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Assessment of the Functional and Technological Properties of Zooprotein Based on Fly Larvae (*Lucilia Caesar*) As Feed Composition

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Abstract

Recently, biotechnologies are gaining large scale as promising areas of science that are exploring the possibilities of using living organisms, systems or products of their vital functions for solving technological problems. The object of present research is the high-protein preparation Zooprotein based on the larvae of the fly species *Lucilia Caesar*. The insect gained wide popularity due to the highly effective bioconversion of various solid organic waste, as well as the high nutritional value of the larvae with the possibility of using fly larvae in farm animals and aquaculture feeding. The high-protein preparation Zooprotein based on the larvae of *Lucilia Caesar* can be considered as a promising functional ingredient for feeding diets for various animal species due to unique chemical composition. Present study provides data on the antimicrobial properties of *Lucilia Caesar* flies from the point of view of the safety in use of the high-protein feed ingredient Zooprotein as feed composition. In addition, the functional and technological properties of Zooprotein were evaluated in comparison with other commonly used ingredients in the feed industry, in particular fish and meat and bone meal. Also, the main basis for its application is the ability to bind moisture and fat, create a specific structure for the finished product, and, finally, the harmlessness of using this product. The main purpose of present research is to study the functional and technological properties of high-protein preparation Zooprotein in comparison with other commonly used feed ingredients.

Keywords: fly larvae, *Lucilia Caesar,* Zooprotein, water-holding capacity, fat-holding capacity, nutrients, feed composition, microbial safety, functional and technological properties, application

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1. Introduction

Modern industrial feed production is a large-scale and multifunctional enterprise, which includes the production of animal feed, feed additives, drugs, etc., and is based on

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modern achievements of microbiological, pharmaceutical, food, chemical and other manufacturing sectors [1].

According to many experts in the field of nutrition of farm animals, the current deficit of protein and energy in diets leads to a large (up to 40%) overspending of feed. In this regard, special attention of both sides native and foreign researchers is paid to studying the potential capabilities of the feed base, as well as finding new feed products and additives that provide a more complete transformation of nutrients in the products [12].

Especially, protein deficiency is observed in poultry farming [24]. The main feed of poultry is cereal grain: wheat, corn, barley, millet, sorghum, oats and etc. They contain all the necessary nutrients, proteins, fats, vitamins, micro-and macro elements, but in an amount not always sufficient for normal growth of young animals and high productivity of adult livestock. In industrial poultry, the problem of protein nutrition is solved by fish, krill, meat and bone meal, sunflower and other meal, feed yeast, but all these products are inaccessible to the population.

In this regard, in recent years, research institutions have been intensively searching for cheap feed products, which by biological value were not inferior to expensive sources of animal and vegetable protein, could serve as a replacement for edible grain and expensive animal feed.

Over the past decade, there has been a worldwide interest in insects as a source of highly digestible feed protein, unique properties of fat, antioxidants, immunomodulators, and raw materials for new drugs [13, 21]. Insects are also a source of chitosan, which can be used to increase the biological value or increase the shelf life of food products [3].

The fundamentals development of the technology for *Lucilia Caesar* fly larvae cultivation, which provides a highly effective protein-lipid component of feed rations, is an urgent topic, the significance of which has significantly increased at present due to the need for import substitution of feed components for farm animals and fish. The fly has gained great popularity in the last decade due to the introduction of feed for reptiles, birds and other animals [13, 21]. First of all, this is due to the high nutritional value of larvae grown on organic waste that contain proteins about 40% and fats about 40%. In the composition of fatty acids, 50% is accounted for by lauric acid, as well as calcium, phosphorus, and iron [7]. The larvae contain beneficial organic compounds, which have commercial and industrial value. Among these elements include: 42.1% -- crude protein; 34.8% -- lipids; 7.0% -- crude fiber; 7.9% is moisture; 1.4% -- free nitrogen extract; 14.6% -- ash; 5.0% -- calcium; 1.5% -- phosphorus [4]. The larvae biomass is used to produce a protein product for feed production. The fly larvae are used in aquaculture systems because the industry is facing a potential shortage of feed protein. Due to the high content of these elements, fly larvae can be used as food for trout. They have a low content of Omega-3 and Omega-6 fatty acids, but, depending on the type of food, the content of these components in the larvae increases [22]. A similar situation in the field of livestock. The geographical isolation of fish production and the decline in world fish stocks may contribute to fishmeal deficiency, which is the main source of protein in animal nutrition. Adding *Lucilia Caesar* fly larvae to cattle and poultry feeds will provide an alternative source of protein in the livestock industry, create an animal nutrition profile, and satisfy by special nutritional needs [17].

