The effect of meal from the larvae of the Black soldier fly (Hermetia Illucens) on the lipid metabolism of breeding chickens of the "Highsex brown" cross

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Abstract. The results of the research presented in the article are devoted to the study of the effect of feeding black soldier fly larvae (Hermetia illucens) instead of soybean meal on lipid metabolism and egg production of breeding hens of the Hisex Brown cross. The inclusion of the new feed (10%) in the diet of hens of the experimental group led to the activation of lipid metabolism. A reliable ($P < 0.05$) increase in the content of total lipids, phospholipids and triglycerides in the blood serum of breeding hens was recorded. The level of low-density lipoproteins decreased to 2.23 mmol / l against 2.56 mmol / l in the control, and high-density lipoproteins increased by 6.43%. The lipid composition of the yolk of hatching eggs of the experimental group also underwent changes: the level of total lipids increased by 9.43% ($P < 0.05$), triglycerides - by 11.30% ($P < 0.05$). A stable increase in phospholipids in the experimental group against the control group by 7.14%, lecithin in their composition by 5.83% was noted. An increase in the intensity of egg production in hens by 2.47%, the yield of incubatory eggs by 1.60, egg hatchability by 2.77%, and chicken hatchability by 2.04% was established. An increase in the intensity of egg production in hens by 2.47%, the yield of incubatory eggs by 1.60, egg hatchability by 2.77%, and chicken hatchability by 2.04% was established.

1 Introduction

The global forecast for the growth rate of egg production in the coming years, due to the growing population worldwide, forces feed specialists to address the issue of providing chickens with a coordinated diet, despite the shortage of animal and vegetable protein, as well as the energy source that ensures lipid metabolism, fat, for many reasons already at present. The supply of hemodynamics with various nutrients predominates in the lipid composition. A positive relationship has been proven between the nutritional components of the substrate, the weight of the larvae, the presence of saturated fatty acids, including lauric acid, and a negative relationship with the content of unsaturated acids. It has also been proven that black soldier fly larvae are capable of independently, regardless of the

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substrate, synthesizing lauric acid from carbohydrates, and the composition of unsaturated carboxylic acids depends directly on the composition of the growing substrate, which suggests that it is possible to regulate, to a certain extent, the composition of fatty acids in larvae [8, 9]. Regardless of the diversity of the fatty acid composition, as a rule, larval fat is considered from the point of view of the presence of lauric acid in it. Provision of hemodynamics with various nutritional components is largely ensured by their absorption by intestinal villi, which can be improved by medium-chain fatty acids. The multifaceted properties of monolaurin and other components of the experimental feed contribute to the destruction of various viral and pathogenic intestinal bacteria. It has been established that monolauric acid stimulates the consumption and assimilation of feed [4, 5, 7, 13]. Monolaurin primarily acts against gram-positive bacteria by destroying their outer lipid membranes, but it also has antiviral properties against lipid-coated viruses. Due to its amphiphilic nature, it forms micelles that provide them with the ability to integrate into the lipid membrane of these microorganisms, thereby changing permeability. Alpha-monolaurin destroys the cell membrane of these bacteria and the lipid coat of viruses, making pathogens permeable [5]. Monolaurin also improves the immune system by stimulating the proliferation of splenocytes and T cells. This shows that monolaurin affects, in particular, T-cell populations. Monolaurin can lead to T-cell proliferation in the phospholipid inositol signaling pathway [6]. Fly larvae are also enriched in mono- and polyunsaturated acids (32 and 23%), among which linoleic acid predominates [11]. The exclusive composition of the fly larvae (Hermetia illucens) feed product allows to compensate for the basic needs of productive animals and birds for such nutrients as proteins, peptides, fats, vitamins, minerals, antioxidants and other biologically active substances [1, 2]. It has been established that alpha-monolaurin has a positive effect on the quality of eggshells due to increased absorption of calcium and phosphorus in the gastrointestinal tract (pH in the upper sections decreases). At the same time, it is extremely important to characterize the metabolism in the body of chickens, and in particular lipids, for comparison and evaluation of data that sometimes present contradictory results in earlier studies by other scientists. Based on the above, the goal was set to study in more detail the effect of biologically active compounds contained in the meal from black soldier fly larvae (Hermetia illucens) on the lipid metabolism of the body of breeding chickens of the Hisex Brown cross.

