



Original Contribution

BLOOD LEVELS OF SOME MACRO AND TRACE ELEMENTS IN MUSCULAR DYSTROPHY TURKEY-BROILERS REARED UNDER THE CONDITION OF HIGH ANIMAL WELFARE OR STRESS

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ABSTRACT

The purpose of the present study was to investigate the alterations in blood plasma concentrations of some principal macro and trace elements in turkey-broilers, reared under conditions of either high animal welfare or stress. The muscular dystrophy was experimentally produced. The animal welfare and stress were determined by environmental factors (temperature, humidity and light). The animals were divided into 4 groups to meet the conditions of animal welfare or stress. Blood concentrations of Ca, Na, K, Cu, Zn and Se were subsequently determined. Results showed that turkey-broilers with muscular dystrophy exhibited increased blood plasma Ca, Na and K levels especially in birds reared under stress whereas the concentrations of trace elements Cu, Zn and Se decreased. After treatment, the concentrations of studied parameters returned to normal. Stress was shown to have a considerable impact on healing process and it exerted a significant effect on the values of studied parameters, demonstrated by their slower return to normal.

Key words: turkey-broilers, muscular dystrophy, macro and trace elements

INTRODUCTION

Myopathies do not account for a high mortality rate, but are responsible for impaired locomotion and lower productivity in birds (1). They are a serious problem in industrial production systems (2, 3)

The majority of authors agree that a new evaluation of the risk factors for this group of diseases is needed and on this basis, an effective strategy for therapy and prevention should be created. Muscular dystrophy is described and reported by many authors (4, 5, 6, 7, 8, 9, 10, 11). Most investigations in this field recommend the determination of indices for preclinical diagnosis of muscular dystrophy. Georgiev (5) reported that the early diagnosis (in the subclinical stage) of muscular dystrophy could be established by hyperkalaemia and increased blood serum activity of glutamate oxaloacetic transaminase in turkey-broilers. In experiments in turkey-broilers from the White Moscow and Bronze breeds, he determined

blood Ca levels between 10 and 12 mg%, of inorganic phosphate – from 5.04 to 5.02 mg% and normonatraemia. Similar changes in macroelements are communicated by Tsokova (12) in alopecia and cannibalism based on Ca, Zn and Cu disequilibrium. Most studies were performed in spontaneous myopathies in broiler chickens and a proper rearing technology. Some questions about the effect of stress factors on the levels of the principal macro- and trace elements in the blood of turkey-broilers suffering from muscular dystrophy, as well as on the healing process, remain however not entirely clear.

The purpose of the present study was to investigate the alterations in blood plasma concentrations of some principal macro-(Ca, Na, K) and trace elements (Cu, Zn, Se) in turkey-broilers, reared under conditions of either animal welfare or stress.

MATERIAL AND METHODS

The experiments were carried out in the Experimental Base of the Department of Internal Diseases, Faculty of Veterinary Medicine, Trakia University with 40 one-day old broiler turkeys from the Stara Zagora-1 hybrid, created in the Hybrid Poultry Centre

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of the Institute of Agriculture – Stara Zagora.

The birds were identified by means of wing marks. From the 1st to the 14th day of life, all turkeys were put under the same regimen of feeding and rearing. By day 14, they were initially divided into 2 groups – controls that were fed with a starter ration and experimental – fed with a balanced but deficient in vitamin E forage, supplemented with 4% oxidized fat with peroxide number 5g (allowed peroxide number 0.20). The prophylactic programme was carried out in both groups but the experimental one was not treated with Seled[®] (*Radomir, Bulgaria*) at a dose of 0.06 mg/kg between 8-13 and 28-30 days of age in order to enhance the development of muscular dystrophy. After the clinical manifestation of the disease, by the 40th day of life the turkeys were divided into 4

groups: group I – control, reared in high animal welfare conditions; group II – control, reared in conditions of stress; group III – diseased, reared in high animal welfare conditions and group IV – diseased, reared under conditions of stress. All groups were housed in the same premises divided into 2 sections. The first section was used for groups I and III, reared in high animal welfare conditions, where the microclimate was within the reference range and the second section – for groups II and IV, reared under stress. The microclimate deviating from the normal one was created by fencing the groups II and IV with polyethylene. Groups I and III and groups II and IV in the first and second sections, respectively, were divided by means of a low barrier.

