BLACK SOLDIER FLY LARVAE MEAL AS FEED INGREDIENT FOR LABORATORY MICE

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Abstract

The condition of laboratory animals chosen for biomedical experiments—such as their appearance, health, genetic consistency, and the care, maintenance, and feeding they receive—has a significant impact on the outcomes and conclusions of experimental research. In experiments testing the effects of various substances, it is often necessary to determine the concentration of the substance being studied. However, animals can sometimes gain excess weight beyond the normal range for their age during feeding, and alterations in internal organs are commonly found during post-mortem examinations.

Laboratory mice have unique dietary requirements that must be met to support their growth, reproduction, and immune responses to pathogens or environmental stress from handling and experimental procedures.

The aim of this study was to examine the changes in growth in mice when black soldier fly (BSF) larvae meal was added to their diet. The results showed that a 4.0% inclusion of BSF larvae meal had a positive effect on the growth of the laboratory mice compared to those fed a standard diet. Overall, BSF larvae could serve as a viable alternative protein source in animal feed.

Key words*: laboratory mice, BSF larvae meal*

INTRODUCTION

The laboratory mice we know today are the result of more than a century of selective breeding aimed at developing desirable traits for research. These mice have played a crucial role in advancing our understanding of biology and remain the preferred model for studying human biology, diseases, and pathological processes. Their use has been fundamental to many significant medical breakthroughs, making them an indispensable tool for researchers across various scientific disciplines [1].

Mice have specific nutritional requirements that must be met to ensure their potential for growth, reproduction, and response to pathogens or various environmental stressors, triggered by handling and experimental interventions. Currently, diets for research mice are commercially produced in an industrial process, where food safety is addressed through the analysis and control of all biological and chemical materials used in various dietary formulations. Similar to human food, mouse diets must be prepared under good sanitary conditions and labeled to provide information about all actual ingredients.

Currently, over 80% of the protein sources needed for animal husbandry in the European Union (EU) are imported from non-EU countries. At the same time, climate change will increase the cost of animal proteins and other feedstuffs due to a higher demand for large areas of land and water for growing feed crops.

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Throughout history, numerous studies have been dedicated to highlighting the role of various dietary components on the physiological state of the organism and the development of different diseases. However, existing feeding systems are not physiologically justified and are far from perfect [2]. The search for ways to utilize by-products with appropriate nutritional value for animal feed, while reducing environmental pollution, is increasing [3, 4].

Insect meal could be a potential substitute for protein ingredients in animal feed. In recent years, insects have been considered one of the most promising alternatives to protein ingredients (such as soybean meal and fishmeal) for animal feed. Following the approval of insect-derived products for use in fish feed, it is expected that in the coming years the EU will extend authorization to other food-producing animal species. Insects are a potential source of protein that could contribute to freeing up land for growing crops needed for direct human consumption [5].

The aim of this research was to study the changes in the growth and food consumption of mice by including different feed components in their diets.

MATERIAL AND METHOD

The study on the impact of feeding with combined feeds on the growth and metabolism of laboratory animals using alternative food resources was conducted as an experiment involving 28 white laboratory mice, divided into 2 groups: the "Control Group" and the "Experimental Group" (with 7 males and 7 females in each). The mice were kept under 12-hour light/dark cycles with a standard diet for rodents.

The mice were housed in standard transparent cages (38 × 22 × 15 cm), with 7 males and 7 females kept separately, having ad libitum access to food and water. The cages were equipped with wood shavings as bedding material and a paper towel as nesting material.

Individual markings were used to identify all animals. The mice maintenance room in the experiment was set to a 12-hour light/dark cycle with lights off at 8:00 AM, a temperature of approximately 22°C, and a relative air humidity of around 50%.

Photo 1. Individual weighing of laboratory mice

In the scientific experiment, the experimental mice were weighed weekly after the first week and before starting different feeding routines. The individuals were weighed using a digital scale (accuracy: 0.1 g; CM 150-1N, Kern, Balligen, Germany).

Autoclaved complete feed was used for feeding the laboratory mice. A typical maintenance diet for laboratory rodents was used, with a metabolizable energy content of approximately 3227 kcal/kg, consisting of 11% fat, 24% protein, and 65% carbohydrates. All experimental procedures were carried out by an experienced researcher who specializes in mouse breeding.

The research conducted within the Animal Resources and Food Safety Department aimed to evaluate the influence of different types of feed on the body weight dynamics and health status of laboratory mice.

