The influence of protein from black soldier fly larvae (Hermetia illucens) on the reproductive functions of roosters

Evgenia Romanenko[1*]

[1]PJSC "Cherkizovo Group", technical regulation area, 5B, st. Lesnaya, business center "White Square", 125047, Moscow, Russia

Abstract. Our research is devoted to studying the possibility of using food from the larvae of black soldier flies (Hermetia illucens) (low-fat flour) in the diets of breeding roosters of the Hisex Brown cross. The inclusion of 7.5% low-fat flour in the diet structure of the roosters of the experimental group led to an increase in the quality indicators of the sperm of breeding roosters, activated metabolic processes and antioxidant protection. By the time of full physiological maturation (25 weeks), the roosters of the experimental group had a superiority ($P<0.05$) over the control in live weight and testes weight by 2.80 and 4.25%. An increase in ejaculate volume was found by 7.55% ($P<0.05$), sperm concentration by 24.36% ($P<0.05$), sperm survival time of up to 139 hours, while reducing the number of morphologically abnormal germ cells. A connection has been established between the amino acid and mineral compositions of the experimental feed and rooster sperm. Bioactive substances of insect larvae are able to activate enzymes of the antioxidant defense of birds. In the blood of experimental roosters, the level of glutathione peroxidase increased by 2.51% ($P<0.05$), superoxide dismutase - by 8.37 ($P<0.01$), and the amount of reduced glutathione by 12.62% ($P<0.01$). A decrease in oxidized glutathione by 29.67% ($P<0.01$) was found. The end product of fat oxidation (malondialdehyde) decreased, relative to the control, by 16.51% ($P<0.01$). The conducted studies have proven the effectiveness of the experimental feed in the nutrition of breeding roosters.

1 Introduction

In the modern world, the use of insect larval protein in feeding farm animals and birds can solve several global problems: an alternative source of protein has been found that is competitive with the resources of the food system, as well as an effective way to dispose of organic waste. However, the scale of insect protein production worldwide is low [1-2]. The main producers of protein (low-fat meal from insects) are the companies Protix (Netherlands), Agriprotein (South Africa), Entoprotek LLC (Russia), Ynsect (France). The first three companies are engaged in the cultivation of black soldier fly larvae (Hermetia illucens), family Stratiomyidae, and Ynsect breeds mealworms.

* Corresponding author: evgenia.romanencko@yandex.ru

The nutritional value of protein from fly larvae, both fresh and dried, instead of protein ingredients, has been widely studied, especially in poultry farming [3-4]. It has been proven that the inclusion of fly larvae meal up to 7% in the diet of broilers improves their growth performance [5], increases carcass yield and reduces abdominal fat mass [6-7], increases the content of lysine and tryptophan in the pectoral muscle, despite unchanged protein content [8], does not have a negative effect on the organoleptic properties of meat [9-10]. The use of up to 10% feed from insect larvae in the structure of the diet of laying hens helps to increase the mass of eggs and the thickness of eggshells [11-13]. At the same time, there are no reports on the use of meal from fly larvae in feeding breeding roosters.

The purpose of our research was to study the possibility of using flour from the larvae of black soldier flies (Hermetia illucens) in feeding breeding roosters of the Hisex Brown cross.

2 Materials and methods

The object of the research was two-line roosters of the "Hisex brown" cross, raised in industrial conditions at the JSC Tulskaya poultry farm in the Tula region. The experimental additive was defatted flour from black soldier fly larvae, produced at the Entoprotek LLC enterprise (Penza region). In the technological process of producing feed protein, plant raw materials (fermented feed) were used as a substrate. The age of the larvae after settling on the extract is 12-14 days. According to the manufacturer, the nutritional value of low-fat flour is as follows: crude protein 62.20%, crude fat – 7.46%, crude fiber – up to 9.5%, moisture – no more than 6-7%, nutritional value – 200 Kcal.

The methodology provided for the formation of two groups of day-old roosters of 30 heads each (control, experimental). During the fattening period (0-25 weeks), the cockerels of the control group were raised on diets standardized according to the standards of nutritional requirements; the cockerels of the experimental group were raised on diets in the structure of which the protein component (soybean, sunflower meal) was replaced by an experimental additive in the amount of 7.5%.

Sperm from roosters was obtained by simultaneous massage of the back and abdomen in the area of the pubic bones. The spermogram was determined in a laboratory for artificial insemination of a poultry farm. Blood parameters were determined in the conditions of the Cherkizovo research complex (Moscow), equipped with all types of the latest equipment (https://cherkizovolab.ru/?ysclid=lrowxevx6k487756140).

