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BEHAVIORAL STUDY OF MULE DUCKS WITH SUBCLINICAL MUSCULAR DYSTROPHY UNDER ECOLOGICAL COMFORT AND STRESS CONDITIONS

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Abstract

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The purpose of the study was to trace behavioral changes of mule ducks during the alimentary induction and treatment of subclinical muscular dystrophy (MD) under conditions of environmental comfort and stress. The study included 40 Mule ducks, divided into 4 groups: group I - control healthy mule ducks, reared under ecological comfort; group II - control healthy mules under ecological stress conditions, group III – mules with provoked MD (MD-mules), under ecological comfort conditions and group IV - MD-mules under stress ecological conditions.

The mule duck behavior was recorded for four consecutive days by video cameras for 12 hours a day: from 58 to 61 days of age (at the beginning of subclinical MD) and from 68 to 71days of age (4 days after the end of treatment). During the subclinical MD significantly differences in mule behavior were found in water intake, in gregarious and aggressive behavior of birds reared under different conditions. In MD-mules, reared under stress conditions less number of standing ($P < 0.01$), moving ($P < 0.05$) and feather cleaning birds ($P < 0.05$) was recorded compared to MD-mules under comfortable conditions. This confirms the aggravating role of stress conditions on the locomotor activity and subclinical course of MD in mule ducks.

The behavioral differences between MD-mules and control mules under comfortable conditions disappeared four days after the end of the MD- treatment. Simultaneously the number of standing ($P < 0.05$) and bathing ($P < 0.01$) MD-mules was lower than that in healthy controls reared under stress conditions. A greater number water drinking birds ($P < 0.001$), more aggression ($P < 0.01$) and less standing birds ($P < 0.001$) were registered in treated MD-mules under stress conditions compared to those under comfort conditions, which confirms the poor locomotor activity and healthy state in treated under stress conditions mule ducks.

Key words: mule ducks, behavior, locomotor activity, mule ducks, subclinical muscular dystrophy, ecological comfort and stress conditions

Introduction

Metabolic musculoskeletal diseases are among the most important challenges to contemporary poultry industry due to the substantial economic losses from lower productivity and lameness (Julian, 2005). Such a metabolic disease is alimentary muscular dystrophy (MD; white muscle disease) which affect all domestic poultry species.

MD is caused by deficiency of selenium (Se) and/or vitamin E and sulfur amino acids, as well as stress factors (Todorovic, 2002; Mench, 2004; Chang et al.,

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2005). It is manifested by dystrophy of skeletal muscles in birds.

Xu and Diplock (1983) reproduced MD in mule ducks by feeding them a diet deficient in vitamin E and Se. Merck Veterinary Manual (2006) confirms that these ingredients play a key role in the metabolism of cysteine and protect birds from MD. McMullin (2004) outlines that the onset of MD is related also to the higher content of rancid dietary fat.

On the other side, the effects of ecological comfort and ecological stress on birds' behaviour are also intriguing (Jones et al., 2005 and Moura et al., 2006), with regard to MD course and treatment (Stoyanchev et al., 2006; Bozakova et al., 2007; Bozakova et al., 2008). There is limited information on the effect of environmental stress (increased air temperature and humidity, ammonia concentrations close to allowances) on the severity of disease and its therapy. Aliev et al. (1985) believe that adverse atmospheric conditions increase energy needs of birds and could provoke signs of muscular dystrophy**.** Georgiev (1979) demonstrated that the transportation of turkeys with MD resulted in more acute and more severe clinical signs of the disease, and sometimes to fatal outcomes.

In previous studies of ours, significant changes in the behavior of turkey breeders, turkey poults, and broiler chickens with alimentary induced muscular dystrophy were shown, both in conditions of ecological comfort and ecological stress (Stoyanchev et al., 2006; Bozakova et al., 2007; Bozakova et al., 2008).