Table 1. Microclimatic conditions of broiler turkeys reared under conditions of high animal welfare or stress

Groups	Temperature (°C)	Humidity, (%)	NH ₃ (µg/l)	Lux (Lx)	Ventilation (m/s)
I. Controls + diseased under conditions of high animal welfare up to the 40 th day	29±0.19	52±0.50	0.005±0.0002	100.0±1.22	0.26±0.004
II Controls + diseased under conditions of stress up to the 40 th day	31±0.25	57±0.43	0.02±0.001	21.0±0.52	0.26±0.004
III Controls + diseased under conditions of high animal welfare up to the 50 th day	29±0.24	52±0.42	0.006±0.0001	120.0±1.16	0.27±0.005
IV. Controls + diseased under conditions of stress up to the 50 th day	34±0.24	55±0.52	0.02±0.001	23±0.54	0.27±0.005
V. Controls + diseased under conditions of high animal welfare up to the 60 th day	28±0.24	51±0.44	0.008±0.0001	100.0±1.5	0.26±0.004
VI. Controls + diseased under conditions of stress up to the 60 th day	35±0.24	55±0.52	0.02±0.001	21.0±0.44	0.26±0.004

The differences in the microclimate between high animal welfare and stress conditions are presented on **Table 1**. The stress in this experimental design was achieved through unfavourable microclimatic conditions (higher temperature, humidity and lower light intensity). The microclimatic parameters were monitored on a daily basis. Each group was subjected to rays from 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. During the first weeks the forage was put into disinfected plastic dishes and thereafter – in a tubular feeder for each group with option for regulation of the height. Thus, a feeding front of not less than 6 cm (Manual B.U.T.2000) ensured conformity with recommendation of the manufacturer of broiler turkeys. The watering during the first weeks was done with

2x2.5 l vacuum watering trays for each group 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 ventilation was natural, by opening the windows, depending on the microclimatic parameters of the premises. The bedding consisted of wood shavings with a thickness of 8-10 cm, with manual cleansing, conforming to zoohygienic requirements. The temperature and the relative humidity of the air were measured with a minimum-maximum recording thermometer; the velocity of the air motion was measured with a catathermometer; the light intensity was measured with a luxmeter, and the concentration of ammonia – with indicator tubes. Prior to the treatment of diseased turkeys, 2 control birds and 2 diseased birds, reared in high animal welfare and 2 birds reared under stress were sacrificed

for determination of changes in musculature and internal organs, characteristic for muscular dystrophy. After manifestation of 80% morbidity rate in group III and 100% in group IV, a treatment was initiated with Seled at a dose of 1 ml/l water orally for 7 days.

At the time of complete clinical manifestation of muscular dystrophy (>80% affected birds) and prior to the treatment, 10 birds of each group were sampled by taking the blood from the wing vein. After the treatment up till age of 58 days when all broiler turkeys were cured, blood samples were obtained once again.

In all groups, blood was sampled from the wing vein after complete manifestation of the disease and after the treatment. The concentrations of macroelements Ca, Na and K and those of trace elements Se, Cu and Zn were determined by atomic absorption

spectrophotometer (Perkin Elmer). The data were statistically processed by ANOVA 2000.

RESULTS AND DISCUSSION

The results are presented on **Tables 2, 3** and **4**. On the 40th day, blood serum Ca in controls reared under conditions of both high animal welfare and stress, was within the reference range: 2.168±0.04 mmol/l and 2.258±0.05 mmol/l, respectively. The higher levels in the latter group were not significant compared to the former one. In turkey poults with muscular dystrophy, Ca concentrations were considerably higher ($p<0.01$) in diseased birds under high animal welfare than diseased and stressed turkey-broilers (8.50±0.12 mmol/l and 9.025±0.75 mmol/l vs the respective control groups).

Table 2. Blood plasma macro and trace elements concentrations in broiler turkeys, either healthy or with muscular dystrophy prior to treatment, reared under conditions of high animal welfare or stress.

