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INFLUENCE OF THE WEIGHT OF TESTES DURING PREFUNCTIONAL PERIOD ON THE MORPHO-PHYSIOLOGICAL AND REPRODUCTIVE INDICES OF ROOSTERS

ВЛИЯНИЕ ВЕСА ЯИЧЕК В ПРЕДОПЕРАЦИОННЫЙ ПЕРИОД НА МОРФОФИЗИОЛОГИЧЕСКИЕ И РЕПРОДУКТИВНЫЕ ОСОБЕННОСТИ ПЕТУХОВ

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The aim of the work was to study the influence of the weight of testes during the prefunctional period on the morpho-physiological and reproductive performance of roosters. It is determined that the use of ultrasound diagnostics is effective for direct visualization of testes in four-month-old Silver Leghorn roosters, allowing to divide the population into M- and B-groups according to the relative weight of testes (up to and more than 40 mg/100 g). Compared to M-roosters, B-roosters are characterized by significantly greater weight of the crests, the concentration and motility of sperm, as well as much weaker severity of correlative connections between the weights of the body, crest and testes. The ratio between the weight of the crest and the weight of the testes (C/T), the numerical expression of which is in an extremely wide range (from 1.6 to 49), can be a new morphometric characteristic of the rooster. Four-month-old progeny of the B- and M-roosters retained the differences between the weights of the testes and the crests, which indicates the possibility of creating a Leghorn population with new morpho-physiological features. The weight of the testes in the prefunctional period is a factor determining the morpho-physiological and reproductive qualities of the rooster.

Key words: *rooster, crest, testes, correlation, fertility.*

Целью работы было изучить влияние массы тестикулов в дофункциональный период на морфо-физиологические и репродуктивные показатели петухов. Установлено, что применение ультразвуковой диагностики эффективно для непосредственной визуализации тестикулов у 4-месячных петухов серебристый леггорн и позволяет разделить

популяцию на М и В-группы по относительной массе тестикулов (до и более 40 мг/100 г). В-петухам свойственны значительно большие, чем М-петухам, массы гребней, концентрация и подвижность спермы, а также значительно меньшая выраженность коррелятивных связей между массами тела, гребня и тестикулов. Отношение массы гребня к массе тестикулов (Г/Т), числовое выражение которого находится в чрезвычайно широком диапазоне (от 1,6 до 49), может быть новой морфометрической характеристикой петуха-производителя. 4-месячные потомки В- и М-петухов сохранили различия между массами тестикулов и гребней, что свидетельствует о возможности создания популяции леггорнов с новыми морфо-физиологическими особенностями. Масса тестикулов в дофункциональний период является фактором, определяющим морфо-физиологические и репродуктивные качества петуха-производителя.

Ключевые слова: *петух, гребень, тестикулы, корреляция, плодовитость.*

Introduction. The fertility of roosters depends on their breed and linear traits and is defined by the characteristics of their testes and the level and quality of sperm. There are scarce data about the characteristics of testes of breeder males. In particular, it is not clear, which weight of testes is optimal to maintain high reproductive activity. The aggravation of fertility at poultry breeding farms is believed to be related to considerable variability of reproductive traits of roosters. Different directions are intensely studied with the purpose of enhancing the fertility indices of roosters, namely, determining and studying genetic factors of mating, hatchability, sperm motility, number of spermatozoa [1], applying histomorphometry of testes to estimate fertility [2], identifying specific structures of spermia, which play a key role in the fertilization process [3], creating transgenic breeders, which are noted for fluorescence of spermatozoa, using non-viral vectors [4], allowing free access to poultry-runs [5]. While selecting breeder roosters, it is important to consider morpho-physiological characteristics of testes in their complex correlation to the sizes and color of the crest as a fertility criterion. So the aim of the work was to study the influence of the weight of testes during the prefunctional period on the morpho-physiological and reproductive performance of roosters.