The obtained data were processed by the method of variation statistics using Microsoft office computer programs, with the calculation of coefficients of variation and determination of the criterion for the significance of the difference according to Student-Fisher at three levels of probability.

3 Research results and Discussion

When raising breeding roosters, their live weight was recorded, which, at all age periods, corresponded to the standard values of the cross. Taking into account the peculiarities of the physiological development of roosters, the weight of the testes was determined at the ages of 14, 17 and 25 weeks (Table 1).

The established difference in live weight in favor of the roosters of the experimental group, relative to the control, at the age of 25 weeks, was 72 g (2.83%; $P<0.05$). The development of the testes corresponded to the age and physiological state of the experimental roosters, but at the same time, the experimental food, in the form of low-fat

flour from the larvae of black soldier flies, caused an increase in their weight, which by the time of puberty was ahead of this indicator in the control group by 1.79 g (4.25 %; P<0.05).

Table 1. Parameters of live weight and testes of experimental roosters.

Age of roosters, weeks	Control group			Experimental group		
	Live weight, g (n=30)	Weight of testes, g (n=5)	% of live weight	Live weight, g (n=30)	Weight of testes, g (n=5)	% of live weight
14	1759±14.25	0.64±0.03	0.036	1778±13.55*	0.75±0.04	0.042
17	1883±15.87	20.84±0.49	1.054	1944±16.95*	22.31±0.52*	1.148
25	2541±16.74	42.12±0.51	1.657	2613±18.06*	43.91±0.47*	1.680

Note: *P<0.05; **P<0.01; ***P<0.001.

In her research, Hussain I. [9], Ushakova N.A. [14], Dillak S.Y.F.G. [5] also revealed a positive effect of various types of insect feeds on live weight during the development of birds, palatability and digestibility, feed conversion, as well as their viability.

In addition to the mass of testes, the reproductive value of roosters is assessed by the quality of sperm production, the fertilizing ability of which depends on a set of physiological properties that determine its biological usefulness (Figure 1).

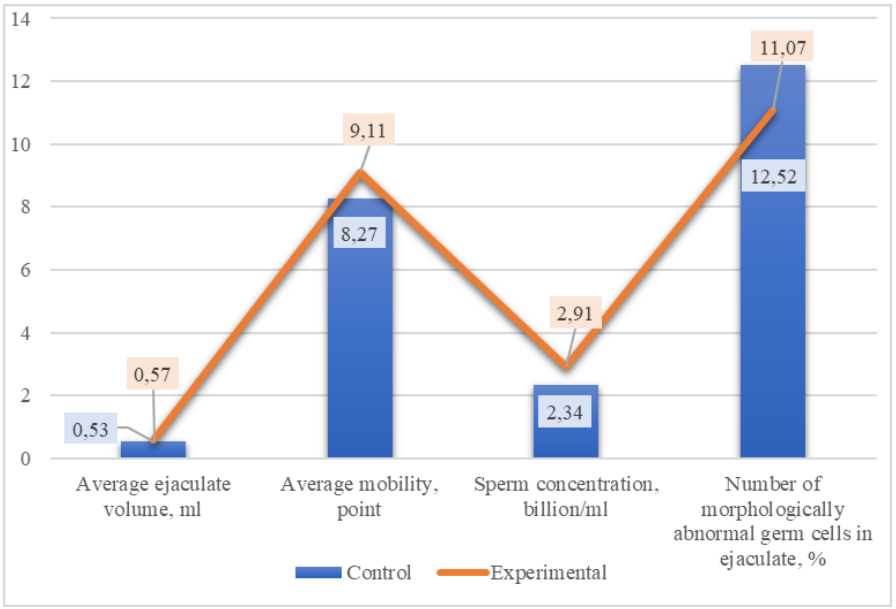

Fig. 1. Qualitative indicators of rooster ejaculate.

The results obtained made it possible to establish an increase in the volume of ejaculate of roosters receiving the experimental food, relative to the control, by 7.55% (P<0.05), the concentration of sperm in the semen also increased by 24.36% (P<0.05), and the number of germ cells with anomalies decreased by 13.09% (P<0.05), and in both groups the color of the ejaculate was certified as milky, and sperm motility was linear and progressive.

