AGRICULTURAL BIOLOGY,
2020, V. 55, № 6, pp. 1233-1244
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

Unconventional feeds

UDC 636.087.6:636.52/.58:591.11

doi: 10.15389/agrobiology.2020.6.1233eng
doi: 10.15389/agrobiology.2020.6.1233rus

THE STANDARDIZED ILEAL DIGESTIBILITY OF AMINO ACIDS FROM PROTEIN CONCENTRATE BASED ON THE LARVAE OF COMMON GREEN BOTTLE FLY *Lucilia* spp. (*Diptera: Calliphoridae*) AND ITS EFFECTS ON THE MORPHOLOGICAL AND BIOCHEMICAL BLOOD INDICES IN BROILERS (*Gallus gallus L.*)

M.S. ZHURAVLEV[1] ✉, V.G. VERTIPRAKHOV[2] ✉, M.V. KOSHCHEYEVA[2], N.P. BURYAKOV[1], M.I. SMAHLIUK[3], A.I. ISTOMIN[3]

[1]*Timiryazev Russian State Agrarian University—Moscow Agrarian Academy,* 49, ul. Timiryazevskaya, Moscow, 127550 Russia, e-mail mikhail.sterh@gmail.com (✉ corresponding author), kormlenieskota@gmail.com;
[2]*Federal Scientific Center All-Russian Research and Technological Poultry Institute RAS,* 10, ul. Ptitsegradskaya, Sergiev Posad, Moscow Province, 141311 Russia, e-mail Vertiprakhov63@mail.ru (✉ corresponding author), lkhasanova@mail.ru, vlk.733@mail.ru;
[3]*OOO «Zooprotein»,* 64 of. 701, Sovetskaya ul., Lipetsk, Lipetsk Province, 398001 Russia, e-mail ms@misma.pro, ais@misma.pro
ORCID:
Zhuravlev M.S. orcid.org/0000-0002-4586-2937
Vertiprakhov V.G. orcid.org/0000-0002-3240-7636
Koshcheyeva M.V. orcid.org/0000-0002-0744-1883
Buryakov N.P. orcid.org/0000-0002-6776-0835
Smahliuk M.I. orcid.org/0000-0003-3676-9746
Istomin A.I. orcid.org/0000-0003-0394-5280
The authors declare no conflict of interests
Acknowledgements:
Supported financially from the Russian Science Foundation for the Project No. 16-16-04089-П "Study of physiological and microbiological aspects of digestion in meat chicken in embryonic and post-embryonic periods to develop diets which fully meet the genetic potential of poultry"
Received September 24, 2020

Abstract

Bioconversion is an ecologically friendly and effective way of the utilization of organic wastes; it involves the use of these wastes as a substrate in the biotechnologies of different products. The larvae of the black soldier fly (*Hermeia illucens* L.) grown on the organic wastes have been earlier studied as a raw material for feed ingredients for poultry; recently the larvae of common green bottle fly (*Lucilia* spp.) draw the attention as a potential source of feed-grade protein for animals and poultry. The dried defatted biomass of the larvae contains crude protein (no less than 62 %), fat (10 %), lysine (no less than 4 %), methionine + cystine (2.0 %) and hence could be a promising protein source in diets for poultry. However, despite the apparent advantages of the bioconversion of organic wastes by insects into the dietary protein sources for poultry there is a scarcity of the data on the quality of these products and on their effects on poultry. Earlier research evidenced the efficiency of the supplementation of diets for growing turkeys with 5.0-7.5 % of dried full-fat *Lucilia* larvae; however, the ileal digestibility of amino acids in turkeys (necessary for the balancing of dietary amino acids) was not assessed. The study presented is a first attempt of the *in vivo* assessment of ileal digestibility of amino acids from *Lucilia* larvae protein concentrate (LLPC) in broilers (*Gallus gallus* L.). The aim of the study was the determination of apparent (AID) and standardized ileal digestibility (SID) of amino acids from LLPC within the experimental diet and its effects on the morphological and biochemical blood indices in broilers. AID and SID were determined in the vivarium of the All-Russian Research and Technological Institute of Poultry in 2019 on broilers (cross Smena 8, 18-42 days of age) with chronic ileal fistulae fed mono-protein diet contained LLPC, dextrose, fiber, and a premix of vitamins and minerals. SID was calculated with the endogenous losses of amino acids taken into account. SID of potentially limiting amino acids were as follows: lysine 82.9 %, methionine 86.6 %; threonine 80.4 %; arginine 89.5 %; isoleucine 80.0 %; leucine 81.9 %; valine 79.9 %; histidine 82.9 %, and phenylalanine 85.7 %. The beneficial effects of LLPC on the blood indices were found: the activity of alanine transaminase was significantly higher by 23.5 % in broilers fed LLPC in compare to control while the activity of aspartate transaminase was lower by 24.6 % ($p < 0.05$) indicating the prevalence of the anabolic processes over the catabolic. The significant increase in total protein concentration in serum (by 20.0 %, $p < 0.05$) and increase in hemoglobin concentration by 4.2 % in compare to control evidenced the activation of the metabolism. The use of SID (instead of AID)

allows for more accurate balancing of dietary contents of available amino acids, more adequate amino acid supply to poultry, and for the reduction of nitrogen emissions into the environment due to the optimization of dietary protein content.

