

## **MORPHOLOGICAL CHANGES OF THE REPRODUCTIVE ORGANS IN DOMESTIC CHICKEN SUFFERED FROM INFECTIOUS BRONCHITIS, BASED ON AN EXCESS OF VITAMIN D3 IN THE DIET**

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### **Abstract**

*In private mini-poultry farms, poultry that have recovered from infectious bronchitis are often not culled. Changes in the organs of the reproductive system of adult chickens and roosters that recovered after an outbreak of infectious bronchitis were studied. It was found that in most of the flock, egg productivity and condition quality of eggs were preserved in laying hens (69%), and in roosters - fertilization ability (after hatching the eggs, hens hatched condition chicks with 80% hatchability). In 31% of hens, egg productivity and/or egg quality was impaired: reduced laying; hens laid eggs with soft and/or deformed shells; there was no clear boundary between the dense and liquid layers of the egg white; the share of second-grade eggs has increased. During the post-slaughter examination of chicken carcasses, various pathologies of the reproductive organs were found: retention of the egg in the oviduct and damage to its walls (it is possible that this is due to calcareous growths on the eggshell); ovarian cysts; atresia of mature follicles.*

**Key words:** domestic chickens, egg production, infectious bronchitis, ovariitis, reproductive system, salpingitis.

### **INTRODUCTION**

Scientists from all over the world are investigating various aspects of infectious bronchitis in chickens (Hoerr, 2021; Shao et al., 2020; Bande et al., 2017; Khataby et al., 2016; Chacón et al., 2014; Cook et al., 2012; Jackwood, 2012; Chousalkar et al., 2007; Raj & Jones, 1997). According to Amarasinghe et al. (2017), Han et al. (2017) and Cavanagh, (2007), the coronavirus, which is the causative agent of the disease, damages the macrophage structures of the poultry body.

Researchers Shahnas et al. (2020) define infectious bronchitis of chickens as a polysystemic disease with suppression of immunity. In case of spontaneous infection of poultry with its causative agent, part of the flock is resistant, which is determined by the status of the immune system (Smith et al., 2015). The instructions for fight against infectious bronchitis in commercial poultry farm, provide for the depopulation of poultry that were infected (Berezovskyi, 2017). The credo of private mini-poultry farms, as a rule, is the search for schemes for poultry saving (rehabilitation). This approach is justified by the fact that after recovery from infectious

bronchitis, the bird have immunity to a specific strain of the causative agent of the disease for a period of at least 5 months (Stehnií et al., 2013). But in such a bird, there are various consequences (long-term and temporary), which indicate that the birds were sick of infectious bronchitis in the past. Among them are changes in the reproductive system of chickens, including impaired or lost egg productivity (Liakhovych et al., 2022; Prokudina, 2015). Dynamic monitoring of birds with infectious bronchitis in the conditions of a specific poultry farm contributes to timely detection and objective assessment of its consequences. Characterization of morphological changes of chickens reproductive organs during infectious bronchitis is an important informative component of understanding the patterns of reproduction processes in reconvalesced birds, the basis for solving practical problems of flock reproduction, assessment and prognosing of egg productivity of flocks.

### **MATERIALS AND METHODS**

We made research by two stages. At the first stage, the level of egg productivity and egg

quality was determined in adult crossbred hens reconvalesced from viral bronchitis due to spontaneous infection (aged from 5 months to 2.5 years). Poultry was kept in a specially equipped place in compliance with standard animal hygiene and veterinary sanitary parameters. The chickens were fed mixtures of cereal grain and/or its waste (shredded wheat, barley, corn); chopped fodder beets, carrots, potatoes; in summer - with green mass of milkweed, cabbage leaves, chopped pumpkins and zucchini. Periodically, the feeding ration was supplemented with a cake of sunflower seeds, flax, fish meal, dairy waste; in order to correct calcium and phosphorus metabolism, which are involved, in particular, in the formation of a strong egg shell, they were fed synthetic preparation of vitamin D. Chickens were supplied with water without restriction. A walking platform was equipped around the perimeter of the aviary. At the second stage, during the post-mortem examination of the slaughtered chickens carcasses (n = 17) and roosters (n = 3), which was performed in accordance with existing requirements (Yatsenko et al., 2015), the organs of reproductive system were examined in detail. Their anatomical preparation was carried out under the control of low magnification optical lenses. The research was conducted according to the scientific topics of the Department of Normal and Pathological Morphology of the State Biotechnology University. Methods of clinical observation, autopsy, anatomical preparation and analysis were used.