At industrial poultry enterprises that have a parent flock and use artificial insemination of chickens, requirements are imposed on hatching eggs, which to a certain extent depend on the quality of sperm production of roosters. These requirements include the survival time of sperm, which, in our experiment, as a result of including 7.5% fly larvae food in the rooster diet, reached 139 hours, which exceeded this figure in the control by 7 hours. The resulting volume of ejaculate from the roosters of the experimental group made it possible

to obtain 2 more sperm doses (14 versus 12 in the control), which is also important from a practical point of view.

The protein of insect larvae has a very high digestibility (95-98.5%), due to the content of individual amino acids (arginine, lysine, tyrosine, phenylalanine, histidine, serine, etc.), which exceeds their presence in fish meal by 1.5 -2 times. We studied the possibility of improving the amino acid composition of the sperm of breeding roosters in the experimental group by feeding them an experimental supplement and the results are illustrated in Figure 2.

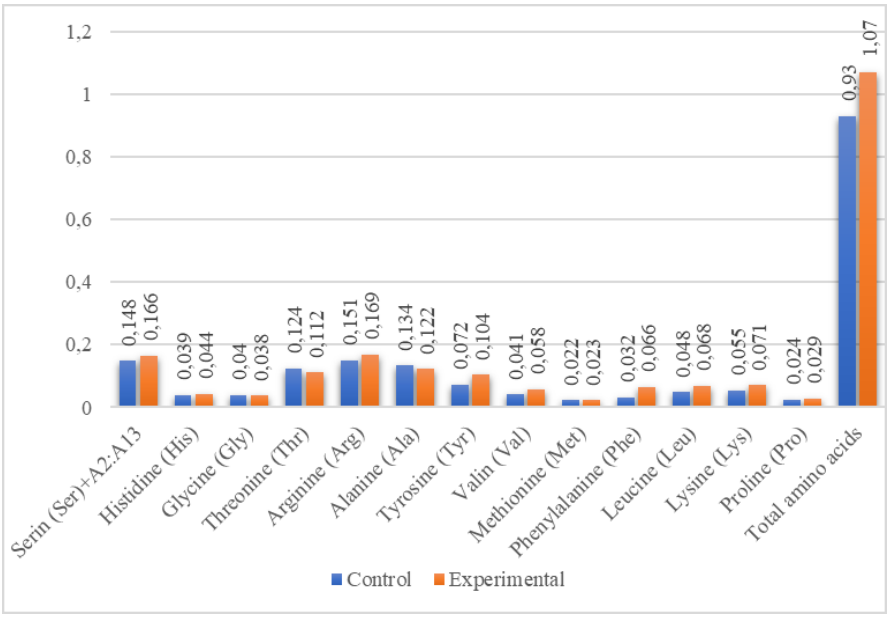

Fig. 2. Amino acid profile of rooster sperm, g/100 g.

A direct relationship between the amino acid composition of the experimental feed and rooster sperm was established. The sperm of roosters in the experimental group significantly improved its amino acid composition, under the influence of dried, defatted flour from black soldier fly larvae: an increase in the total amino acid content by 15.05% ($P<0.01$) is due to a significant increase ($P<0.01$) in the amount of lysine, phenylalanine, tyrosine, serine and ($P<0.05$) valine, leucine, arginine, histidine, relative to the control group.

This may be due to the higher amino acid profile and high protein digestibility (98.5%) of black soldier fly larvae meal [15-16].

Considering the significant presence of mineral elements in low-fat flour from black soldier fly larvae, their content in the sperm of breeding roosters was studied (Figure 3).