Groups	n	Ca mmol/l	Na mmol/l	K mmol/l	Cu µmol/l	Zn µmol/l
I. Controls-high animal welfare	10	2.168±0.043	158.21±0.815	5.322±0.066	4.790±0.194	62.872±1.480
II. Controls-stress	10	2.258±0.054	160.04±1.090	5.613±0.039	4.170±0.038	56.930±1.068
III. Diseased – high animal welfare	10	8.500±0.128	176.25±1.497	6.405±0.240	3.095±0.338	32.770±0.956
IV. Diseased-stress	10	9.025±0.750	179.97±0.570	8.108±0.271	2.050±0.013	26.250±0.597

Table 3. Blood plasma macro and trace elements concentrations in broiler turkeys, either healthy or with muscular dystrophy after treatment, reared under conditions of high animal welfare or stress.

Groups	n	Ca mmol/l	Na mmol/l	K mmol/l	Cu µmol/l	Zn µmol/l
I. Controls-high animal welfare	9	2.161±0.028	159.00±0.966	5.330±0.057	5.000±0.100	63.483±0.496
II. Controls-stress	9	2.240±0.047	157.05±1.915	5.530±0.042	4.200±0.036	59.525±0.572
III. Diseased – high animal welfare	8	3.308±0.347	166.60±1.829	5.633±0.061	3.930±0.061	46.740±3.330
IV. Diseased-stress	8	3.475±0.444	168.66±1.200	6.750±0.166	4.078±0.068	54.660±2.247

After the therapy, Ca levels (**Table 3**) in both diseased groups sharply decreased, but remained still higher than controls. In diseased turkeys reared under conditions of high animal welfare they were 3.308±0.34 mmol/l, compared to 8.50±0.12 mmol/l prior

to treatment ($p<0.01$). In diseased and stress turkeys, Ca concentration was 3.475±0.44 mmol/l, also significantly lower than in the respective pretreatment period (9.025±0.75 mmol/l, $p<0.01$).

Table 4 . Blood plasma Se concentration in broiler turkeys, either healthy or with muscular dystrophy prior to treatment and after treatment with Seled-0,06 mg/kg per os for 7 days, reared under conditions of high animal welfare or stress

Groups	n	Prior to treatment			After treatment			
		$\bar{x} \pm Sx$	P<	n	$\bar{x} \pm Sx$	P<		
		ppm			ppm			
I. Controls -high animal welfare	10	0.898±0.061	I-II	P<0.01	9	0.897±0.061	I-II	P<0.01
II. Controls-stress	10	0.722±0.009	III-IV	P<0.01	9	0.719±0.003	III-IV	P<0.01
III. Diseased–high animal welfare	10	0.413±0.0004	I-III	P<0.01	8	0.544±0.002	I-III	P<0.01
IV. Diseased-stress	10	0.267±0.001	II-IV	P<0.01	8	0.389±0.009	II-IV	P<0.01

Similar trend was observed in blood plasma Na of turkey-broilers by the 40th day. In control birds reared under conditions of high animal welfare it was normal – 158.21±0.81 mmol/l. In controls reared under stress, Na concentrations were slightly higher (160.04±1.09). In both groups of birds with muscular dystrophy, Na levels were considerably elevated (p<0.01): 176.25±1.49 mmol/l (in group I) and 179.97±0.570 mmol/l (group II). After the therapy, Na concentrations decreased significantly (p<0.05) in both experimental groups (166.6±1.82 mmol/l and 168.6±1.2 mmol/l) compared to pretreatment periods. At the same time, the birds reared under stress, exhibited again higher Na blood levels than those, reared under conditions of high animal welfare (p<0.05). The role of Na and K ions in pathogenesis of skeletal and muscle damage in broiler chickens was studied by Sandercock (10). The authors performed an *in vitro* experiment with muscle preparation. The results showed that along with Na ions increase, Ca ions in muscle were also elevated resulting in damage of musculo-skeletal membrane. That is why the authors assume that Na and Ca ions are directly interacting and redistributed via a mutual influence. For prevention of myopathies in chickens, they recommended the avoidance of conditions that could result in increased Na and Ca ions – such as stress, excessive muscular activity etc. Muscle cells contain a large amount of Na (6). After their destruction, the Na is released and enters the bloodstream. This is an explanation of increased blood Na levels in our study. The aforementioned authors consider that the changes in those 2 parameters characterize muscular dystrophy at an early stage, prior to its clinical development.

