Study on the level of natural humoral immunity in turkey-broilers with muscular dystrophy, reared under conditions of either animal welfare or stress

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SUMMARY

The aim of the present study was to reproduce experimentally muscular dystrophy in 40 broiler turkeys under condition of high animal welfare or stress and to study blood serum lysozyme concentrations (µg/ml) and APCA activity (CH50). The muscular dystrophy was induced via supplementation of 4% fat with peroxide number 5.0 g to a balanced, but deficient in vitamin E and selenium (Se) starter forage for broiler turkeys divided into 4 groups: group I - control, reared under conditions of high animal welfare; group II - control, reared under conditions of stress; group III - diseased, reared in high animal welfare and group IV - diseased, reared in stress. The results indicated clearly that lysozyme concentrations in broiler turkeys with experimental muscular dystrophy were significantly higher than in healthy birds. In broiler turkeys reared under stress, lysozyme concentrations’ increase was at a lesser extent. Complement activity was also significantly higher in diseased turkey compared to healthy birds.

Keywords : Muscular dystrophy, broiler turkeys, welfare, stress, lysozyme, complement.

Introduction

Musculoskeletal diseases related to reduced locomotor activity of meat type fowl, are a serious problem for their welfare and productivity [18]. The author assumes that the aetiology of these disorders is not completely clear and therefore, an efficient approach based on the evaluation of risk factors for their reduction and elimination is considered necessary.

WHITEHEAD et al. [33] also emphasized the problem with musculoskeletal diseases whereas AVANZO et al. [1] described the effect of Se and vitamin E deficiency upon antioxidant systems of defense, related to systemic dystrophy. The muscular dystrophy is also described by GABRASHANSKI et al. [9]. GEORGIEV [10] reported this disease in turkeys from the White Moscow and Bronze breeds.

The effect of lysozyme against Gram-positive bacteria [4, 8, 15, 23], as well as complement against bacteria, viruses and some parasites [11, 13, 14, 20] are well known. There are significant phenotypic variations in lysozyme and complement activities depending on the breed of turkeys [26]. The influence of the raising system on lysozyme and complement activities was followed out in broiler chickens, bred in batteries and on a hard floor [29] and in broiler turkeys [34]. Taking into consideration that muscle dystrophy is not an infectious (provoked by a given antigen) but a dystrophic disease, it was appealing to find out the changes in systemic factors of defense, more precisely the humoral factors of innate immunity (lysozyme and complement) throughout the course of muscle dystrophy in birds.

The aim of the present study was to study the effect of muscle dystrophy upon the activity of non-specific factors of systemic defense - lysozyme and complement in turkey-broilers,
Material and Methods