Nowadays, secondary raw ingredients of animal and vegetable origin are widely used in feed production: whey, buttermilk, meat, bone, blood and fish meal, vegetable oil cake, etc. [20].

Feed meal is an additional resource, especially protein nutrition, meat and bone meal have a high nutritional value and is used both in natural form and as a premix for the manufacture of animal feed [25].

An important protein component of animal feed is meat and bone meal. It is produced mainly in meat processing plants, in technical areas, as well as in poultry farms [5]. Meat and bone meal are a protein feed of animal origin intended for poultry, pigs. Its addition to the feed allows increasing their productivity, enriches with proteins, useful minerals and vitamins, reduces the cost of feed, thus it is important to monitor the quality of meat and bone meal [6].

Feed meal of animal origin is produced in loose and granulated form, in accordance with the requirements of GOST 17536-82 "Feed flour of animal origin" according to technological instructions in compliance with sanitary and veterinary-sanitary rules approved in the prescribed manner (GOST (Russian National State Standard) 17536--1982).

If the conditions for obtaining and storing secondary resources of both vegetable and animal origin are not followed, the development of undesirable microflora and its dissemination of finished feed are most often observed. This represents a real danger to animal health and the human life quality [15, 19].

Researchers are focusing increasingly on antibacterial substances that can suppress the growth of pathogenic, commensal and putrefactive microorganisms. According to studies, during the fly larvae growth, a significant number of pathogens (*Escherichia coli, Salmonella Enterica*) are suppressed [8], which explains the presence of antimicrobial proteins in insects [11]. Another group of scientists has proven the antibacterial properties of the larvae of *Lucilia caesar* flies and their metabolic products [4].

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Currently, many programs have been proposed involving the development of non-traditional methods, when insect larvae utilizing organic animal waste are used as sources of feed protein. The use of this method, along with the production of valuable protein feed, makes it possible to protect the environment from pollution by livestock waste from farms and poultry farms, to control the number of flies, to improve the sanitary and hygienic situation, and to reduce the epidemiological and epizootological hazards at livestock facilities.

In this regard, the search for new forms of high-quality and safe raw materials are the overriding priority of productive feed production.

The functional and technological properties should be taken into account while feed production. The functional and technological properties of the product, such as water-holding capacity and fat-holding capacity are important from the point of view of finished product consistency. Of great importance are the functional properties of protein preparations, characterizing their interaction with fats and surface-active properties. Water-holding capacity is the property of protein preparations to absorb and retain water due to the presence of hydrophilic groups. The water-holding ability characterizes the properties of the protein product to firmly bind free moisture during the technological processing of the food product. This property allows predicting the content of protein products in the formulation to provide the necessary water-holding and rheological properties of the product, its consistency, increase yield, reduce losses and waste during processing. Fat-holding capacity characterizes the ability to absorb and retain fat. On the surface of the protein molecule are hydrophilic and hydrophobic groups, thanks to hydrophobic bonds, the protein molecule has the ability to retain fat molecules. The fat-holding ability can also be explained by the physical capture, binding and retention of oil by a protein molecule that has a porous structure [2].

Thus, when considering Zooprotein as a feed additive, it is necessary to take into account the functional and technological properties of the product [16].

In present study, we assess the functional and technological properties of high-protein preparation Zooprotein in comparison with other widely used raw ingredients, in particular, fish meal and meat and bone meal.

2. Methods and Equipment

2.1. Sampling

As objects of research was used following raw ingredients:

- fish meal in accordance with GOST 2116-2000 "Feed meal from fish, marine mammals, crustaceans and invertebrates".

- meat and bone meal in accordance with GOST 17536-1982 "Feed flour of animal origin".

- The protein-lipid preparation (commercial name -- ``Zooprotein'') was manufactured at the enterprise ``New Biotechnologies'' LLC (Lipetsk) according to the Technical Specifications. This company specializes in the production of high-protein product from dried and crushed fly larvae of the species *Lucilia Caesar*.

2.2. Determination of the water-holding capacity of samples

For the study, a certain portion of the previously prepared crushed dry mix was measured in an amount of 2 g. The measured portion of the dry mix was transferred to a special centrifuge tube, where it was mixed with 25 g of distilled water and vigorously stirred for 1 minute until a homogeneous suspension with using a vortex magnetic stirrer. After that, the prepared samples were centrifuged on a SIGMA 3-16L equipment at 4115 rpm for 10 minutes. At the end of the centrifugation, the amount of released supernatant was measured. The results of the experiment were calculated by the formula:

$$WHC\,(g/100g) = \frac{watermass\,(g) - supernatantmass\,(g)}{dryweight\,(g)} \cdot 100 \quad (1)$$

For the value of the WHC used the average amount of water (in grams), which was kept dry mixture in the process of the experiment in terms of 100 g of dry mixture.

