The importance of microelements in forming duck liver morphology

L V Kletikova¹, A N Martynov¹, G A Fedorov² and V I Garkun¹

¹ State Agricultural Academy, 45 Sovetskaya str., Ivanovo, 153012, Russia
² Shuy branch of Ivanovo State University, 24, Cooperative str., Ivanovo region, Shuya, 155908, Russia

E-mail: doktor_xx1@mail.ru

Abstract. Microelement composition in a diet influences the morphology of duck liver is described. Microelement composition of the duck food was studied in accordance with GOST (State Standard Specification), which states the selenium content amounting to 0.06 mg/kg for the growing ducks and 0.14 mg/kg for adult ducks. The experiment on Pekin ducks aged from 1 to 120 days implied that the control group received basic diet and the experimental group received DAFS-25k (Diacetofenilselenide) in accordance with the product instruction during the whole period of raising. The studies were undertaken at an interval of 15 days. Liver structure of one-day ducks had typical anatomy. Connective tissue was moderately marked, tubular structure was clearly marked, hepatocytes had polygon shapes, the nuclei were oval or round, located centrally, they contained from one to four nucleoli. Vacuolated cytoplasm was observed for 15-day ducks from the control group. For the experimental group: hepatocyte nuclei had the same size, cytoplasm was homogeneously coloured, sinusoidal capillaries with red blood cells were clearly marked. In critical periods of raising, namely on the 30th day, when neoptile was replaced with primary plumage, and on the 75th day, during post-juvenile moult, for the control group we could observe granular cytoplasm structure, which is a characteristic feature of granular degeneration; for the experimental ducks: liver structure had definitive structure, morphofunctionally active. For 120-day ducks from the experimental group: the liver retained its tubular structure, had singular fat build-ups; for the control group: clear signs of hepatosteatosis. DAFS-25k did not have negative influence on morphofunctional activity of the organ; the selenium content in the liver of 120-day ducks from the experimental group amounted to 0.52 mcg/kg compared to 0.31 mcg/kg for the control group.

1. Introduction

Healthy growing ducks and further productivity is determined by well-balanced diet, as it influences functioning of organs and systems. Early period of post-embryonic development is characterized by adaptive processes activation in the setting of rapidly changing environment. [1]. The load received by the liver increases in such conditions. The liver is the largest organ in the abdomen for newly born individuals. Normally, it provides homeostasis of organism [2, 3]. The problem of liver formation at the early stage of development is associated with immaturity of liver enzymes and continuation of morphological differentiation of structural elements [4], which influences metabolic process and clearance of potentially toxic compounds, both endogenous and exogenous [5]. Liver cooperative cell system is comprised of hepatocyte — Kupffer's cell — endotheliocyte — lipocyte — Pit-cell. In this
cell cooperation, Kupffer's cells represent the system of mononuclear phagocytes. They act as blood–liver barrier; by interacting with immune system, they establish the basis of maintaining consistent connective tissue of liver with the help of monokines and collagenase. They influence the processes of hepatocyte regeneration. Transport and metabolic functions are fulfilled by endotheliocytes, Kupffer's cells and hepatocytes [6], which are directly connected to blood vessels and bile capillaries. It determines histological liver structure as compared to any other gland [7]. As opposed to other species, bird liver is delicate and fragile, it is easily damaged by applying pressure [8]. Adult birds have dark brown liver, growing birds after hatching have clayish-ochreish liver with a faint tinge of pink. Such liver colour for growing birds is the result of the following factors: high content of fat, which came from yolk-bag; destruction of fetal haemoglobin and red blood cells [9]; more developed vascular tree [10, 11].

For the majority of species, regardless of their habitat, we can observe liver dynamic stability, i.e. morphological and functional structure supported by plastic dynamism of the organ [12]. Nevertheless, every species is characterized by unique type of metabolism, which is determined by such factors as species, breed, age, sex, heredity, etc. [13]. Besides, morphological structure of organs reflects deficiency of amino acids, vitamins and mineral substances [14-18].

2. The objective of the research
The objective is to study morphological features of Pekin duck liver in relation to age at the setting of consuming DAFS-25k in the province distinguished by selenium deficiency of the soil.