Keywords: broilers, poultry farming, alternative protein sources, ileal digestibility, insects, zooprotein

A large volume of organic waste and by-products of food production creates problems of the natural environment pollution – water, air, and soil. Bioconversion is an ecologically friendly and effective way of the utilization of organic wastes; it involves the use of these wastes as a substrate in the biotechnologies of different products for various purposes. One of the promising directions is the growing of fly larvae of the order *Diptera* on such waste, from which it is possible to produce a feed protein supplement [1, 2] and bioactive substances [3]. Dipterans have some advantages in agriculture as waste processors: they produce much fewer greenhouse gases and ammonia than traditional farm animals [4], require less space for growing [5], feed conversion is more efficient [6], while they can convert unsuitable for animal and human nutrition by-products and wastes into high-protein raw materials and energy [7].

The black soldier fly *Hermetia illucens* L. is considered one of the most studied insects used for poultry feeding [8]. The use of dried and partially defatted fly larvae of this species as a source of protein for broilers [9], laying hens [10], and meat quails [11] is surveyed in this article. The use of live black soldier fly larvae in turkey feeding has also been investigated [12]. In addition to poultry farming, *H. illucens* larvae can be used in pig farming [13] and aquaculture [14, 15].

Currently, the investigations are in progress to search for and study other insects as promising sources of protein for animal feeding. In the work by Huis and Oonincx [16], the experiments on the replacement of fish meal in the diets of farm animals with insect protein were analyzed and it was concluded that a partial replacement was justified. A meta-analysis of 75 studies [17] showed that when feeding poultry, insect protein, in general, did not have a statistically significant adverse effect on the live weight gain, feed consumption and conversion but more than 10% of insect biomass in the diet led to a decrease in the average daily growth.

In addition to studying insects as a source of protein, regulatory issues and the use of insect products are discussed. For example, in the article by Sogari et al. [18], the issues of the regulatory framework of the European Union, North America, and some Asian countries on the use of insects in feed production and their consumer evaluation were considered. The authors concluded that these objects were still underutilized in the production of animal feed but it was expected that with the development of commercial insect growing, their use and evaluation by the end-user would increase. Such potentially useful insects in animal feeding include green bottle flies (*Lucilia* L., *Diptera: Calliphoridae*) [19].

The possibility of using dried full-fat larvae of the *Lucilia* genus for weaning piglets (1-2% of the diet) was studied in Russia [20]. Previously [21], 5-7.5% of dried full-fat *Lucilia* spp. larvae was proved to be effective in the diets for fattened turkeys. However, the assessment of the ileal digestibility of amino acids from these sources, which is necessary for rationing diets for amino acids, was not carried out. The digestibility of amino acids of a feed component is one of the key indicators that determine its quality [22]. The coefficient of standardized ileal digestibility allows the most accurate calculation of the percentage of amino acids digestibility in the intestine, taking into account their endogenous losses in the body and the use of ceca or the large intestine by microorganisms [23]. It should be noted that in general, despite all the advantages of growing insects on organic

waste to obtain raw materials for the production of feed, information about the quality characteristics of such sources of feed protein is still limited.

This paper presents the first data on the ileal digestibility of amino acids of protein concentrate from the larvae of *Lucilia* spp. flies, which was 79.3% for apparent digestibility, and 80.7% on average for standardized digestibility. The used supplement had a beneficial effect on the metabolism due to changing the ratio of aminotransferases, increasing the total blood protein and hemoglobin in the of poultry.

The objective of the paper was to evaluate the ileal digestibility of amino acids of protein concentrate from larvae of *Lucilia* spp. flies and their influence on the biochemical and morphological parameters of blood in broilers to optimize the regulations for the use of the larvae of common green bottle fly as a promising source of feed protein.

Methods. The protein concentrate has been produced by the Zoprotein group of companies (Lipetsk, Russia) from the larvae of *Lucilia* spp. flies. After hatching, the larvae were grown for 4 days on a substrate of food products with an expiring shelf life (meat and its processed products), before pupation, the larvae were separated from the substrate and dried for 1 hour at 110 °C. The obtained protein-lipid concentrate (43.8% crude protein, 23.5% crude fat) was defatted in a cold press for oilseeds, the obtained cake was crushed in a hammer mill to a loose powder state (62.70% crude protein, 13.46% crude fat, 4.09% lysine).

Experiments were performed on six 18-42-day old broilers (*Gallus gallus* L.) of cross Smena 8. Poultry were grown in a vivarium (All-Russian Research and Technological Poultry Institute RAS, Sergiev Posad, Moscow Porvince, December 2019—January 2020) according to the recommendations developed for the cross.