In fowl, the clinical MD is characterized by ataxia, stiffed gait, paresis, paralysis and death (Whitehead, 2003; Mench and Lameness, 2004). In mule ducks with alimentary MD, the disease course was subclinical, without these signs. Despite the subclinical course of the disease, there were considerable differences in the locomotor activity and the behavior of mule ducks with induced MD, reared under ecological comfort or stress. This was the incentive of the present experiment.

The purpose of the study was to trace behavioral changes of mule ducks during the alimentary induction and treatment of subclinical muscular dystrophy (MD) under comfort and stress environmental conditions.

Material and Methods

The field experiment took place in the Experimental Base of the Department of Internal Diseases, Faculty of Veterinary Medicine, and Trakia University with 40 one-day-old Mule ducks. The identification of birds was done with wing marks.

From day 1 to 15, all ducks were fed and housed identically. To induce alimentary muscular dystrophy (MD), on the 16^{th} day the birds were divided into 4 groups (n=10) housed in two facilities with a different microclimate. In one facility, the healthy controls (group I) and experimental birds (MD-mules) with alimentary MD (group III) were reared in conditions compliant to the hygienic norms for this category of birds (ecological comfort) (Table 1). In the other facility, the group of

Table 1
Microclimatic conditions for Mule ducks after induction and treatment of muscular dystrophy reared under comfortable and stress ecological conditions

Rearing conditions	Days of age	Temperature, ^0C	Humidity, %	NH_3, ppm	Light intensity, lx	Air velocity, m/s
Comfortable microclimatic conditions (for I &III groups)	1-14	28±1.16	65.6±0.54	6.56±0.26	70±6.77	0.26±0.004
	15-28	20.2±0.20	69±0.71	6.56±0.13	52.5±1.44	0.26±0.004
	29-71	18.14±0.14	70±0.82	9.21±0.13	42.0±1.44	0.28±0.004
Ecological stress conditions (for II & IVgroups)	1-14	28±1.16	65.6±0.54	6.56±0.26	70±6.77	0.26±0.004
	15-28	34.00±0.24	79.20±0.92	15±0.13	23.0±0.5	0.27±0.005
	29-71	35.00±0.24	82.4±1.68	15±0.13	21.0±0.4	0.26±0.004
Reference values	1^{st}-15^{th} days	35-21	65 – 70	15	minimum 15 lx	0.2-0.3
	16^{th}-28^{th} days	20	65 – 70	15		0.2-0.3
	after 28^{th} days	15- 18	65 – 70	15		0.8-1.0

*Reference values as per Ordinance 44/ 2006 healthy controls (group II) and MD-mules (group IV) were housed under ecological stress conditions: high ambient air temperature and humidity, ammonia concentrations close to the upper limit (Table 1).

Each group of mule ducks was housed in a separate section with area of 4 m^2 (0.4 m^2/ bird vs norm of 0.303 m^2/ bird). The floors of sections were covered with 10 cm clean dry straw bedding. In each section, a swimming pond of 0.8 m^2 was provided for demonstration of the natural behavior of mule ducks (Holderread, 1992).

Mule ducks were fed balanced pelleted compound feed – prestarter, starter and grower, produced by PROVIMI ZARA – Stara Zagora. The feed given to birds from group III and IV (MD mules) was balanced with respect to the energy content, but deficient in in selenium, vitamin E, sulfur-containing amino acids, and supplemented with 4% oxidized fat with peroxide number 200.0 (allowed peroxide number 0.20) for induction of MD.

A prophylactic programme was carried out in the four groups but the MD-mules (groups III and IV) was not treated with *Seled* at a dose of 1 ml/l water between days 22-27 and 36-41 in order to enhance the development of muscular dystrophy.

The treatment of MD-mules began at the age of 63 days with *Seled-hydro*, containing sodium selenide and vitamin E, made by Biovet-Peshtera at a dose of 1 ml/l drinking water for 5 days (Debski et al., 2005**;** Nier et al., 2006).

Microclimatic conditions were determined by routine methods. The temperature and the relative humidity of air were measured with a weekly thermohygrograph (Type 405, Germany); the velocity of the air motion – with a catathermometer, the light intensity – with a electronic luxmeter (Taschen-Luxmeter LM37, Germany), the concentration of ammonia – with indicator tubes and Drager ammonia sensor and calculated in ppm.