3. Materials and methods
The study was undertaken in 2016-2019. The subjects of the study are Pekin ducks aged from 1 to 120 days, the object is duck liver and particular microelement content of duck food. Using analogue method, we formed two groups of one-day ducks. The groups were formed with the consideration of live weight (weight variation within 0.5%), each group consisted of 250 ducks. The first group is the control group, which received basic diet; the second is the experimental group, which received DAFS-25k feed additive apart from basic diet in accordance with the product instruction, dose: 1.6 mg/kg of the food given as per the duck weight. A preparation containing moderate doses of selenium is capable of preventing oxidative destruction of cell membranes and symptoms of hypoxia. It neutralizes toxic action of heavy metals and stimulates activity of enzymes and hormones; it increases the effectiveness of digestive bronchopulmonary system diseases treatment. It is capable of improving the functions of phagocytosis and modulations of apoptosis. [19, 20, 21, 22, 23, 24, 25].

The iron, zinc, copper, manganese, nickel, cobalt, cadmium and lead concentration in combined fodders for adult and growing ducks was estimated at Federal State Budgetary Institution 'Ivanovskaya Station of Agrichemical Service' (SAS Ivanovskaya) by means of atomic adsorption spectroscopy using spectrophotometer Kvant-2A; sample ashing in accordance with GOST 30178-96. The iodine and selenium content was analysed at Federal Research Centre 'All-Russia Research and Development Technological Institute of Poultry Breeding' within Russian Academy of Sciences, in accordance with GOST Р52471-2005.

Ducks at the age of 1, 15, 30, 45, 60, 75, 90, 105 and 120 days old underwent morphological examination of liver. Samples of the organ were fixed in 10% preparation of neutral formalin. The material was processed with the application of tissue processor TLP-720 (Russia, Mt Point TM), and embedded in paraffin using tissue embedding station ESD-2800 (Russia, Mt Point TM). Sections with the thickness of 5-8 μm were prepared at rotary semi-automatic microtome RMD-3000 (Russia, Mt Point TM), stained with haematoxylin and eosin using automatic linear stainer ALS-96 (Russia, Mt Point TM). The preparations were studied using microscope Micmed-6 (Russia, LOMO). We used E31S video camera (China) and TopView software with the amplification of x100 and x400 for measurements and photographic documentation. A measuring scale of the camera was calibrated using object micrometre of transmitted light OMP (Russia, LOMO). The volume of hepatocyte and nuclei of hepatocytes were calculated using the formula:
in which $\pi = 3.14$, Dm is minor cell (nucleus) diameter and Db is major cell (nucleus) diameter. Cytoplasm volume represents the difference between hepatocyte volume and nucleus volume.

Weight content of selenium in the liver of 120-day ducks was determined by means of atomic adsorption spectroscopy with decomposition of samples in closed vessels (Federal Service for Veterinary and Phytosanitary Surveillance, 2001) in the modification of Ivanovo State University of Chemistry and Technology, 2004.

We used Microsoft Excel-2010 for statistical data processing. We used Student's t-test to estimate the statistical significance of differences between the parameters (G. F. Lakin, 1980).

4. Results and discussion
Ducks are characterized by high sensitivity to excessive amount or deficiency of mineral substances. Before introducing biologically active microelements into the food, we analysed the diet. As a result, we established the following content of substances for the adult and growing ducks: copper 3.40-3.68 mg/kg, zinc 34.32-36.70 mg/kg, iron 115-185 mg/kg, manganese 68-92 mg/kg, cobalt 0.27-0.36 mg/kg.

In the combined fodder for growing ducks the iodine content amounted to 0.69 mg/kg. Fodder for adult ducks contained submicrograms of the microelement. The concentration of such dangerous pollutants as lead and cadmium did not exceed the maximum permissible concentration and amounted to 2.12 mg/kg and 0.024 mg/kg for growing ducks; 2.31 mg/kg and 0.040 mg/kg for adult ducks respectively. The nickel content in fodder for growing and adult ducks amounted to 1.80 - 2.00 mg/kg, which exceeded ecologically permissible concentration by 35-50% ($p \leq 0.05$). The concentration of selenium in combined fodder for growing and adult ducks amounted to 0.06 mg/kg and 0.14 mg/kg.