At the age of 15 days, all broilers were surgically fitted with ileal fistulae [25] in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes [24]. The poultry had no feed 12-18 hours before surgery. All surgical manipulations were performed using painkillers (analgin and diphenhydramine), xylazal (0.2 ml) was used for immobilization. Broilers were fixed in the left side position on a special operating table, a 0.5% novocaine solution was used for conducting anesthesia and infiltration anesthesia (into the abdominal cavity along the incision line). Through an incision on the right side behind the last rib in the caudal direction at a distance of 4-5 cm slightly above the edge of the lateral process of the thorax, the caudal part of the ileum was removed and, by retreating cranially from the confluence of the ceca 1-2 cm, an incision of the intestine was made. The ceca were washed with a disinfectant solution and ligated to stop their activity completely. A seromucous purse suture was made at the caudal part of the intestine, and then, after a serous suture on top, the previous suture was immersed inside. A small hole was made in the abdominal wall, retreating 4-5 cm below and to the right of the cloaca, and the cranial segment of the ileum was sewn to the resulting hole with interrupted sutures, by forming an artificial anal orifice. After wound closing, a 1.5-2.0 cm long PVC tube was sewn into the hole with interrupted sutures. The post-surgery recovery period lasted 3-5 days.

Experiments on the digestibility of amino acids and the assessment of the physiological state of poultry were performed on broilers with ileal fistulae ($n = 6$), from which groups were formed ($n = 3$ in each). To obtain reliable results, feeding experiments were performed at least 3 times on each broiler, by replacing the groups according to the Latin square scheme for the following periods: 3 days for the control diet, 2 days for the transition period, 3 days for the test period

(feeding with a mono protein diet based on a concentrate from the larvae of *Lucilia* spp. flies).

The standard and test diets were composed in such a way that they had the same crude protein content (23.6%). The experimental feed was prepared in such a way that the only source of amino acids of the feed was protein concentrate from fly larvae in an amount corresponding to 23.6% of crude protein. Dextrose (Roquette Freres SA, France) was used as the main source of energy. The feed was balanced in calcium, phosphorus, vitamins, and trace elements according to the norms of Federal Scientific Center All-Russian Research and Technological Poultry Institute RAS (2014), the necessary amount of fiber was provided by chitin protein concentrate from fly larvae and the addition of cellulose of the Arbocel trademark (J. Rettenmaier & Söhne GmbH + Co KG, Germany).

Blood (2-3 ml samples) was taken from the axillary vein in the morning before feeding the poultry. As an anticoagulant, a 3.8% solution of sodium citrate was used in a volume ratio of 1:10 with a blood sample. The sample was centrifuged at 3000 rpm for 5 min to separate the plasma from the formed elements. The resulting plasma was examined using a Sinnowa BS-3000P semi-automatic flow analyzer (SINNOWA Medical Science & Technology Co., Ltd., China) using biochemical kits (DIAKON-VET, Russia), the total protein, uric acid concentration, alanine-, aspartate aminotransferase, and trypsin activity were determined [26]. In feeding trials, the amount of feed consumed and the excreted brood was recorded. All the excrement during the 3-day trial period was collected, packed, stored at –20 °C in a laboratory freezer, after which it was lyophilized and analyzed for the amino acids.

The content of amino acids in the feed and chyme of the ileum was evaluated by ion-exchange chromatography with post-column derivatization with ninhydrin reagent and subsequent detection at $\lambda = 570$ nm (for proline $\lambda = 440$ nm). The analyses were performed using the YL 9100 HPLC System for high-performance liquid chromatography (Young Lin Instrument Co., Ltd., Korea).

The values of apparent (AID) and standardized (SID) ileal digestibility were calculated using the formulas:

$$AID = \frac{\text{AA consumed} - \text{AA in SI chyme}}{\text{AA consumed}} \times 100\ \%,$$

$$SID = \frac{\text{AA consumed} - (\text{AA in SI chyme} - \text{basal EL})}{\text{AA consumed}} \times 100\ \%,$$

where AA is amino acids, SI is small intestine, EL is endogenous losses.

To convert AID to SID, the average values of the main endogenous losses of amino acids [27] in the ileal chyme of broilers obtained on a nitrogen-free diet [9] (mg/kg of dry matter consumed) were used: 9234 for crude protein, 255 for lysine, 79 for methionine, 169 for cystine, 571 for threonine 82 for tryptophan, 216 for arginine, 390 for isoleucine, 381 for leucine, 449 for valine, 209 for histidine, 237 for phenylalanine, 280 for glycine, 1023 for serine, 580 for proline, 301 for alanine, 612 for aspartic acid, and 1037 for glutamic acid.

For statistical processing of the results, JMP Trial 14.1.0 software (SAS Institute, Inc., USA) was used. The mean values (M) and standard errors of means ($\pm$SEM) are presented. To compare the obtained values of protein concentrate digestibility with the traditionally used feed raw materials, the AminoDat 5.0 database (Evonik Industries AG, Germany) was used which represents the average values of the content of standardized ileal amino acid digestibility for a global selection.

Results. The experimental feed composition is shown in Table 1.