## RESULTS AND DISCUSSIONS

Evaluation of egg productivity and egg quality of the researched laying hens showed a decrease in the amount of conditioned eggs and, accordingly, an increase in non-conditioned and low-grade eggs (nutritional inferiority of eggs with shell defects; II category eggs weighing at least 44 g; eggs weighing not less than 43 g, which are sold in Ukraine under the name "small" (Tiutiun, & Pototskyi, 2011) (Figure 1). According to Quinteros et al. (2022), a quarter of chickens infected with the infectious bronchitis virus laid eggs with a soft shell, and 10% - with a rough surface.



Figure 1. The appearance of substandard eggs from chickens that were reconvalesced from infectious bronchitis

With the appearance of small eggs, hens were suspected of egg-laying abnormalities, in particular, due to stenosis of the oviduct. A narrowing of the oviduct lumen in its cranial part, caused by post-necrotic scarring of the wall, was diagnosed during the post-mortem examination of the culled chickens. There were violation of the formation of the eggshell, including, with the absence of its calcification in 20% of the studied chickens (Figure 2). Such eggs were easily broken immediately in the nest, leaving corresponding traces of fragments of the inner and outer layers of the shell and the moist sticky mass of protein and yolk. To obtain native preparations of these eggs, the corresponding female chickens were isolated in separate sectors of the poultry house, provided with a nest with soft porous filling, in which the egg retained its integrity after laying.



Figure 2. Appearance of eggs with non-calcified shells from chickens after infectious bronchitis. Native sample

Chickens ate the remains of uncalcified eggs. As it known, chicken eggs contain various hormones: estrogen, progesterone, testosterone (Aslam et al., 2013). Chicken egg is rich in proteins, vitamins, minerals, fatty acids (Milinsk et al., 2003; Qi & Sim, 1998). An excess of these substances, in particular, steroid hormones and vitamin E, is harmful to the reproductive system of the bird. It was established that in some chickens there was an overdose of synthetic vitamin D. This was evidenced, in particular, by the thickening of the eggshell (it reached 0.43 mm in some parts of it) and hilly chalk growths on it. Such an eggshell injured the mucous membrane of the

oviduct of chickens, disturbed its peristalsis, which caused a delay in laying the egg. Complicated egg laying took place. Calcified outgrowths interfered with the effect of sliding on the mucous membrane, so egg-laying caused pain in female hens. In some culled chickens, post-mortem examination showed signs of delayed egg laying due to hypercalcified and deformed shell (Figure 3).

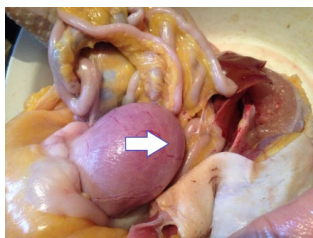


Figure 3. Visceral organs of domestic chicken. Autopsy. Retention of the egg in the uterus (arrow)

The uterus stretched and lowered, its wall became thinner and lost tone (due to the delay of the egg in fallopian tube (blood stasis developed in its venous vessels) (Figure 4). The egg retained its integrity after laying.



Figure 4. A fragment of the oviduct and uterus with a retained egg in a domestic hen reconvalesced from infectious bronchitis. Native sample

Due to the slow movement of the egg in the fallopian tube, the secretion of the glandular structures of the mucous membrane was disturbed. The delay of the egg in the uterus of the fallopian tube had a particularly negative effect on the neurovascular layer of its muscular membrane. It is located between the inner (circular) and outer (longitudinal) layers and is a wide layer of fibrous connective tissue (Zhyhalova et al., 2008; Bakst, 1998). These violations led to: atony and stretching of the wall and other parts of the fallopian tube due to compressive atrophy of the muscular elements; the mesentery of the fallopian tube was also stretched (Figure 5).



