The results of the study of the amino acid composition of compound feeds during the extrusion of wheat grain with the addition of Black Soldier Fly Larvae (Hermetia illucens L.)

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Abstract. The production of quality and inexpensive feed based on agricultural raw materials is the main way to increase the profitability and competitiveness of livestock production, ensuring its import substitution and high quality. It is known that in the structure of the cost of livestock products, 50-70% of all costs are accounted for by feed. Three variants of a mixture of crushed wheat grain and biomass of the black soldier fly larvae (Hermetia illucens) were extruded with the content of the latter 10, 15, and 20% by weight at different temperatures. The content of amino acids in raw materials and finished extrudate was determined. It was found that the feed mixture of crushed grain and black soldier fly larvae can be successfully extruded at a temperature of 121-135 °C. With an increase in the extrusion temperature in the range of 115-140 °C, the amino acid content in the finished extrudate decreases. The change in the content of insect larvae in the feed mixture does not affect the nature of the dependence of the amino acid content in the extrudate on the extrusion temperature and the course of the process. It was found that the content of amino acids in the extruded feed decreases with increasing speed with increasing temperature, regardless of the content of insect biomass. The rational range of the extrusion temperature of the feed mixture from wheat grain and insect larvae was determined – 121-127 °C, which ensures a reduction in the content of essential amino acids in the extrudate by no more than 30%. The extruded feed, which includes 15% of the biomass of insect larvae, contains 9.6 ± 0.13% amino acids, including 4.38 ± 2.01% of essential amino acids. The extrusion of insect larvae in a mixture with seeds of grain crops is a promising direction for improving the production of feed for fish and farm animals.

1 Introduction

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According to Rosstat, the production of high redistribution products in Russia — fish meat (including minced meat) — increased by 61.4% or 15.5 thousand tons in 11 months of 2021 and reached 40.7 thousand tons.

Increasing fish meat production (including minced meat) is due, among other things, to the commissioning of 21 out of 24 fish processing enterprises as part of the implementation of the investment quota program.

Today, the new plants can collectively process about 3.3 million tons of aquatic biological resources from more than 5 million tons extracted in 2021.

The industry faces the task of globally updating and modernising production facilities in order to eventually produce final products. As part of the investment quota, it is planned to build another 8-10 large fish processing plants in the Far East.

Also, in January-November 2021, the production of frozen fish fillets increased by more than 23%, or 38.5 thousand tons — to 204 thousand tons. Fish, including fillets, smoked — by almost 3%, or 1.7 thousand tons and exceeded 59 thousand tons.

The increase in processed products caused, among other things, an increase in the volume of exports. Thus, the export supplies of fish fillets increased by 38.5 thousand tons in 2021 — up to 143.8 thousand tons.

Due to the growth rate of fish production, there is a need to increase the production of high-protein feed products.

Along with the increase in the volume of feed production, prices for them are increasing. Over the past 5 years, feed prices have increased more than 2 times. This is primarily due to the rising prices of raw materials for their production. In addition, compound feeds used in Russia for aquaculture are mainly imported, which significantly affects the increase in the cost of final products.

Proper nutrition of animals plays a key role in maintaining their health and well-being and in the production of safe and high-quality animal products. The growing demand for animal proteins leads to the intensification of livestock production, mainly based on the use of compound feeds produced by industry. This has led to the increasing use of certain types of feed grains and feed seeds, pesticides and fertilizers, as well as new and non-standard materials in the production of feed, such as by-products in the production of biofuels and a number of other agro-industrial by-products. In recent years, a number of episodes related to food contamination have drawn attention to the importance of ensuring feed safety and the need to prevent and control the presence of both traditional and new harmful factors, such as dioxin, aflatoxins and other unwanted impurities.

The link between safe animal feed and safe food is now well established. In particular, the modern approach to food safety provides for measures aimed at minimising and preventing the ingress of hazardous substances at the early stages of the food chain, including the primary production of feed grains and forage. Thanks to this, producers of primary products, including farmers and feed producers, have become more aware of their responsibility for the production of safe and high-quality food.

The assessment of the current state of fisheries and its role in the Russian economy is formulated in the Strategy for the Development of the Fishery Complex of the Russian Federation for the period up to 2030. At its core, the Strategy assumes an increase in the extraction of aquatic biological resources, the production of fish products and, as a result, an increase in the production of feed products.