ANIMALS AND PROTOCOL DESIGN

The experiments were carried out in the Experimental Base of the Department of Internal Diseases, Faculty of Veterinary Medicine, Trakia University. They were conducted on 40 one-day old broiler turkeys from the Stara Zagora-1 hybrid, created in the Hybrid Poultry Centre of the Institute of Agriculture, Stara Zagora. The birds were identified by wing marks. From the 1st to the 14th day of life, all turkeys were put under the same regimen of feeding and rearing (Table 1). By day 14, they were initially divided into 2 equal groups (n=20): the control birds were fed with a starter ration whereas in the assay group, birds were fed with a vitamin E deficient forage and they were supplemented with 4% oxidized fat (a fat which stayed under the direct influence of air oxygen and sunlight), with peroxide number 5 (allowed peroxide number 0.20). The prophylactic program was carried out in both groups except that Seled (at a dose of 0.06 mg/kg) was not administered to the experimental group during period 2 (period 1- days 8-13; period 2 - days 28-30) in order to enhance the development of muscular dystrophy. When turkeys were 40 day-old and the clinical signs of muscular disease were evident, each group (control and assay) was divided again into 2 equal groups (n=10) according to animal welfare conditions (Table 1). The group I (stemming from the control group - healthy birds) and the group III (stemming from the assay group - birds with muscular dystrophy) were reared under high welfare condition (favorable microclimatic conditions: the temperature, humidity, ammonia concentrations and light intensity ranged between 28-30°C, 51-52%, 5-8 mg/l and 100-120 lux, respectively, Table 1). On the other hand, groups II (healthy birds) and IV (diseased group) were submitted to an environmental stress - unfavorable microclimatic conditions: high temperature (31-35°C), high humidity (55-57%), high ammonia concentrations (20 µg/l) and weak light intensity (21-23 lux). The microclimatic parameters were monitored on a daily basis. In the centre of each section there was an infrared lamp with a power of 250 W with option for regulation of the height and the power depending on the air temperature for the respective period. The ventilation was natural, by opening the windows, depending on the microclimatic parameters of the premise. The temperature and the relative air humidity were measured with a minimum-maximum recording thermometer, the velocity of the air motion - with a catathermometer, the light intensity - with a luxmeter, the concentration of ammonia - with indicator tubes. During the first weeks the foliage was put into disinfected plastic dishes and thereafter - in a tubular feeder for each section with option for regulation of the height. Thus, a feeding front not less than 6 cm was ensured conforming to the manufacturer recommendations (Manual B.U.T.2000). The watering during the first weeks was done with 2 x 2.5 l vacuum watering trays and afterwards - with two large watering trays of 10 l each ensuring a drinking front of 3.5 cm vs. the recommended 3 cm (Manual B.U.T.2000). The bedding consisted of wood shavings with a thickness of 8-10 cm, conforming to zoohygienic requirements with manual cleansing. Diseased turkeys were treated with Seled (Vet Prom JSC, Radomir, Bulgaria - Natrii selenis α-tocopheroli acetas - 2,5 g; D-3-α tocopheroli acetats - 2.5 g; cholecalciferolom - 0.063 g and excipientes ad 100 ml) at a dosage of 0.06 mg/kg was initiated in group III (morbidity rate: 80%) and group IV (morbidity rate: 100%) per os, for 7 days. Control groups (I and II) were not treated with Seled.

Blood for analyses (5 ml) was sampled from v. subcutaneous ulnaris. The blood was allowed to clot for one hour at room temperature (25°C) and the samples were centrifuged at 2000 g for 10 min. After treatment, when all broiler turkeys were cured, the birds were 58 days old and blood samples were obtained once again.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Temperature (°C)</th>
<th>Humidity (%)</th>
<th>NH3 (µg/l)</th>
<th>Lux (Lx)</th>
<th>Ventilation (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40 days</td>
<td>29.00 ± 0.19</td>
<td>52.80 ± 0.50</td>
<td>5.0 ± 0.2</td>
<td>100.0 ± 1.2</td>
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<tr>
<td></td>
<td>40-50 days</td>
<td>29.00 ± 0.24</td>
<td>52.00 ± 0.42</td>
<td>6.0 ± 0.1</td>
<td>120.0 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>50-60 days</td>
<td>28.00 ± 0.24</td>
<td>51.00 ± 0.44</td>
<td>8.0 ± 0.1</td>
<td>100.0 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>40 days</td>
<td>31.00 ± 0.25</td>
<td>57.00 ± 0.43</td>
<td>20.0 ± 0.10</td>
<td>21.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>40-50 days</td>
<td>34.00 ± 0.24</td>
<td>55.00 ± 0.52</td>
<td>20.0 ± 1.00</td>
<td>23.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>50-60 days</td>
<td>35.00 ± 0.24</td>
<td>55.00 ± 0.52</td>
<td>20.0 ± 1.00</td>
<td>21.0 ± 0.4</td>
</tr>
</tbody>
</table>

Table 1. Microclimatic conditions for healthy groups (I and II) or birds with muscular dystrophy (groups III and IV) during growth period. Results are expressed as means ± standard errors.
DETERMINATION OF SERUM LYSOZYME CONCENTRATIONS