Materials and methods. The work was performed using the roosters of Birkivska barvysta population in accordance to the feeding ratios and light regime, selected to breed Silver Leghorn roosters. In the first experiment, 80 four-month-old roosters were divided by the relative weight of their testes (up to and over 40 mg/100 g) into M-roosters and B-roosters respectively. The weight of the crest and the correlations between live bodyweight, weights of testes and crests as well as individual ratios between the weight of the crest and the weight of the testes were determined. The following experiment was aimed at determining incubation indices of egg qualities for two groups of hens, 32 birds in each, while keeping them with roosters, selected by live bodyweight, sizes of the crest and testes. The sizes of the tes-

tes were determined at the age of 4 months using the Logiq e ultrasound scanner with the 4–10 MHz micro-convex probe. At the lateral abdominal access, the liver served as an "acoustic window" to find the spleen, which, in its turn, is a reference point to finding testes. The roosters with the testes of 12–17 and 7–8 mm were divided into the first and the second group of hens, respectively. Two loads of 280–300 eggs from each group were incubated. The biological control of egg fertilization was conducted by egg windowing. The roosters, bred by this incubation, were kept till the age of four months. The indices of sperm quality were determined at the end of the productive period. The work was performed in accordance to the bioethics norms concerning animals, which comply with the Law of Ukraine "On Protection of Animals from Cruelty", dated February 21, 2006, meeting the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and general ethical principles of experiments on animals, approved at the National Bioethics Forum (Kyiv, 2001).

Results and discussion. B-roosters and M-roosters, which were divided at the age of 4 months by the relative weight of testes (up to and over 40 mg/100 g), were significantly different by their morphological and reproductive specificities (Table 1).

Table 1. **Morphometric indices of 4-month-old roosters**

Indices	Whole group	B-roosters	M-roosters
n	80	36	44
Live bodyweight, g	1665 ± 20	1732 ± 25**	1610 ± 31
Weight of crest, g	10 ± 0.52	12.2 ± 0.9**	8.29 ± 0.54
Relative weight of crest, mg/100 g	602 ± 32	712 ± 54**	512 ± 33
Weight of testes, g	1.1 ± 0.16	1.93 ± 0.31**	0.44 ± 0.02
Relative weight of testes, mg/100 g	66.1 ± 9.7	113 ± 19**	27 ± 1
Relative weight of crest/ Relative weight of testes	15.1 ± 1.0	9.6 ± 1**	19.5 ± 1.2

*P< 0.05

Compared to M-roosters, B-roosters had larger live bodyweight (by 7.6 %), weight and relative weight of the testes (4.4 and 4.2 times), and the weight and relative weight of the crest (1.5 and 1.4 times.) The difference between the ratios of weights of the crest and the testes was on average much higher for M-roosters than for B-roosters.

The selection of breeder roosters does not take into consideration the characteristics of the testes and the level and quality of sperm products; it is not clear which weight of the testes is optimal to maintain high reproductive activity of breeder roosters. It was reported that the weight of testes of 15-week-old roosters of meat breeds was 0.5 g on average, i.e. it does not exceed 30–40 mg / 100 g of the bodyweight. Expressed heterogeneity in terms

of the weight of testes – from 0.9 g to 44 g (i.e. from 18–22 to 880–1100 mg/100 g) – was found in roosters of the meat breed with the live bodyweight of 4–5 kg [6]; this fact demonstrates that the variability in the weight of testes is remarkable for roosters of different age groups.

The sizes (weight) of the rooster crest are conditioned by genetic factors and are in positive correlation with the level of fertility [7]. The form of a crest is determined by genes of two types – *pea comb* and *rose comb*; crests are an index of resistance to different diseases; roosters with large crests are notable for high immune status [8–9].

The morpho-physiological condition of the testes depends on the breeding conditions, the availability of biologically active components in fodder. Photostimulation of hypothalamus (due to longer days) leads to the secretion of gonadotrophin-releasing hormone (GRH). Under the impact of GRH, the anterior lobe of hypophysis releases luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH stimulates Leydig cells in the interstitial tissue of the testes to produce testosterone. The number of Leydig cells is in positive correlation with the weight of the testes and the indices of sperm production. Testosterone is the main androgen for adult male birds, defining their behavior and the sizes and color of the crest. The size and uniform development of the crest is a relevant characteristic of the breeder rooster; the sizes of the crest are related to the weight of the testes [10]. The hypophysis controls the amount of testosterone in blood, thus creating the inverse relationship to maintain the level of hormones in a certain range. FSH impacts the structures, producing spermatozoa. The ability to produce spermatozoa in the seminiferous tubules of testes is related to the reproduction of Sertoli cells. The number of Sertoli cells is in proportion to the weight of the testes, larger testes produce more spermatozoa. The main relationship between the testes and the crest is kept via testosterone; while producing testosterone, the testes regulate the development of the crest; on the other hand, the crest does not affect the characteristics of the testes; there have been no previous reports on the ratio between the weight of the crest and the weight of the testes of male birds. It was established in our case that roosters were different in their ratio between the weight of the crest and the weight of the testes (C/T). These ratios were in the range from 1.6 to 49; the weight of testes in any Leghorn rooster was at least 1.6 times less than the weight of its crest. This index may be a significant characteristic of breeder roosters on condition of defining optimal thresholds for C/T ratio for specific breed and age groups.

