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MORPHOFUNCTIONAL EVALUATION OF THYMUS IN PEKIN DUCK DURING DIETARY INCORPORATION OF ORGANIC SELENIUM FEED ADDITIVE DAFS-25k

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SUMMARY

The histological study results of the thymus structure of Pekin ducks during administration of DAFS-25k feed additive are presented. It has been established that the thymus is a morphologically mature and actively functioning organ in one-day-old Pekin ducklings. With age an increase in the size of thymus lobules was observed in ducklings in the control and experimental groups but under the influence of selenium the cortical area increased more intensively. 45-day-old ducklings in the experimental group demonstrated a reliable increase in the ventricular-brain ratio mainly due to the expansion of the cortical area. The surface of the cortical substance did not change in intact ducklings, the tendency of extension in the surface of the brain substance was observed, which at that age is a sign of an accidental involution associated with the onset of a plumage change from juvenile down to primary feathers. Under the influence of selenium in the thymus of the ducklings of the experimental group the processes of accidental involution are leveled, the processes of synthesis of thymopoietins and proliferation of thymocytes are more active. Up to age of 75 days there is active proliferation of thymocytes in the thymus of ducklings of both groups; however reliably higher parameters of ventricular-brain ratio under the influence of selenium were observed in the experimental group, there was a clear boundary between them, as well as significantly higher quantity and size parameters of Hassal's corpuscles were noted. From the age of 90 days signs of age involution in the organ were observed, and in the experimental group these processes were going more physiologically in accordance with the age of the birds. Introduction of the organic and selenium additive DAFS-25k in the diet of ducklings at a dose of 1.3 mg/kg of feed promoted the increase of the functional activity of the thymus that leveled the development of accidental involution and optimization of age involution.

Key words: thymus, Pekin duck, DAFS-25k, Hassal's corpuscles, thymocytes.

INTRODUCTION

Poultry farming is one of the leading industries in meat production, flexible enough to adapt for consumers' demands within the short period of time [2]. In the current agricultural industry structure, the duck meat production is equal to about 5% [3]; however, this sector is rather promising, because duck farming requires low costs, and duck meat is of high consumer's performance [1]. In practical poultry farming stresses, caused by intensive production technologies, dietary and managing malpractice, leading to immune deficits, characterized, among other things, by the accidental thymic involution, prevent complete unleashing of birds' genetic potential [4]. Morphology of avian immunocompetent organs raises interest among many scientists [5, 7, 8]. The publications on duck thymus studies at different ontogeny stages are available [6], but there are still no studies, devoted to the effect of organoselenium feed additive DAFS-25k on duck thymic microstructure.

In this light, the target of the study was to analyze Pekin duck thymic histostructure when using DAFS-25k feed additive.

MATERIALS AND METHODS

Thymic material for the study was taken from 1–120-day-old Pekin ducks, raised on "Romashino" farm (Moscow Oblast), free from infectious and invasive diseases.

Diet and management conditions were in line with animal health and technical standards, recommended for this species by the All-Russian Poultry Production Research and Technological Institute.

Quantification of selenium in feeds, used on the duck farm, was made using MGA-915MD atomic adsorption spectrometer in the Kostroma Oblast Veterinary Laboratory. The test revealed selenium deficiency in the diet equal to 1.3 mg per kg of feed.

In order to study selenium effect on the thymus development and functional activity, production and research trial was conducted on "Anisimov" farm (Vladimir Oblast). Two groups of one-day old ducks were formed for this purpose. Group 1 (test birds) received DAFS-25k feed additive, containing organic selenium at the deficiency correcting dose. The feed additive was administered by multistage mixing with feeds. DAFS-25k is a powder of white to light yellow color with a slight specific odour, non-soluble in water and soluble in vegetable oil; it contains at least 95% of the active ingredient diacetone-phenonyl-selenide with 25% selenium by mass; selenium availability from DAFS-25k is estimated at 76–100%. Group 2 (control birds) received a usual on-farm diet. In order to study selenium effect on thymic morphofunctional condition, 5 birds from each group were killed and autopsied (according to the common practices) every 15 days.