In the experiment, the mice were divided into 2 groups: Group 1 was the control group and received a basic compound feed, which included a plant-based fishmeal substitute as the protein component. The second group of mice (EG – experimental group) received the same basic compound feed, but the plant-based fishmeal substitute was replaced with larva meal. All experimental groups of mice were provided with food allocations in accordance with official regulations on the feeding of laboratory animals.

The condition of the laboratory animals selected for the scientific experiment-their appearance, health, genetic homogeneity, as well as care, maintenance, and feeding conditions-largely determines the actual results and conclusions of the experimental work [6, 7].

The animals were fed according to the standard ration formulated for laboratory mice [8]. The determination of the nutritional value of the feeds, as well as the compound feeds used in the diet of the laboratory mice, can be conducted following the analysis of their chemical composition. This analysis was carried out within the Animal Resources and Food Safety Department at the Faculty of Agricultural, Forestry, and Environmental Sciences, UTM.

Based on the obtained data, the nutritional value was calculated, and using e Hybrimin software, the rations for the laboratory mice used in the research experiments were determined. These experiments included the incorporation of non-traditional feed obtained from black soldier fly larvae (Hermetia illucens larva meal).

Similarly, in the experiments conducted, the determination of macroelements was carried out at the Institute of Chemistry of the State University of Moldova, with research conducted on a contractual basis, where the levels of Ca and P in the feeds used for laboratory animals were assessed.

The compound feeds used in the research were produced by the feed manufacturing enterprise AO "Biochimica" in Bălți, Moldova, according to recipes developed by the Department of Animal Resources and Food Safety at the Technical University of Moldova. This was done for conducting an experiment on laboratory mice to evaluate the effectiveness of their use when supplemented with various non-traditional feeds.

The data obtained during the research were statistically analyzed using the method of variation statistics as described by Plohinschi N., 1969 [9].

RESULTS

Insect meal (photo 2) could represent a potential substitute for protein ingredients in animal feed.

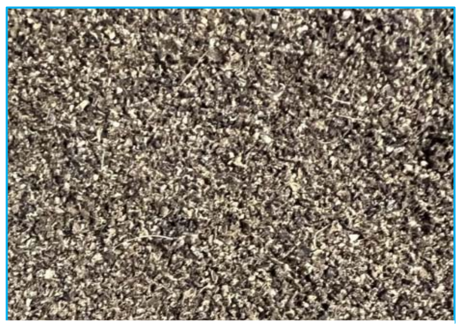

Photo 2. Larva meal from *Hermetia illucens*

In recent years, insects have been considered one of the most promising alternatives to traditional protein ingredients (such as soybean meal and fishmeal) for animal feed. Following the approval of insect-derived products for use in fish feed, it is expected that the EU will extend authorization to other food-producing animal species in the coming years. Insects are a potential source of protein that could help free up land for growing crops needed for direct human consumption [10].

Insect proteins have the potential to support a circular economy within the human food chain, as insects can be raised on organic waste from the human food supply chain. This waste recycling capability utilizes resources more effectively and reduces the volume of waste [11].

The larvae (photo 4) contain proteins—up to 50% of their weight—and can be transformed into a variety of products. The main product is the meal (photo 3), which is widely used as a food source for pigs, poultry, aquaculture, etc. The meal obtained from larvae can replace fishmeal, reducing feed costs, as it is significantly less expensive while still providing valuable protein.

Photo 3. Larvae of the *Hermetia illucens* (black soldier fly) * [14]

Larva meal from black soldier flies is already being produced on a moderate industrial scale, but the use of insect meal in Europe and the UK is currently limited to high-margin niches in the pet food sector [12].

Various types of rations are available for laboratory animals. The ideal ration for a particular animal species depends on the production environment or the experimental objectives. Additionally, the ration should be palatable enough to ensure adequate food intake and nutritionally balanced to provide the necessary nutrients to achieve the proposed goals. The diets used in studies should also be easy to reproduce, allowing results to be confirmed through further research.

The choice of the most suitable type will depend on the degree of control over the composition of necessary nutrients, the need to add substances for testing, the potential effects of microbes in the animal feed, the diet's acceptability to the animals, and cost. Waste is also an issue with certain types of rations, which can be a disadvantage when trying to measure quantitative intake accurately.

When establishing target concentrations of nutrients, the estimated requirements for nutrients, potential nutrient losses during production and storage, nutrient bioavailability in ingredients, and potential nutrient interactions must be considered.

