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Original Contribution

HEREDITARY MUSCULOSKELETAL DISEASES AND CHANGES IN BIOCHEMICAL PARAMETERS IN HEALTHY AND DISEASED LIGHT (LL) AND HEAVY (HM) TURKEY PARENTS AND TURKEY BROILERS DURING GROWTH UNDER ENVIRONMENTAL STRESS AND COMFORT

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ABSTRACT
The aim of this study was to determine the affect of hereditary musculoskeletal diseases (HMSD) to the blood serum parameters and enzyme activity in turkey parents from light laying and heavy meat breed, as well as the impact of conditions of environmental comfort and environmental stress on biochemical changes in turkey broilers. Muscular dystrophy expression was on the 80-th day, and on the 46-th day in parents and broiler, respectively. Data on the level of liver enzyme creatine kinase (CK) in the group of turkeys with HMCD showed a decrease compared with control group. The increase is observed in the change of the enzyme activity of aspartate aminotransferaze (ASAT) and alanine aminotransferaze (ALAT). In turkey broilers under the stress conditions, the changes of enzymes were more severe, instead of the lower rate of muscular dystrophy compared to parents.

Key words: turkey, muscular dystrophy,ALAT, ASAT, CK

INTRODUCTION

Blood enzyme activity is used in a routine assay for detection of animal's pathology, but in poultry there is a little information of the enzyme level in plasma and serum as well the level in the tissue of internal organs. Franson (1) believes that prior to use, as a diagnostic criterion is necessary to establish normal enzyme levels in plasma, serum and tissues of the poultry. Gee et al. (2) reported serum levels of enzymes in 12 bird's species, including geese, wild ducks, crane and etc. Franson (1) determined the activity of enzymes ALAT, ASAT, LDH, cratine kinase (CK), GGT (gamaglutamil transferees) in plasma, liver and kidney on the black and wild duck. Due to the strong variation of enzyme activity in liver and kidney, the author recommended a plasma or serum activity of these enzymes to be used as a diagnostic criterion for tissue damage in clinical pathology of birds. High activity of CK in the plasma of the wild ducks establishes Driver (3). Coles (4) considers the increase of ALAT activity in the serum of mammals as indicator for liver damage.

Some serum enzymes may be measured and used to detect changes prognosticating muscular dystrophy (MD). ASAT is a main indicator of MD.

In biochemical studies of muscular dystrophy in humans and animals was found increases of serum enzymes, decrease in muscle enzyme activity and increased creatine excretion (5). These changes are the result of muscle destruction. Mitchell et al (6) by "in vitro" technichue explains the mechanism of "leakage" of SC from muscle cells through the involvement of Ca^{2+} in sarcoplasma. Pathological increase sarcoplasmatyc Ca^{2+} in chicken skeletal muscle induced by specific Ca^{2+} ionophoresis causes clear increase of enzyme losses of intracellular CK. Increase in plasma or serum activity of this enzyme is a diagnostic indicator of skeletal damage (7). Clear increase in plasma CK activity in poultry is related to stress-induced myopathy (8; 9). Leaking of CK enzyme from skeletal muscle cells in this case is a result of changes in

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musculoskeletal membrane integrity, induced by increased Ca^{2+}in sarcoplasma (10; 11; 12).
Dabbert and Powell (13) determined the serum concentrations of the enzymes ASAT and CK as an indicator of muscle damage in wild ducks.
Stewart et al. (14) established increases in CK activity in embryonic skeletal muscles of chickens with hereditary muscular dystrophy (HMD) of a BB breed and hybrids. Enzyme activity progressively increased during incubation and 1 week before hatching CK muscle activity was 97% of the total muscle activity in either genotype. After hatching the total CK activity significantly decreased in distrophyc pectoral muscle.
The literature review shows that some enzymes as CK, ASAT, ALAT were examined in mice and birds, as the norm and their change by hereditary and nutritional MD. The results regarding the activity of enzymes are controversial. There are no reports of comparative studies on enzyme activity by light laying turkeys, heavy turkey parents for meat and broiler turkeys as well as the impact of conditions of environmental comfort and environmental stress on the changes of these enzymes in hereditary muscular diseases. This was the subject of the present study.