The mule duck behavior was recorded four consecutive days by video cameras for 12 hours a day: from 58 to 61 days of age (at the beginning of subclinical MD) and from 68 to 71 days of age (4 days after the end of treatment). During the ethological study was counted the number of birds exhibiting a specific pattern of behavior: ingestive behavior − intake of food and water), gregarious behavior - lying, standing, walking, bathing, swimming and preening and aggressive behavior (Popova-Ralcheva et al., 2002). Ethological forms were determined from 58 to 61 days of age (at the beginning of subclinical MD) and from 68 - to 71- age of days (4 days after the end of treatment).

Statistical processing of the results was performed by means of one-way ANOVA using the GraphPad InStat 3.06 software to determine the level of significance among mean values. raphPad InStat 3,06 software at level of significance $P<0.05$.

Results

During the entire experimental period, the microclimatic parameters in the facility where the birds from groups I and III were reared were compliant to the hygiene norms specified by Ordinance 44/ 2006 (Table 1).

At the same time, within the age period 15-71 days, the birds from groups II and IV were reared under air temperature and humidity higher than the normal values. Ammonia levels in the living area were near to the upper allowance of 15 ppm. Light intensity and air velocity during the entire period of the study were within the reference ranges.

Observing the changes in the behaviour in the subclinical MD (Table 2) significantly differences in mule behaviour were found in water intake, in gregarious and aggressive behaviour of birds reared under different conditions. The onset of MD was by the age of 57 days, where in 20% of birds reared under ecological stress (IV group), the test for muscular dystrophy was positive (Georgiev, 1979).

At the same time, the number of lying down ($P<0.001$) moving ($P<0.01$), swimming ($P<0.05$) and bathing ($P<0.01$) MD mules (group III) was lower as compared to control birds under ecological comfort (group I).

MD mules reared under environmental stress (group IV) exhibited higher number of lying down ($P<0.001$) and lower number of standing ($P<0.05$), moving ($P<0.01$), swimming ($P<0.05$) and bathing ($P<0.001$) birds than controls under stress conditions (group II).

Table 2
Mule duck`s behaviour during induction and treatment of muscular dystrophy (MD) under comfort and stress rearing conditions. Data are presented as Mean ± SEM, n=10

Behaviour	Groups	Sub clinical MD	%	4 days after the end of MD -treatment	%
Feeding	I	1.63±0.42	16.25	2.00±0.45	20.00
	II	1.63±0.34	16.25	1.63±0.35	16.25
	III	1.83±0.44	18.33	1.96±0.44	19.58
	IV	1.83±0.34	18.33	2.21±0.30	22.08
Drinking	I	0.67±0.13	6.67	0.79±0.16	7.92
	II	1.58±0.18###	15.83	1.58±0.18	15.83
	III	0.75±0.12	7.50	0.67±0.13	6.67
	IV	1.92±0.16”””	19.17	1.83±0.22”””	18.33
Lying	I	0.38±0.12	3.75	0.33±0.12	3.33
	II	0.33±0.12	3.33	0.33±0.12	3.33
	III	2.33±0.27***	23.33	0.25±0.11	2.50
	IV	2.71±0.23***	27.08	0.50±0.10	5.00
Standing	I	2.13±0.26	21.25	1.86±0.25	18.75
	II	1.50±0.19#	15.00	1.50±0.19	15.00
	III	1.79±0.23	17.92	2.33±0.30	23.33
	IV	1.04±0.14*””	10.42	1.08±0.13*”””	10.83
Movement	I	2.13±0.22	21.25	2.04±0.21	20.42
	II	1.63±0.16#	16.25	1.63±0.16	16.25
	III	1.46±0.16**	14.58	2.04±0.23	20.42
	IV	1.04±0.11**”	10.42	1.96±0.20	19.58
Swimming	I	0.63±0.17	6.25	0.58±0.16	5.83
	II	0.50±0.15	5.00	0.50±0.15	5.00
	III	0.21±0,08*	2.08	0.54±0.16	5.42
	IV	0.17±0.08*	1.67	0.42±0.12	4.17
Bathing	I	2.21±0.19	12.08	1.13±0.17	11.25
	II	1.08±0.15	10.83	1.08±0.15	10.83
	III	0.67±0.12***	6.67	0.92±0.19	9.17
	IV	0.54±0.10**	5.42	0.54±0.12**	5.42
Aggression	I	0.17±0.08	1.67	0.17±0.08	1.67
	II	0.92±0.17###	9.17	0.92±0.17	9.17
	III	0.21±0.08	2.08	0.13±0.07	1.25
	IV	0.33±0.10**	3.33	0.67±0.16””	6.67
Feather cleaning	I	1.00±0.21	10.00	1.00±0.21	10.00
	II	0.88±0.20	8.75	0.88±0.20	8.75
	III	0.79±0.16	7.92	1.08 ±0.21	10.83
	IV	0.46±0.10”	4.58	0.83±0.20”	8.33

