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THE ADRENAL GLAND MORPHOLOGY IN LAYING HENS WITH NONSPECIFIC STRESS SYNDROME

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ABSTRACT

The use of a coniferous feed additive for laying hens with nonspecific stress syndrome allows improving the general condition of the poultry and normalizing the secretory activity in the adrenal glands. Sixty days after the beginning of the experiments, a decrease in the number of chromaffinocytes and lipid inclusions in the adrenal glands was noted. These changes indicate the development of protective adaptive reaction in laying hens and the effectiveness of treatment.

KEY WORDS
laying hens, stress, chromaffinocytes, lipid inclusions, adaptive reaction, biohacking

INTRODUCTION

Stress is a borderline state between the pathology and the norm, the diseased and the healthy state, which allows considering it as a general nonspecific adaptive syndrome, which, according to H. Selye, proceeds in stages [1-3]. In the case of stress in animals and poultry, the hypothalamo-pituitary-adrenal axis activates, which leads to significant releases of the adrenocorticotropic hormone (ACTH) and glucocorticosteroids (cortisol), and suppresses the output of the sex hormones and the growth hormone [4-6]. Morphofunctional characterization of the adrenal glands in laying hens is a direct method for diagnosing stress syndrome and is an important criterion for assessing the treatment made [7, 8].

It has been established that under stress, lipid inclusions, which are glucocorticosteroids (GCS), accumulate in the adrenal glands of laying hens. The use of a coniferous feed additive allows reducing the amount of lipid inclusions in the adrenal glands and decreasing the production of GCS.

The research was aimed at studying the morphological structure of the adrenal glands of laying hens to assess the protective adaptive response of the bird's body. In this regard, the following tasks were to be solved:

- to assess the main structures of the adrenal glands in laying hens by the development and distribution of chromaffin tissue, regulating the production of glucocorticoids, in the medullary and cortical substances;
- to identify lipid inclusions in the adrenal glands using the histological preparations at the beginning of the experiments and after 60 days of using the coniferous feed additive.

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MATERIALS AND METHODS

The experiments were performed at a poultry farm with the laying hens that had symptoms of stress syndrome. For this purpose, two groups of laying hens were selected, 20 birds in each group. The laying hens in the experimental group received the coniferous feed additive together with the feed at the dosage of 800 g per 1 ton of the feed, while the laying hens in the second group were used for reference. The hens were observed for 60 days.

The basis of the coniferous feed additive was the biologically active substances of woody greens, extracted by the composition of polyhydric alcohols widely used in the food industry and showing no negative side effects, when compared to many other similar products. Excellent taste improved the appetite of animals and poultry and improved the palatability of feed.

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The coniferous feed additive contained glycerin and coniferous paw natural carrier. Glycerin was rapidly and completely absorbed in the gastrointestinal tract of animals and served as a source of glucoplastic substance. Coniferous paw was the main carrier of natural bioregulators in the coniferous feed additive. Coniferous paw was comprised of cut young shoots of softwoods covered with needles. The maximum

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diameter of the shoots at the cut was 6-8 mm. They were harvested from the crowns of freshly cut trees and processed in a short time. Coniferous paw storage at the positive temperature for more than 7 days could lead to the loss of its valuable properties, the destruction of vitamins, carotene, chlorophyll, etc.

The dry matter of coniferous paw contained fat- and water-soluble vitamins. The maximum content of carotene and other vitamins in needles was from October to May. In addition, the needles contained the following microelements: iron, manganese, cobalt.

The coniferous feed additive was used for glucose synthesis and direct energy production. It normalized various types of metabolism: carbohydrate, fat and energy, restrained the development of fatty hepatosis, prevented the development of ketosis, promoted the rapid recovery of reproductive function after calving, normalized the production of sex hormones, which reduced the duration of the service period and the risk of complications with a new pregnancy, satiated the animal organism with vitamins, micro- and macroelements. In addition, glycerin in the additive had antibacterial properties that ensured the preservation of consumer qualities of the product for a long period [9].