MATERIAL AND METHODS

The experimental trials were carried out at the base of the Agricultural Institute Stara Zagora, and poultry farm in village Krushovitza - Vratsa. Parents of 700 oviparous light turkeys (LL) and 700 heavy turkey for meat (HM) were reared in conditions of environmental comfort on litter floor with the poultry density of 1.43/m^2 (normal density rate according to Anonnymous (15) is 1.7/m^2. As environmental comfort is a considered optimum microclimatic condition.
One day old turkey broilers (n=1200) were divided in two groups and reared under environmental conditions for comfort and environmental stress up to age of 70-th day.
Microclimatic parameters were determined by routine methods. The temperature and relative humidity were determined weekly by termohigrograf, ventilation by katatermometar, the intensity of light by luksmetar, the concentration of ammonia by indicator tubes and apparatus by Dregerov. Relative counts were calculated by formula in ppm.
Hereditary musculoskeletal diseases were obtained for the period from first day to 1 year in turkey parents and up to age of 70 days in turkey broilers.
Determined were blood parameters: blood sugar test by Roche-Diagnostic and enzymes ASAT, ALAT and creatine kinasis (CK) with an automatic biochemical analyzer "Reflotron-plus" Germany.

RESULTS AND DISCUSSION

Differences in microclimatic parameters under conditions of environmental comfort and environmental stress are presented in **Table 1**. Within the normal rates (15) were averages of temperature, relative humidity, concentration of ammonia, light intensity and speed of air movement (ventilation) in the living area of turkey broilers reared under conditions of environmental comfort (Table 1). But during the period from the 11th to the 70th day were recorded deviations from normal microclimatic conditions (Table 1). The results indicate that during this period turkeys were kept under the conditions of the environmental stress.

Table 1. *Microclimatic conditions in houses for turkey parents and turkey broilers.*

Groups	Age, (days)	Temperature (°C)	Humidity, (%)	NH_3, (ppm)	Lux, (lx)	Ventilation, (m/s)
Turkey parents	320-330 days	16.20±0.25	62.12±0.50	10.93±0.31	49.10±1.76	0.28±0.02
Turkey broilers in comfort conditions	1- 10 days	32,6±0,82	70,9±0,38	1,2±0,2	70±6,77	0,16±0,004
	11- 20 days	29.5±0.42	68±0,12	5,2±0.1	52,5±1,44	0.18±0.004
	21-40 days	24,0±0.56	65±0,82	8,5±0.1	47,5.0±1,44	0.27±0.005
	41-70 days	18.5±0.44	61,8±0,76	10,82±0.1	42.0±1.44	0.26±0.004
Turkey broilers in stress conditions	1- 10 days	35,6±0,82	70,9±0,38	5,2±0,2	70±6,77	0,16±0,004
	11- 20 days	35.5±0.42	78±0,12	15,2±0.1	52,5±1,44	0.18±0.004
	21-40 days	28±0.33	72±0.50	15,02±0.1	23±0.54	0.27±0.005
	41-70 days	27,5±0.45	71±0.51	15,22±0.1	21,0±0.44	0.26±0.004

The results of the expression of hereditary musculoskeletal diseases (HMCD) in turkey parents and turkey broilers show the following: In turkeys parents for the first time HMCD are expressed at age of 80-th day with a lower rate in oviparous light turkey (LL) - 5.0%, compared with heavy turkey (HM) - 7.0%. However, in turkey broilers HMCD are expressed from 46 days age with 6, 0% in conditions of environmental comfort, and 8% in environmental stress.

Olson (16) also observed HMCD in 3-4 month old chicken broilers, despite being fed with -α-tocopherol acetate (100 mg/kg).

The results for the level of enzymes CK, ASAT and ALAT in turkey parents are presented in **Table 2.** Data on the level of liver enzyme CK in group of turkeys with HMCD showed a decrease compared with control group. The lowest level of this enzyme was in heavy meat turkeys (HM) - 244,0±4,3 compared with control group (428,4±6,7; p<0,01). In a lesser was reduced the level in oviparous light (LL) group (310,4±3,9) compared with control group (417,2±4,7), but the difference was significant (p <0,01).

Significant was the difference in enzyme CK levels (P <0.01) between two control groups of health oviparous light (LL) - 417,2±4,7 and heavy meat turkeys (HM) - 428,4±6,7 and also between diseased patients, respectively 310, 4 ± 3,9 and 244,0 ± 4,3 (P <0.01).

***Table 2**. Level of blood glucose, activity of CK, ASAT and ALAT in turkey parents from oviparous light (LL) and heavy meat (HM) breed with and without hereditary musculoskeletal diseases (HMCD)*

Groups	**n**	**Blood glucosae**	**CK**	**ASAT**	**ALAT**
		$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
Control (LL)	10	14,4±0,01	417,2±4,7	267,7±1,63	3,3±0,04
Control (HM)	10	16,4±0,01	428,4±6,7	280,2±3,27	3,8±0,03
HMCD (LL)	10	13,7±0,01	310,4±3,9	301,0±1,19	4,2±0,07
HMCD (HM)	10	11,9±0,02	244,0±4,3	337,0±3,45	4,8±0,04
		I-II p<0.01	p<0.01	p<0.01	p<0.01
		I-III p<0.05	p<0.01	p<0.01	p< 0,05
		II-III p<0.01	p<0.01	p<0.01	p<0.001

Significant was the difference in enzyme CK levels (P <0.01) between two control groups of health oviparous light (LL) - 417,2±4,7 and heavy meat turkeys (HM) - 428,4±6,7 and also between diseased patients, respectively 310, 4 ± 3,9 and 244,0 ± 4,3 (P <0.01).

