Effects on the environmental stress on experimentally induced muscular dystrophy in broiler turkeys

K. STOYANCHEV

Department of Internal Diseases and Clinical Toxicology
Faculty of Veterinary Medicine, Trakia University, 6 000 Stara Zagora, BULGARIA

Corresponding author: E-mail: ksto@abv.bg

SUMMARY

The aim of the present study was to investigate the effects of environmental stress (unfavourable microclimatic conditions) on broiler turkeys with experimentally induced muscular dystrophy throughout the determination of biochemical markers of muscular injury (glycaemia and plasma AST, ALT and CK activities) on days 50 and 58 and throughout examination of morphological lesions at necropsy. For that, muscular dystrophy has been induced in 20 birds by dietary supplementation with 4% oxidized fat (peroxide number: 5) and by vitamin E and selenium (Se) deficiencies in diet regimen, whereas healthy birds (n = 20) were fed with starter forage and were supplemented by Seled (vitamin E: 2.5%; Se: 0.06%, 0.06 mg/kg/day). When birds were 40 day old, they were divided in 4 equal groups (n = 10) according to the housing conditions: Turkeys from the group I (healthy) and from the group III (diseased) were reared under high welfare conditions whereas animals from the groups II and IV (healthy and diseased respectively) were reared under unfavourable microclimatic conditions. Muscular dystrophy was confirmed by clinical signs (weakness to paralysis) and by anatomopathological lesions (muscle and heart necrosis) and these manifestations were exacerbated into the group IV. Plasma AST, ALT and CK activities were dramatically enhanced while a significant hypoglycaemia was observed in birds with muscular dystrophy (p < 0.01). Again, stressed birds (group IV) exhibited the greatest biochemical alterations (group III vs. group IV: p < 0.01 except for glycaemia and CK activity). In healthy 50 and/or 58 day old birds, environmental stress induced significant increases of glycaemia and of plasma enzyme activities (p < 0.01). After Seled treatment of diseased birds (0.06 mg/kg for 7 days), glycaemia was totally restored and enzyme activities were strongly reduced but they remained significantly higher than control values (p < 0.01, except for ALT). Again, stressed animals exhibited higher values of glycaemia and of CK activity than animals in high welfare conditions (group I vs. group II and group III vs. group IV: p < 0.001). These results clearly show that vitamin E / Se deficiency coupled to dietary supplementation with oxidized fat induces muscular dystrophy in turkeys and that an environmental stress aggravates muscle injury revealed by increases of ALT, AST and mainly CK activities in plasma and by an increase of blood glucose consumption.

Keywords: muscular dystrophy, broiler turkeys, welfare, stress, glycaemia, CK, AST, ALT.

RESUME

Effets d’un environnement stressant sur des dindonneaux atteints de dystrophie musculaire induite expérimentalement