The morphological status of the adrenal glands of the laying hens was assessed with the use of the histological preparations prepared according to the methods and recommendations several investigators [10- 13]. To identify lipid inclusions in the adrenal glands of the laying hens, staining of histological sections with Sudan III was used. For staining slices, pieces of the adrenal glands after formalin fixation were used, as well as fresh organs. The procedure of organ staining with Sudan III included the following stages: washing frozen slices (fresh organs and organs fixed in neutral formalin) in 50 % ethyl alcohol, staining for 15 – 30 minutes in an alcohol solution of Sudan III, rinsing in 50 % ethyl alcohol, washing in distilled water, nuclei staining with Ehrlich's hematoxylin, and rinsing and placing the slices in the glycerin-and-gelatin mixture. The histological preparations were studied using a Motic BA310 Digital microscope with a built-in digital camera. During the research all the legal and ethical standards of using laying hens in the experiments were observed.

RESULTS AND DISCUSSION

Clinical status assessment

At the beginning of the experiments, for assessing the general state of the laying hens, attention was paid to their behavioral reactions. The overall excitement of the poultry was noted. The hens made rapid aimless movements around the cage, some of them showed aggression against the nearby hens and the hens in the neighboring cells. Most hens showed signs of cannibalism. Pecking of the eye area, the scallop, and the cloaca occurred. Panic fear was noted in the hens when someone passed between the cages. The hens crowded at the wall opposite to the passage and remained motionless for 4 – 7 seconds. After that, the fright passed, and the signs of excitation and anxiety repeated.

The examinations of the feather and skin cover revealed extensive areas of alopecia, which was a consequence of premature molting. Some laying hens had no feather cover at all. Most hens showed a loss of feathers in the area of the neck, the back, and the abdomen. The examinations of the skin revealed various wounds in the area of scallop and jowls, which arose from mechanical injuries from metal parts of the cells. Some hens had hematomas in the subcutaneous tissue of the neck, the base of the wings, and the abdomen.

Several cases of wing bone fractures were also noted. These changes could occur in the chickens due to their aggressive behavior and the struggle for leadership. In assessing the behavioral reactions during feeding, it was found that not all laying hens had free access to the feeder due to dense placing. Most injuries and aggressive behavior incidents occurred near the feeders during feeding. In general, the overall anxiety and excitement of the laying hens also persisted in the dark, which prevented the hens from resting. The skeletal muscles in most laying hens were well developed. There was a normal ossification of the middle processus of the keel bone, the tracheal rings were strong and did not compress. The skin was pale; the scallops were pale pink to bright red.

Thirty days after the beginning of the experiments, the overall state of the laying hens changed. They had no panic fear anymore. They behaved less aggressively with each other. In the experimental group, the laying hens took food calmly. No anxiety was observed. Extensive areas of alopecia remained in all the laying hens. Sixty days after the beginning of the experiments, the laying hens adequately reacted to each other and the service personnel. No signs of excitement were noted. The feather cover had completely recovered. Egg productivity over the 60 days of the experiments was 93.6 %. In the reference hens, this value was 62.6 %.

In the reference hens, the signs of aggression and anxiety remained. Some cages contained the hens that had died of pecking. Egg productivity sharply reduced. It was decided to slaughter the entire livestock of this poultry house.

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Morpho-functional characteristics of the adrenal glands in laying hens

The adrenal glands of the laying hens were dark brown. They were located on both sides of the abdominal aorta on the ventral surface of the kidneys. The weight of the right adrenal gland was approximately 0.19 – 0.37 g, of the left one — 0.12 – 0.27 g. The left adrenal gland received blood through the artery that started from the aorta or the renal artery. The right adrenal gland received blood through the branch starting from the right renal artery. There is no central vein in the adrenal glands of poultry; venous blood flows out of the adrenal glands through several vascular branches [14, 15]. On the outside, the adrenal glands were covered with a thin translucent shell consisting of collagen and elastic fibers. The cells of the cortex and the medulla of the adrenal glands formed cords that intertwined with each other; therefore, there was no clear separation of the layers in poultry, unlike the adrenal glands of mammals. The effect of the coniferous feed additive on the adrenal glands of the laying hens with nonspecific stress syndrome was assessed by observing the presence of lipid inclusions and chromaffin cells in the studied tissue.