1. **Experimental mono-protein diet for Smena 8 cross broiler chicks (*Gallus gallus* L.) based on *Lucilia* spp. fly larvae concentrate** (vivarium of the Federal Scientific Center All-Russian Research and Technological Poultry Institute RAS, Sergiev Posad, Moscow Province, 2019)

Ingredient composition	Content, %
Protein concentrate from fly larvae	37.5
Dextrose	52.5
Cellulose (Arbocel)	1.5
Sunflower oil	1.5
Dicalcium phosphate	3.0
Sodium bicarbonate	1.5
Potassium chloride	1.0
Vitamin and mineral premix	1.5

The amino acid analysis shows that the control and experimental feed differed in the content of some amino acids (Table 2). In the experimental feed, the glutamic acid was 1.46% lower, arginine 0.43% lower, proline 0.37% lower, cystine 0.17% lower, and methionine 0.17% lower than in the control feed, although the proportion of each of these amino acids in the protein concentrate from the fly larvae was relatively high. However, the experimental feed was superior to the control feed in the levels of alanine (by 0.50%), phenylalanine (by 0.36%), and histidine (by 0.30%).

2. **Amino acid composition** (%) **of the pooled sample of feed for Smena 8 cross broiler chicks (*Gallus gallus* L.) and the protein concentrate from *Lucilia* spp. fly larvae** (vivarium of the Federal Scientific Center All-Russian Research and Technological Poultry Institute RAS, Sergiev Posad, Moscow Province, 2019)

Ingredient	Feed		Protein concentrate from fly larvae
	control	experimental	
Amino acid:			
aspartic acid	2.20	2.23	5.98
threonine	0.74	0.8	2.30
serin	0.91	0.86	2.47
glutamic acid	4.33	2.87	7.9
glycine	1.01	0.94	2.74
alanine	0.97	1.47	4.22
valine	1.07	1.16	3.26
isoleucine	0.93	0.89	2.48
leucine	1.54	1.37	3.76
tyrosine	0.73	1.42	4.21
phenylalanine	1.01	1.36	3.75
histidine	0.54	0.84	2.26
lysine	1.42	1.46	4.09
arginine	1.50	1.07	2.99
proline	1.29	0.92	2.57
cystine	0.36	0.19	0.51
methionine	0.75	0.58	1.48
Crude protein	23.61	23.63	62.79
Crude fiber	3.52	7.65	3.56

Table 3 shows coefficients of apparent and standardized ileal digestibility of amino acids in protein concentrate from the fly larvae.

3. **Apparent ileal digestibility** (AID) **and standardized ileal digestibility** (SID) **of amino acids in protein concentrate from *Lucilia* spp. fly larvae for Smena 8 cross broiler chicks (*Gallus gallus* L.)** (n = 6, M±SEM, vivarium of the Federal Scientific Center All-Russian Research and Technological Poultry Institute RAS, Sergiev Posad, Moscow Province, 2019)

Amino acids	AID, %	SID, %
Lysine	82.3±1.47	82.9±1.46
Methionine	86.1±1.30	86.6±1.29
Cystine	50.0±0.60	53.5±0.56
Threonine	77.9±1.63	80.4±1.58
Arginine	88.8±0.51	89.5±0.51

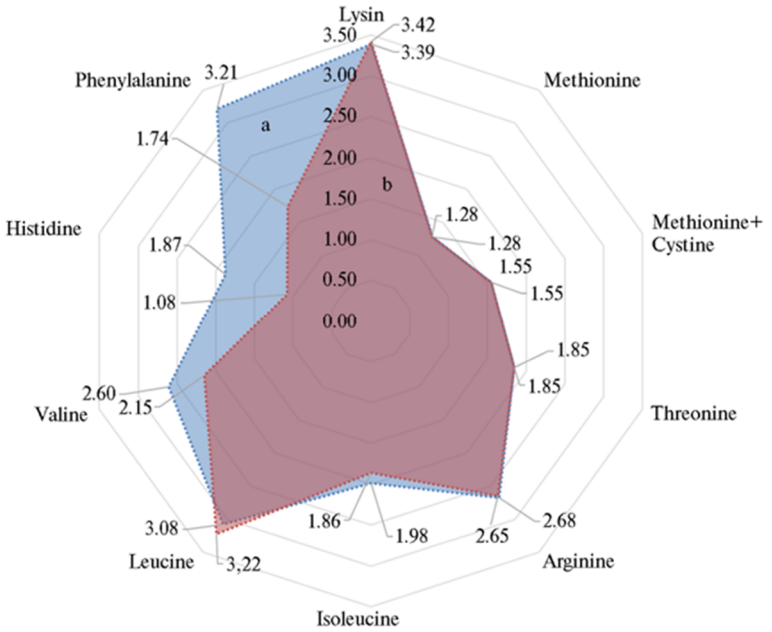
Continued Table 3

Isoleucine	78.4±1.31	80.0±1.28
Leucine	80.9±1.23	81.9±1.21
Valine	78.5±1.47	79.9±1.44
Histidine	82.0±0.85	82.9±0.84
Phenylalanine	85.1±0.35	85.7±0.35
Tyrosine	90.3±0.51	90.3±0.51
Glycine	65.4±3.51	66.4±3.46
Serin	80.9±1.91	85.0±1.82
Proline	79.3±1.27	81.6±1.23
Alanine	78.9±1.70	79.6±1.68
Aspartic acid	80.7±1.21	81.7±1.19
Glutamic acid	82.6±0.85	83.9±0.84