Le but de cette étude est d’évaluer les effets d’un environnement stressant (conditions climatiques défavorables) sur des dindonneaux présentant une dystrophie musculaire expérimentale en analysant les variations de marqueurs biochimiques d’une atteinte musculaire aux jours 50 et 58 (glycémie, activités plasmatiques de l’ALT, l’AST et de la CK) ainsi qu’en recherchant des lésions anatomopathologiques à l’autopsie. La dystrophie musculaire a été induite par une alimentation enrichie en graisses oxydées (indice en peroxydes : 5) et carencée en vitamine E et en Sélénium (Se) alors que les contrôles ont été nourris par un aliment de démarrage spécifique des dindonneaux et supplémentés par du Seled (vitamine E: 2.5%; Se: 0.06%, 0.06 mg/kg/jour). A l’âge de 40 jours, les oiseaux ont été répartis en 4 groupes égaux selon les conditions d’élevage : les conditions d’ambiance étaient correctes pour les dindonneaux des groupes I (contrôles) et III (malades) alors qu’elles étaient défavorables pour les groupes II (contrôles) et IV (malades). La dystrophie musculaire a été confirmée par les signes cliniques (faiblesse locomotrice jusqu’à une paralysie) et l’autopsie (nécrose musculaire et cardiaque) et ces manifestations étaient exacerbées dans le groupe IV. Les activités plasmatiques de l’ALT, l’AST et de la CK ont été fortement augmentées tandis qu’une hypoglycémie significative a été observée chez les oiseaux malades (p < 0.01). A nouveau, les altérations biochimiques ont été maximales chez les animaux stressés (groupe III vs. groupe IV : p < 0.01 sauf pour l’activité CK et la glycémie). Chez les contrôles de 50 et/ou de 58 jours, un environnement stressant a induit des augmentations significatives de la glycémie et des activités enzymatiques dans le plasma (p < 0.01). Après traitement des malades par le Seled (0.06 mg/kg pendant 7 jours), la glycémie a été totalement restaurée et les activités enzymatiques ont été fortement réduites mais elles sont restées plus élevées que chez les contrôles (p < 0.01 excepté pour l’ALT). Les animaux stressés ont présenté des valeurs plus élevées de la glycémie et de l’activité CK que ceux soumis à un environnement sain (groupe I vs. groupe II et groupe III vs. groupe IV : p < 0.01). Ces résultats montrent clairement qu’une double carence en vitamine E et Se couplée à un apport excessif en graisses oxydées induit une dystrophie musculaire chez le dindonnoe et qu’un environnement stressant aggrave les lésions musculaires révélées par l’augmentation des activités plasmatiques de l’ALT, de l’AST et surtout de la CK et par une consommation accrue du glucose sanguin.

Mots-clés : dystrophie musculaire, dindonneaux, bien-être, stress, glycémie, CK, AST, ALT.

Introduction

Musculoskeletal diseases considered as metabolic disorders are not characterized by a high death rate in broilers and turkeys, but cause lameness and decreased productivity [12]. Frequently encountered in production herds, musculoskeletal disorders induce great economic posses, particularly in meat production poultry [25]. According to MENCH [16], the aetiology of these diseases is not completely understood although the genetic selection in breeding stocks has reduced...
The incidence. SANDERCOCK [22] has described a monensin-induced myopathy in broilers with body weight reduction, decreases of blood inorganic phosphorus and glucose concentrations and of ALAT activity and increase of ASAT activity, haemoglobin concentration and erythrocyte, lymphocyte and thrombocyte counts. By contrast, JACOBSEN et al. [11] clearly evidenced the protective effect of vitamin E upon oxidative stress: treatment of broiler chickens with different doses of alpha, gamma and delta tocopherol acetate did not affect the body weight, the CK, glutathione peroxidase, citrate synthase, total LDH activities, or blood concentrations of fatty acids, but induced significant decrease of plasma ASAT activity.

GEORGIEV [6] has studied some clinical and laboratory parameters (total protein, vitamins, ubiquinone concentrations and several enzyme activities) in an experimental model of muscular dystrophy in 40-day old turkeys from the Moscow White and Bronze turkey breeds, induced by administration of irradiated fish oil. Three months after the beginning of the experiment, the clinical signs characteristic for muscular dystrophy were observed and were associated with some biochemical changes. The same author [6] studied the influence of the transport stress (induced by transportation of birds on 200 km by car) onto turkey-chickens with symptoms of muscular dystrophy. The results demonstrated a deepening of clinical symptoms and death in some birds, but the mechanism causing that is still unclear.

Consequently, the aims of the present study are to explore the effects of environmental stress on muscle integrity and to look for an aggravation of experimentally induced muscular dystrophy by unfavourable microclimatic housing conditions in broiler turkeys by measurement of glycaemia and of biochemical markers (plasma AST, ALT and CK activities).

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 (figure 1). On day 14, they were initially divided into 2 equal (n  20) groups: the control 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 conditions (favourable microclimatic conditions: temperature, humidity, ammoniac concentrations and light intensity ranged between 28-30°C, 51-52%, 5-8mg/l and 100-120 lux respectively, Table I). On the other hand, the groups II (healthy birds) and IV (diseased group) were submitted to an environmental stress (unfavourable microclimatic conditions: high temperature (31-35˚C), high humidity (55-57%), high ammoniac concentrations (20 µg/l) and weak light intensity (21-23 lux)). The microclimatic parameters were monitored on a daily basis. In the center 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 forage 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 then 6 cm was ensured conforming to the manufacturer recommendations (Manual B.U.T.2000).

