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THE INFLUENCE OF LONG TERM SOUND STRESS ON HISTOLOGICAL STRUCTURE OF IMMUNE ORGANS IN BROILER CHICKENS

ABSTRACT: The aim of this paper was to examine the effect of different duration sound stress on immune organs of broiler chickens of different age. Nine groups, with 10 chickens in each group were included in experiment. The histological structure of bursa of Fabricius, thymus, and spleen were analyzed. The results indicated that the bursa of Fabricius, in relation to the other examined organs, was the most sensitive to this kind of stress. Histological changes of spleen and thymus were also observed, but less prominent except in chickens after more than 30 days of exposure to stress. According to our results, degree of histological changes of immune organs under the influence of sound stress depends on the length of exposure and age of chickens.

KEY WORDS: sound stress, broiler chickens, immune organs

INTRODUCTION

Stress is the reaction of the organism to stimulus, which disturbs physiological equilibrium, usually with harmful consequences. This disturbance of homeostasis has resulted in changes in the concentration of large number of different hormones that have a crucial role in the regulation of immune system function.

The link between neuroendocrine and the immune system, is first reflected in the existence of receptors for a number of chemical mediators in immune cells. These chemical modulators, actually hormones, are ACTH, glucocorticosterids, vasoactive intestinal peptide (VIP), substance P, prolactin, growth hormone, sex steroids, catecholamine, acetylcholine, releasing hormones, and opioid molecules (K h a n s a r i et al., 1990).

Immunomodulatory effect of these hormones can be direct, when they affect biochemical reactions that are responsible for cell proliferation, differentiation, and their functions. In addition, indirect influence is reflected in the

modulation of production and/or activities of lymphokines and monokines (Munck et al., 1984; Johnson and Torres, 1985; Ooi et al., 1987).

Furthermore, in stress reaction autonomic nerve system (ANS) is activated. Importance of that system is in his connection with lymphoid organs, which are innervated by ANS. In that way, ANS is involved in process of immunomodulation (Ackerman et al., 1989; Ackerman et al., 1991; Felten and Felten, 1991; Vizi et al., 1995).

Results of different investigations show that stress in broiler chickens has significant influence on immune system and immune organs. Lazarević et al. (2000) reported that sound stress has immunosuppressive effects on broiler chickens. Heat stress has also led to significant immunosuppression in broiler chickens (Al-Ghamdi, 2008). Social stress induces decreasing ratio between weight of bursa of Fabricius and body weight (Mohamed and Hanson, 1980). Investigating the nutritional stress on the bursa of Fabricius and thymus of chickens Griffiths et al. (1985) point out that stress induces thymus atrophy and reduction in bursa of Fabricius weight. Chickens exposed to stress promoted by vaccination and overpopulation had smaller weight of the bursa of Fabricius, thymus and spleen compared to control group (Awadalla, 1998). Pardue et al. (1985) investigated influence of high temperature on broiler chickens and point out that thermal stress decreased weight of bursa of Fabricius and spleen. ACTH infusion induces decreasing of bursa of Fabricius weight in chicken. Same response of that organ was observed in *Gallus domesticus* after implantation of pellets with corticosterone and cholesterol (Davison et al., 1985). In addition, exogenous cortisol and treatment with combination of cortisol and thermal stress decrease Bursa of Fabricius and spleen weight in broiler chickens (Brake et al., 1988).

The aim of our investigation was to estimate the influence of long-term sound stress on morphology of immune organs (thymus, spleen, and Bursa of Fabricius) in broiler chickens.

MATERIALS AND METHODS

The experiments were conducted on 90 HYBRO broiler chickens, divided into nine groups (each group consisting of 10 birds) as follows:

$C_{1—15}$ — control (non exposed) group
$O_{1—15}$ — chickens exposed to the sound stress from 1st to 15th day of life
$C_{1—30}$ — control (non exposed) group
$O_{1—30}$ — chickens exposed to the sound stress from 1st to 30th day of life
$O_{15—30}$ — chickens exposed to the sound stress from 15th to 30th day of life
$C_{1—45}$ — control (non exposed) group
$O_{1—45}$ — chickens exposed to the sound stress from 1st to 45th day of life
$O_{15—45}$ — chickens exposed to the sound stress from 15th to 45th day of life
$O_{30—45}$ — chickens exposed to the sound stress from 30th to 45th day of life

The control and exposed chickens were kept in two different buildings under same conditions of temperature, light, humidity, and number of birds per m^2. They were fed *ad libitum* and had free access to water. According to the experimental schedule, chickens were moved from the building without noise to the building where they were subjected to sound stress. The chickens were exposed to sound stress in sound attenuated building using a fire alarm bell (95 dB) for stress sessions lasted 120 min., every day. The bell was programmed to ring for 5 sec in a variable interval schedule (5 to 115 s) through signals generated by PC software.