It is expected that the intensification of aquaculture will reduce dependence on feed, which is based on fish meal and fish oil, which are produced mainly by processing low-value commercial species of pelagic fish. Against the background of the recognition of the nutritional value of aquaculture products, which are directly consumed by humans, the demand for fishmeal and fish oil far exceeds the supply.

The world leaders in the production of fishmeal are Latin America. It is the main center of fishmeal production, which is geographically far from Chinese and European consumption centers. This significantly affects its cost, which increases due to the costs of its transportation. The world leaders in the production of fishmeal are South American and Asian countries, the Russian Federation is only in 11th place in this indicator. It should be noted that the production of fishmeal in Russia from 1987 to 2022 decreased by 5 times from 800 million tons to 160 million tons per year [1].

Thus, there is an obvious need to find alternative sources of protein and fat for feed production, which in their composition would not be inferior to fishmeal.

As a result, alternative proteins and fats, primarily of vegetable origin, are increasingly used in the composition of feed. However, the widespread use of such feeds in the cultivation of omnivorous species can affect their well-being and weaken the immune system. Currently, work is underway at an accelerated pace on new sources of proteins and fats – these are insects (Figure 1) (black soldier fly larvae (Hermetia illucens), mealworms, rice grashopper (Oxya fuscovittata), seaweed (Figure 2) (chlorella, scenedesmus, spirulina), fungi (Paecilomyces varioti) (Figure 3), the production of which as feed is rapidly becoming profitable. It is important to ensure that a wide transition to such feeds does not affect the nutrient content of aquaculture products [2, 3].

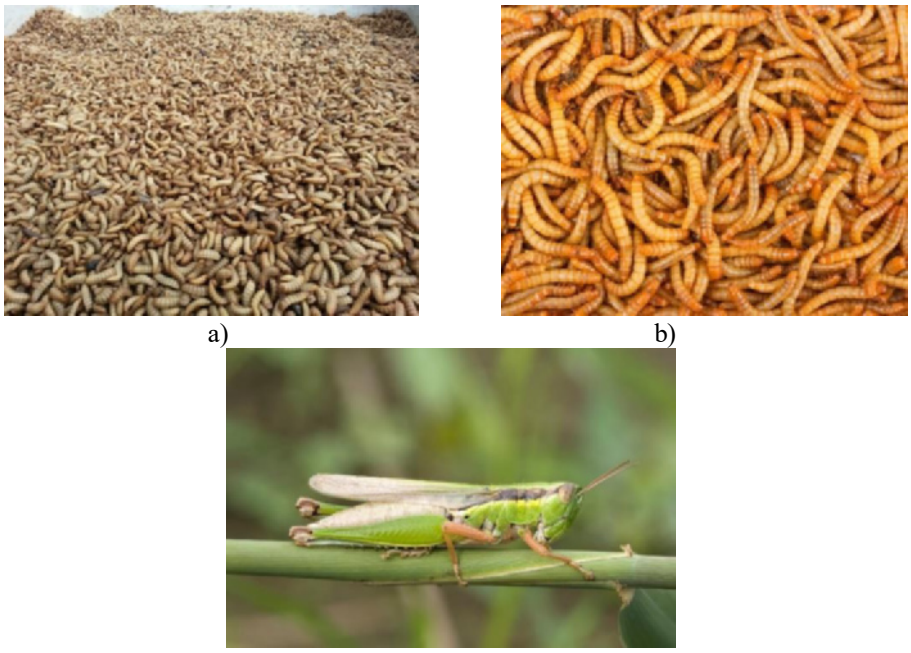
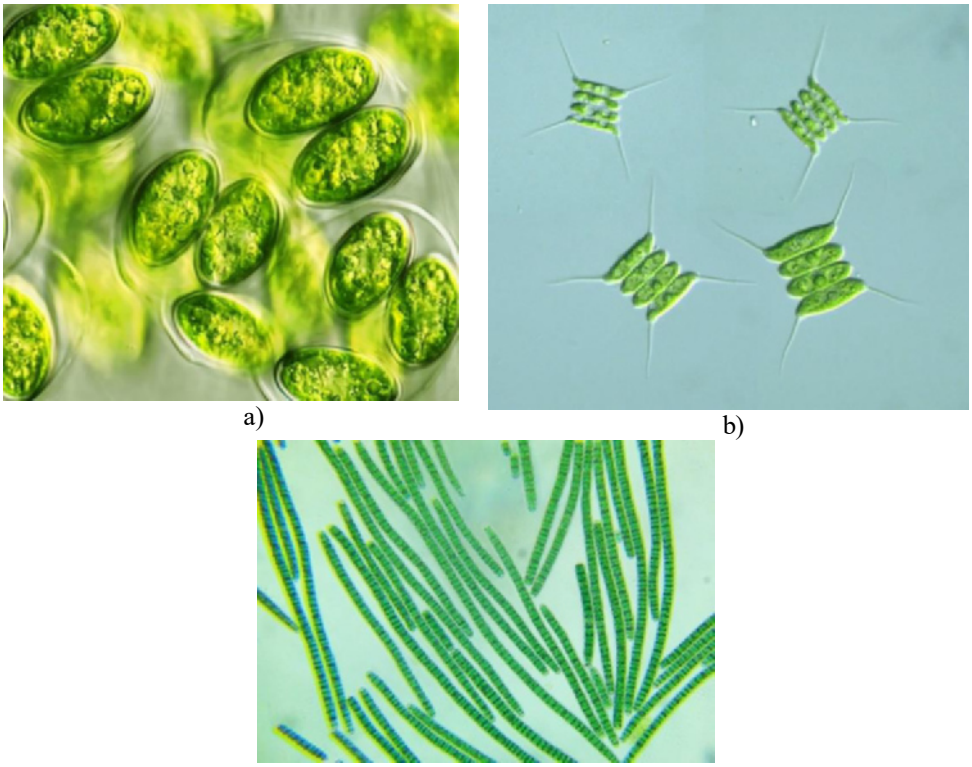