*P<0.05; **P<0.01; ***P<0.001: statistically significant difference between healthy and MD-provoked mule ducks under comfortable or stress-ecological conditions;

#P<0.05; ##P<0.01; ###P<0.001 statistically significant difference between healthy mule ducks under comfortable and stress-ecological conditions;

“P<0.05; “”P<0.01; “””P<0.001 statistically significant difference between MD-provoked mule ducks under comfortable and stress-ecological conditions.

In MD-mules, reared under stress conditions (group IV) more water drinking birds (P<0.001) and less standing (P<0.01), moving (P<0.05) and feather cleaning birds (P<0.05) were recorded compared to MD-mules under comfortable conditions (group III).

The environmental stress influenced also the number of water drinking birds which was higher (P<0.001) but resulted in less standing (P<0.05) and moving (P<0.05) birds, strongly increased the acts of aggression (P<0.001) in healthy mules from group II compared to those reared under comfort (group I).

Four days after the end of the 5-day treatment of MD birds with *Seled-hydro*, the behavior of mule ducks was altered (Table 2). Because of the treatment, in birds reared under ecological comfort (group III), no more behavioral differences were observed compared to controls (group I). At the same time, in group IV, there were less standing (P<0.05) and bathing birds (P<0.01), compared to controls reared under stress (group II). There were also more birds drinking (P<0.001) and higher level of aggression (P<0.01), but less standing birds (P<0.001) compared to treated birds reared under ecological comfort (group III).

Discussion

The microclimatic parameters (Table1) during the experimental period for groups I and III were within the ecological comfort norms specified for this category birds.

Mule ducks from groups II and IV were under the complex influence of several adverse stressors – high air temperature and relative air humidity, ammonia concentrations close to the upper allowances, i.e. under ecological stress.

The subclinical course of alimentary MD in ducks was accompanied by substantial changes in their behavior under both microclimatic conditions (Table 2) as seen from the water drinking, gregarious and aggressive behaviors.

The higher number of lying MD mules (P<0.001), reared in comfort and the lower number of moving (P<0.01), swimming (P<0.05) and bathing birds (P<0.01) than controls corresponded to the data of Mench (2004), who reported a reduced locomotor activity secondary to musculoskeletal diseases in meat type birds. Mench and Siegel (1997), Tankson et al. (2001), Mench (2002), established more lying broiler chickens, ducklings and turkey poults with myopathies. This could be attributed to the dystrophy of skeletal muscle fibrils in birds and thus, to the easier exhaustion (Surai, 2002; Nier et al., 2006). Xu and Diplock (1983) observed the development of skeletal muscle lesions in Peking ducks with alimentary MD, whereas Reddish et al. (2005) demonstrated vacuolization of microscopic and small areas of the thoracic muscle sarcolemma in chickens.