A significant morphological characteristic of roosters is found in the indices of correlations between the weights of the body, crest, testes, and the ratio between the weight of crest and testes (C/T). B- and M-roosters have evident differences in the manifestation of the correlations between the weight of the body, crest and testes: all three correlations are insignificant in

B-roosters, whereas in M-roosters such bonds are statistically significant, which can be explained by unequal levels of the sensitivity of roosters to stress factors (Table 2).

Table 2. The correlation coefficients of morphometric indices of 4-month-old roosters

Morphometric indices of roosters	B-roosters (n=36)	M-roosters (n=44)
Live bodyweight / weight of crest	-0.139	0.378*
Live bodyweight / weight of testes	-0.171	0.48**
Live bodyweight/ C/T	0.08	0.068
Weight of testes / weight of crest	0.20	0.42**
Weight of testes/ C/T	-0.80**	-0.22
Weight of crest/ C/T	0.43*	0.76**

*P< 0.05; **P<0.01

The initial reaction of the organism to the effect of external factors is manifested in the proportional change in the level of correlations between morphological characteristics, in particular, the aggravation of conditions results in stronger correlation between the weights of organs. Strong correlations between the weights of the body, crest and testes in M-roosters are in contrast with the corresponding insignificant correlations in B-roosters which indicates a probable advantage of B-roosters in terms of adaptability.

In B- and M-roosters, the correlation of the relative weights of crests and C/T is positive, statistically significant.

The correlation of the weight of the testes and C/T in both groups is negative, however, in B-roosters it is statistically significant degree correlation, and in M-roosters – weakly expressed linear correlation (Fig. 1). Hypothetically, C/T may be one of the criteria in selecting 4-month-old Silver Leghorn breeder roosters as the largest weight of the testes was found for roosters with the indices, not exceeding 10; the indices in the range of 10–50 are notable for males with small testes – under 1 g.

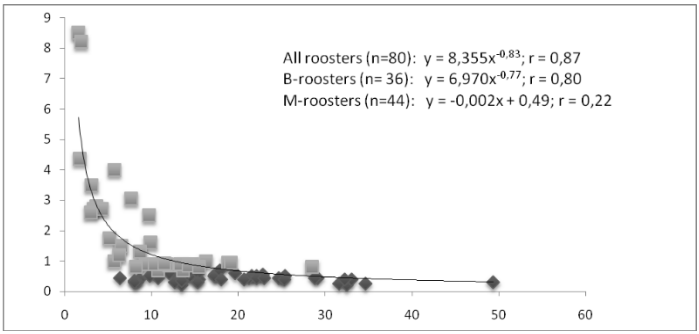

Fig. 1. The correlation of the weights of testes (g, vertical) and the C/T ratio (horizontal) in B- (gray boxes) and M-roosters (black boxes)

Thus, these observations demonstrated significant inequalities in 80 Leghorn roosters regarding the weight of such important organs as the testes as well as the corresponding crest and an extremely wide range of indices C/T – from 1.6 to 49, which indicated the reasonability of studying the correlations between the weights of the testes and the crest in prefunctional period and reproductive indices of roosters.

Ultrasound diagnostics was applied to determine the size of testes in roosters; here the testes were viewed as echopositive formations of ovoid form and even structure; the echogenicity of the testes and spleen was the same (Fig. 2).

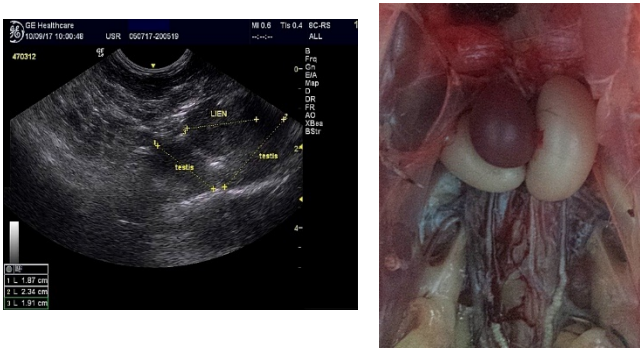

Fig. 2. The spleen and testes of a 4-month-old Silver Leghorn rooster (ultrasound image is on the left)