Fig. 1. Insects used in fish feed: a) Black soldier fly larvae (Hermetia illucens), b) mealworms, c) rice grasshopper (Oxya fuscovittata).



a) b) c)

Fig. 2. Weeds used in fish feed: a) chlorella, b) scenedesmus, c) spirulina.

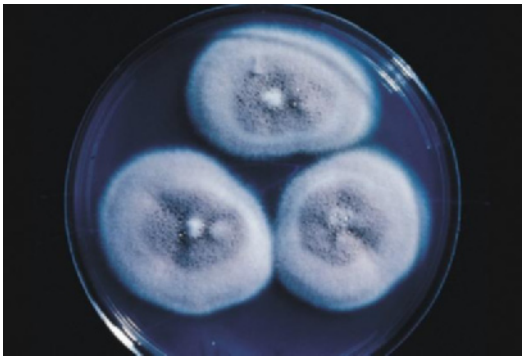


Fig. 3. Paecilomyces varioti fungus (for producing Pekilo single-cell protein).

Over the past decade, there has been a transition from the use of insects as a whole to their processing in order to ensure long-term preservation, ease of introduction into the composition of compound feeds and food products, and increase digestibility. They are dried, crushed, and also processed into protein-containing flour and fat, after which they are often mixed with other raw materials. Insects are successfully included in the diets of poultry, pigs, young cattle and various types of fish. It has been established that insect flour can replace fish meal in animal diets, corresponding to its amino acid profile. Insects are successfully included in the composition of food products for people, such as snacks [4, 5, 6].

Insects differ in their composition and structure from traditional types of feed and food raw materials. Therefore, their processing in order to prepare for inclusion in the composition of feed and food products requires the use of technological operations other than those used in the processing of traditional raw materials. Active research on insect processing technologies has been conducted only in the last eight years (2014-2022), but this topic is still insufficiently studied, and there is no generally accepted opinion of the scientific community about the optimal technological techniques for this.