Figure 5. A fragment of an eviscerated fallopian tube with a mesentery of an adult domestic chicken reconvalesced from infectious bronchitis (venous stasis of vessels of the serous membrane and mesentery; stretching of the wall). Native sample

We found changes classified as vascular congestion (post-compressive internal) and muco-catarrrhal ovariitis during macroscopic examination of the oviduct mucous membrane in slaughtered hens with a history of complicated egg-laying due to large egg size and/or deformed hypercalcified shell (Figure 6).

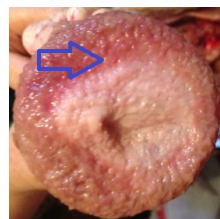


Figure 6. View of the mucous membrane surface of the oviduct fragment of the domestic hen with signs of local internal post-compressive congestion and mucocatarrrhal ovariitis (arrow) due to egg retention. Native sample

It is known that in birds, the uterus of the oviduct narrows at the border with its vagina and forms a uterine-vaginal junction, which plays an important role in sperm deposition (Wahabu, 2016; Kot, 2011; Bondarenko, 1998; Bakst, 1998). Delay of the egg in the fallopian tube due to stretching and loss of tone of its wall leads to a violation of this depositing function (as a result - loss of fertility in females). Normally, in chickens, only one (the largest) follicle per day is ready for ovulation: preovulatory follicles ovulate in order of size due to a surge of luteinizing hormone (Cunningham et al., 1984). Signs of edema developed in the vascular membrane of preovulatory follicles according to delay of the formed egg in the egg-bearing tract of chickens (Figure 7).

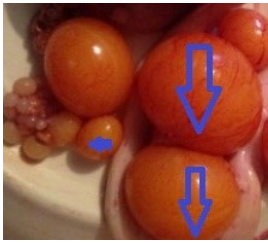


Figure 7. Preovulatory follicles (down arrow): blood stagnation in the vascular membrane; small yellow follicles during egg retention in chickens (left arrow)

In individual chickens, double oviposition per day took place (double oviposition on one day) with the laying of eggs of various sizes, including small and/or without calcified shells. Three one-and-a-half-year-old hens with stopped egg-laying had increased abdominal volume and difficulty breathing. They were culled, and during the post-mortem examination, changes typical for yolk peritonitis were diagnosed, with the presence of sticky, thin, dirty-yellow contents in the abdominal cavity (due to the rupture of yolk follicles). Defective, compacted, stone-like yolk follicles in the capsule, which were easily ruptured, were also found (Figure 8).



Figure 8. The content of the encapsulated yolk follicle in an adult chicken suffering from infectious bronchitis

Adhesion ovarioperitonitis was diagnosed postmortem in one slaughtered chicken; the other two have salpingoperitonitis with adhesive obliteration of the peritoneum and the serous lining of the intestinal tract. In these chickens, patho-anatomical signs of non-specific intoxication of the body of varying severity (general cyanosis, toxic liver dystrophy, ascites, pericarditis, myocardial dystrophy, granular renal dystrophy) were detected. A post-mortem examination revealed a monocystic lesion of the oviduct in one 5.5-month-old immature hen, which had no clinical manifestations characteristic of infectious bronchitis (Figure 9). At the same time, hyperplasia of the bursa of Fabricius was

observed, which indicates a sufficient immune status in this individual bird.

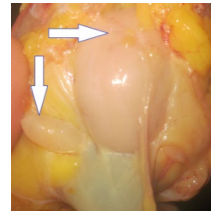


Figure 9. An oviduct cyst (down arrow) and a bursa of Fabricius (right arrow) in a 5.5-month-old hen which has not yet laid eggs

Polycystic ovary was diagnosed in three culled hens with stopped egg-laying during the post-mortem examination (Figure 10). Multiple follicular cysts of different sizes surrounded by a compacted capsule of connective tissue elements were found. Their lumen contained a liquid or oily mass. Torsions of individual cysts with rupture of the capsule were observed.