The liver of 1-day Pekin ducks had typical structure; stroma was represented by connective tissue of capsule and interlobular partitions. The connective tissue was moderately marked and could be observed only at the periphery of the organ, where it formed a thin capsule, and at the area of triads; interlobular connective-tissue partitions were not observed. Tubular structure was clearly marked, hepatic tubules were positioned radially and had branching, curvy and sometimes glomerular form. Thickness of tubules amounted to 18.43±0.40 μm, sinusoidal lumen was 4.46±0.19 μm. Blood corpuscles could be observed in the lumen of central veins and the branches of portal vein. Branches of portal vein with extended lumens could be encountered. The boundaries of hepatocytes were moderately marked, cells had polygon shape, their volume amounted to 553.51±42.23 μm3. The nuclei were located centrally, in some places moved to the periphery; intensively coloured; round-oval shape; contained from one to four nucleoli. The nuclear volume amounted to 38.73±2.00 μm3. The cytoplasm was coloured inhomogeneously and had granular structure; its volume amounted to 383.16±12.45 μm3. Nuclear-cytoplasmic proportion amounted to 0.12±0.01 μm3 (figure 1).

By the age of 15 days the volumes of hepatocytes significantly increased for the control and the experimental groups by 12.7% and 27.4% respectively. The increase in the volumes of hepatocytes was more prominent for the experimental group, the difference in the amount of cells was 14.7%. The volume of hepatocytes increased due to cytoplasm; the nuclear volume remained unchanged. For the control group, the cytoplasm was coloured inhomogeneously and had foamy structure due to its vacuolization.

For the experimental group, hepatocyte nuclei were clearly marked, had the same size, cytoplasm was coloured homogeneously, sinusoidal capillaries with red blood cells were clearly marked. Red blood cells could be observed in the central vein as well. Cells of mononuclear phagocyte system were activated, which is an indicative feature of protection from the activity of toxic substances.

For 30-day ducks from the control and the experimental groups we could observe the decrease in the volume of hepatocytes by 7.4% and by 6.3% respectively. At the same time, in comparison with the previous age, the difference between the control groups is significant ($p \leq 0.05$). The decrease in the volume of hepatocytes is associated with the critical period in the development of ducks, when neoptile
is replaced with primary plumage. For the control group, the cytoplasm had granular structure, vacuoles still remained in cytoplasm.

By the age of 45 days we could observe the increase in the volume of hepatocytes in comparison with the previous age. For the experimental group, the volume of hepatocytes was 17.3% higher ($p \leq 0.05$) than for the control group. For the control group, the cytoplasm coloured in homogeneously, with clearly marked granular structure, which is a characteristic feature of granular degeneration (figure 2). For the experimental group, cell boundaries were well-observed, the cytoplasm was homogeneous, the nuclei contained from 1 to 4 nucleoli; sinusoidal capillaries contained red blood cells. For the experimental group, cytoplasm volume was 19.8% higher (figure 3), than for the control group.

For 60-day ducks from the control group, the cytoplasm dye was heterochromous, inhomogeneous; in some places the cytoplasm was vacuolated. The volume of hepatocytes increased significantly due to the increase of the volume of nuclei and cytoplasm by 9.2% and 20.6% respectively. Liver structure of experimental group of ducks was characterized by tubular structure. Boundaries between hepatocytes
were observed; cytoplasm dye was homogeneous; nucleoli could be distinguished in the nuclei. The tendency for the increase in hepatocyte volume, due to the increase in nuclear volume, is observed.

For the 75-day ducks of both control and experimental groups, we could observe the tendency for decrease in the volume of hepatocytes by 8.0% and 2.6% respectively. It is explained by the second critical period in the development of ducks, namely with the post-juvenile moult. For the control group, the signs of hepatosteatosis remained in liver parenchyma. Despite the critical period, for the experimental group of birds we could observe that the volume of hepatocytes and cytoplasm was 8.4% and 14.2% higher respectively compared to the control group.