In the production of compound feeds for farm animals and fish, as well as many food products, some or all of their components are mixed and extruded to improve the structure and increase the digestibility of nutrients [7, 8]. Extrusion processing of raw materials in the production of compound feeds has recently become a generally accepted standard. As part of this approach, insects are also extruded in a mixture with other types of raw materials, most often of plant origin. At the same time, insects are treated in two different forms. In the first case, they are used in the form of fat-free and dehydrated protein-containing flour or dried and crushed mass without fat separation, i.e. completely processed insect biomass is used. In another variant, they are used in the form of non-processed biomass. It should be noted that in the course of well-known studies of the extrusion of feeds containing grain and insect larvae, the latter were introduced into the feed in the form of fat-free and dehydrated protein-containing flour [4, 5, 6] or in the form of dehydrated and crushed mass [9]. Studies on the extrusion of larvae without their pretreatment or with minimal treatment (chitin isolation) have not been carried out before [10, 11, 12].

2 Materials and methods

A small single-screw extruder developed jointly with “Donskoy” ASC was used for the preparation of feed. Its maximum capacity is 80 kg/h, the electric motor power is 7.5 kW. The screw rotation speed is 240-360 s^{-1}. The extruder's loading hopper was equipped with a volumetric dispenser for uniform feed of raw materials. The ratio of the screw length to the diameter was L/D = 6:1 at D = 55 mm, the diameter of the outlet of the spinneret die was 10 mm.

Crushed wheat grain and larval biomass were mixed with a screw dispenser. Each variant of the feed mixture was prepared in an amount of 1000 g. The mixture was loaded into an extruder and extruded at a given value of the screw rotation speed. At the exit from the matrix, the extrudate bundle was crushed, resulting in granules with a length of 100 ± 5 mm. The pellets were cooled to room temperature by natural cooling, then packed in plastic bags and sent for analysis.

Three variants of the feed mixture were extruded with the following composition and component ratio (by weight): I – black soldier fly larvae + wheat grain in a ratio of 10:90. II – black soldier fly larvae + wheat grain in a ratio of 15:85. III – black soldier fly larvae + wheat grain in a ratio of 20:80. For comparison, a feed containing only crushed wheat grain (control sample) was also extruded.

The rotation frequency of the screw was changed using a frequency converter in the range of 240-360 s^{-1}. An increase in the rotation speed of the extruder screw led to an increase in the extrusion temperature. The value of the screw rotation speed 240 s^{-1} corresponds to the temperature of the extrudate 115 ± 0.5 °C, and 360 s^{-1}, to 140 ± 0.5 °C. The temperature of the extrudate was measured at the outlet of the spinneret die of the extruder matrix with a thermocouple.

The study of extruding each variant of the feed mixture at a certain speed of rotation of the extruder screw was carried out in three repetitions. The measurement results are presented in the form: average value ± standard deviation. The reliability of the mean

differences was determined by the method of one-factor analysis of variance using a posteriori analysis by the Tukey`s criterion at $p < 0.05$.

Samples of feed mixtures and extrudates were taken according to GOST ISO 6497-2014 and prepared for analysis according to GOST ISO 6498-2014. The obtained samples of feed mixtures and finished extrudates were subjected to chemical analysis according to standard methods, determining the total protein and fat content, as well as humidity. The diameter of the extrudate granules to determine its expansion index was measured with a caliper.

The criteria for the possibility of extruding a feed mixture of a certain composition and satisfactory flow of the process were the absence of jamming of the extruder screw, burning of the product, reduction in the productivity of the extruder, as well as the even passage of raw materials through the extruder, obtaining a good quality extrudate. The quality of the extrudate was evaluated by the organoleptic method. The extrudate of good quality should have a dense porous homogeneous structure, even color and pleasant smell, its granules should have sufficient strength [13, 14].

The content of various amino acids in feed mixtures and extrudates was determined by capillary electrophoresis [15] according to GOST R 55569-2013. The samples were examined in the laboratory “Biochemical and spectral analysis of food products” of DSTU using the device SKE “Kapel-104T”.

The method of capillary electrophoresis consists in the decomposition of feed samples by acid hydrolysis, which causes the transition of amino acids into free forms, the production of FTC derivatives and their electrophoretic separation [15]. Quantitative determination of amino acid content was performed by analysing electrophoregrams. The total content of aspartic acid and asparagine, glutamic acid and glutamine, leucine and isoleucine were determined without their separation.