Every 15 day, birds were sacrificed as show in above schedule. For histological investigation samples of thymus, spleen and bursa of Fabricius were fixed in Bouin solution for 24 h, and after standard histological procedure of dehydration, organs are embedded in paraffin. Serial cuts, 5 μm thickness, were made by microtome and stained with hematoxylin and eosin. Histological analysis was performed with light microscope Leica DLMS connected with camera (Leica DC-300). Software for image analysis was IM 1000 (Leica Imaging Systems Ltd, Cambridge, UK).

RESULTS

Bursa of Fabricius

The typical changes of bursa of Fabricius in all groups of chickens exposed to sound stress are reflected in the follicles atrophy and increase of connective tissue (Figure 1A-F).

The degree of changes was in direct correlation with the duration of exposure to sound stress. In the group that was exposed to stress from day 1 to day 45 a complete atrophy of lymph follicles, the large amount of connective tissue and appearance of cysts in epithelium was observed.

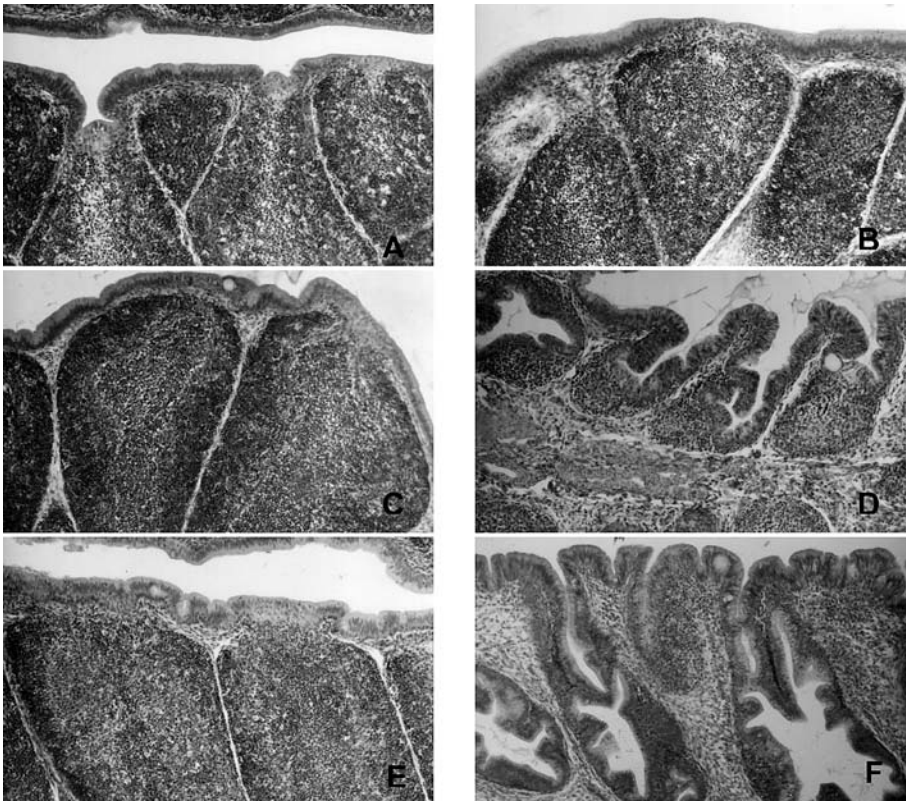

Fig. 1 — Microphotographs of chicken bursa of Fabricius (A — control group, 15 days old; B — sound stress from day 1 to day 15; C — control group, 30 days old; D — sound stress from day 1 to day 30; E — control group, 45 days old; F — sound stress from day 1 to day 45 (HE; 150x)

Thymus

The chronic action of sound stress did not cause significant changes in the histological structure of thymus. Discrete changes are observed only in the group of chickens that was exposed to chronic sound stress from 1st to 30th day of life (Figure 2A-B). Thymus of these animals is characterized by the presence of a larger number of macrophages in the cortex and the more developed connective tissue, but the basic structure of organ was unchanged.