The watering during the first weeks was done with 2 × 2.5 l vacuum watering trays and afterwards – with two large tubes. During the first weeks the forage 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 then 6 cm was ensured conforming to the manufacturer recommendations (Manual B.U.T.2000).

The watering during the first weeks was done with 2 × 2.5 l vacuum watering trays and afterwards – with two large tubes. During the first weeks the forage 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 then 6 cm was ensured conforming to the manufacturer recommendations (Manual B.U.T.2000).

Figure 1: Experimental protocol: duration and daily induction of muscular dystrophy in broiler turkey chickens.

* : Induction of muscular dystrophy by deficit in Seled (Na2Se2: 0.06%, α tocopherol acetate: 2.5%, cholecalciferol: 0.063%, Vet Prom JSC, Radomir, Bulgaria, 0.06 mg/kg/day) during days 28-30, vitamin E deficiency and by dietary supplementation with 4% oxidized fat. HWC: High welfare conditions; SC: Stress conditions. T: oral Seled treatment (0.06 mg/kg/day) of diseased animals.
soft and later liquid faeces appeared. On the 40th day, the muscular disease in our experiment: firstly, on the 35th day, significant effect was evidenced by ANOVA. Differences were comparing the different groups between them when a significant

Results

We observed the following clinical manifestations of the muscular disease in our experiment: firstly, on the 35th day, soft and later liquid faeces appeared. On the 40th day, the young turkeys exhibited weakness in the extremities, followed by unconfident walking and frequent sitting. They stood up again after 3 to 4 minutes, making only a few steps before sitting again. Some of them moved slowly and with difficulty towards the feeding dish, and ate in a crouching position. Appetite persisted within this period. Walking appeared stiff. Palpation of the neck showed that it was stiff in some of the birds. In the group IV, where the ill animals were held under stress conditions, the weakness in the extremities gradually turned into paralysis. They lied on one side and remained in that position, with stretched extremities and opistotonus, and one of the turkeys died on the 54th day. In the group III (high animal welfare), there were no severe case and no dead animal.

During necropsy, a rapid examination of the bodies confirmed the birds were properly fed. After removal of the skin, muscles in the area of the legs were pale in appearance (easily distinguished among the other muscles). Necrosis into breast and tight muscles of the birds was evidenced. The liver showed signs of parenchyma dystrophy. In one of the examined pullets, the heart exhibited signs of fatty dystrophy. The cuticum of the gizzard was cracked and was easy to remove with a great number of grey coloured lesions (spots). All other organs and systems appeared normal.

Blood glucose concentrations and AST, ALT and CK activities prior to the muscular dystrophy treatment are presented in Table II. In birds with muscular dystrophy (groups III and IV) glycaemia was significantly depressed compared to the healthy controls whatever the housing conditions (groups I and II, p < 0.01), whereas plasma AST, ALT and CK activities were dramatically increased (p < 0.01).

In stressed healthy birds (group II), glucose concentrations and enzyme activities were significantly increased compared to birds reared in high welfare conditions (group I vs. group II: p < 0.01) except for AST activity, which did not significantly differ between the 2 groups (Table II). Nevertheless, a weak enhancement of AST activity was also noticed in the group II. In the same way, marked elevations of plasma AST and ALT activities were also evidenced in diseased birds reared under stress conditions (group IV) compared to birds reared in favourable climatic conditions (group III vs. group IV: p < 0.01). For CK activity, the same tendency was also observed but difference was not statistically significant, because of the great dispersion of the values recorded in the group IV (Table II). By contrast, stress conditions did not significantly affect glycaemia in birds with muscular dystrophy (group III vs. group IV: p > 0.05).

### Table 1: Microclimatic conditions for healthy broiler turkeys (groups I and II) or birds with muscular dystrophy (groups III and IV) during the growth period.