The most pronounced changes in the behavior were observed in MD-mules, reared under stressful microclimate. The increased number of lying (P<0.001) and the lower number of standing (P<0.05), moving (P<0.01), swimming (P<0.05) and bathing (P<0.001) MD-mules compared to respective controls could be explained by the more severe skeletal muscle dystrophy under the combined influence of the disease and environmental stress. These data correlate to those of Scheele (1997), who stated that adverse atmospheric conditions could trigger clinical signs in subclinical MD in birds and exacerbate muscle dystrophy.

The lower number of standing (P<0.01), moving (P<0.05) and feather cleaning (P<0.05) MD-mules reared under stress compared to diseased birds under comfort emphasizes the role of the environment for the development of this disease. These data came in support of the data provided by Georgiev (1979) that in White Moscow and Bronze turkeys with MD, the transportation stress worsened the clinical manifestations of the disease and even resulted in a lethal issue in some birds. Aliev et al. (1985) presented similar data as well.

All these facts confirmed the exacerbating effect of stress on impaired locomotor activity in birds with alimentary MD.

The rearing stress resulted in lower number of healthy birds standing on their feet (P<0.05), moving (P<0.05) but markedly increased the number of drinking (P<0.001) and fighting birds (P<0.001) compared to those reared in comfort. According to Shields et al. (2004), Gentile (2006), Dixon (2008), ecological stressors increase the aggressive acts in birds, which could be related to the excess secretion of ACTH and corticosterone (Cymering et al., 1998; Tsai et al., 2002), provoking stress and anxiety.

Just as our observations on increased water intake in mule ducks, Sherwin and Kelland (1998), Bozakova et al. (2009), Bozakova (2010) established more turkey breeders or turkey poults drinking water under heat stress. The more intensive drinking during the hot period is a thermoregulatory response aimed at attaining a higher rate of heat elimination in order to restore the systemic fluid balance under high ambient temperatures (Smith & Teeter, 1981).

The 5-day treatment of MD-mules with *Seled-hydro*, those reared under ecological comfort exhibited no behavioural differences compared to controls. This confirmed the efficacy of the treatment performed (Surai, 2002**;** McMullin, 2004; Nier et al. 2006) for restoration of skeletal muscle dystrophic events and reinstatement of the normal locomotion. After subcutaneous injection of sodium selenide, Reddish et al. (2005) observed restoration of dystrophic vacuolated areas of thoracic muscle sarcolemma in chickens with induced MD.

The MD mules reared under environmental stress, the lower number of standing and bathing birds indicated slowing down of regeneration events and impossibility for restoration of locomotor activity to that in healthy controls. This confirms the environmental impact upon the course of the treatment on one hand, and the subtle mechanisms at the background of the various types of locomotor activity in birds on the other. In support of this hypothesis, Sherwin and Kelland (1998), Pereira et al. (2011) underlined that standing and bathing in birds were reliable indices of good health and welfare.

The results allowed to conclude that ecological stress increased the susceptibility of birds to muscular dystrophy and delayed the healing process.

Also, the lower number of MD-mules standing on their feet and the higher level of aggression confirmed the worse locomotor activity and general condition of birds treated under conditions of environmental stress as compared to these treated in comfortable conditions.

As could be seen from the present results, the subclinical course of alimentary MD in mule ducks reared either under ecological comfort or stress was related to permanent changes in their behavior, which should be acknowledged in order to evaluate the accurate diagnosis, the proper treatment, and the time healing occurs.

Conclusions

Alimentary induced muscular dystrophy (MD) in mule ducks under comfort or stress had a subclinical course and was manifested by reduced locomotor activity i.e. higher numbers of lying and lower numbers of standing, moving, swimming and bathing birds compared to healthy ducks. Stress aggravated the course of disease, manifested by less standing, moving and feather cleaning bird's vs ill birds housed in comfortable conditions.

The environmental stress in treated MD-mules impeded the restoration of locomotor activity, expressed by preserved lower number of standing and bathing birds compared to healthy controls.

Compared to treated birds housed in ecological comfort conditions, stress conditions negatively influenced on a behavior normalizing of treated MD-mules, which was manifested by preserving of the lower number of standing birds and the higher frequency of aggression acts.

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