Serum lysozyme concentrations were determined by the method of LIE et al. [17]. Briefly, 20 ml of 2% agarose (ICN, UK, Lot 2050) dissolved in phosphate buffer (0.07 M Na2HPO4 and NaH2PO4, pH = 6.2) were mixed with 20 ml suspension of 24 hours culture of *Micrococcus lysodeicticus* at 67°C. This mixture was poured out in Petri’s dish (14 cm diameter). After solidifying at room temperature 32 wells were made (5 mm diameter). Fifty microliters of undiluted sera were poured out in each well. Eight standard dilutions (from 0.025 to 3.125 mg/l) of lysozyme (Veterinary Research Institute, Veliko Tarnovo) were used in the same quantity as well. The samples were incubated for 20 hours at 37°C and lytic diameters were measured.

DETERMINATION OF ALTERNATIVE PATHWAY OF COMPLEMENT ACTIVATION

The alternative pathway of complement activation (APCA) was studied by the method of SOTIROV [24]. For this aim we used veronal-veronal Na buffer (85 g NaCl - High School of Biotechnology, Bulgaria; 3.75 g 5,5-Diethylbarbitur-saure Natrium salz - Loba - Chemie, Austria; 5.75 g 5,5-Diethylbarbituric acid - Reanal, Hungary; 0.01 M EGTA - Sigma, USA; 0.008 M MgCl2 - Polskie Odczynniki Chemiczne, Poland. All of these chemicals were diluted in 2 l distilled water with pH = 7.5. This buffer must be diluted 1:5 before use.) Then 100 µl from each serum sample were mixed with 300 µl buffer. From these mixtures using U bottomed microplates (Flow Laboratories, UK) seven dilutions were made - 70 µl diluted serum (dil. ser.) + 30 µl buffer; 60 µl dil. ser. + 40 µl buffer; 50 µl dil. ser. + 50 µl buffer; 40 µl dil. ser. + 60 µl buffer; 30 µl dil. ser. + 70 µl buffer; 20 µl dil. ser. + 80 µl buffer; 10 µl dil. ser. + 90 µl buffer and 50 µl buffer were additionally added to each well. 100 µl of 1% rabbit erythrocyte suspension to each dilution were dropped and were incubated at 37°C for 1 hour. Optical density were measured by "Sumapelz" ELISA reader (Karl Zeiss, Germany) at 540 nm. Lysozyme content and APCA activity were calculated using special computer programmes developed in Trakia University.

STATISTICAL ANALYSIS

The data were statistically processed by Student’s test for comparing the different groups between them when a significant effect was evidenced by ANOVA.

Differences were considered as significant when p < 0.05.

Results

The results of lysozyme activities in control turkey-broilers and those suffering from muscle dystrophy, reared under animal welfare or stress prior to the treatment are presented in Table 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Prior to treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SE</td>
<td>VC (%)</td>
</tr>
<tr>
<td>I. Controls - high animal welfare</td>
<td>10</td>
<td>0.872 ± 0.013</td>
<td>4.35</td>
</tr>
<tr>
<td>II. Controls - stress</td>
<td>10</td>
<td>0.426 ± 0.013</td>
<td>9.07</td>
</tr>
<tr>
<td>III. Diseased - high animal welfare</td>
<td>10</td>
<td>***1,500 ± 0.011</td>
<td>2.32</td>
</tr>
<tr>
<td>IV. Diseased - stress</td>
<td>10</td>
<td>***1,325 ± 0.028</td>
<td>6.34</td>
</tr>
</tbody>
</table>

*p<0.05; **p<0.01; ***p<0.001 vs. healthy controls

TABLE 2: Serum lysozyme levels (mg/L) in broiler turkeys, either healthy or with muscular dystrophy prior to treatment and after treatment, reared under conditions of high animal welfare or stress. SE - standard error; VC - variation coefficient.