<table>
<thead>
<tr>
<th>Time (day)</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>High welfare (groups I, III)</td>
<td>40</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>
</tr>
<tr>
<td></td>
<td>40-50</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</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>Stress conditions (groups II, IV)</td>
<td>40</td>
<td>31.00 ± 0.25</td>
<td>57.00 ± 0.43</td>
<td>20.0 ± 0.1</td>
<td>21.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>40-50</td>
<td>34.00 ± 0.24</td>
<td>55.00 ± 0.52</td>
<td>20.0 ± 1.0</td>
<td>23.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>50-60</td>
<td>35.00 ± 0.24</td>
<td>55.00 ± 0.52</td>
<td>20.0 ± 1.0</td>
<td>21.0 ± 0.4</td>
</tr>
</tbody>
</table>

**BIOCHEMICAL ANALYSIS**

The blood glucose concentrations were determined by diagnostic kits of Roche-Diagnostic (0744948202, Mannheim, Germany) and plasma activities of enzymes AST (0745120202, Manheim, Germany), ALT (0745138202, Manheim, Germany) and CK (1126695, Manheim, Germany) were analyzed on an automated biochemical analyzer (Reflotron – plus, Germany).

**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 values were less than 0.05.
After oral Seled treatment for 7 days, glycaemia has significantly increased in diseased and cured turkeys (p < 0.01) and has become similar to control values measured in 58 day old healthy controls (group I vs. group III and group II vs. group IV: p > 0.05, Table II). In parallel, the treatment of the diseased birds has also clearly induced significant reduction of plasma AST, ALT and CK activities (p < 0.01 compared to pre-treatment values). But, except for ALT activity, these enzyme activities remained higher in diseased and treated animals (group III and IV) than in the respective controls (group III vs. group I: p< 0.01 for AST and CK activities; group IV vs. group II: p< 0.01 for CK activity). By contrast, the ALT activities were comparable in all the groups. Moreover the significant effect of environmental stress on glucose concentrations and on plasma CK activity was again evidenced not only in older controls but also in diseased and treated animals: glycaemia and CK activities were significantly higher in stressed birds (group I vs. group II and group III vs. group IV: p< 0.01, Table II). The AST activity also remained more elevated in stressed controls compared to healthy birds reared in high welfare conditions (p< 0.01), but in diseased and cured birds, values were similar whatever the microclimatic conditions. By contrast, the ALT activities were identical in all groups: even in healthy turkeys, no significant difference was obtained between stressed and not stressed animals.

### Discussion

The muscular dystrophy experimentally induced by vitamin E deficiency and excess of oxidized fat in the present study in broiler turkeys was characterized by exsudative degenerescence of skeletal muscles and heart and was associated with blood biochemical alterations (marked decrease of glycaemia and remarkable elevations of plasma AST, ALT and CK activities in plasma).

Vitamin E [1] as an oil soluble intracellular antioxidant is responsible for stabilizing unsaturated fatty acids by suppressing toxic peroxides building. Vitamin E is localized in lipid layer of cell membranes, banded with poly-unsaturated fatty acids. By this way, it provides protection to peroxides. Therefore in our study, its deficiency together with oxidized oil feeding causes lipid peroxidation and cell membrane disruptions, occurring during muscular dystrophy. Furthermore, Selenium was also associated with antioxidant systems as cofactor of glutathione peroxidases, which catalyze the reduction of hydroperoxides into alcohols. Selenium associated with vitamin E stabilizes cell membrane of the muscle fibers. The oxidative stress induced by dietary selenium and vitamin E deficiencies can lead muscle to apoptotic pathway [21].

As muscles contain large amounts of AST, ALT and CK enzymes, the increases of these enzyme activities in plasma can reflect muscular injury with a relative high sensibility. Moreover, the specificity of CK measurement to monitor muscle damage is very high, because of its remarkable quantity in skeletal muscle compared to all other body tissues [4]. Differences in CO₂, glucose and Se concentrations and in glutathione peroxidase, AST, ALT and CK activities in plasma were found in chicks with myopathy exsudative diathesis, a condition caused by Se and vitamin E deficiency [3]. As stated [1], as early as the initial stage of the disease in lambs, a 100-fold elevation of blood AST, ALT, ALP and CK activities was evidenced not only in older controls but also in diseased and treated animals: glycaemia and CK activities were significantly higher in stressed birds (group I vs. group II and group III vs. group IV: p< 0.01, Table II).