As a result of processing the analysis results, the quantitative content of each of the following amino acids in the sample was determined: alanine, arginine, asparagine, valine, histidine, glycine, glutamine, leucine, lysine, methionine, proline, serine, tyrosine, threonine, tryptophan, phenylalanine, cystine. Based on the data obtained, the mass fraction of each amino acid in the studied feed sample, expressed as a percentage, was determined. The obtained values were summed up by determining the total content of amino acids in the feed and separately the total content of essential amino acids (arginine, valine, histidine, leucine, lysine, methionine, threonine, tryptophan, phenylalanine).

When processing the experimental results, the influence of the extrusion temperature and the content of larval biomass in the feed mixture on the content of amino acids in the finished extrudate was evaluated. The total content of amino acids in the extrudate and the content of essential amino acids in it were used as the main indicator of the quality of the extruded feed. As auxiliary indicators of the quality of the extrudate, its humidity and expansion index were used.

In this study, the extrusion temperature of the feed mixture was estimated by measuring the temperature of the extrudate when it exits the die, since its determination does not cause significant difficulties. The temperature of the finished extrudate is lower than the temperature in the working chamber of the extruder, but it is directly dependent on it.

To assess the effect of temperature on the extrusion process of the feed mixture, based on experimental data, the instantaneous rate of change in the amino acid content was calculated, which shows how much percent the amino acid content in the extrudate changes when the extrusion temperature changes by one degree (%/ºC).

3 Results

The amino acid content in the feed feed mixture is shown in the table 1.

Table 1. Amino acid content of the feed mixture from wheat grain and black soldier fly larvae (Hermetia illucens).

Variant of feed mixture	Amino acids content, %	Indispensable amino acids content, %
I – Larvae biomass 10 % + wheat grain 90 %	13.25□0.12	5.05□0.08
II – Larvae biomass 15 % + wheat grain 85 %	13.71□0.15	5.28□0.09
III Larvae biomass 20 % + wheat grain 80 %	14.12□0.13	5.63□0.07
Control sample (wheat grain 100 %)	11.81□0.13	3.93□0.08

The introduction of larvae into the feed (10-20%) increased the total amino acid content by 1.44-2.31%, and essential amino acids – by 1.12-1.7% compared to wheat grain (before heat treatment).

As a result of experiments, it was found that the feed mixture consisting of wheat grain and the black soldier fly larvae can be extruded in a single-screw extruder.

The process of extrusion of the feed mixture proceeds satisfactorily at a temperature of 121-135 °C. At a temperature of less than 121 °C, the process proceeds inefficiently, the extrudate turns out to be of unsatisfactory quality with a sharply heterogeneous structure, loose and fragile. At an extrusion temperature of more than 135 °C, the extrudate burns, accompanied by a significant decrease in the protein content.

As a result of extrusion of a mixture of wheat grain and biomass of Black Lion fly larvae at a temperature of 121-135 ° C, an extruded feed of good quality was obtained. The finished extrudate had a homogeneous porous structure; it was dense and durable. The extrudate containing the larval biomass had a darker color than the extrudate from wheat grain alone. Figure 2 shows samples of extrudate from wheat grain and black soldier fly larvae (option II: larvae 15% + wheat grain 85%) obtained at different temperatures.

Fig. 4. Samples of the extrudate containing black soldier fly larvae biomass and wheat grain (Variant II: larvae biomass 15 % + wheat grain 85 %) obtained at the temperature: 1, 2 – 115 ºC; 3, 4 – 118 ºC; 5, 6 – 121 ºC; 7, 8 – 124 ºC; 9, 10 – 127 ºC; 11, 12 – 130 ºC; 13, 14 – 132 ºC; 15, 16 – 135 ºC; 17, 18 – 138 ºC; 19, 20 – extrudate containing only wheat grain obtained at the temperature of 124 ºC (control sample).

The change in the extrusion temperature did not have a significant effect on the fat content in the finished extrudate, which practically did not change compared to the content in the initial feed mixture.

It was found that the addition of biomass of black soldier fly larvae to wheat grain in an amount of 10 to 20% by weight (at a temperature of 124 °C) increases the total amino acid content in the extruded feed from 68 to 105% compared with the extrudate only from wheat grain (Table 2). At the same time, the content of essential amino acids in the extrudate increases in the range from 112 to 165%.