Histological material was processed in the FGBI "ARRIAH" Preclinical Research Centre, operating in compliance with the OECD Good Laboratory Practice (GLP). The samples were fixed in 10% neutral buffered formalin. The test was performed using Tissue-Tek® Xpress™ X50 Continuous

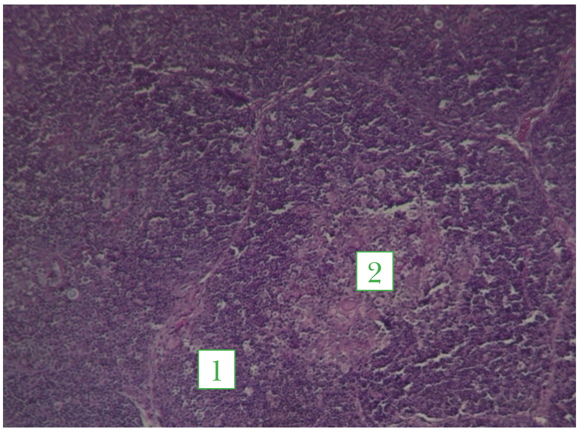

Fig. 1. Thymic cortex (1) and medulla (2) of day-old ducklings (stained with hematoxylin and eosin, ×100 magnification)

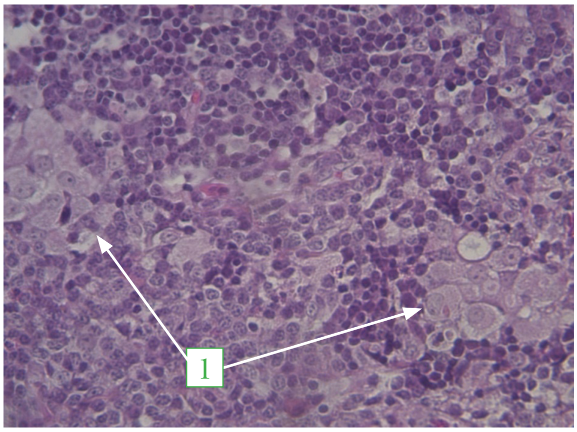

Fig. 2. Hassall's corpuscles development (1) in medulla of day-old ducklings (stained with hematoxylin and eosin, ×400 magnification)

Rapid Tissue Processor. Then samples were paraffin embedded using Leica EG1160 Histoembedder. Using Leica RM2125 RT rotary microtome, samples were sectioned (section thickness 4–5 μm) into paraffin blocks. After deembedding, sections were stained with haematoxylin and eosin and coverslipped using Leica ST5010 Autostainer XL/CV5030 with BioMount medium. Then they were analyzed with Leica DM1000 and Leica DMB microscopes and photographed.

Digital data were statistically processed by biometric technique. The arithmetic mean and deviation were calculated ($M \pm m$):

$$M = \frac{\sum X}{n};\; m = \frac{\sum (X - M)^2}{n},$$

where M – arithmetic mean of the data available;
X – digital value of the object under study;
m – deviation from the arithmetic mean.

RESULTS AND DISCUSSION

Based on our own research, it was established that thymic lobes in day-old ducklings are covered with a connective tissue capsule, with trabeculae extending from it and dividing it into lobules. Each lobule could be clearly divided into medulla and cortex (Fig. 1). Cortex was located in the peripheral part of the lobules, darker in staining and consisted of small, medium and large thymocytes. The latter were predominantly located in the peripheral part of the lobule, while small and medium size thymocytes were not characterized by specific locations. Cortex area was 1.76×10^5 μm², medulla area was 1.05×10^5 μm², the ratio was 1.68 ± 0.11 (Table). Thymocytes were located compactly, densely to each other in the cortex, that's why histologically it looked darker than the medulla. In medulla thymocytes were less dense, reticular-epithelial cells forming the organ stroma, were well noticed. Hassal's corpuscles were identified, both juvenile and mature (Fig. 2), which were concentrically arranged epithelial reticular cells with well-defined deplanate nuclei and acidophilic cytoplasm. Prevalence of the cortex area over medulla area, presence of a distinct boundary between them, Hassal's corpuscles, all suggest that day-old ducklings' thyme is a mature, well-developed organ, which synthesizes thymopoietins and lymphocytes actively.

Control and test 15-day-old ducklings demonstrated growth of medulla and cortex areas. Moreover, in test birds, cortex area increased more intensely due to thymocyte proliferation and Hassall's corpuscles developed more actively, and were insignificantly larger than those of controls (Table).