2 Materials and methods

The studies were conducted on breeding chickens of the "Hysex Brown" cross, in the conditions of the Tulskaya Poultry Farm JSC in the Tula Region, and black soldier fly larvae meal produced in the conditions of Entoprotek LLC (Penza Region) was used as a new experimental feed.

Blood and hatching egg studies were conducted at the Cherkizovo Research Complex (Moscow), equipped with all types of the latest equipment (https://cherkizovolab.ru/?ysclid=lrowxevx6k487756140).

The digital values obtained during the research were processed using the "ONLYOFFICE" computer program.

3 Research results and discussion

As is known, the structure and nutritional value of the chicken diet, along with the physiological state of the body, coordinate metabolism, including lipid metabolism, and the level and ratio of different classes of lipids in the blood serum is determined by the level of

total lipids [3, 5]. The presence in insect-based feed products, in addition to the protein component, of fats with a unique composition, where saturated lauric acid dominates [8], allows us to consider this feed not only as a source of lipids, but also as an immunomodulator.

At the end of the experiment, the effect of dried flour from black soldier fly larvae on lipid metabolism in the blood serum of chickens in the experimental group was established (Figure 1).

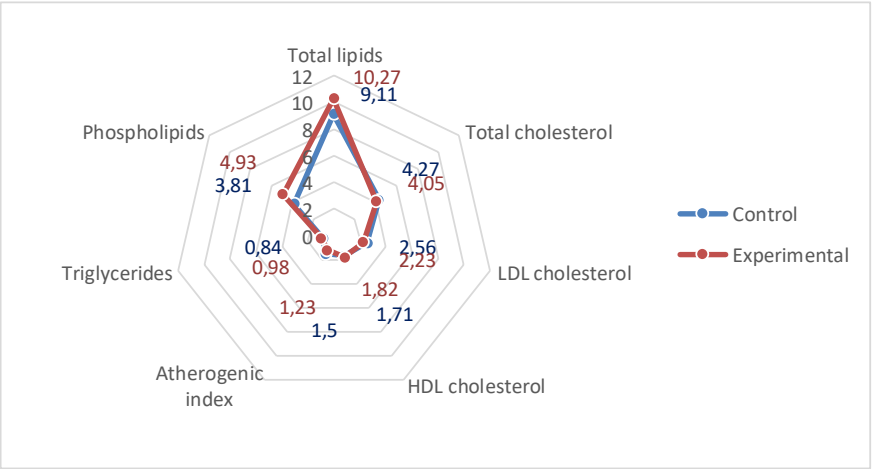

Fig. 1. Lipid metabolism parameters, mmol/l.

The synergistic effect of the feed ingredient components, including monolaurin and other fatty acids, was manifested in the increase in the total lipid content in the blood serum of breeding chickens, which significantly exceeded this indicator in the control by 12.73% ($P<0.05$), while the variation did not go beyond the physiological values. Significant changes in the content of phospholipids and triglycerides were also established in the experimental group against the control (29.39%; $P<0.01$ and 15.47%; $P<0.05$). The cholesterol level decreased by 5.43% in the experimental group, but the difference was not significant, while low-density lipoproteins (LDL) decreased to 2.23 mmol/l against 2.56 mmol/l in the control (14.80%; $P<0.05$), and the content of high-density lipoproteins (HDL) increased by 6.43% (statistically not significant). As a result, the changes that occurred in the ratio of lipoproteins of different densities had a positive effect on the atherogenicity index, which decreased from 1.50 in the control to 1.23 units (21.95%; $P<0.01$) in the experimental group.