At the beginning of the studies, the following changes were found using histological preparations from the adrenal glands of the reference laying hens. On the outside, the adrenal glands were covered with a single-layer connective tissue capsule [Fig. 1a]. Epithelial strands departed from it and formed glomeruli containing cells of loose connective tissue. In poultry, unlike mammals, the adrenal glands are not pronouncedly divided into cortical and medullary substances. A definite tendency in the development of the connective tissue cords, the location of chromaffin cells, and the presence of powerful blood vessels in the center of the adrenal glands indicate the structuredness of the adrenal glands in poultry. It should be noted that 30 days after the beginning of the experiments, no differences from the initial data were noted in the adrenal gland ultrastructure of the laying hens. Under stress reactions, redistribution of chromaffin tissues occurs in poultry. Chromaffin cells are subdivided into adrenocytes and noradrenocytes, which produce adrenaline and noradrenaline, respectively. In healthy poultry, chromaffin cells are located mainly in the adrenal medulla. When the organism is exposed to harmful factors, chromaffin cells leave the adrenal cortex. The number of chromaffinocytes depends on the severity of the stress factor and its duration. In the studies, chromaffin cells had high density of distribution throughout the organ tissue [Fig. 1b]. At the end of the experiments, their number reduced. Sixty days after the beginning of the experiments, the number of chromaffinocytes in the experimental laying hens decreased, which indicated the development of the adaptive capabilities of their organisms [Fig. 1c]. The positive effect of the coniferous feed additive on the adrenal glands of the laying hens was assessed by the content of large lipids which were inclusions of GCS (cortisol, corticosterone, and hydrocortisone). The most important is cortisol. Numerous light vacuoles of dissolved lipid inclusions in the adrenal glands signify an increased production of GCS in the poultry organism and the severity of the stress syndrome [Fig. 1d, 1e]. Sixty days after the beginning of the experiments, the number of light vacuoles of dissolved lipid inclusions in the adrenal glands of the laying hens in the experimental group decreased, compared with the reference hens [Fig. 1f, 1g]. These changes may indicate the development of the protective adaptive reaction in the laying hens and a decreased production of GCS.

Using the histological preparations stained with Sudan III, lipid inclusions of various localizations were identified. On the 30th day after the beginning of the experiments, lipid inclusions in the adrenal glands in all the laying hens were identified both in the lumen of the blood vessels and in the form of separate lumps in the perivascular space. The lipids were placed the most densely in the adrenal cortex [Fig. 2a, 2b]. Sixty days after the beginning of the experiments, large lipid inclusions in both the cortical and medullary substances of the adrenal glands were found in the laying hens in the reference group [Fig. 2c, 2d]. By the end of the experiments, a decrease in the number and size of lipid inclusions in the perivascular space was observed in the adrenal glands of the experimental laying hens. Lipids were mainly detected in the adrenal cortex [Fig. 2e]. In all the experimental laying hens, disruption of fats metabolism was observed, which was manifested by the deposition of lipids in the lumen of blood vessels. For instance, in the laying hens in the experimental group, a decrease in the number of lipid inclusions in the perivascular space was noted along with an increase in their number in the lumen of the blood vessels. These changes were manifested in the adrenal cortex [Fig. 2f].

At the end of the experiments, only single lipid inclusions in the perivascular space and blood vessels of the adrenal medulla were detected in the laying hens in the experimental group [Fig. 2g].