By the age of 30 days, lobules enlarge in the birds of both groups, most lobules were polyangular, but sometimes oval and roundish lobules were observed. Often trabeculae did not reach the lobule central parts and merged in their roots. Lobules were enlarged due to cortex and medulla growth, but cortex grew more intensely in test birds, and medulla in controls. Cortex/medulla ratio value in test birds was statistically higher than the one of test birds. The size of Hassall's corpuscles and their number also prevailed over the analogous parameters of controls ($p \leq 0.05$; Table). The abovementioned suggests a higher thymus functionality of test birds, induced by selenium.

At 45 days, test ducklings demonstrated a statistically higher cortex/medulla ratio value compared to the previous age, predominantly due to cortex area growth. Cortex area did not expand in intact ducklings, but medulla area tended to grow, which suggests accidental thymic involution at this age, likely associated with juvenal plumage replacement by primary feathers. A higher density of Hassall's corpuscles in thymic lobules of test ducklings, statistically larger than those in controls, was noted (Table). Hassall's corpuscles, both juvenile, in the form of epithelial reticular cell layers, in the cytoplasm of which unstructured acidophilic mass was accumulated, and mature bodies, containing deplanate surface and aged central cells, as well as blood capillaries, more frequently observed in test birds, were found in birds of both groups. According to some authors [3, 8], Hassall's corpuscles also play endocrine, immunity modulating role. This means selenium effect on thymus of tests ducklings involved smoothing of accidental thymic involution processes, activating thymopoetin synthesis and thymocyte proliferation.

Larger cortex areas in tests birds compared to controls remained to be found at 60 days too. In controls, the boundary between cortex and medulla was blurred; the latter contained Hassall's corpuscles of different size with large bodies, consisting of hypertrophied epithelial reticular cells with blurred and lysed nuclei prevailing. A statis-

Table
Morphometric description of Pekin duck thymus by ages, $M \pm m$

Days	Cortex area, $\mu m^2 \times 10^5$		Medulla area, $\mu m^2 \times 10^5$		Cortex/medulla ratio		Trabecular average thickness, μm		Hassal's corpuscles number		Hassal's corpuscles average diameter, μm	
	C	T	C	T	C	T	C	T	C	T	C	T
1	1.76 ± 0.12		1.05 ± 0.08		1.68 ± 0.11		2.12 ± 0.12		4.18 ± 0.33		6.58 ± 1.23	
15	1.94 ± 0.14	2.32 ± 0.16	1.06 ± 0.05	1.13 ± 0.08	1.83 ± 0.14	2.05 ± 0.11	2.91 ± 0.13	2.76 ± 0.18	5.68 ± 0.25	6.18 ± 0.26	8.16 ± 2.23	8.78 ± 2.09
30	2.14 ± 0.16	2.75 ± 0.13*	1.53 ± 0.11	1.46 ± 0.12	1.40 ± 0.08	1.88 ± 0.07*	2.72 ± 0.15	2.46 ± 0.18	6.92 ± 0.24	8.54 ± 0.56*	8.65 ± 0.23	9.37 ± 0.12*
45	2.12 ± 0.13	3.19 ± 0.22*	1.75 ± 0.11	1.45 ± 0.08*	1.21 ± 0.07	2.20 ± 0.12*	3.14 ± 0.21	3.65 ± 0.18	8.72 ± 0.31	10.47 ± 0.46*	9.34 ± 0.32	10.58 ± 0.66*
60	4.03 ± 0.14	5.37 ± 0.21*	2.67 ± 0.18	2.04 ± 0.18*	1.51 ± 0.11	2.38 ± 0.18*	3.23 ± 0.22	3.76 ± 0.21	8.83 ± 0.56	10.45 ± 0.40*	10.81 ± 0.38	12.40 ± 0.32*
75	4.68 ± 0.32	5.78 ± 0.43*	2.83 ± 0.12	2.25 ± 0.20*	1.65 ± 0.11	2.57 ± 0.18*	3.22 ± 0.18	3.09 ± 0.26	7.24 ± 0.31	8.86 ± 0.22*	10.59 ± 0.32	12.15 ± 0.18*
90	3.33 ± 0.21	4.35 ± 0.17*	4.07 ± 0.16	3.20 ± 0.19*	0.82 ± 0.07	1.34 ± 0.12*	3.84 ± 0.21	3.60 ± 0.19	7.13 ± 0.22	8.02 ± 0.19*	9.79 ± 0.19	11.37 ± 0.27*
105	3.54 ± 0.11	4.10 ± 0.21*	4.46 ± 0.16	3.52 ± 0.14*	0.79 ± 0.06	1.16 ± 0.11*	3.82 ± 0.13	3.54 ± 0.14	6.42 ± 0.32	7.83 ± 0.21*	9.58 ± 0.17	10.72 ± 0.10*
120	3.09 ± 0.21	3.68 ± 0.11	5.19 ± 0.18	4.67 ± 0.31	0.59 ± 0.02	0.79 ± 0.06*	4.67 ± 0.22	4.69 ± 0.19	6.23 ± 0.21	7.62 ± 0.30*	9.46 ± 0.18	10.70 ± 0.22*