The determination of absolute sizes is complicated due to the limited acoustic window and limited movements of the probe, but the comparison of their sizes against the sizes of the spleen allows determining the gradations of the testes: large, medium or small. According to the ultrasound imaging, large testes have the sizes (27–30 mm; 7–9 g), which exceeds the sizes of spleen (22–24 mm; 2.5–3 g), with clear visualization. In ultrasound imaging, medium testes (20–21 mm; 3–4 g) have the sizes which are similar to or somewhat smaller than the sizes of the spleen, their visualization is satisfactory. According to the ultrasound imaging, the size of small testes is significantly (almost twice) smaller than the size of the spleen, their visualization is very complicated.

Ultrasound imaging is a non-invasive and relatively cheap method of direct visualization of the testes of roosters and allows dividing roosters into groups with large, medium, and small testes in a non-invasive way at an early stage (prefunctional period) and tracing the changes in their sizes. High sensitivity (84 %) and specificity (89 %) of ultrasound method, envisaging life-time determination of the sizes of testes, allows selecting roosters

with hypergonadism with high precision. Ultrasound visualization of morphological and functional changes in gonads of mature birds was demonstrated [11], but there have been no reports about applying ultrasound imaging to determine the sizes of testes in roosters. As chickens have high heredity of morphological characteristics of the crest and testes, the application of ultrasound imaging may promote the establishment of populations with certain specificities of these organs in roosters.

Keeping roosters together with hens demonstrated that in the group, where the testes of 4-month-old B-roosters were 12–17 mm, egg fertilization (in one of two incubations) and the hatchability was higher than in the group where the testes of 4-month-old M-roosters were 7–8 mm.

According to the results of 12 determinations, average volumes of ejaculate in the groups for both types of roosters were the same, but in B-roosters the concentration and motility of spermia was much higher (44 and 35 %, respectively) compared to M-roosters. Four-month-old rooster progeny of both types did not differ by average live bodyweight. The progeny of B-roosters had much larger (1.63 and 1.8 times respectively) relative weights of the crest and testes compared to the progeny of M-roosters (Table 3).

Table 3. **Comparative characteristics of B- and M-roosters**

Indices	B-roosters	M-roosters
Sizes of testes (by ultrasound imaging), mm	12–17	7–8
Sizes of crest, mm	80–100	55–60
The ratio between the sizes of crest and testes	5–6.7	7.5–8.6
Incubation 1: Egg fertilization, % Hatchability, %	95.8* 87.3	87.5 72.1
Incubation 2: Egg fertilization, % Hatchability, %	95.8 90.8	96 82.7
Sperm quality: motility, points volume, ml concentration, billion/ml	8.76 ± 0.14** 0.74 ± 0.07 1.81 ± 0.26**	6.5 ± 0.96 0.75 ± 0.12 1.26 ± 0.33
Indices of progeny (4-month-old roosters):		
Live bodyweight, g	1632±24 (1070-2150)	1627±48 (1180-1850)
Weight of crest, g	19.1±1.1 (2.2-42.5)*	11.7±1.1 (3.9-19.2)
Relative weight of crest, mg/100 g	1146±64 (133-3457)*	699±57 (331-1089)
Weight of testes, g	2.8±0.36 (0.4-13.2)*	1.6±0.3 (0.2-4.5)
Relative weight of testes, mg/100 g	167±20 (21.5-880)*	93±15 (17-249)
Relative weight of crest/ Relative weight of testes	11.7±1 (2-40.6)*	9.9±1 (4.6-19.5)

* – $P < 0.05$

Conclusions. The use of ultrasound diagnostics is effective for direct visualization of testes in four-month-old Silver Leghorn roosters, allowing to divide the population into M- and B-groups according to the relative

weight of testes (up to and more than 40 mg/100 g). Compared to M-roosters, B-roosters are characterized by significantly greater weight of the crests, the concentration and motility of sperm, as well as much weaker severity of correlative connections between the weights of the body, crest and testes. The ratio between the weight of the crest and the weight of the testes (C/T), the numerical expression of which is in an extremely wide range (from 1.6 to 49), can be a new morphometric characteristic of the rooster. Four-month-old progeny of the B- and M-roosters retained the differences between the weights of the testes and the crests, which indicates the possibility of creating a Leghorn population with new morpho-physiological features. The weight of the testes in the prefunctional period is a factor determining the morpho-physiological and reproductive qualities of the rooster.

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