CONCLUSION

The use of the coniferous feed additive promotes the development of an adaptive response in the laying hens with nonspecific stress syndrome. Coniferous feed additive allows improving the general condition of laying hens in case of nonspecific stress syndrome. By the 60th day of administering the coniferous feed additive, the birds showed an adequate reaction to each other, their appetite improved, and the egg productivity increased. The use of a coniferous feed additive has a positive effect on the morphofunctional state of the adrenal glands of laying hens in case of nonspecific stress syndrome. The number of chromaffin cells and lipid inclusions in the adrenal glands decreases, which is evidence of a decrease in glucorticoids. The research results have demonstrated that administering the coniferous feed additive to laying hens allows normalizing their general condition in case of nonspecific stress syndrome. Evaluation of the clinical status of the experimental bird after 60 days of using the coniferous feed additive indicated

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a decrease in signs of arousal and aggression. Laying hens calmly accepted food without fighting for the feeding front. An increase in egg productivity and egg quality was noted.

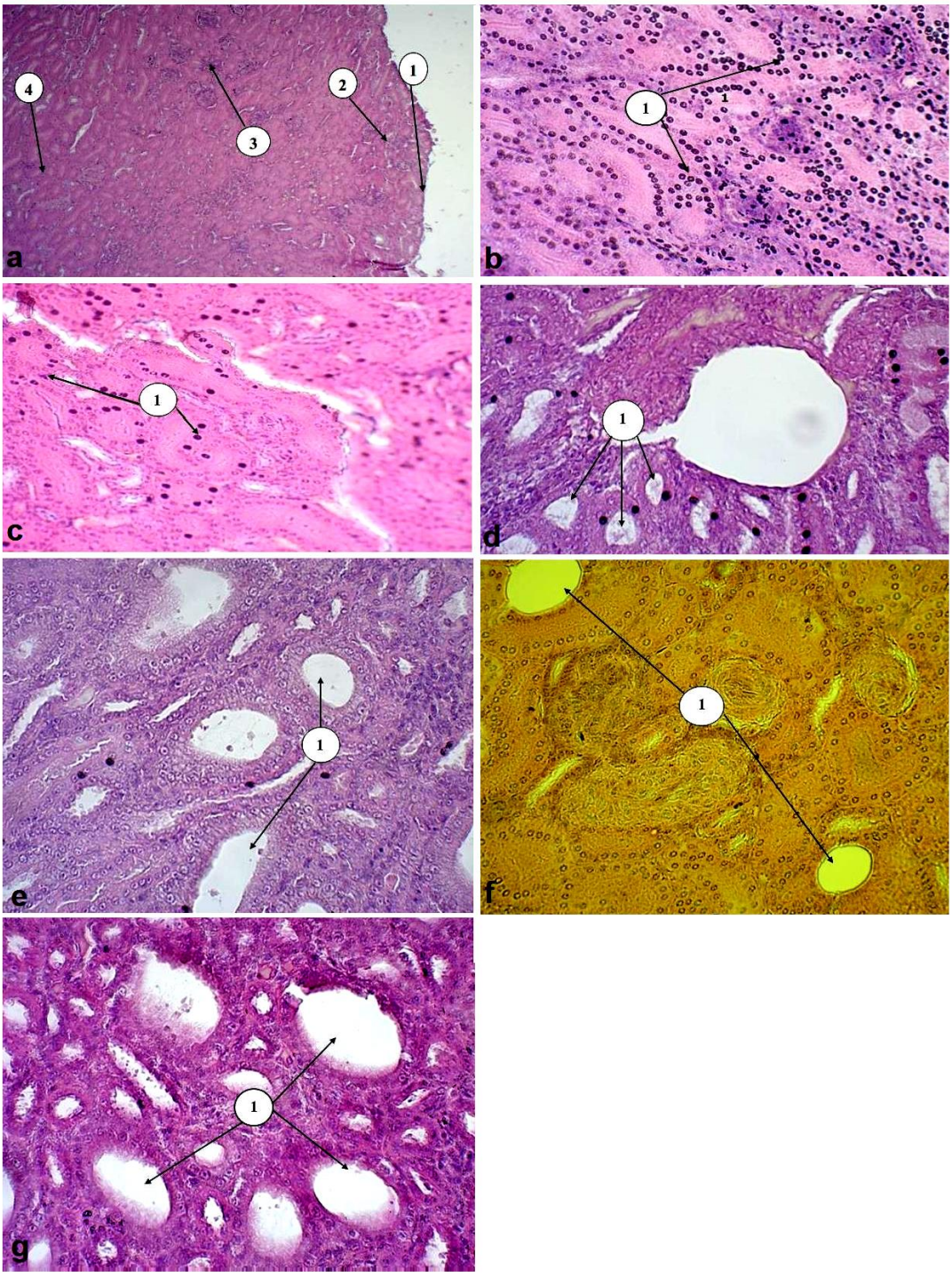