The basic compound feed had the same characteristics as the standard diet recommended for laboratory mice [13].

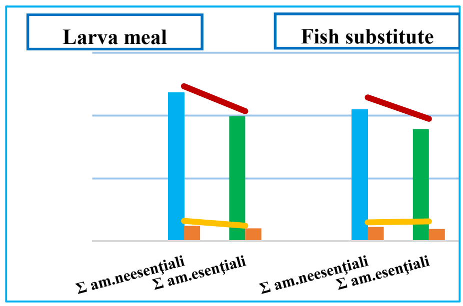

Fig. 1. Content of essential and non-essential amino acids in larva meal and

Table 1. Composition of the compound feeds for laboratory mice in the experiment

Ingredients	CF, % / Control group	CF2, % / Experimental group
Extruded corn	36,0	36,0
Extruded wheat	32,0	32,0
Barley	13,0	13,0
Soybean meal	8,5	8,5
Fish substitute	4,0	4,0*
Skim milk powder	3,5	3,5
Chalk	0,84	0,84
Monocalcium phosphate	0,74	0,74
Salt	0,42	0,42
Premix	1,0	1,0

**- Larva meal*

All the nutritional parameters of this diet comply with the NRC recommendations for rats and mice (Tables 1 and 2).

Table 2. The main nutritional indicators of compound feed

Ingredients	%
Crude protein	19,0
Crude fiber	3,0
Crude fat	5,5
Ca	0,9
P	0,6
Na	0,18
Metabolizable energy, kcal/100 g	330,0

The diet was formulated as a general diet for the growth of all strains of mice. The total fat content was kept at approximately 5% to maximize the long-term productivity of most animals. Nutritional factors are sources of variability for research, which can also affect behavior. As such, the food provided to research animals should be palatable, uncontaminated, and nutritionally adequate.

DISCUSSIONS

Research conducted within the Animal Resources and Food Safety Department aimed to evaluate the influence of different types of feed on the body weight dynamics and health status of laboratory mice. The accepted norm in most laboratories worldwide is to feed laboratory mice ad libitum, although several health deficiencies are well established. In contrast, reducing the body weight of animals by feeding them no less than once a day (referred to as a 24-hour feeding schedule) has been shown to improve lifespan and reduce susceptibility to diseases.

Against this backdrop, our study aimed to investigate the effects of feeding laboratory mice with different compound feeds. Therefore, the effect of *ad libitum* feeding in groups fed with various compound feeds was established by determining the body weight development of the animals and assessing the health status of both male and female mice.

To establish the feeding characteristics affecting the physiological status of the laboratory mice, their weights were recorded weekly.

Regardless of the feeding method used for the laboratory mice described above, the component feeds in the compound rations positively influenced the weight gain of the animals. Specifically, mice that consumed the compound feed supplemented with black soldier fly larvae meal achieved greater body weight gain compared to the control group.