* $p \leq 0.05$ compared to controls;
C – controls;
T – test birds.

tically significant cortex/medulla ratio, which was higher than that of control birds, clear boundary between them, predominance of mature Hassall's corpuscles in the medulla suggest a higher thymic morphological functionality of test ducklings, induced by organoselenium feed additive, DAFS-25k.

Thymus of 75-day-old test ducklings still demonstrated signs of accidental thymic involution, triggered by the peak of moulting, being the stress factor for birds. Thymic involution manifested as the absence of clear boundary between cortex and medulla, and lack of thymocytes in both areas (Fig. 3). Selenium positively influenced on thymic morphological physiology in test birds (Fig. 4), which was supported by a higher cortex/medulla ratio value, clear boundary between them and statistically higher numbers and sizes of Hassall's corpuscles (Table).

Involution processes were observed in 90-day-old ducklings of both groups, associated with the puberty onset; however those processes were more distinct in test birds in connection with accidental transformation. For the first time during the study period, the medullary area was larger than the cortical one in the test group, due to the decrease in thymocyte proliferation. In test birds selenium prevented early thymic involution, modulated the thymic functional morphology and had a moderate immunostimulating effect, manifested as higher corticomedullary ratio values, larger numbers and sizes of Hassall's corpuscles and clear corticomedullary boundary. A higher density of thymus cortical cells suggests active thymocyte proliferation and differentiation.

Thymic involution processes increased in 105-day-old control ducklings: corticomedullary boundary was blurred, medulla area was larger than cortical one. Hassall's bodies were single, roundish, consisted of concentric aggregations of elongated spindle-shaped cells with large nuclei and acidophilic cytoplasma. Test ducklings of the same age also demonstrated reduction in corticomedullary area. Corticomedullary boundary of thymus was clear, with large Hassall's bodies in the medulla being conglomerates of two or three smaller merged bodies. Normal deplanate epithelial cells were located on their periphery, and dystrophic cells in the centre. A big number of blood capillaries were found near Hassall's corpuscles with stromal epithelial cells adjacent and surrounding them. According to some authors, perivascular space is located between epithelial membrane and capillaries, filled with tissue liquid, containing lymphocytes, macrophages and plasma cells. As a result, a hematothymic barrier is formed between tissues and thymic blood channels. Exactly this barrier prevents penetration of antigens being at the same time penetrable for lymphoid cells [3, 5]. Thus, when using selenium, the histological picture of test duckling thymus suggests a positive dynamics in thymocyte proliferation, thymocyte active migration into thymus-dependent areas of immuniocompetent organs through the medulla capillary network.

At the age of 120 days, medulla area was larger than cortical one in ducklings of both groups with no distinct boundary between them; interlobular stromal elements tended to enlarge and Hassall's bodies reduced in the number and size (Fig. 5, 6). Notwithstanding the onset of active involution related to duck puberty, test bird thymus has a better morphofunctional structure, facilitating the stabilization of the immune response, thymocyte proliferation and maturation.

CONCLUSIONS

In day old ducklings thymus is a morphologically mature and actively functioning organ, which is characterized by a larger cortical area than the medulla area, distinct boundary between them, presence of Hassall's corpuscles of different maturity.

Up to 75 days of age active thymocyte proliferation is observed in duckling thymus, characterized by a more intensive growth of cortex compared to medulla, formation of Hassall's bodies of different shapes and sizes and thymocyte density suggesting thymus morphological functionality.