Table 2. Quality parameters of the extruded feed containing black soldier fly larvae biomass and wheat grain (produced at the temperature of 124 ºC).

Variant of feed mixture	Amino acids content, %	Indispensable amino acids content, %	Expansion index
I – Larvae biomass 10 % + wheat grain 90 %)	7.85□0.13	3.51□0.09	1.62□0.2
II – Larvae biomass 15 % + wheat grain 85 %)	8.55□0.14	3.89□0.09	1.53□0.24
III – (larvae biomass 20 % + wheat grain 80 %)	9.6□0.13	4.38□0.1	1.41□0.19
Control sample (wheat grain 100%)	4.68□0.16	1.65□0.11	1.79□0.22

4 Discussion

The analysis of experimental data showed that the total content of amino acids in the extrudate produced at a temperature of 121 °C, on average, is 69% of their initial content before extrusion. The content of essential amino acids in such an extrudate is 78.6% of the original. Thus, the extrusion of the feed mixture in the temperature range of 118-121 °C reduces the content of amino acids by 31% (essential by 21.4%).

The total content of amino acids in the extra date produced at a temperature of 135 °C is on average 50.8% of their initial content in the initial feed mixture, the content of essential amino acids is 52.1% of the original. Thus, the extrusion of the feed mixture in the temperature range of 121-135 °C reduces the content of amino acids by about 20% more (irreplaceable by 26%).

At an extrusion temperature of more than 135 °C, the amino acid content decreases significantly faster, amounting to 41.7% of the original at a temperature of 139 °C (for irreplaceable 42.7%).

There were no statistically significant differences between the variants of the feed mixture in terms of the decrease in the amino acid content compared to the initial one ($p > 0.05$). This also confirms that the content of insect larvae in the feed mixture does not affect the preservation of amino acids as a result of extrusion. It can also be concluded that the extrusion should not be carried out at the temperature of the extrudate more than 135 ° C, as this will lead to a decrease in the content of amino acids in the finished feed by more than half compared to the original one.

The instantaneous rate of change in the content of amino acids in the extrudate allows to visually assess the effect of temperature on the process of extrusion of the feed mixture (Table 3).

Table 3. Instantaneous rate of amino acids content change in the extrudate containing black soldier fly larvae biomass and wheat grain at varying extrusion temperature (in relation to the general content of different substances in the extrudate), %/ºC.

Range of temperature, ºC	I variant (larvae biomass 10 % + wheat grain 90 %)		II variant (larvae biomass 15 % + wheat grain 85 %)		III variant (larvae biomass 20 % + wheat grain 80 %)	
	for all amino acids	for indispensable amino acids	for all amino acids	for indispensable amino acids	for all amino acids	for indispensable amino acids
115-121	0.03	0.02	0.04	0.03	0.03	0.02
121-135	0.17	0.06	0.14	0.07	0.17	0.07
135-140	0.34	0.17	0.37	0.2	0.34	0.23

When the temperature changes in the range of 115 – 121 °C, the average instantaneous rate of change in the total amino acid content is 0.033% / °C, and essential amino acids – 0.023% / °C. Such an insignificant rate of change in the amino acid content indicates an inefficient extrusion process.

When the temperature changes in the range of 121 – 135 °C, the average instantaneous rate of change in the total amino acid content increases and amounts to 0.16% / °C, and essential amino acids – 0.007% / °C. This indicates the effective course of the extrusion process of the feed mixture.

When the temperature changes in the range of 135 – 140 °C, the average instantaneous rate of change in the total amino acid content increases significantly and amounts to 0.35% / °C, and essential amino acids – 0.2% / °C. Thus, in the range of 135 – 140 °C, with an increase in temperature, the total amino acid content decreases on average 2.5 times faster than in the range of 121 – 135 °C. The content of essential amino acids decreases on average 2.85 times faster. This confirms the irrationality of extrusion of the feed mixture in this temperature range due to poor preservation of amino acids.