In addition, enzymes (glutathione peroxidase, superoxide dismutase, ceruloplasmin) and malondialdehyde, characterizing the degree of fat oxidation in the blood serum, as expected, showed changes in the experimental group compared to the control: glutathione peroxidase increased by 4.21% ($P<0.05$), superoxide dismutase - 7.43% ($P<0.01$), ceruloplasmin - by 8.27% ($P<0.01$), and malondialdehyde decreased its activity by 4.38% ($P<0.01$), which is consistent with the findings of Fikri F. et al. [15]. Since lauric acid indirectly affects the immune system and the body's natural resistance, it is logical to test the parameters of immunocompetent cells. Cellular immunity increased in the experimental group of chickens due to the growth of T-lymphocytes by 4.98% ($P<0.05$), B-lymphocytes - by 3.42% ($P<0.05$) relative to the control. Humoral factors, which reflect the immunoglobulin profile of blood serum, in our experiment depended on feeding conditions. In the experimental group, where the chickens were fed, as part of the combined feed, the

studied additive of black soldier larvae, the IgG level dominated over the control by 5.12 (P<0.01). Autosynthesis of immunoglobulins of the IgA and IgM classes in the blood serum of experimental chickens was also more intense than in the control, but the difference did not reach reliable values. The factors of natural resistance, which also determine the immune status of birds, include the level of phagocytic activity of leukocytes, which increased by 2.04% (P<0.01).

During the experiment, we determined the lipid composition of the yolk of hatching eggs (Figure 2).

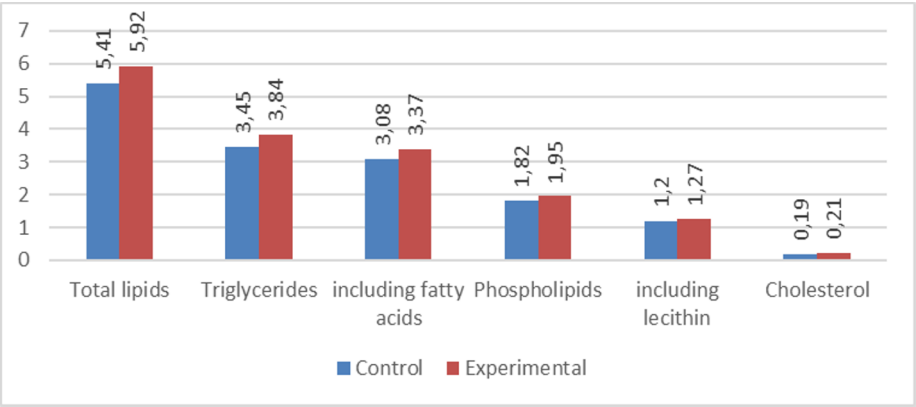

Fig. 2. Lipid composition of yolk of hatching eggs, g.

It should be emphasized that the high fat content in the studied feed did not significantly affect either the increase in the weight of laying hens or the weight of hatching eggs.

The lipid composition of the yolk of the hatching eggs of the experimental group also underwent certain changes under the influence of the feed from the black soldier fly larvae. The level of total lipids in the yolk of the eggs of the experimental group increased by 9.43% (P<0.05) relative to the control group. The content of triglycerides increased by 11.30% (P<0.05), including fatty acids - by 9.42% (P<0.05). There was a steady increase in phospholipids in the experimental group against the background of the control by 7.14%, lecithin in their composition - by 5.83% (the difference is insignificant).

During the research, the content of essential fatty acids in the yolk, which affect the quality of hatching eggs, was determined (Figure 3).

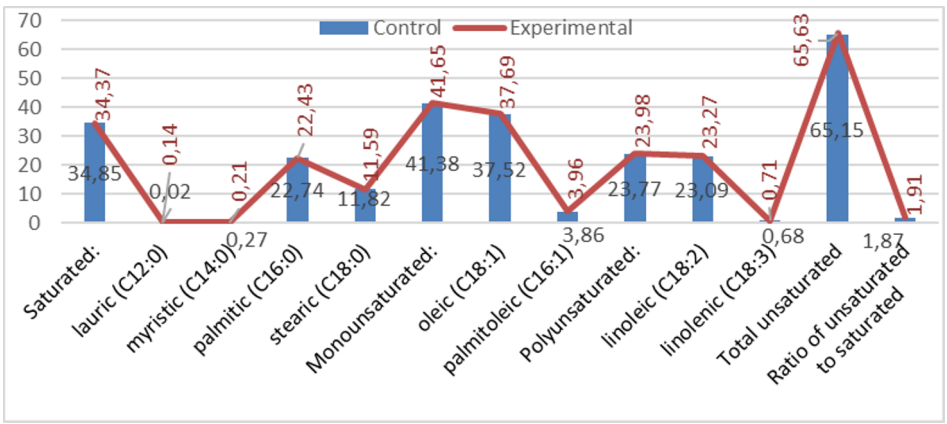

Fig. 3. Fatty acid composition of the yolk of hatching eggs, %.