Fig. 1: Staining with hematoxylin and eosin. a) The adrenal gland of an experimental laying hen. Magnification x40, (1 — adrenal capsule, 2 — subcapsular zone, 3 —chromaffinocytes, and 4 — the inner zone of the interrenal tissues). **b)** The adrenal gland of an experimental laying hen at the beginning of the experiments. Magnification: x100, (1 — chromaffinocytes). **c)** The adrenal gland of an experimental laying hen 60 days after the beginning of the experiments. Magnification: x100, (1 — single chromaffinocytes). **d)** The adrenal gland of an experimental laying hen at the beginning of the experiments. Magnification: x100 (1 – light vacuoles of dissolved lipids). **e)** The adrenal gland of an experimental laying hen at the beginning of the experiments. Magnification: x100 (1 — light vacuoles of dissolved lipid inclusions in the adrenal cortex). **f)** The adrenal gland of an experimental laying hen 60 days after the beginning of the experiments. Magnification: x40 (1 — light vacuoles of dissolved lipid inclusions). **g)** The adrenal gland of a reference laying hen 60 days after the beginning of the experiments. Magnification: x100 (1 — light vacuoles of dissolved lipid inclusions).

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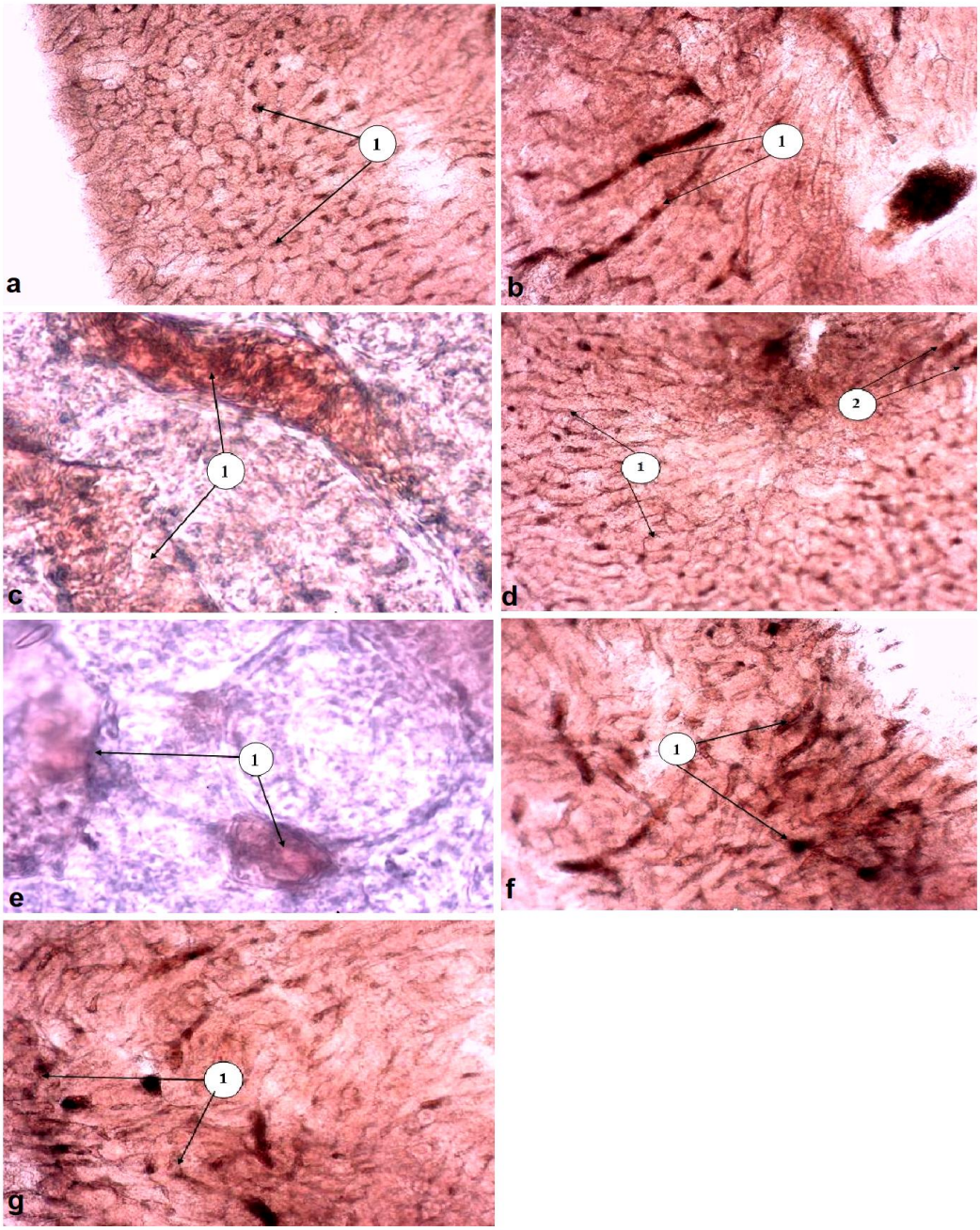