**Table 2: Blood biochemical parameters (glycaemia and plasma AST, ALT, CK activities) in healthy broiler turkeys (groups I and II) and in turkeys with muscular dystrophy (groups III and IV) housing in favourable microclimatic conditions (HWC, group I and III) or in unfavourable microclimatic conditions (SC, groups II and IV) before (birds are 50 day old) or after oral treatment with Seled (0.06 mg/kg) for 7 days (birds were 58 day old). Results are expressed as means ± deviation errors.**
occurred with higher changes in CK concentration, because this is markedly present in muscle tissue and is released during muscular dystrophy. TSOKOVA et al. [24] have also reported increased ALT, AST and alkaline phosphatase activities in layer hens with alopecia and cannibalism. GEORGIEV [6] has also reported a significant increase of ASAT in birds with muscular dystrophy whereas for ALT, the differences between diseased and control birds was not statistically significant. The elevated CK activity in broiler turkeys with muscular dystrophy in our opinion was not only due to alteration of cell membrane permeability but also to increased levels of the substrate creatine. The higher substrate concentration induces the synthesis of the enzyme CK on the basis of the genetic regulation of enzymatic activity [2]. It is clear that this regulatory mechanism was triggered in birds with muscular dystrophy and that it could explain the higher CK activities in blood. This is also confirmed by the experiments of other authors [2, 5-10, 14, 17-19], who reported considerable alterations in this enzyme after intramuscularly injection of various medications in different domestic animal species. Our data showing marked increases of plasma AST, ALT and CK activities in broiler turkeys with muscular dystrophy reared in high animal welfare or stress, allowed us to recommend them as markers of muscular damage characterizing this disease, whereas the changes in the MDA concentrations and in some antioxidant enzyme activities (SOD and CAT) are recommended as markers for radical-induced damage [4, 13]. The hypoglycaemia observed during muscular dystrophy in the present study may be related to the increase of glucose consumption by still intact muscle fibers and to the decrease of pyruvate and α-amino-acids release from muscles leading to a drop of liver neoglucogenesis.

After oral Seled treatment, enzyme activities were significantly lowered and glycaemia was markedly increased. However, AST and CK activities still remained elevated in diseased and treated animals compared to healthy controls. These findings suggest that this treatment may attenuate the muscular activity [2]. It is clear that this regulatory mechanism was not only due to alteration of cell membrane permeability but also to increased levels of the substrate creatine. The higher substrate concentration induces the synthesis of the enzyme CK on the basis of the genetic regulation of enzymatic activity [2]. It is clear that this regulatory mechanism was triggered in birds with muscular dystrophy and that it could explain the higher CK activities in blood. This is also confirmed by the experiments of other authors [2, 5-10, 14, 17-19], who reported considerable alterations in this enzyme after intramuscularly injection of various medications in different domestic animal species. Our data showing marked increases of plasma AST, ALT and CK activities in broiler turkeys with muscular dystrophy reared in high animal welfare or stress, allowed us to recommend them as markers of muscular damage characterizing this disease, whereas the changes in the MDA concentrations and in some antioxidant enzyme activities (SOD and CAT) are recommended as markers for radical-induced damage [4, 13]. The hypoglycaemia observed during muscular dystrophy in the present study may be related to the increase of glucose consumption by still intact muscle fibers and to the decrease of pyruvate and α-amino-acids release from muscles leading to a drop of liver neoglucogenesis.

As conclusions:

Muscular dystrophy in broiler chickens combined with stress, decreased blood sugar concentrations whereas after treatment, it was elevated because of the environmental stress;

The activities of enzymes AST, ALT and CK were elevated in muscular dystrophy and these changes were amplified with unfavourable microclimatic conditions;

After the Seled treatment, broiler turkeys with muscular dystrophy exhibited similar plasma AST and ALT activities compared to the corresponding control groups whereas the CK activity persisted considerably higher under stress.

References