The decrease in the content of palmitic and stearic acids in the yolk of eggs of the experimental group by 0.31 (P<0.05) and 0.23% (P<0.05), as well as some decrease in myristic acid caused a decrease in the total composition of saturated fatty acids by 0.48% (P<0.01) compared to the control group. It is necessary to emphasize the fact that the experimental sample of yolk contained lauric acid (0.14%), which was practically absent in the control. This convincingly proves that lauric acid synthesized by black soldier larvae from carbohydrates of the substrate accumulates in the yolk of eggs during metabolism in the body of hens [9]. In the experimental group, among monounsaturated fatty acids Oleic acid showed a significant increase of 0.17% (P<0.05) compared to the control, which, together with a slight increase in the level of palmitoleic acid, was reflected in the predominance of the total content of monounsaturated acids by 0.27% (P<0.05). The level of polyunsaturated acids in the experimental group also increased by 0.21% (P<0.05), mainly due to an increase in linoleic acid by 0.18% (P<0.05) relative to the control. The change in the content of fatty acids in the egg yolk in the experimental group affected the ratio of unsaturated fatty acids to saturated ones (1.91 in the experimental group versus 1.87 in the control).

Taking into account the vitamin composition of the experimental feed, it is advisable to test the content of fat-soluble vitamins in the egg yolk, the reference values of which increased against the control background: vitamin A - by 1.96 μg / g (20.85%; P<0.01), vitamin E - by 10.89 μg/g (7.23%; P<0.05). The concentration of carotenoids, as a mandatory criterion for assessing the yolk of hatching eggs, increased by 2.90 μg / g (20.42%; P<0.01). The acid number in the yolk part of the eggs decreased from 4.19 to 3.61 mg KOH/g, or by 16.07% (P<0.05).

During the experiment, the egg production of breeding hens was studied (Table 1).

As shown by the results of the taken into account zootechnical indicators of productivity of hatching eggs, 6964 eggs were obtained from the hens of the experimental group during the experimental period (15 weeks), and 210 eggs less from the hens of the control group during the same period, respectively, the intensity of egg production of the hens of the experimental group exceeded the control by 2.47%. Of the number of eggs obtained in the experimental group, the yield of hatching eggs exceeded the control by 314 pieces or by 1.60%, which is due to a significant improvement in the quality indicators of eggs, including the thickness of the shell, which increased by 9 microns (2.51%). Due to the improvement in the indicators of dense protein of hatching eggs, the main protein indicator increased - the HAU unit, which in the experimental group increased by 4.62% (P<0.05).

Table 1. Egg production of hens for 15 weeks of the experiment.

Indicators	Control	Experimental
Average number of hens, heads	69.7	70
Survivability, %	98.57	100
Total eggs received, pcs., including hatching:	6754	6964
pcs.	6521	6835
%	96.55	98.15
From the average laying hen, pcs.	96.9	99.5
Egg-laying intensity, %	92.28	94.75
Feed costs per 10 eggs, kg.	1.32	1.27

As already noted, no reliable difference in the weight of hatching eggs between the experimental and control groups was established, but a stable tendency to increase in favor of the experimental group by 0.86 g was achieved. Breeding chicken eggs, as well as eggs, are identified with a source of nutrients and plastic substances for the developing embryo, and the degree of its provision with nutrients largely determines the hatching of day-old