Plasma K levels in controls (groups I and II) were within the reference range by the 40th day (5.322±0.06 mmol/l and 5.613±0.039 mmol/l) as well as by the 58th day (5.330±0.05

mmol/l and 5.530±0.04 mmol/l). In turkey-broilers with muscular dystrophy, its levels increased in both groups, prior to the treatment (**Table 2**), to 6.405±0.24 mmol/l and 8.108±0.27 mmol/l, respectively. This was quite significant compared to the controls (p<0.01). A particularly high K levels was determined in stressed group (8.108±0.27 mmol/l) with significant difference compared to the group reared under high animal welfare (6.405±0.24 mmol/l; p<0.01). After therapy, the levels in experimental group I (5.633±0.06 mmol/l) decreased and were similar to levels in controls. K levels in the second experimental group, despite the considerable decrease (6.750±0.16 mmol/l) compared to pretreatment levels (8.108±0.27 mmol/l), were outside the normal range.

These facts showed that the stress in turkeys did not allow the normalization of blood K concentrations as in the group reared under high animal welfare. According to Aliev (6) the K was deposited in liver and mainly in muscle cells that enclose about 75% of total K contents. During extensive muscle work or other type of stress, K levels increase (6). The obtained data showed that dystrophic processes in muscle cells in turkey-broilers with muscular dystrophy are the cause for release of K from cells into the bloodstream and explain the elevated blood K concentrations. The combination with stress enhances this process. Georgiev (5) supposed that hyperkalaemia was a characteristic index for dystrophic events in muscles of calves, lambs and chickens. Georgiev (5) described hyperkalaemia as a typical trait of muscular dystrophy in White Moscow and bronze turkeys, reared under industrial conditions. With regard to Na levels, he reported also an increase but lower Ca concentrations in diseased purebred turkeys.

In our experiments, aside the described macroelements we studied also the microelements Cu and Zn as components of

the enzyme Cu, Zn-superoxide dismutase that are involved in muscular dystrophy.

The blood plasma Cu content in both control groups, reared under conditions of either animal welfare or stress on the 40th day was $4.790 \pm 0.19 \mu\text{mol/l}$ and $4.170 \pm 0.03 \mu\text{mol/l}$, respectively. In turkey-broilers with muscular dystrophy, Cu levels decreased ($p < 0.01$) to $3.095 \pm 0.33 \mu\text{mol/l}$ in those reared under high animal welfare and were almost twice lower in stressed and diseased birds ($2.05 \pm 0.01 \mu\text{mol/l}$) compared to controls. The difference between experimental groups I and II was also significant ($p < 0.05$). After the treatment, the values increased in birds healed from muscular dystrophy but unlike all observed tendencies, the elevation in groups reared under high animal welfare conditions was less – $3.93 \pm 0.06 \mu\text{mol/l}$ and did not reach the control levels by day 58 ($5.00 \pm 0.10 \mu\text{mol/l}$). At the same time, in the stressed group, the strongly reduced Cu levels prior to the therapy ($2.050 \pm 0.01 \mu\text{mol/l}$) normalized to $4.078 \pm 0.06 \mu\text{mol/l}$ after treatment and attained control levels.

A similar trend was observed in blood plasma Zn concentrations. On the 40th day, in affected birds reared under both high animal welfare and stress, Zn levels were considerably lower ($32.770 \pm 0.95 \mu\text{mol/l}$ and $26.250 \pm 0.59 \mu\text{mol/l}$; $p < 0.01$) compared to respective control groups ($62.87 \pm 1.48 \mu\text{mol/l}$ and $56.93 \pm 1.06 \mu\text{mol/l}$). At the same time, stressed controls also exhibited decreased Zn levels ($56.930 \mu\text{mol/l}$) than controls reared under high animal welfare ($62.872 \mu\text{mol/l}$; $p < 0.05$), indicating that stress alone exerted an influence and decreased Zn concentrations. After treatment, blood Zn values increased, compared to pretreatment values, but the group reared under high animal welfare displayed again lower levels than stressed group ($46.74 \pm 3.33 \mu\text{mol/l}$ and $54.66 \pm 2.24 \mu\text{mol/l}$, respectively).