The activity of lysozyme in control group of healthy turkey poults reared under stress was almost twice lower (0.426 ± 0.013) than the corresponding concentrations in the group reared under high animal welfare (0.872 ± 0.013), the differences being highly statistically significant. This fact showed that stress factors on their own, without occurrence of disease, reduced one of the factors of natural defense: the lysozyme.

In diseases turkeys with muscle dystrophy, lysozyme activity increased sharply up to 1,500 ± 0.011 under high animal welfare conditions and to 1,325 ± 0.028 under stress, the difference between these groups as well as between each of them and the respective controls being statistically significant too. Again, this trend was confirmed in turkey-broilers with muscle dystrophy - stress decreased the systemic factors of defense.
After the treatment of diseased turkeys, lysozyme activity was not normalized compared to pretreatment values. It decreased from 1,500 ± 0,011 (prior to treatment) to 1,238 ± 0,017 (after treatment) in the high animal welfare group and from 1,325 ± 0,028 (prior to treatment) to 1,011 ± 0,015 (after treatment) in stressed group. The tendency for a lower lysozyme activity in birds reared under stress compared to controls was however preserved.

Following out the data about the pretreatment activity of complement (Table 3) in controls reared under high animal welfare, the values were slightly higher (572,60 ± 8,92) compared to the groups under stress (547,40 ± 8,57), the difference being statistically insignificant.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Prior to treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Controls-high animal welfare</td>
<td>10</td>
<td>Mean ± SE</td>
<td>VC (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>572,6 ± 8,22</td>
<td>4,31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>576,0 ± 6,66</td>
<td>3,46</td>
</tr>
<tr>
<td>II. Controls-stress</td>
<td>10</td>
<td>Mean ± SE</td>
<td>VC (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>547,4 ± 8,57</td>
<td>4,88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>557,2 ± 4,32</td>
<td>2,33</td>
</tr>
<tr>
<td>III. Diseased - high animal welfare</td>
<td>10</td>
<td>*643,7 ± 4,72</td>
<td>2,20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>573,4 ± 3,50</td>
<td>1,83</td>
</tr>
<tr>
<td>IV. Diseased-stress</td>
<td>10</td>
<td>*609,5 ± 8,35</td>
<td>4,11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>551,3 ± 3,89</td>
<td>2,11</td>
</tr>
</tbody>
</table>

*p<0,05 vs. healthy controls

| TABLE 3: APCA activity (CH50) in broiler turkeys, either healthy or with muscular dystrophy prior to treatment and after treatment, reared under conditions of high animal welfare or stress. SE - standard error; VC - variation coefficient.

In turkey-broilers with muscle dystrophy, complement activity increased in both groups compared to controls: 643,70 ± 4,72 (animal welfare) and 609,50 ± 8,35 (stress), the difference being statistically significant at p<0,05 although at a lower magnitude than that between lysozyme levels. At the same time, the diseased turkeys reared under stress exhibited lower complement activity (609,50 ± 8,35), than high animal welfare birds (643,70 ± 4,72; statistically significant at p<0,05). After treatment, the activities returned to normal compared to pretreatment values - 573,40 ± 3,50 (animal welfare) and 551,30 ± 3,83 (stress), the activity in the latter group being the lowest. In both groups reared under stress (control and diseased), the post treatment activities were statistically significantly lower compared to respective groups reared under high animal welfare standards (p < 0,05).

These data showed that the activity of complement did not increase at the same extent as lysozyme levels did in turkey pouls with muscle dystrophy. It is known that unlike lysozyme, complement activity is a factor not only of non-specific, but also of specific systemic defense and the muscle dystrophy was not an infectious disease and thus, did not activate complement specifically and strongly. Simultaneously, stress factors showed a weak tendency to induce complement activity prior to treatment, but it was well expressed after the treatment.