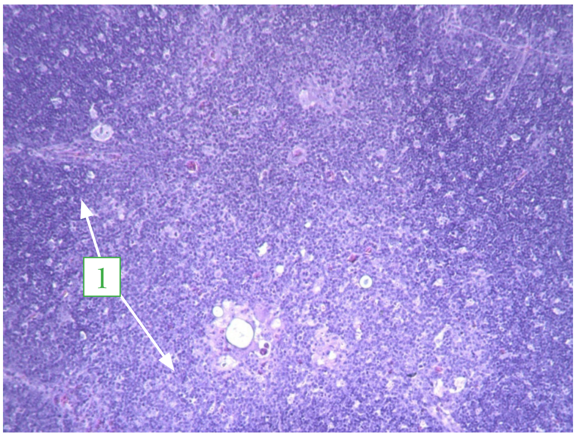

Fig. 3. Thymus of 75-day-old control ducklings. Signs of accidental thymic involution (1) (stained with hematoxylin and eosin, ×200 magnification)

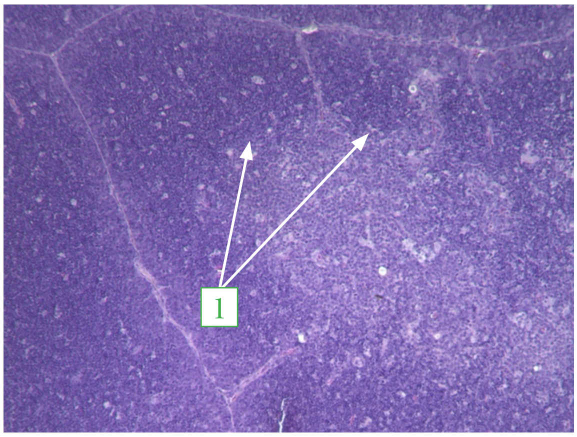

Fig. 4. Clear cortico-medullary boundary (1) in the thymus of 75-day-old test ducklings (stained with hematoxylin and eosin, ×200 magnification)

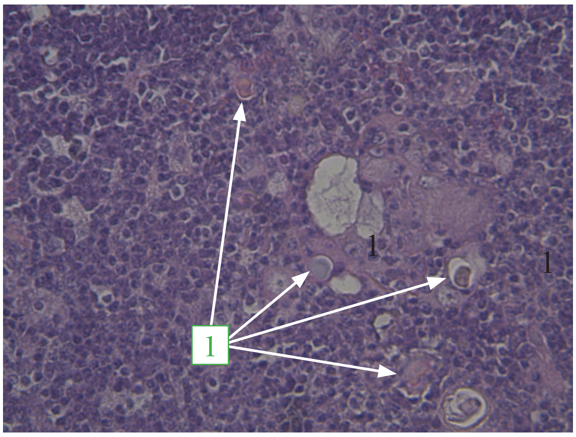

Fig. 5. Hassall's bodies (1) in thymic medulla of 120-day-old control ducklings (stained with hematoxylin and eosin, ×400 magnification)

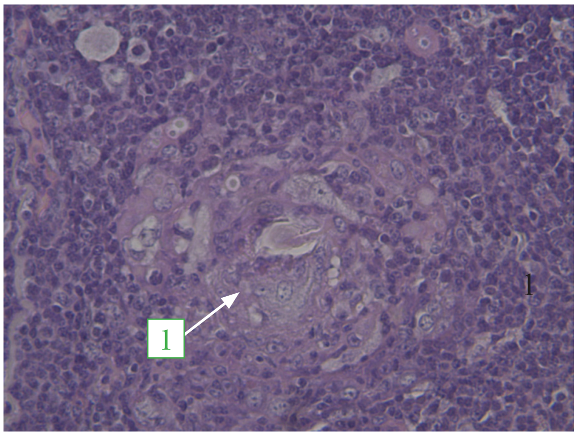

Fig. 6. Hassall's bodies (1) in thymic medulla of 120-day-old test ducklings (stained with hematoxylin and eosin, ×400 magnification)

Starting from 90 days of age signs of thymic involution are observed, triggered by the puberty onset and followed by the decrease in cortical area, blurring of corticomedullary boundary, reduction of thymocytes in parenchyma and number and sizes of Hassall's bodies as well as thickening of trabeculae.

Use of organoselenium feed additive DAFS-25k at the dose of 1.3 mg/kg in duck diet had a positive impact. Test ducks at all studied ages showed a higher thymic functionality, manifested as higher medulla/cortex ratio values, larger numbers and sizes of Hassall's bodies and higher thymocyte density. Accidental thymic involution processes were smoothed in test birds too.

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