The post treatment results showed that on one part, stress decreased Zn levels in control groups whereas in experimental, the levels were higher, with experimental groups II being closer to reference values than experimental group I. Increased Zn levels in diseased turkey-broilers after therapy could explain its impaired absorption on the background of Ca excess, reported in the studies of Aliev (6). In birds with muscular dystrophy, the significantly higher Ca concentrations result in impaired Zn absorption. According to Bozkaya et al. (13), Cu and Zn participate in antioxidant enzyme

composition and especially in that of Cu, Zn superoxide dismutase, thus ensuring protection against cellular damage. The authors observed lower activities of antioxidant enzymes and particularly of Cu, Zn superoxide dismutase in groups with feed Ca deficiency.

Those data and our results made us consider that in turkey-broilers with muscular dystrophy, trace elements Cu and Zn could play an essential role in the elimination of hydroperoxides obtained as a result of the used experimental design (supplementation of feed with 4% fat with peroxide number 5,00 g). Therefore, both these trace elements and the enzyme Cu, Zn superoxide dismutase in which composition they participate, would be lower. This hypothesis entails future studies on the activity of this enzyme in turkey-broilers with muscular dystrophy.

Plasma Se concentrations in control and diseased turkey-broilers prior to and after treatment, are presented on **Table 4**. Both before and after the treatment, Se concentrations in controls reared under high animal welfare were similar: 0.898 ± 0.061 prior to and 0.897 ± 0.061 post treatment. In stressed controls Se levels were lower than in controls living under high animal welfare: 0.722 ± 0.009 before and 0.719 ± 0.003 after the therapy, the differences compared with the respective animal welfare groups being significant: 0.898 ± 0.061 and 0.897 ± 0.061 ($p < 0.01$). The differences between control groups prior to and after the treatment were not significant. In turkey-broilers with muscular dystrophy, Se sharply decreased up to 0.413 ± 0.0004 (high animal welfare) and 0.267 ± 0.001 (stress), with significant differences compared to the respective control groups ($p < 0.1$ and $p < 0.01$ respectively). After treatment, this tendency was maintained despite that Se in healed birds increased to 0.544 ± 0.002 (high animal welfare) and 0.389 ± 0.009 (stress). The differences between post- and pretreatment periods were significant ($p < 0.01$). Those between healed affected birds and the respective controls were also considerable: 0.897 ± 0.061 – in high animal welfare and 0.719 ± 0.003 – in stress ($p < 0.01$).

Our data showed that muscular dystrophy led to considerable reduction in Se concentrations, more evident in birds reared under stress than in those reared under high animal welfare conditions. Although the treatment with Seled raised Se levels in healed turkeys, they did not attain the levels in corresponding controls and it remained the

lowest in healed diseased and stressed birds. According to Gabrashanski et al. (4), two mechanisms are responsible for the onset of myopathies in animals: (a) impaired equilibrium between unsaturated fatty acids and systemic antioxidants, resulting in increased peroxides content in mitochondria and (b) Se deficiency. The authors emphasize the inexplicable aspects of this relationship where several enzymes are involved. Later in his observations on muscular dystrophy in lambs, Gabrashanski et al. (4) noticed Se levels of 0.04 to 0.18 ppm in healthy and 0.05 ppm in lambs with muscular dystrophy. Also, in some diseased lambs, Se was not detected at all.

The reduced Se concentrations in turkey-broilers with muscular dystrophy in our experiment could be explained by the aforementioned mechanisms. Being an antioxidant, Se is used for neutralization of peroxides generated in the organism of turkey-broilers with muscular dystrophy following the addition of oxidized fat to their feed and, consequently, its blood plasma levels decreased.

CONCLUSION

In turkey-broilers suffering from muscular dystrophy, blood plasma levels of macroelements Ca, Na and K increased. After the therapy with Seled[®] (Radomir, Bulgaria), Ca and Na levels normalized and this outcome was more evident in birds reared under high animal welfare conditions. The levels of trace elements Cu, Zn and Se decreased in turkey-broilers with muscular dystrophy, more distinctly in stressed birds. Stress exerted a significant effect upon the values of studied parameters, demonstrated by their slower return to normal.

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