A comparison of the results for different variants of the feed mixture shown in Table 3 shows that the instantaneous rate of change in the amino acid content during extrusion does not depend on the biomass content of larvae in the mixture and is determined only by the extrusion temperature. Analysis of the experimental results shows that there are no factors limiting the possibility of effective extrusion of a feed mixture of wheat grain and biomass of black soldier fly larvae with an insect content of up to 15%, and possibly more. It can be assumed that the patterns established as a result of experiments are also valid for cases of extrusion of feed mixtures from grain and other protein-rich raw materials of animal origin. The effective implementation of the process of extrusion of feed mixture from grain and insect biomass depends on temperature.

An extrudate of satisfactory quality can be obtained at a temperature from 121 to 135 °C. But with increasing temperature, the content of amino acids in the finished extrudate steadily decreases. Therefore, it is necessary to determine a rational range of the extrusion temperature of the feed mixture, at which the process proceeds satisfactorily, but there is still no significant decrease in the content of amino acids in the extrudate.

As an indicator determining the rational temperature range of extrusion, it is best to use the content of essential amino acids in the finished extrudate. In our opinion, for extrusion, such temperature values should be used at which the content of essential amino acids in the extrudate is no more than 30% lower than in the initial feed mixture. The extrusion temperature range of 121 – 127 °C corresponds to this requirement. This range was found for a feed mixture with a larval content of up to 15% (by weight). However, it can be assumed that the range of 121 – 127 °C will be rational for the extrusion of feed mixtures with a high content of black soldier fly larvae. It can also be assumed that it is possible to efficiently produce extruded feed from a mixture of insect larvae and grains of other cereals, such as barley or sorghum.

5 Conclusions

As a result of experimental studies, it was found that the feed mixture consisting of crushed wheat grain and biomass of black soldier fly larvae (10-20% by weight) can be successfully extruded at a temperature of 121 – 135 °C (the temperature of the finished extrudate). In this temperature range, a good quality extrudate was obtained.

The addition of insect larvae to the grain increases the protein content, including essential amino acids, in the finished feed by 68 – 105%. The extruded feed can be used for feeding farm animals, as a whole, or as one of the components of compound feed. With an increase in the mass fraction of the biomass of insect larvae, such food can be used to feed fish.

It was found that with an increase in the extrusion temperature in the range of 115 – 140 °C, the content of amino acids in the finished extrudate from wheat grain and insect larvae decreases. The change in the content of insect larvae in the feed mixture does not affect the nature of the dependence of the amino acid content in the extrudate on the extrusion temperature and the course of the extrusion process.

It is established that there are three temperature ranges in which the process of extrusion of the feed mixture proceeds differently. At a temperature of 115 – 121 °C, the amino acid content in the finished extrudate decreases slightly, but the extrusion process proceeds ineffectively, without resulting in a homogeneous extrudate. In the range of 121 – 135 °C, a more significant decrease in the amino acid content is observed, the extrusion process proceeds efficiently, resulting in a homogeneous extrudate. The range of 135 – 140 °C is characterised by a sharp decrease in the content of amino acids in the extrudate and its burning.

It was found that the content of amino acids in the extruded feed decreases with increasing speed with increasing temperature, regardless of the content of insect biomass in the feed mixture. The instantaneous rate of change in the amino acid content during the extrusion process has no significant dependence on the biomass content of larvae in the mixture and is determined only by the temperature of the process.

The data obtained are consistent with the results of studies by Ottoboni et al. [16], who found that during the extrusion of a mixture of wheat and dried larvae, upon reaching a certain temperature, the amino acid content decreases linearly. In our experiments, such a phenomenon was observed during extrusion in the range of 121 – 135 °C.

The rational range of the extrusion temperature of the feed mixture from wheat grain and insect larvae is determined: 121 – 127 °C, which ensures a reduction in the content of

essential amino acids in the extrudate by no more than 30% compared with their content in the feedstock. The extruded feed obtained with these parameters, which includes 15% of insect biomass, contains 9.6% amino acids, including 4.38% essential, which is 105% and 165% more than in wheat-only extrudate.

The proposed technology for processing insect larvae and their introduction into the feed is much simpler and, therefore, cheaper than the technology used for separating insect biomass into fat and protein fractions, followed by drying the latter to obtain protein flour. Thanks to this, small enterprises, such as aquaculture farms, can also produce such feed.

Extrusion of the black soldier fly larvae in a mixture with grain of grain crops is a promising direction for improving the production of feed for fish and farm animals.

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