For the 90-day ducks of both control and experimental groups we did not observe any differences in the sizes of the described structures. However, the tendency for development of the features of hepatosteatosis still remained. This is a regular phenomenon for productive birds and is considered to be a positive factor in terms of nutritional quality; at the same time, it is definitely a negative factor in terms of health (figure 4). In comparison with the previous point of study, for the control group, the volume of hepatocyte increased by 7.9% due to the increase of cytoplasm volume. For the experimental group, the liver structure was characterized by the definitive structure, morphofunctionally active; the tendency for increasing height of sinusoidal capillaries and trabecules was observed. The tendency for the increase in hepatocyte volume, due to the increase in nuclear volume, still remained (figure 5).

For 105-day and 120 day ducks, the micrometrical parameters of liver structure did not change significantly as compared to the previous age. However, for the control group, in liver parenchyma the signs of hepatosteatosis were clearly marked. It had a foamy structure due to micro- and macrocellular fat infiltration. The nuclei of the cells were moved to the periphery (figure 6).

DAFS-25k prevented hepatosteatosis; the liver retained its tubular structure. Occasional build-ups of fat could be observed; cytoplasm was coloured homogeneously (figure 7).

In accordance with the existing data, DAFS-25k stimulates productivity and accumulation of selenium in egg albumen and yolk, in thigh and chest muscles, in blood and liver of birds [26, 27, 28, 29].

Selenium concentration for 120-day ducks of the control group amounted to 0.31±0.07 mcg/kg, for the experimental ducks — 0.52±0.04 mcg/kg (p≤0.05).
Figure 6. Liver of 105-day ducks of the control group. Haematoxylin and eosin. Ocular lens x10. Objective x40.

Figure 7. Liver of 120-day ducks of the experimental group. Haematoxylin and eosin. Ocular lens x10. Objective x40.

5. Conclusion
We established that the concentration of nickel in duck fodder exceeded ecologically permissible amount. It amounted to 1.80-2.00 mg/kg. The concentration of selenium is obviously insufficient. Combined fodder for growing ducks contained 0.06 mg/kg of selenium; for adult duck fodder it amounted to 0.14 mg/kg.

For 30-day ducks of the experimental group, during the first critical period, the volume of hepatocyte and the size of trabecules exceeded the same parameters for the experimental group. For 75-day ducks of the experimental group, during the second critical period, cytoplasm volume of hepatocytes, the size of trabecules and sinusoidal capillaries were higher than for the control group. For the experimental group, by the period of attaining physiological maturity, the volume of hepatocyte, nucleus and cytoplasm was higher than for the control group. Nuclear-cytoplasmic proportion amounted to 0.10.

For both groups of 120-day ducks we could observe the increase in the volume of hepatocyte. In comparison with the results established for 1-day ducks, for the control group the hepatocyte volume increased by 35.5%, nuclear volume increased by 18.4%, cytoplasm volume increased by 37.7%. For the experimental group, hepatocyte volume increased by 36.9%, nuclear volume increased by 19.0%, cytoplasm volume increased by 38.9% (p≤0.05).

For the experimental group, selenium concentration in the liver is 67.7% higher than for the control group and amounted to 0.52±0.04 mcg/kg.

The data analysis established that introduction of organic selenium in the form of DAFS-25k into the diet of ducks (in the dose which is recommended by the manufacturer), does not lead to any pathological changes and stimulates morphofunctional activity and selenium content in the liver.