2.3. Determination of the fat-holding capacity of samples

For the study, a certain portion of the previously prepared crushed dry mix was measured in an amount of 2 g. The measured portion of the dry mix was transferred to a special centrifuge tube, where it was mixed with 20 ml of vegetable oil (corn oil with a density of 0.92 g / ml) and thoroughly mixed for 1 minutes to a homogeneous suspension using a vortex magnetic stirrer. After that, the prepared samples were centrifuged at 4115 rpm for 10 minutes and then the volume of the supernatant of the studied samples was measured.

The results of the experiment were calculated by the formula:

$$FHC\,(g/100g) = \frac{oilmass\,(g) - supernatantmass\,(g)}{dryweight\,(g)} \cdot 100 \quad (2)$$

The amount of oil (g) in the dry mixture was taken as the averaged amount of fat-holding capacity (FHC), which was held by the dry mixture during the experiment in terms of 100 g of dry mixture.

The composition balance index with a different combination of raw ingredients was evaluated by 3-th constituents (n) according to formula 3 (Lisin, 2018). Optimum product balance is achieved when indicator Di is equal 1.

$$D_i = \sqrt[n]{\Pi_{i=1}^{n} U_i} = \sqrt[n]{U_{WHC} \cdot U_{FHC} \cdot U_{CP}} \quad (3)$$

Where,

U_{WHC} -- *index of the water-holding capacity (WHC);*

U_{FHC} -- *index of the fat-holding capacity (FHC);*

U_{CP} -- *index of the cost price (CP).*

On condition:

$U_i = \left(\frac{u_i}{u_d}\right)$, *if* $u_i \leq u_d$

$U_i = \left(\frac{u_d}{u_i}\right)$, *if* $u_i \geq u_d$

Where,

u_i -- *indicator of WHC, FHC and CP in samples;*

u_d --*desirable indicator of WHC, FHC and CP;*

i=1 is WHC; i=2 -- FHC, i= 3 -- CP.

The logistic function of E.K. Harrington known as the scale of desirability was used for the analysis of the calculated data. The scale of desirability is divided in the range from 0 to 1 into five sub-ranges: [0-0.2] as ``very bad''; [0.2-0.37] as ``bad''; [0.37-0.63] as ``satisfactory''; [0.63-0.8] as ``good''; [0.8-1] as ``very good'' (Pichkalev, 2012).

2.4. Statistical analysis

Studies of the functional and technological properties of the samples were carried out in triplicate, the data were processed by the method of mathematical statistics with finding the confidence interval with a probability of 0.95 using MS Excel.

3. Results

The larvae of flies of the species *Lucilia Caesar* are unpretentious to the conditions of cultivation, in particular, are grown on food waste as a substrate. Despite this, the results of a previous study were obtained, which showed the antimicrobial activity of *L.*

caesar larvae using indicator microorganisms, which are pathogenic for humans and birds (*Salmonella enteritidis, Staphylococcus aureus, Enterococcus faecium, Listeria monocytogenes, Clostridium perfringens, Escherichia coli 015, Pseudomonas aeruginosa*). Growing *L. caesar* fly larvae on forcemeat, artificially contaminated, caused growth inhibition of pathogenic bacteria from the substrate. The obtained results on the antimicrobial activity of *L. caesar* larvae towards a number of bacterial infections in humans and birds allows considering the protein-lipid preparation from dried and crushed fly larvae of the species *Lucilia Caesar* as a promising feed production resource agreeable to the safety standards [23].

In addition, it is important to evaluate raw materials for feed production from the point of view of functional and technological properties, such as water-holding capacity (WHC) and fat-holding capacity (FHC). The water-binding capacity of the feed meal determines its quality during processing. It is difficult to obtain high-quality products from feed meal with a low water-holding capacity, since the loss of moisture and, accordingly, soluble substances are high during processing. As a result, the rapid determination of the water-holding capacity of raw materials is very important in the practice of feed production. High fat-holding capacity provides a uniform texture of products, eliminates the separation of fat, reduces losses during the process.