Increase is observed in the change of the enzyme ASAT. In turkeys with HMCD it is increased (301,0±1,19 in LL; 337,0±3,45 in HM turkeys) compared to control groups (267,7±1,63 in LL and 280,2±3,27 in HM control turkeys). The significant differences are found between diseased and control groups (p <0,01) and also between the two LL and HM control groups (p <0,01). More pronounced is the increase in the enzyme ASAT in HM turkeys with HMCD.

Analogous is observed and the change in the enzyme ALAT, which is more increased (p <0,01), in HM turkeys with HMCD (4,8±0,04), compared to control group (3,8±0,03) and a little bit lower (p <0,05) in LL turkeys (4,2 ± 0,07) and their controls (3,3±0,04). Significant difference (p <0,01) in ALAT level was found between the groups with HMCD (4,2 ± 0,07 in LL, and 4,8±0,04 in HM).

Our results correlate with results obtained by Stoyanchev (17), where by feed intended muscular dystrophy in turkey broilers was detected increases in ASAT, ALAT and CK. But in HMCD we found decrease of CK. Macrae et al. (18), which also investigated enzymes CK, LDH and ASAT as indicators of muscle damage and nutrient myopathies in broilers, broiler parents and oviparous breed and authors establish their high content in the blood. The recovery process after diseases was expressed by decrease in CK, LDH and ASAT. The decrease in the enzyme in studied by us HMCD despite it increase in nutrient muscular dystrophy, gives us reason to recommend it to differentiate the two types of diseases. We share the opinion of Hotton & Watts (19) who consider that hereditary muscular dystrophy is characterized by genetic changes in muscular CK. This is probably the reason for decreased activity of CK in turkeys suffering HMCD.

Analyses of enzymes in turkey broilers with HMCD show the same correlation as in parents in increase of ASAT and ALAT and reduction of CK activity. Similarly to parents, in turkey broilers these parameters are more pronounced when poultry growing under conditions of environmental stress as with those grown under conditions of environmental comfort. In terms of environmental stress the enzyme level was 241,6±3,54 for CK; 348,3±4,0 for AST and 4,9±0,31 for ALAT. In terms of environmental comfort the same enzymes rate was, 304,0±12,9; 302,0±0,71 and 4,6±0,71 respectively **(Table 3)**.

It is clear, that in broilers with HMCD in conditions of environmental stress changes in the enzyme activity were greater than those in turkey parents.

We share the opinion of the Franson (1), where due to the strong variation of enzyme activity in liver and kidney, the author recommended in poultry pathology for tissue damage to be used plasma or serum activity of these enzymes.

***Table 3**. Level of blood glucose, activity of CK, ASAT and ALAT in turkey broilers with and without hereditary musculoskeletal diseases (HMCD) growing under condition of environmental comfort and stress*

Groups	**n**	**Blood glucose**	**CK**	**ASAT**	**ALAT**
		$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$	$\bar{x} \pm S\bar{x}$
Control	10	14,6±0,10	429,2±7,30	265,8±0,86	3,2±0,07
Turkey broilers in environmental comfort	10	13,16±0,09	304,0±12,9	302,0±0,71	4,6±0,71
Turkey broilers in environmental stress	10	12,0±0,07	241,6±3,54	348,3±4,0	4,9±0,31
		I-II p<0.01	p<0.01	p<0.01	p<0.01
		I-III p<0.01	p<0.01	p<0.01	p< 0,05
		II-III p<0.01	p<0.01	p<0.01	n.s
		p<0.01	p<0.01	n.s	

CONCLUSIONS

1. In turkey parents with hereditary musculoskeletal diseases (HMCD), was found increased levels of enzymes ASAT and ALAT and decreased activity of CK, which is more pronounced in heavy turkey parents for meat (HM), compared with oviparous light (LL) turkey parents. The same correlation was detected between turkey broilers growing under the conditions of environmental stress and ones under conditions of environmental comfort.
2. Changes in enzyme activity were more pronounced in broilers, compared with parents in which the rate of disease was higher.
3. Decreased activity of CK in HMCD can be used as a diagnostic parameter differentiating hereditary from nutrient related muscular diseases under which activity of CK increases.

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