young or juveniles [5]. In turn, the nutritional value of the cell for the development of a full-fledged embryo in it directly depends on the quality composition of the feed. The study of the effect of the experimental feed on the chemical composition of breeding eggs revealed an increase in the protein part of the eggs of the experimental group of dry matter and protein by 0.09 and 0.07%, in the yolk part, in addition to an increase in the level of dry matter and protein by 0.22 and 0.06%, an increase in the concentration of fat by 0.13% was recorded, which proves the effect of feed from black soldier larvae on the nutritional value of eggs. Close attention is paid to the composition of the protein of chicken eggs, since its amino acid composition is close to ideal and any changes occurring under the influence of feeding must be monitored, especially since the experimental feed used in our experiment has a wide range of essential amino acids. It was experimentally established that the protein of the eggs of the experimental group differed favorably from the control sample in a number of amino acids (lysine, arginine, tyrosine, histidine, methionine ($P<0.05$) and others), which affected the increase in the total amount of amino acids by 3.22 ($P<0.01$). The amino acid spectrum of the protein of the yolk part of the eggs was even more significantly adjusted for the following amino acids: lysine, leucine and isoleucine ($P < 0.01$), methionine, threonine, alanine and glutamic acid ($P<0.05$), which, as a result, increased the total amount of amino acids by 6.84% ($P < 0.001$) in relation to the control.

Feed costs for 10 eggs also turned out to be the most optimal in the experimental group - 1.27 kg, which is lower than in the control group by 0.05 kg. After completion of the experimental studies of feeding the new feed ingredient, incubation of eggs obtained from the hens of the experimental groups was carried out in the amount of 300 pieces from each group. The results of incubation showed an increase in egg hatchability by 2.77% due to an increase in fertilization under the influence of the active substances of the experimental feed and a decrease in the death of embryos at an early stage of development, respectively, the hatching of chickens increased by 2.04% relative to the control group. The day-old chickens obtained in the experimental group turned out to be more conditioned, as evidenced by the high survival of the livestock up to 10 days of age, which amounted to 98.05%, exceeding the control by 1.47%.

4 Conclusion

It has been proven that the feed product we are studying (black soldier fly larval meal) is a promising, unique product with high feed conversion efficiency, nutritional composition suitable as feed for animals and birds, which needs to be popularized and production scale increased.

Our research has established that the inclusion of 10.0% defatted black soldier fly larval meal (Hermetia illucens) in the diet of breeding chickens of the Hisex Brown cross instead of soybean meal led to the activation of lipid metabolism and enzymes characterizing the degree of fat oxidation in the blood serum of chickens in the experimental group. Cellular and humoral immunity also depended on the feeding factor, as evidenced by the growth of T- and B lymphocytes, the level of IgG and the phagocytic activity of leukocytes, relative to the control.

The experimental egg yolk sample was found to contain lauric acid (0.14%), which was virtually absent in the control (0.02), which convincingly proves the relationship between this fact and the presence of lauric acid in the feed, which is transformed into the yolk during metabolism in the body of hens. The fact of an increase in fat-soluble vitamins (A, E) in the yolk of hatching eggs was recorded. The effect of black soldier fly larvae feed on the amino acid spectrum of the yolk part of eggs was established, which was significantly adjusted for the following amino acids: lysine, leucine and isoleucine ($P<0.01$), methionine, threonine, alanine and glutamic acid ($P<0.05$), which ultimately increased the total amount

of amino acids by 6.84% (P<0.001) compared to the control. Feed conversion per 10 eggs improved by 0.05 kg.

All these factors led to an increase in the egg productivity of hens, the yield of hatching eggs and their quality, an increase in egg hatchability by 2.77% and the hatching of chicks by 2.04%.