Discussion

The presented data about lysozyme showed higher values in turkey-broilers with muscle dystrophy compared to healthy birds. This was probably due to one or several of metabolic products throughout the course of disease. The specific cause was not elucidated. FANG et al. [7] identified in dystrophin-deficient mouse muscles increased expression of murine monocye chemoattractant protein-1 (JE/MCP-1), cathepsin S, UPIX-1, nmb, cathepsin B, and lysozyme M mRNAs. Other authors reported similar results [7, 19, 30, 31, 32]. The increased lysozyme M mRNAs level is a parameter of increased lysozyme concentration in muscle tissue and most probably, plays an important role for overcoming of disease. It is known that lysozyme is a factor not only of natural immunity [4]. He plays other essential biological functions - increased the motility of spermatozoa and improves their fertility in men and turkeys [16, 27]. The negative effect of stress upon lysozyme concentration could be explained by the possible increase in blood cortisol concentrations. PANARELLI et al. [21] explained the mechanisms of this decrease by the glucocorticoid-mediated inhibition of the lysozyme gene transcription. They reported that the minimum blood concentrations of dexamethasone or cortisol for inhibiting lysozyme synthesis should be 1 mmol/l. By contrast, acute and short-time stress challenges induced elevation of lysozyme concentrations instead of decreases because glucocorticoid concentrations were probably not or weakly modified during such stimuli and were kept below 1 mM, they did not alter lysozyme synthesis [34].

The cause for increased complement activity in myelodysrophy was explained by a number of authors. ENGEL and BIESECKER [6] detected a membrane-attacking complex (MAC) of complement in necrotic muscle fibres. C3 and C9 are in significant amounts, but the contents of C1q and C4 are variable. CORNELIO and DONES [5] localized calcium and albumin as endogenous markers of extracellular fluid penetration and C3 and C9 complement components as markers of muscle fibre necrosis. In both Duchenne dystrophy and congenital muscular dystrophy, a significant percentage of fibres were overloaded with calcium and penetrated by albumin. C3 and C9 complement components appeared only in necrotic fibres, which invariably were also penetrated by albumin. These observations support previous findings that muscle fibre necrosis is linked to massive inflow of extracellular fluid and complement activation. SEWRY et al. [22] found necrotic fibres labelled intensely with C9 and C8 but intensities varied with the different monoclonal antibodies. This was thought to result from differences in the polymeri-
sation of the C9 molecule in the membrane attack complex. The results obtained by SPULER and ENGEL [28] do not support the role for antibody-dependent complement-mediated muscle fibre injury in the major inflammatory muscle diseases. The cause and pathogenetic significance of the C+ fibres in the different types of muscular dystrophies remains to be elucidated. HAYASHI et al. [12] found out in 52 day old baby prominent massive muscle cell degeneration occurred in association with the deposition of the C5-9 complement membrane attack complex (MAC). Most of the MAC-positive muscle fibres showed a severely deranged immunoreaction to dystrophin, dystroglycans, and other sarcosomal proteins. The effect of stress upon the reduced complement activity in these groups could be explained by the results of PANARELLI et al. [21]. BOZAKOVA [3] did not observe statistically significant differences in complement activity in turkey-broilers, reared in two production systems - on a litter and on a slatted floor. The author explained this fact with the lack of strong stimuli upon complement activity, as the birds were reared with constant control of the optimal zoohygienic parameters and the prophylactic programmes.

Our results and the data of the aforementioned authors confirmed the important role of complement in the pathogenesis of muscle dystrophy. At the same time, stress factors inhibited the complement system, but at a lower extent than lysozyme.

Conclusions

1. The dystrophic products in turkey-broilers with muscle dystrophy increased lysozyme activity at a higher extent and complement activity - at a lower one.

2. In turkey-broilers reared under stress, lysozyme activity decreased significantly whereas that of complement - at a lesser extent.

3. The applied treatment protocol in turkey-broilers normalized completely the blood serum lysozyme concentration, whereas the complement the normalization was less manifested.

References