Given the indicators of apparent (AID) and standardized (SID) ileal digestibility, the amino acids of the protein concentrate from the fly larvae were absorbed on average by 79.3% and 80.7%, respectively. The obtained experimental data on the digestibility of amino acids in protein concentrate were compared to the average values of the SID content of amino acids in the global selection from the AminoDat 5.0 database (Evonik Industries AG, Germany). Among the essential amino acids, the lowest SID value was found in valine (79.9%), which is 0.9% higher than its digestibility from rapeseed meal (79.0%), and the highest in arginine (89.5%), which is similar to corn gluten (89.0%). Among the nonessential amino acids, cystine had the lowest SID value (53.5%), which, however, is higher than the digestibility of cystine from feather flour (48.0%), but slightly lower than for poultry meat and bone meal (56.0%). Tyrosine was best absorbed from the protein concentrate (90.3%), which is comparable to its absorption from fish meal.

The digestibility of the main limiting amino acids was quite high, i.e., 82.9% for lysine (higher than for corn gluten, 80.0%) and 86.6% for methionine (the digestibility corresponds to that of fish meal, 86-87%). By the amount of digestible essential amino acids, protein concentrate from the larvae of *Lucilia* spp. flies is close to salmon fish meal (comparison of amino acid profiles of essential amino acids is shown in the figure). In the global selection, salmon fish meal contains on average 3.42% of the digestible lysine (according to SID), 1.55% of methionine + cystine, 1.85% of threonine, 2.68% of arginine, which is consistent with the average content of the digestible amino acids in the protein concentrate from fly larvae in the protein concentrate is 0.12%, 0.45%, 0.79%, and 1.47% higher, respectively. In terms of digestible leucine content, the protein concentrate is inferior to the salmon fish meal by 0.14%.

The content of amino acids in the protein concentrate, taking into account their ileal digestibility, is presented in Table 4. It is worth noting that in the fish meal taken for comparison, according to a selection of 33 analyses, the proportion of crude protein is on average 54.71%, while in the protein concentrate from the larvae of *Lucilia* spp. flies it averaged 62.79%. Such quantity difference in crude protein is due not only to the higher digestibility of amino acids but also to the fact that the crude protein index in the protein concentrate from fly larvae includes protein nitrogen and a large amount of chitin and melanin nitrogen, which makes it difficult to use the classical nitrogen-to-protein conversion factor equal to 6.25. Janssen et al. [28] propose a nitrogen-to-protein conversion factor, taking into account chitin nitrogen and other nitrogen-containing compounds, for protein concentrates from fly larvae, equal to 5.60±0.39. In the protein concentrate from the larvae of *Lucilia* spp. flies used in the study, when using a refined coefficient, the content of crude protein adjusted for chitin is 56.26%, which is consistent with the content of amino acids in the fish meal compared to it, taking into account the digestibility of these feed ingredients.



The average content of SID amino acids in protein concentrate from *Lucilia* spp. fly larvae (a)**, used in the preparation of experimental feed for Smena 8 cross broiler chicks (*Gallus gallus* L.), and salmon fish meal** (b) (data from AminoDat 5.0, Evonik Industries AG, Germany)**.**

4. Amino acid contents in protein concentrate from *Lucilia* spp. fly larvae with regard to standardized ileal digestibility (SID) **for Smena 8 cross broiler chicks (*Gallus gallus* L.)** (*M*±SEM, vivarium of the Federal Scientific Center All-Russian Research and Technological Poultry Institute RAS, Sergiev Posad, Moscow Province, 2019)

Amino acids	In total, %	With regard to SID, %
Lysine	4.09	3.39
Methionine	1.48	1.28
Methionine + cysteine	1.99	1.55
Threonine	2.3	1.85
Arginine	2.99	2.68
Isoleucine	2.48	1.98
Leucine	3.76	3.08
Valine	3.26	2.60
Histidine	2.26	1.87
Phenylalanine	3.75	3.21
Tyrosine	4.21	3.80
Glycine	2.74	1.82
Serin	2.47	2.10
Proline	2.57	2.10
Alanine	4.22	3.36
Aspartic acid	5.98	4.89
Glutamic acid	7.9	6.63

We also compared blood biochemical and morphological parameters of birds, reflecting the state of their metabolism, when using protein concentrate from the larvae of *Lucilia* spp. flies in the diet (Table 5). The results of the study show that the metabolism of broilers when the protein concentrate from fly larvae was fed changed towards increasing anabolic and reducing catabolic processes. This is evidenced by a decrease in the De Ritis ratio by almost 1.7 times compared to the control group. A 4.2% ($p \leq 0.05$) increase in the hemoglobin level indicates the intensity of oxidative processes in broilers fed the experimental feed. Thus, the use of protein concentrate from the larvae of *Lucilia* spp. flies in the diet for broilers has a positive effect on the metabolism due to a significant increase in the activity of alanine aminotransferase (by 23.5%), a decrease in the activity of aspartate

aminotransferase (by 24.6%), a 20.0% increase in the total protein, and a 4.2% increase in the hemoglobin level compared to the control group.