References

1. V. Zagorovskaya, Feed alternative. Feed protein from insects; prospects of this direction, Agrotechnics and technology, **2** (2020) https://www.agroinvestor.ru/animal/article/33400-kormovaya-alternativakormovoy-belok-iz-nasekomykh-perspektivy-etogo-napravleniya/
2. R.V. Nekrasov, M.G. Chabaev, A.A. Zelenchenkova, A.I. Bastrakov, N.A. Ushakova, Nutritional properties of Hermetia Illucens L. larvae - a new feed product for young pigs (Sus Scrofa Domesticus Erxleben), Agricultural biology, **2(54)**, 316-325 (2019) doi: 10.15389/agrobiology.2019.2.316rus
3. M.M. Orlov, V.V. Zaitsev, M.S. Seitov, L.M. Zaitseva, N.M. Orlov, Effect of the mineral preparation Vermiculax on the content of total lipids, triglycerides, phospholipids and cholesterol in the blood serum of laying hens, Bulletin of the Oryol State Agrarian University, **6(98)**, 306-310 (2022)
4. M.G. Teymurazov, E.A. Svetoch, O.Yu. Manzenyuk, O.I. Tazina, T.V. Fedorov, I.I. Istomin, A.I. Istomin, S.S. Yakovlev, Antimicrobial activity of Lucilia Caesar larvae against bacteria pathogenic to humans and poultry, Veterinary Science, **2**, 9-13 (2018)
5. N.A. Ushakova, E.S. Brodsky, A.A. Kovalenko, A.I. Bastrakov, A.A. Kozlova, D.S. Pavlov, Features of the lipid fraction of black soldier fly larvae Hermetia illucens, Reports of the Academy of Sciences, **4(468)**, 462-465 (2016) doi: 10.7868/S0869565216160258
6. M.S. Zhang, A. Sandouk, J.C.D. Houtman, Glycerol Monolaurate (GML) inhibits human T-cell signaling and function by disrupting lipid dynamics, Scientific Reports, **6(1)**, 1-13 (2016) doi:10.1038/srep30225
7. L. Borrelli, L. Varriale, L. Dipineto, A. Pace, L.F. Menna, A. Fioretti, Insect derived LA as a promising alternative strategy to antibiotics in the antimicrobial resistance scenario, Front. Microbiol, **12**, 620798 (2021) doi.org/10.3389/fmicb.2021.620798/
8. N. Ewald, A. Vidakovic, M. Langeland, A. Kiessling, S. Sampels, C. Lalander, Fatty acid composition of black soldier fly larvae (Hermetia illucens) - Possibilities and limitations for modification through diet, Waste Management, **102**, 40-47 (2020) doi: 10.1016/j.wasman.2019.10.014
9. M. Spranghers, C. Ottoboni, A. Klootwijk, S. Ovyn, B. Deboosere, J. De Meulenaer, M. Michiels, P. Eeckhout, S. De Clercq, De Smet Nutritional composition of black soldier fly (Hermetia illucens) prepupae reared on different organic waste substrates, J. Sci. Food Agric., **97**, 25942600 (2017)
10. C. Lalander, S. Diener, M.E. Magri, C. Zurbrügg, A. Lindström, B. Vinnerås, Faecal sludge management with the larvae of the black soldier fly (Hermetia illucens) - From a hygiene aspect. Sci. Total Environ, **458**, 312-318 (2013)
11. N.S. Liland, I. Biancarosa, P. Araujo, D. Biemans, C.G. Bruckner, R. Waagbo, B.E. Torstensen, E.-J. Lock, Modulation of nutrient composition of black soldier fly (Hermetia illucens) larvae by feeding seaweed-enriched media, PLoS One, **12(8)**, 0183188 (2017) doi: 10.1371/journal.pone.0183188

12. X. Liu, X. Chen, H. Wang, Q. Yang, U.K. Rehman, W. Li, M. Cai, Q. Li, L. Mazza, J. Zhang, Dynamic changes of nutrient composition throughout the entire life cycle of black soldier fly. PLoS ONE, **12**, e0182601 (2017)
13. S. Scieuzo, F. Giglio, R. Rinaldi, M.E. Lekka, F. Cozzolino, V. Monaco, M. Monti, R. Salvia, P. Falabella, In Vitro Evaluation of the Antibacterial Activity of the Peptide Fractions Extracted from the Hemolymph of Hermetia illucens (Diptera: Stratiomyidae), Insects, **14(5)**, 464-480 (2023) https://doi.org/10.3390/insects14050464
14. N.A. Ushakova, A.E. Dontsov, N.L. Sakina, E.S. Brodsky, I.A. Ratnikova, N.N. Gavrilova, A.I. Bastrakov, A.A. Kozlova, R.V. Nekrasov, Melanin properties at the different stages towards life cycle of the fly hermetia illucens, Ukrainian Journal of Ecology, **7(4)**, 424-431 (2017) doi:10.15421/2017_137
15. F. Fikri, A. Purnomo, S. Chhetri, M.T.E. Purnama, H. Calışkan, Effects of black soldier fly (Hermetia illucens) larvae meal on production performance, egg quality, and physiological properties in laying hens: A meta-analysis, Veterinary World, **17**, 1904-1913 (2024) 10.14202/vetworld.2024.1904-1913