Fig. 2: Staining with Sudan III. a) The adrenal gland of an experimental laying hen 30 days after the beginning of the experiments. Magnification x10 (1 — lipid inclusions in the adrenal cortex). **b)** The adrenal gland of an experimental laying hen 30 days after the beginning of the experiments. Magnification x100 (1 — lipid deposits in the vessels of the adrenal cortex). **c)** The adrenal gland of a reference laying hen 60 days after the beginning of the experiments. Magnification x100 (1 — lipid inclusions in the adrenal cortex). **d)** The adrenal gland of a reference laying hen 60 days after the beginning of the experiments. Magnification x40 (1 — lipids in the adrenal cortex; 2 — lipids in the blood vessels of the adrenal medulla). **e)** The adrenal gland of an experimental laying hen 60 days after the beginning of the experiments. Magnification x100 (1 — lipid inclusions in the adrenal cortex). **f)** The adrenal gland of an experimental laying hen 60 days after the beginning of the experiments. Magnification x40 (1 — lipids in the vessels of the adrenal cortex). **g)** The adrenal gland of an experimental laying hen 60 days after the beginning of the experiments. Magnification x40 (1 — lipid inclusions in the adrenal medulla).

When assessing the morphological structure of the adrenal glands after 60 days of administering the coniferous feed additive, a decrease in the number of chromaffinocytes as well as their redistribution were revealed. At the beginning of the experiments, they were located both in the medulla and in the adrenal cortex. By the end of the experiments, most of the chromaffin cells were found in the adrenal medulla. Important changes were observed in relation to lipid inclusions in the adrenal glands. When stained with hematoxylin and eosin, they were white vacuoles, while staining with Sudan III revealed them as yellow-red inclusions. At the beginning of the experiments, lipid inclusions were detected in the adrenal medulla and cortex. After 60 days of using the coniferous feed additive, lipid inclusions were detected in the cortex in

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large quantities, and only single lipid inclusions were found in the medulla. A decrease in the number of lipid inclusions may indicate the normalization of the production of glucocorticoids of steroid nature.

CONFLICT OF INTEREST

The authors declare no competing interests in relation to the work.

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