5. Blood biochemical and morphological parameters in Smena 8 cross broiler chicks (*Gallus gallus* L.) fed protein concentrate from *Lucilia* spp. fly larvae (n = 6, M±SEM, vivarium of the Federal Scientific Center All-Russian Research and Technological Poultry Institute RAS, Sergiev Posad, Moscow Province, 2019)

Indicator	Group	
	control	test
Total protein, g/l	25±0.4	30±0.4*
Uric acid, μM/l	79±4.4	90±3.4
Trypsin, units/l	59±2.4	65±3.3
Alanine aminotransferase, units/l	6.8±0.72	8.4±0.25*
Aspartate aminotransferase, units/l	338±15.9	255±18.6*
De Ritis ratio	50	30
Erythrocytes, $\times 10^{12}$/l	1.8±0.03	1.9±0.04
Hemoglobin, g/l	94±0.5	98±1.2*
Leucocytes, $\times 10^{9}$/l	31.2±1.00	30.9±1.00

* Differences with the control group are statistically significant at $p \leq 0.05$.

The data obtained are consistent with other studies on the protein digestibility of fly larvae of other species. In dried full-fat larvae of the housefly *Musca domestica* L. (*Diptera: Muscidae*) grown on broiler brood [29], the percentage of apparent ileal digestibility of amino acids averaged 83.16% vs. 79.30% for *Lucillia* spp., and the AID values for lysine, methionine, threonine, valine, and arginine were 87.0, 88.0, 78.0, 81.0, and 88.0% vs. 82.3, 86.1, 77.9, 78.5, and 88.8%. In our test, the digestibility was lower, which is probably due to the different age of the poultry in the experiments, the biological characteristics of flies of different species, as well as the product form. Hall et al. [29] used another drying technology to produce full-fat biomass of larvae, i.e., 65 °C for 3 hours and then 40 minutes at 95 °C vs. 1 hour at 110 °C in this study. Heat treatment of insect larvae plays an important role in digestibility. Thus, a comparison of the digestibility of crude protein in vitro, depending on the method of preparation (cooking, frying in a pan, cooking in a vacuum, and cooking in an oven) of yellow mealworm beetle *Tenebrio Molitor* L. (*Coleoptera: Tenebrionidae*) [30] showed its improvement with any heat treatment, although there are also opposite effects [31], they were obtained when using higher temperatures of prolonged heating. It is shown that prolonged heat treatment increases the number of disulfide bonds in protein molecules and accelerates their oxidation, thereby changing the conformation of proteins and reducing the digestibility of peptide bonds for enzymes [31].

The protein digestibility of the black soldier fly *Hermetia illucens* L. (*Diptera: Stratiomyidae*) larvae has been compared in numerous publications. Thus, the average percentage of apparent ileal digestibility of amino acids from *H. illucens* larvae grown on grain waste and dried at a temperature of 60 °C for 20 hours without further defatting was 68% [32], which is significantly lower than the value obtained for *Lucillia* spp. and is not consistent with the results of another study [33], in which AID and SID were obtained for a defatted protein concentrate from *H. illucens* equal to 80.7 and 86.0%, respectively. In the work of Mwaniki et al. [34], the values of apparent ileal digestibility for some amino acids are close to those obtained by us, e.g., the AID of lysine, methionine, threonine, valine, arginine is 83.9, 85.3, 78.2, 85.0, and 88.7% for *H. illucens* and 82.3, 86.1, 77.9, 78.5, and 88.8% for *Lucillia* spp., respectively. The data of Mwaniki et al. [34] are also confirmed by the paper of Schiavone et al. [35] in which, when using defatted black soldier fly flour (65.5% crude protein) dried at 60 °C for 20 hours, the average apparent ileal digestibility of amino acids was 80%, and AID for lysine, methionine, threonine, valine, and arginine was 80, 78, 77, 91, and 80%, respectively. That is consistent with the results of this study.

Thus, it is found that the protein of *Lucilia* spp. larva is a valuable source of digestible amino acids. For the first time, the ileal digestibility of amino acids of protein concentrate from the *Lucilia* L. larvae was determined (the apparent and standardized average was 79.3 and 80.7%). The obtained data on the standardized ileal digestibility will be useful for innovative poultry feed formulations. The protein concentrates from fly larvae in the diet for broilers positively affected the metabolism by increasing the activity of alanine aminotransferase (by 23.5%, $p \leq 0.05$), reducing the activity of aspartate aminotransferase (by 24.6%, $p \leq 0.05$), and increasing ($p \leq 0.05$) the total blood protein level (by 20.0%) and hemoglobin (by 4.2%) compared to the control group.