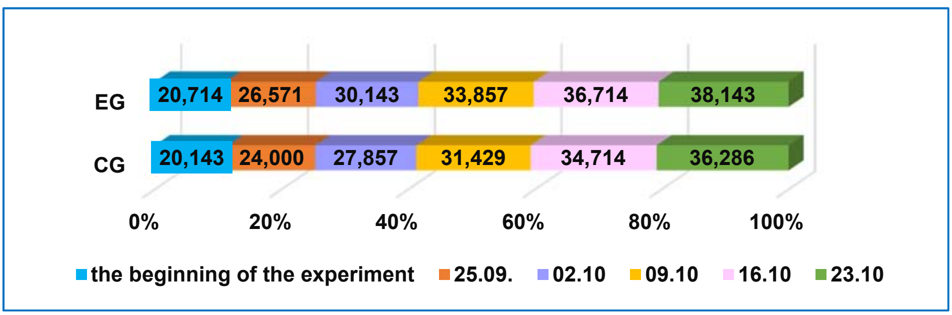

Fig. 2. Body weight of males in the experimental groups by week, g

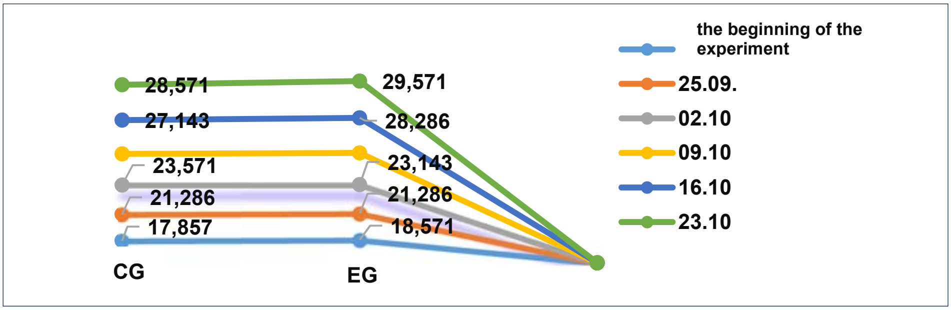

Fig. 3. Body weight of females in the experimental groups by week, g

In the experimental group, a higher body weight was observed at the end of the experiment, averaging 38.143 g for males and 29.571 g for females. This indicator reflects the positive influence of the supplemented unconventional feeds in the compound diet administered to the laboratory mice. The average daily gain, closely correlated with body weight, showed lower values at the end of the experiment in the experimental group compared to the control group.

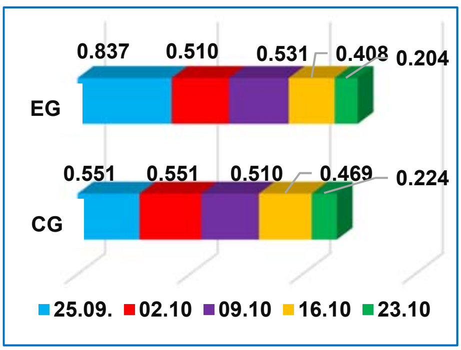

Fig. 4. Average daily gain of experimental mice, males, g

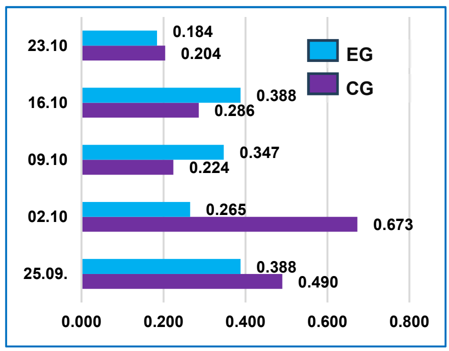

Fig. 5. Average daily gain of experimental mice, females, g

If we analyze the data obtained by sex groups, at the beginning of the experiment, the animals in the control group had a higher average daily gain compared to the experimental group. However, this gain decreased toward the end of the experiment, remaining low until the conclusion of the study. Similarly, these parameters were 0.184 for females compared to 0.204 for the control group.

From the data collected, we can also conclude that females and males grow at different rates. From the research data, we can further conclude that females and males grow at different rates. Then was examined the consumption of compound feed across the groups to assess how unconventional feeds impacted the feed intake capacity of laboratory mice.

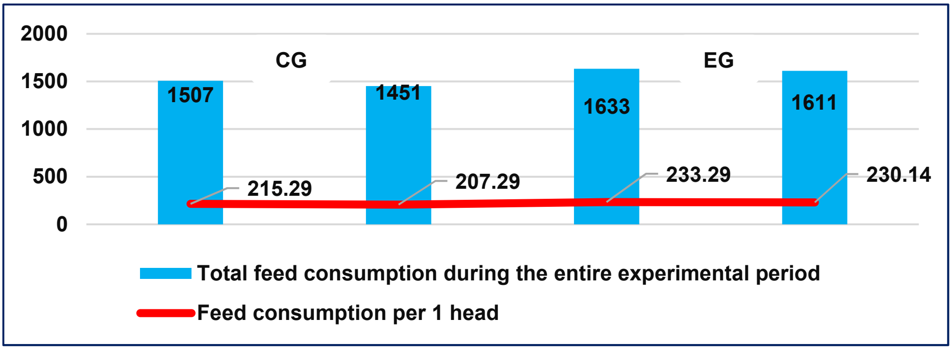

Fig. 6. Feed consumption during the experimental period, g

CONCLUSIONS

The unconventional feed, black soldier fly larvae meal, did not have a negative impact on the growth of laboratory mice and, in fact, had a positive effect as follows:

- the body weight of laboratory mice receiving the compound feed supplemented with unconventional feed was higher in the experimental group compared to the control group. Specifically, by sex groups, the difference was 1.86 g for males and 1.00 g for females compared to the control group (CG), which corresponds to an increase of 5.12% and 3.50%, respectively;
- the average daily gain, which is correlated with body weight, was also greater in the experimental group.

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