Figure 10. Polycystic ovary an adult chicken suffering from infectious bronchitis

A follicular ovarian cyst indicates a violation of the normal ovulation process: when a mature follicle does not ovulate due to the death of an egg in it. Disintegration of follicles at different stages of their development was detected. This pathology was classified as follicular atresia. Similar changes are described by researchers Hassan et al. (2021), Pereira et al. (2019), Boroomand et al. (2012), in particular, in the experimental infection of chickens with a highly pathogenic strain of infectious bronchitis virus. Scientists Mueller et al. (2022), Zhong et al. (2016) indicate the pathogenicity of the virulent infectious bronchitis virus isolate YN on the ovary and oviduct of chickens. Zhang et al. (2020) observed changes in the reproductive system and egg production of chickens infected with infectious bronchitis virus TW type I. A peculiar finding was the changes in oviduct mucous membrane during the preparation of

the oviduct of a 2.5-year-old hen, culled due to cessation of egg-laying (Figure 11). Necrotic salpingitis was diagnosed.



Figure 11. A fragment of the dissected oviduct (from the mucous membrane side) of an adult hen with stopped egg-laying after remissions of infectious bronchitis: necrotic salpingitis (arrow)

This individual had classic respiratory signs of infectious bronchitis during his lifetime. After the first two remissions of the disease, the hen had a temporary cessation, and later - a resumption of egg laying. There were hyperpigmented small spots of dark brown-red color on the shell of the laid eggs.

Under the dynamic monitoring of productivity indicators and the morphological state of the reproductive organs of the studied poultry, these indicators were evaluated in roosters. After the slaughter of a 7-month-old rooster, an examination of the organs of the thoraco-abdominal cavity revealed that the testicles are anatomically correct (at the level of the last three ribs), and have an ovoid shape; the right testicle is smaller than the left; the color of the surface of the testes is pale yellowish (Figure 12), the consistency is elastic.



Figure 12. View of the testicles of a 6-month-old, immature rooster reconvalesced from infectious bronchitis

The indicated parameters of the macroscopic characteristics of the testicles of the investigated rooster coincide with the data of Althnaian (2022), Mfoundou et al. (2022), Razi et al. (2010). According to Mfoundou et al. (2022), in immature roosters the testes are

yellowish or pink in color and the left testis is always larger than the right.

Fertilizing ability was preserved in the two studied adult roosters: under natural conditions of incubation of eggs by hens, 80% hatching of chicks with high viability took place. A postmortem examination of a one-and-a-half-year-old rooster revealed that the testicles were anatomically placed correctly, had a bean-like shape, and a slightly increased volume (this indicator was determined by the following test, which is used in the macroscopic diagnosis of the parameters of bean-shaped organs: the testicle was dissected from its convex part to 2/3 of the depth of the organ, after which the dissected halves were compared - they deviated somewhat from the cut line, which indicates an increase in the organ).

This increase in testes is a morphological criterion, in particular, of spermatogenic activity. Dyscirculatory disorders were observed in the vascular membrane of the testicular capsule: hyperemia, hemostasis with the effect of small hemorrhages (Figure 13), which developed under general cyanosis in the background of keeping this male rooster in hypodynamic conditions.



Figure 13. View of the testicles of a one-and-a-half-year-old rooster: hemodynamic disorders of the choroid

## CONCLUSIONS

The study of the correlation of egg productivity and the morphological state of the reproductive organs of chickens helps to objectively assess the influence of various factors and their relationship on the bird's body. With an excess of vitamin D in the diet of the researched reconvalesced from infectious bronchitis egg-laying hens, hypercalcification and deformation of the eggshell were observed. This, in combination with morphological changes in the oviduct of chickens caused by



the influence of the infectious bronchitis virus (stenosis on the background of scarring), provoked complicated egg laying. Analysis of the effects of factors affecting the reduction, disruption or cessation of egg-laying in hens reconvalescent from infectious bronchitis contribute to the optimization of the production process, prognosing the reproductive and productive qualities of the flock. Also, this information supplements the data on the differential diagnosis of pathologies of the reproductive organs of poultry.

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