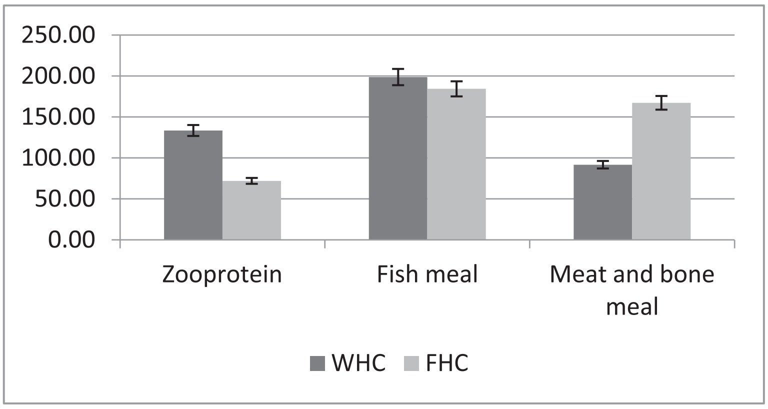

Figure 1: Comparative analysis of functional and technological properties of feed raw materials.

For this purpose, a comparative analysis of the functional and technological properties of Zooprotein was carried out in comparison with the most common raw materials for feed production, in particular, fish meal and meat and bone meal. The results of the study are shown in Figure 1.

4. Discussion

Based on the obtained data, it can be stated that fishmeal has the most intense water- and fat capacity compared to other raw ingredients. It is worth noting that Zooprotein is inferior to fishmeal in water-holding capacity (by 65.23), but surpasses meat and bone meal in this indicator (by 41.77). At the same time, Zooprotein has the least intense fat-holding capacity, which is 112.33 and 95.28 lower than this indicator for fish and meat and bone meal, respectively.

Table 1: The functional and technological properties of feed raw materials.

Samples	Research indicators			
	WHC, g/100g	FHC, g/100g	CP, rub/kg	D_i
Zooprotein	133.50	72.00	50.00	-
Fish meal	198.73	184.33	40.00	-
Meat and bone meal	91.73	167.28	27.00	-
1:1:1	141.32	141.20	39.00	0.81
1:2:1	155.67	151.98	39.25	0.86
1:1:2	128.92	147.72	36.00	0.78
2:1:1	139.36	123.90	41.75	0.77

A comparative analysis of the samples cost price was examined, in particular, the indicator for fish meal was 40 rub/kg, for meat and bone meal -- 27 rub/kg, Zooprotein -- 50 rub/kg.

Consequently, Zooprotein has the highest cost, which is 23 and 10 rubles per kg higher than the cost of meat and bone meal and fish, respectively.

Based on the results of the present study, it was revealed that each studied raw material resource has a number of advantages and disadvantages. It is proposed to consider Zooprotein, fish and meat and bone meal in various combinations: 1:1:1; 1:2:1; 1:1:2; 2:1:1 to determine a rational combination of raw materials. For these combinations of raw ingredients, the studied parameters were identified, in particular, WHC, FHC and CP. Assessment of the quality indicators of the samples was carried out according to three indicators, in particular, the WHC index, the FHC index and the CP index. The highest values of raw materials were used as the desired indicators of WHC and FHC, equal to 198.73 (for WHC) and 184.33 (FHC), which are common to fish meal. The lowest cost value for meat and bone meal, equal to 27 rubles per kg, was considered as the desired cost indicator. According to the results of the present study, it was found that all combinations have a fairly high index of balance. Samples with a combination of raw materials Zooprotein: fish meal:meat and bone meal in a ratio of 1:1:2 and 2:1:1 have balance indices of 0.78 and 0.77, respectively, which are rated as ``satisfactory'' according to Harrington's desirability function. Samples with a combination of raw materials in the ratio 1:1:1 and 1:2:1 showed higher balance indices equal to 0.81 and 0.86, respectively, which are rated as "very good" according to Harrington's desirability function.

5. Conclusion

As a result of the present research, the functional and technological properties of raw ingredients for feed production and their combinations are determined. The mathematical method of analysis established the balance index of the composition of various combinations for raw ingredients, such as Zooprotein, fish meal, meat and bone meal. The assessment of the numerical values of the balance index of various samples combinations on the Harrington desirability scale showed that a sample with a combination of 1:2:1 had the highest rate of 0.86. The evaluation of the balance index for various combinations of raw ingredients showed a satisfactory balance level for all studied samples. The competitive cost of the considered combinations of samples allows considering them as the basis for the preparation of animal feed formulations, which is more relevant for cats as carnivores.

Conflict of Interest

The authors have no conflict of interest to declare.

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