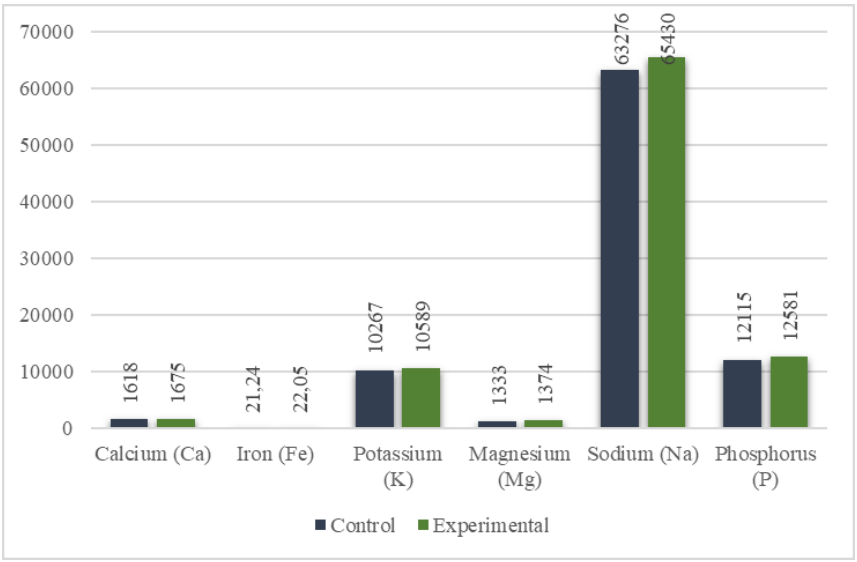

Fig. 3. Saturation of rooster sperm with macroelements, mcg/g.

According to the results of the research, a significant increase ($P<0.05$) was recorded in the presence of the studied macroelements in the sperm of roosters in the experimental group compared to the control group in values from 3.07 to 3.85%.

The improvement in sperm quality as a result of the use of experimental feed can be motivated by the activation of metabolic processes in the body of roosters. We recorded changes in the presence of blood cells in experimental roosters (Figure 4).

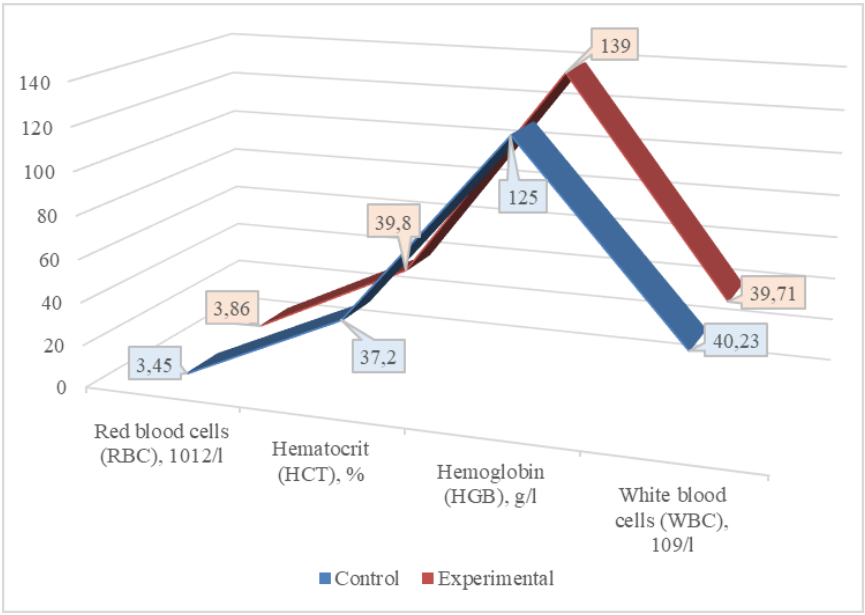

Fig. 4. Morphological composition of rooster blood.

The values of the indicators characterizing the morphological picture of blood correspond to the abstract values for the type and age of the experimental birds, but at the

same time, an increase in the number of erythrocytes in the blood of roosters of the experimental group was recorded by 11.88% (P<0.05), hematocrit - by 6.99% (P<0.05), hemoglobin - by 11.20% (P<0.05) compared with similar indicators in the control group.

The main indicators characterizing protein metabolism in the blood serum of experimental roosters are shown in Figure 5.

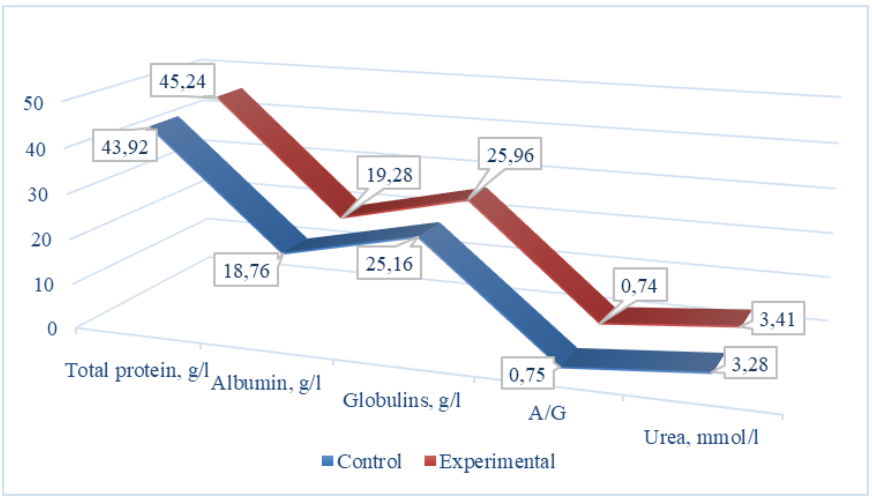

Fig. 5. Metabolism of protein and its fractions in the blood serum of roosters.