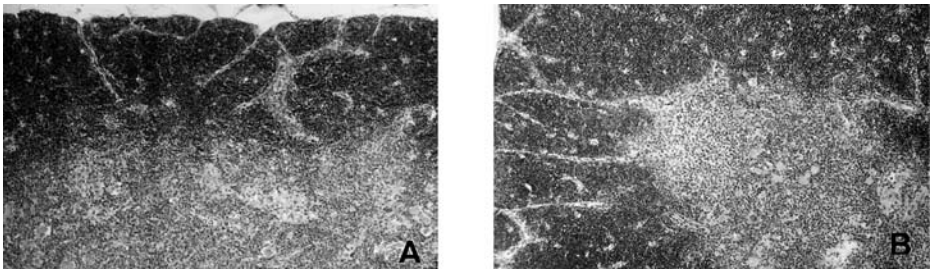

Fig. 2 — Thymus of 30-day-old chicken. Control (A) and chicken exposed to sound stress from day 1 to day 30 (B) (HE; 150x)

Spleen

The changes in histological structure of chicken spleen exposure to chronic sound stress, like in thymus, were not significantly expressed. At the largest number of chickens, spleen was without changes and the follicles development degree was in accordance with their age (Figure 3). The only change was observed in chickens exposed to sound stress from day 1 to day 30. Spleen of these animals was characterized by a smaller number of follicles and less developed of periarteriolar lymphoid sheath (PALS) (Figure 3).

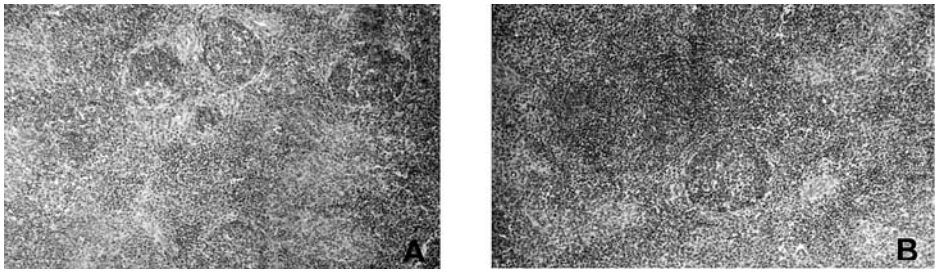

Fig. 3 — Spleen of 30 day old chicken (A) and spleen of chicken exposed to sound stress from 1st to 30th day of age (B) (HE; 150x)

DISCUSSION

Histological analysis of broiler chickens immune organs showed significant differences in structure of bursa of Fabricius in chicken groups exposed to chronic sound stress. In younger chickens, changes were slightly expressed and reflected in the better-developed connective tissue. However, in older chickens, changes were intensive, and reflected in the progressive reduction of the lymphoid follicles size, increasing of connective tissue amount and appearance of the cyst in both epithelium and in the follicle.

On the base of our findings could be concluded that bursa of Fabricius is very sensitive organ to the sound stress. In accordance with our findings are

the results of other authors who have examined the impact of social stress (Mohamed and Hanson, 1980), nutritive stress (Griffiths et al., 1985), stress caused by vaccination (Awadalla, 1998) and thermal stress (Pardue et al., 1985; Brake et al., 1988) on the bursa of Fabricius; they point out that stress induces atrophy of that organ which was reflected by organ weight decreasing. Stock densities, also, have effects on the bursa of Fabricius structure (Muniz et al., 2006).

Our results point out that duration of stress has significant influence on degree of histological changes. What was obvious in comparison of its histological changes in groups exposed to stress 15, 30, and 45 days. Higher degree of follicular atrophy was in groups that were exposed to sound stress in longer period. In addition, age of chicken had influence at the degree of histological changes of bursa of Fabricius. At the same duration of sound stress, older compared to younger chickens have much more prominent structural changes of the bursa of Fabricius.