REFERENCES

1. Dedyaeva V.V., Istomin A.I., Argunov M.N., Zhukov I.V., Stepanov V.A. *Materialy mezhdunarodnoi nauchno-prakticheskoi konferentsii, posvyashchennoi 90-letiyu fakul'teta veterinarnoi meditsiny i tekhnologii zhivotnovodstva, provodimoi na baze FGBOU VO Voronezhskii gosudarstvennyi agronomicheskii universitet* [Proc. Int. Conf., dedicated to the 90th anniversary of the Faculty of Veterinary Medicine and Animal Husbandry Technology, the Voronezh State Agronomic University]. Voronezh, 2016: 87-90 (in Russ.).
2. Nekrasov R.V., Chabaev M.G., Zelenchenkova A.A., Bastrakov A.I., Ushakova N.A. Nutritional properties of *Hermetia illucens* L., a new feed product for young pigs (*Sus scrofa domesticus* Erxleben). *Agriculktural Biology [Sel'skokhozyaistvennaya biologiya]*, 2019, 54(2): 316-325 (doi: 10.15389/agrobiology.2019.2.316eng).
3. Bastrakov A.I., Dontsov A.E., Ushakova N.A. *Izvestiya Ufimskogo nauchnogo tsentra RAN*, 2016, 4: 77-79 (in Russ.).
4. Oonincx D.G., Van Itterbeeck J., Heetkamp M.J., Van Den Brand H., Van Loon J.J., Van Huis A. An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PLoS ONE*, 2010, 5(12): e14445 (doi: 10.1371/journal.pone.0014445).
5. Oonincx D.G.A.B., De Boer I.J.M. Environmental impact of the production of mealworms as a protein source for humans — a life cycle assessment. *PLoS ONE*, 2012, 7(12): e51145 (doi: 10.1371/journal.pone.0051145).
6. Van Huis A. Potential of insects as food and feed in assuring food security. *Annual Review of Entomology*, 2013, 58: 563-583 (doi: 10.1146/annurev-ento-120811-153704).
7. FAO. *Global food losses and food waste — extent, causes and prevention.* Rome, 2011.
8. Barragan-Fonseca K.B., Dicke M., van Loon J.J. Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed — a review. *Journal of Insects as Food and Feed*, 2017, 3(2): 105-120 (doi: 10.3920/JIFF2016.0055).
9. Dabbou S., Gai F., Biasato I., Capucchio M.T., Biasibetti E., Dezzutto D., Schiavone A. Black soldier fly defatted meal as a dietary protein source for broiler chickens: effects on growth performance, blood traits, gut morphology and histological features. *Journal of Animal Science and Biotechnology*, 2018, 9(1): 49 (doi: 10.1186/s40104-018-0266-9).
10. Maurer V., Holinger M., Amsler Z., Früh, B., Wohlfahrt J., Stamer A., Leiber F. Replacement of soybean cake by *Hermetia illucens* meal in diets for layers. *Journal of Insects as Food and Feed*, 2016, 2(2): 83-90 (doi: 10.3920/JIFF2015.0071).
11. Woods M.J., Cullere M., Van Emmenes L., Vincenzi S., Pieterse E., Hoffman L.C., Zotte A.D. *Hermetia illucens* larvae reared on different substrates in broiler quail diets: effect on apparent digestibility, feed-choice and growth performance. *Journal of Insects as Food and Feed*, 2019, 5(2): 89-98 (doi: 10.3920/JIFF2018.0027).
12. Veldkamp T., Van Niekerk T. Live black soldier fly larvae (*Hermetia illucens*) for turkey poults. *Journal of Insects as Food and Feed*, 2019, 5(4): 301-311 (doi: 10.3920/JIFF2018.0031).
13. Newton G.L., Booram C.V., Barker R.W., Hale O.M. Dried *Hermetia illucens* larvae meal as a supplement for swine. *Journal of Animal Science*, 1977, 44(3): 395-400 (doi: 10.2527/jas1977.443395x).
14. Caimi C., Renna M., Lussiana C., Bonaldo A., Gariglio M., Meneguz M., Dabbou S., Schiavone A., Gai F., ConcettaElia A., Prearo M., Gasco L. First insights on Black Soldier Fly (*Hermetia illucens* L.) larvae meal dietary administration in Siberian sturgeon (*Acipenser baerii* Brandt) juveniles. *Aquaculture*, 2020, 515: 734539 (doi: 10.1016/j.aquaculture.2019.734539).
15. Belghit I., Liland N.S., Gjesdal P., Biancarosa I., Menchetti E., Li Y., Lock E.J. Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (Salmo salar). *Aquaculture*, 2019, 503: 609-619 (doi: 10.1016/j.aquaculture.2018.12.032).
16. Huis A., Oonincx D. The environmental sustainability of insects as food and feed. A review. *Agronomy for Sustainable Development*, 2017, 37(5): 43 (doi: 10.1007/s13593-017-0452-8).
17. Moula N., Detilleux J. A meta-analysis of the effects of insects in feed on poultry growth

