementation significantly decreased the FCR at all ages ($p < 0.01$).

Ascites mortality was markedly higher in TE₁ group (13.2%) compared to TE₂ (8.9%) and TE₃ (9.52%) groups ($p < 0.01$; Table 2). Affected birds had moderated to severe ascites with clots of fibrin floating in the fluid and covering the liver. The heart was flaccid due to RVH and dilation. The lungs and kidneys were congested and sometimes haemorrhagic. The liver was either congested or nodular. Hematocrits were significantly higher in ascitic birds (TE₁) compared to treated (TE₂ and TE₃) birds. During the week of death, ascitic birds had extremely high hematocrits in compare with TE₂ and TE₃ groups ($p < 0.05$; Table 3).

As expected, the mean value of RV/TV ratio in RVF (Right Ventricle Failure) turkeys was significantly ($p < 0.01$) higher compare with treated turkeys. Terbutaline supplementation of the diet significantly decreased the weight of the right ventricle ($p < 0.01$), the total ventricular weight ($p < 0.05$) and RV/TV ($p < 0.05$) in survived birds (Table 3).

Administration of β_2 adrenergic did not affect the plasma T₃ levels, but it changed significantly the plasma T₄ concentration ($p < 0.05$; Table 3) at the end of experiment.

Increased susceptibility of broilers to ascites has previously been linked with intensive growth (Luger *et al.*, 2001). However, in the present study, we demonstrated that during the whole period of trail the weight gain and feed intake of the individuals that suffered from the syndrome was significantly lower.

Hassanzadeh *et al.* (2001) reported that adrenergic receptors are dynamically regulated by many factors; as previously described in hypertrophied ventricles, changes in receptor's density with hypoxia may be due to changes in the rate of receptor synthesis and/or degradation or modulation by the neural or hormonal environment. Hypoxia could directly affect cardiac muscle cell membranes (Hassanzadeh *et al.*, 2001), leading to a decrease in the density of receptors due to a loss of functional cell surface area. Increased availability of oxygen free radicals either due to increased production or relative deficit in antioxidant, could also lead to degradation of myocardial β_2 -adrenergic receptors in hypoxic conditions (Dhalla *et al.*, 1992).

The affinities of cardiac β_2 -adrenergic receptors did not alter in the failing hearts compared with normal hearts, which confirming with an earlier report on mammals (Dhalla *et al.*, 1992).

The density of myocardial β_2 -adrenergic receptors was not associated with magnitudinal differences in protein recovery. Differences in heart tissue composition (e.g., endothelial cells, fibroblasts and inflammatory cells) may collectively alter the β_2 -adrenergic receptor characteristics (Hassanzadeh Ladmakhi *et al.*, 1997).

Hassanzadeh *et al.* (2001) found that the density of β_2 -adrenergic receptors was reduced in heart failure birds as has already been reported in mammals (Brodde, 1991). In mammals, an increase in

activity of the sympathetic nervous system in compensation for the reduced cardiac output seems to be a mechanism of the organism to help the failing heart (Browne *et al.*, 1997), but subsequent downregulation of cardiac β_2 -adrenergic receptors could be due to an increase in receptor occupancy (Mardon *et al.*, 1998). Previous studies in birds showed that the binding capacity and affinity of cardiac β_2 -adrenergic receptors in ascites-sensitive broilers was slightly higher than receptors in ascites-resistant broilers (Hassanzadeh *et al.*, 1997). By considering the greater values for the ventricular and cardiac indices, it is possible to speculate that these variables may have contributed to the reduced mortality due to ascites. As implied by Wideman *et al.* (1997), a more balanced distribution of intraventricular pressures and volumes may be achieved in the clenbuterol-treated animals, which, in turn, allows the birds to withstand the cardiovascular changes ascribed to the ascites syndrome.

Exposure to cold conditions enhances the imbalance between oxygen supply and oxygen needs. Moreover some changes have been observed in the cardiovascular system to accommodate the needs for oxygen under those conditions. Increases in blood volume, hematocrit and hemoglobin concentration have been observed in broilers acclimated to low ambient temperature (Wideman *et al.*, 1998). The lower PCV induced by terbutaline may decrease blood viscosity in the narrow capillaries of the lungs and, consequently, could reduce pulmonary hypertension and ascites (Hassanzadeh *et al.*, 2000).

According to the strong relationship between thyroid hormone activity and ascites incidence reported previously (Decuypere *et al.*, 1994, 2000; Hassanzadeh *et al.*, 2000, 2004, 2005) it can be inferred that ascites-susceptible broiler chickens have an altered thyroid hormone metabolism (Scheele *et al.*, 1992). Therefore, the changes in thyroid hormone activity observed in this study could contribute to metabolic disorders of broiler chickens, resulting in higher incidence of right ventricular hypertrophy and ascites. In present study T_4 levels were significantly higher. Hassanzadeh *et al.* (2002) showed that the significantly higher T_4 levels in the atenolol group are unclear. This may be due to higher T_4 output by thyroid glands or by a reduced T_4 degradation rate to reverse- T_3 .

Although this study showed that supplemented diet with terbutaline (7.5 and 15 mg kg⁻¹) decreased heart failure syndrome in birds, more studies should be carried out for the better understanding of the exact mechanisms that could contribute to change in cardiac function and, consequently, pulmonary hypertension in turkeys. By considering the effective role of both levels of terbutaline in reducing the incidence of ascite, it is suggested that using of 7.5 mg kg⁻¹ is economical.

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