The established excess of total protein levels in the experimental group over the control group was 3.01% (P<0.01). At the same time, a narrowing of the ratio of albumin to globulin fractions was recorded, which characterizes the improvement of immunity in the body of birds of the experimental group.

During the period of growing and puberty of roosters, the formation of resistance of the body occurs due to the functioning of the endocrine glands. These studies showed high protective functions in the body of cockerels of both groups, but in the experimental group, the immune system stimulated by insect food was superior to the control: bactericidal activity increased by 4.22% (P<0.05), lysozyme activity - by 3.84 % (P<0.05), phagocytic – 4.87% (P<0.01).

It has been proven that biologically active substances (antimicrobial peptides, fatty acids and polysaccharides) contained in insect feeds stimulate the innate immune system and exhibit antimicrobial activity [17-20]. In this context, it is important to note that the lowest ratio between albumin and globulin was recorded in broilers fed black soldier fly meal [21-22], which indicates better disease resistance and immune response in birds. The authors explain this phenomenon by the prebiotic effect of biologically active substances produced by insects. At the same time, no negative changes were recorded in the liver and muscle cells (the levels of AST and enzymes that lower antioxidant status were within the physiological norm), which once again proves the uniqueness of the protein of their insects.

The inclusion of a new experimental additive in the diet of roosters during the period of their rearing and physiological maturation requires studying the level of antioxidant defense of the body (Figure 6).

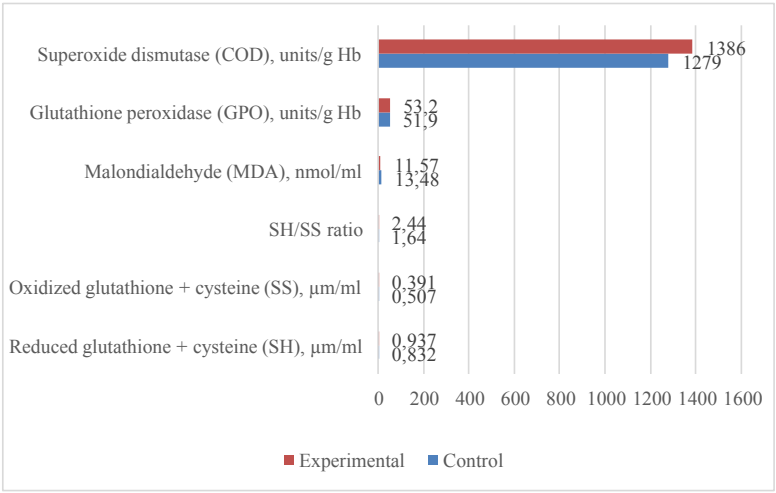

Fig. 6. Functional state of the antioxidant system in the blood of roosters.

In the blood of roosters in the experimental group, under the influence of the experimental supplement, a glutathione-saving effect was recorded: the amount of reduced glutathione increased by 12.62% ($P<0.01$), and oxidized glutathione decreased by 29.67% ($P<0.01$), according to compared to the control. Enzymes of the body's antioxidant defense were also activated: glutathione peroxidase - by 2.51% ($P<0.05$), superoxide dismutase - by 8.37 ($P<0.01$). The end product of fat oxidation (malondialdehyde) decreased, relative to the control, by 16.51% ($P<0.01$).

At the end of the experiment, eggs from chickens inseminated with sperm from experimental roosters were incubated. The hatching of chickens in the experimental group exceeded the control by 1.89% and amounted to 85.12%.

4 Conclusion

It has been proven that protein from insects is a promising, unique product that can replace animal and vegetable protein in the diets of animals and birds, which needs to be popularized and the scale of production increased.

Our research has proven that the inclusion of low-fat flour from the larvae of black soldier flies (Hermetia illucens) in the amount of 7.5% in the diet of double-line roosters of the “Hisex brown” cross resulted in an increase in the quality of sperm, activated metabolic processes and antioxidant protection.

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