These results confirm that the sound stress causes immunosuppression. We assume that the basic mechanism of action of all kind of stressors on the immune system is basically the same, what result in immunosuppressive action of glucocorticoids on this system.

Researching the effects of glucocorticoids on the bursa of Fabricius, Compton et al. (1990) found that dexamethasone causes regression and reduction in its cell activity. These authors also found that the regression of bursa of Fabricius was a consequence, first of programmed cell death (apoptosis) activated by steroids, and second as a consequence of the migration of lymphocytes from the tissue caused by action of dexamethasone.

Unlike the bursa of Fabricius, chronic sound stress caused less prominent changes of thymus structure. Higher degree of follicular atrophy was noticed in chickens longer exposed to sound stress. In thymus tissue were found the larger number of macrophages and higher amount of connective tissue in chickens of the age of 30 days than in control animals.

Atrophy of chicken thymus, which is in connection with reduction of organ weight, is found in nutritive stress (Griffiths et al., 1985). Reduction of thymus weight was also found in the chickens exposed to stress due to vaccination and overpopulation (Awadalla, 1998). These finding indicate that stress affect the thymus, as the primary immune organ, but that the degree of its change depends of animal strain, type of stressor, the length of exposure, etc.

Increase of macrophages number and increase in amount of connective tissue in chickens thymus in our experiment indicate that the thymus react to stress. These findings are in accordance with the results of Compton et al. (1990), which indicate the important role of macrophages in the inactivation of lymphocytes after glucocorticoides treatment.

Like in thymus, changes in the spleen structure of chicken exposed to sound stress were not significantly expressed. In the largest number of animal, spleen normal structure is preserved, and development of follicles is in accordance with age of chickens.

The only deviation in spleen structure in chickens exposed to sound stress observed at the age of 30 days. In these animals, spleen is characterized by a

smaller number of follicles and less developed PALS. Stress caused by vaccination and overpopulation, as well as high temperature, were resulted in the reduction of the spleen weight of broiler chickens (Pardue et al, 1985; Awadalla, 1998). Researching the impact of stress on the spleen, Davison et al. (1985) found that ACTH infusion causes reduction of spleen weight. In addition, treatment with cortisol and cortisol in combination with the heat stress causes the reduction of body mass in broiler chickens (Brake et al., 1988).

Histological changes in the spleen showed a lower sensitivity of this organ on sound stress compared to the Bursa of Fabricius, which is in fully agree with the results of experiments that are performed by Compton et al. (1990). They showed that organs where mature lymphocytes were located are less sensitive on the influence of glucocorticoides.

CONCLUSION

Chronic sound stress causing significant changes in histological structure of bursa of Fabricius in broiler chickens. These changes are reflected in the follicles atrophy, development of connective tissue and appearance of cysts in epithelium and in the follicles.

In broiler, thymus exposed to sound stress, minor changes are visible, exactly increasing number of macrophage and more connective tissue then in control group at 30 days of age.

In the same period, minor changes were observed in the spleen structure of broiler chickens exposed to sound stress then in control group. These changes are shown by smaller number of follicles and less developed of periarteriolar lymphoid sheath (PALS).

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УТИЦАЈ ДУГОТРАЈНОГ ЗВУЧНОГ СТРЕСА НА ХИСТОЛОШКУ ГРАЂУ ИМУНОЛОШКИХ ОРГАНА КОД БРОЈЛЕРСКИХ ПИЛИЋА

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Резиме

Циљ рада је да се испита утицај звучног стресора на имунолошке органе бројлера који су били излагани у различитим узрастима и различитом трајању стресора. Формирано је девет група, а свака група је имала по 10 пилића. Испитивана је хистолошка грађа Фабрицијеве бурзе, грудне жлезде и слезине. Резултати указују да је Фабрицијева бурза, у односу на остале испитиване органе, најосетљивија на деловање ове врсте стресора. Промене се уочавају и код грудне жлезде и слезине, али су мање и јављају се након излагања стресору више од 30 дана. Степен промена у хистолошкој грађи имунолошких органа под утицајем звучног стресора зависи од дужине излагања као и узраста пилића.