performances. *Animals*, 2019, 9(5): 201 (doi: 10.3390/ani9050201).
18. Sogari G., Amato M., Biasato I., Chiesa S., Gasco L. The potential role of insects as feed: a multi-perspective review. *Animals*, 2019, 9(4): 119 (doi: 10.3390/ani9040119).
19. Romanenko E., Istomin A., Zhukov I., Argunov M., Dedyaeva V. *Kombikorma*, 2018, 7: 79-81 (in Russ.).
20. Nekrasov R., Chabaev M., Zelenchenkova A., Zhuravlev M. *Kombikorma*, 2019, 3: 41-43 (doi: 10.25741/2413-287X-2019-03-3-052) (in Russ.).
21. Romanenko E.A., Istomin A.I. Intensivnost' rosta i razvitiya indyushat krossa big-6 pri ispol'zovanii belka iz lichinok mukh populyatsii *Lucilia caesar*. *Politematicheskii setevoi elektronnyi nauchnyi zhurnal Kubanskogo gosudarstvennogo agrarnogo universiteta*, 2020, 157: 136-144 (doi: 10.21515/1990-4665-157-011) (in Russ.).
22. Dalibard P., Paillard E. Use of the digestible amino acid concept in formulating diets for poultry. *Animal Feed Science and Technology*, 1995, 53(2): 189-204 (doi: 10.1016/0377-8401(95)02010-W).
23. Yapontsev A.E. *Ptitsevodstvo*, 2016, 2: 35-37 (in Russ.).
24. *Evropeiskaya konventsiya o zashchite pozvonochnykh zhivotnykh, ispol'zuemykh dlya eksperimentov ili v inykh nauchnykh tselyakh (ETS № 123) (Strasburg 18.03.1986)* [The European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS № 123) (Strasburg 18.03.1986)]. Available: http://www.conventions.ru/view_base.php?id=19432. No date (in Russ.).
25. Vertiprakhov V.G., Grozina A.A., Kislova I.V., Rebrakova T.M. *Mezhdunarodnyi vestnik veterinarii*, 2019, 4: 125-131 (in Russ.).
26. Vertiprakhov V.G., Grozina A.A. *Veterinariya*, 2018, 12: 51-54 (doi: 10.30896/0042-4846.2018.21.12.51-54) (in Russ.).
27. Golian A., Guenter W., Hoehler D., Jahanian H., Nyachoti C. Comparison of various methods for endogenous ileal amino acid flow determination in broiler chickens. *Poultry Science*, 2008, 87(4): 706-712 (doi: 10.3382/ps.2007-00330).
28. Janssen R.H., Vincken J. P., van den Broek L.A., Fogliano V., Lakemond C.M. Nitrogen-to-protein conversion factors for three edible insects: *Tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens*. *Journal of Agricultural and Food Chemistry*, 2017, 65(11): 2275-2278 (doi: 10.1021/acs.jafc.7b00471).
29. Hall H.N., Masey O'Neill H.V., Scholey D., Burton E., Dickinson M., Fitches E.C. Amino acid digestibility of larval meal (*Musca domestica*) for broiler chickens. *Poultry Science*, 2018, 97(4): 1290-1297 (doi: 10.3382/ps/pex433).
30. Megido R.C., Poelaert C., Ernens M., Liotta M., Blecker C., Danthine S., Francis, F. Effect of household cooking techniques on the microbiological load and the nutritional quality of mealworms (*Tenebrio molitor* L. 1758). *Food Research International*, 2018, 106: 503-508 (doi: 10.1016/j.foodres.2018.01.002).
31. Poelaert C., Beckers Y., Despret X., Portetelle D., Francis F., Bindelle J. In vitro evaluation of fermentation characteristics of two types of insects as potential novel protein feeds for pigs. *Journal of Animal Science*, 2016, 94: 198-201 (doi: 10.2527/jas.2015-9533).
32. Bax M., Aubry L., Ferreira C., Daudin J., Gatellier P., Rémond D., Santé-Lhoutellier V. Cooking temperature is a key determinant of in vitro meat protein digestion rate: investigation of underlying mechanisms. *Journal of Agricultural and Food Chemistry*, 2012, 60(10): 2569-2576 (doi: 10.1021/jf205280y).
33. De Marco M., Martínez S., Hernandez F., Madrid J., Gai F., Rotolo L., Kovitvadhi A. Nutritional value of two insect larval meals (*Tenebrio molitor* and *Hermetia illucens*) for broiler chickens: apparent nutrient digestibility, apparent ileal amino acid digestibility and apparent metabolizable energy. *Animal Feed Science and Technology*, 2015, 209: 211-218 (doi: 10.1016/j.anifeedsci.2015.08.006).
34. Mwaniki Z., Neijat M., Kiarie E. Egg production and quality responses of adding up to 7.5 % defatted black soldier fly larvae meal in a corn-soybean meal diet fed to Shaver White Leghorns from wk 19 to 27 of age. *Poultry Science*, 2018, 97(8): 2829-2835 (doi: 10.3382/ps/pey118).
35. Schiavone A., De Marco M., Martínez S., Dabbou S., Renna M., Madrid J., Gasco L. Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (*Hermetia illucens* L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. *Journal of Animal Science and Biotechnology*, 2017, 8(1): 51 (doi: 10.1186/s40104-017-0181-5).