References
[1] Rovensky R V 2001 Determining of functional condition of liver for newly-born calfs and its role in the immunity formation Author's abstract. Candidate of veterinarian science (16.00.01) Belgorod p 30
[2] Litvitckii P F, Maltseva L D and Morozova O L 2018 Typical forms of children's liver pathologies Issues of Modern Pediatrics 17(1) 38-5
[3] Sokolov M N 2018 Pharmacological and toxicological features of Heprasan and its application to poultry farming Author's abstract. Candidate of veterinarian science (06.02.03) Krasnodar p 28
[4] Poretskova G Yu 2004 Specific features of protein-synthetic liver function of premature neonates with physiological and aggravated course of post-natal adaptation Author's Abstract. Candidate of medical science (14.00.09) Samara p 28
[5] Hepatobiliary diseases 2002 ed. V T Ivashkin (Moscow. M-Vesti) p 416
[6] Drozdova L I, Kundraukova Yu I 2010 Bird liver – live laboratory of estimation of quality of feeding and maintenance Agricultural Journal of Urals 5 68-70
[7] Grishina D Yu 2009 Morphology of broiler chicken liver in early post-natal development Author's Abstract. Candidate of biological sciences (16.00.02) Orenburg p 17
[8] Bessarabov B F, Alekseeva S A and Kletikova L V 2011 Aetiopathogenesis, diagnostic and prevention of metabolic disorders of domestic birds (Moscow: Zoomedit.) p 296
[9] Garkun V I, Kletikova L V and Pronin V V 2019 Anatomic and morphological characteristics of Pekin duck liver Hippology and veterinary medicine 2 17-22
[10] Vrakin F V, Sidorova M V 1984 Aetiopathogenesis, diagnostic and prevention of metabolic disorders of domestic birds (Moscow: Kolos) p 156-62
[11] Hochleithner M 2005 Evaluating and Treating the Liver Clinical Avian Medicine 1 441-9
[12] Krasnikova L V, Fomenko L V 2014 Species-determined features of bird liver structure Omsk State Agrarian University Journal 2 58-60
[13] Kuznetsov S L, Mushkambarov N N 2005 Histology, cytology and embryology (Moscow: Medical Information agency) p 600
[14] Skovorodin E N, Davletova V D and Diudbin O V Influence of 'Solmivin Selen' and 'Selamag' preparations on growth and development of Muscovy ducks 3 54-8
[15] Sobolev A I 2012 Meat productivity of ducks determined by introduction of selenium in combined fodder Kursk State Agricultural Academy Journal 5 56-8
[16] Gadiev R R 2018 The efficiency of Sel-Plex selenium preparation in the diet of parental flock of ducks Orenburg State Agrarian University Journal 4 312-4
[17] Ermaskevich E I, Kletikova L V 2016 Evaluation of the effectiveness of phytocomponents in the course of treatment of chicken liver dysproteinosis by biochemical blood tests Orel State Agrarian University Journal 6(63) 112-7
[18] Pronin V V, Kletikova L V, Yakimenko N N and Fedorov G A 2018 Integration of biological metals of fodders in organism of cattle Buryat State Academy of Agriculture Journal 1(50) 73-8
[19] Lukashiv O Ya, Bolnar O I and Grubinko V V 2016 Prospects of use and influence of selenium-containing bioadditives on metabolic processes in the body Bulletin of problems of biology and medicine 2-3(130) 30-4
[20] Marshania I V 2019 Dynamics of changes in morphological and biochemical blood parameters and cellular protection factors of growing geese Kurgan State Agricultural Academy by T.S. Maltsev 32 42-6
[21] Marushko Yu V, Ostopenko Yu Yu 2012 The importance of selenium in clinical practice Children's 5(18) 32-6
[22] Treyak L N, Gerasimov S M 2007 Specific features of influence of selenium for human and animal organism (with respect to the problem of creation of nutrition products with selenium content) Orenburg State University Vestnik 12 136-45
[23] Shvydkov A N, Lantseva N N and Ryabukha L A 2016 Biological role of selenium in poultry meat, liver and eggs Innovation and food security 34(14) 27-31
[24] Hermes-Lima M, Storey J M and Storey K B 2001 Antioxidant defenses and animal adaptation to oxygen availability during environmental stress (Amsterdam: Elseiver Press Bessarabov) 2 p 263-87
[25] Osoba I A 2009 Features of organism antioxidant protection system functioning Fisheries Science of Ukraine 1 133-9
[26] Marmurova O M 2006 Productivity, egg-meat quality of hens in lay when using DAFS-25 during the finishing period of egg laying Author's abstract. Candidate of Agricultural Science (06.02.04; 16.00.06) Voronezh p 22
[27] Rubtsov V V 2007 Correction of immune protection of chickens with selenium deficiency using selenium-organic preparations (Ivanovo) p 22
[28] Shatsky E V 2009 *Physiological explanation of using different form of selenium compounds for feeding broiler-type chickens* (Borovsk) p 48

[29] Shishkina D A, Shatsky E V 2016 *Morphology of the liver of Chinese gray geese against the background of the use of selenium-organic preparation DAPS-25K* (Moscow) p 20