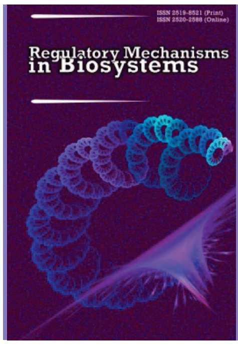

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Effects of nutrient medium on various-age larvae of *Hermetia illucens* (Diptera, Stratiomyidae)

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The saprophage *Hermetia illucens* (Linnaeus, 1758) (Diptera, Stratiomyidae) plays a crucial role in the processing of organic wastes, thus contributing to the achievement of the global goals of sustainable development. However, its introduction in the European Union led to intense discussions about the expended application of this insect in new spheres. The article considers a laboratory culture of the fly *H. illucens* as an innovative nutritional product. The objective of the presented study was assessing the influence of nutrition medium on the dynamics of gains in the mass and biochemical composition of live larvae of the fly. In particular, we measured the mean gain in mass of the larvae over the period of consuming the diet, vitality of the larvae, and the content of protein, fat, ash, and water. In the studies, we tested seven diets for fly larvae in relation to the dynamics in their mass gain. The studies revealed that wheat bran and soybean meal led to the highest parameters of mass of the larvae at all development stages, with the highest gains measuring 10.15 and 10.76 g, respectively. Based on those results, the diet of larvae was further optimized: the main component, wheat bran, was supplemented with the following additional ingredients: soybean meal, yeasts, glucose, and vitamin B9. Such a composition of diet promoted gain in the mass of the larvae, which on average accounted for 14.06 g. The same changes were observed in the accumulation of proteins and fats – 16.8% and 20.4%, respectively, which may suggest heightened nutritional value of the larvae. Due to the high adaptability and varying abilities of biochemical composition, one can select an artificial medium in cases of different proportions. This study confirmed that larvae of the fly can be a source of protein and fatty acids for the food industry in the future and thus improve the qualitative and nutritional characteristics of end products.

Keywords: saprophage; insect breeding; gain in mass; chemical composition; adaptation; food products.

Introduction

One of the most important tasks worldwide is improvement of the nutritional structure of the population by increasing the production of mass-market foods with high nutritional and biological values, which correspond to the modern demands in the level of safety. Recent medical-physiological studies and theories about nutritional needs as the basis of vital activity of the human organism have led to the revision of both nutrition in general and production of individual food products. Most of the population of Ukraine consumes an unbalanced diet: researchers state the deficiency of full-protein, water- and fat-soluble vitamins, and a number of mineral compounds. At the same time, there is excessive consumption of products with high levels of animal fats, simple hydrocarbons, and synthesized food supplements (Gahukar, 2011; Slobodianiuk et al., 2018).

Currently, there is a deficit of complete-protein food products associated with prices for meat products rising during market transformations and emergence of low-quality products, which are poorly metabolized in the organism. Furthermore, production of foods of high-quality natural raw material is economically not profitable all around the world. This has led to a tendency of producing herodietic (health-improving) nutrition or functional products of special purpose, which combine the meat raw material with low-value raw materials of animal and plant origins. The special health-improving products help correct different deficits of biologically active compounds in humans (Peshuk, 2017). Recently, combining proteins of plant and animal origins has become widely popular in the production of meat products, because it allows broadening the raw material resources, and also improves the quality of food products, raw materials, and – to a substantial degree – the technological regimes of obtaining protein products (flour, concentrates, isolates) (Nadeau, 2015). Therefore, the search for new sources of protein and the study of the products resulting from their processing are of great scientific and practical interest and is a relevant task in the food industry.

In the modern world, scientists have taken a broad interest in promising insects that are used in various applications, including beekeeping, silk production, and plant protection (Laurenza & Carreno, 2015; Wang et al., 2017; Statkevych & Drozda, 2020). At the same time, insects are also considered a source of food protein and fats with unique properties. In this case, this is a widely known fly *H. illucens* (Salomone et al., 2017; Liu et al., 2020). The natural range of the fly's population includes the regions with subtropical climate, particularly, in North and South America. The peculiarity of this insect is its ability to develop in a pure culture throughout the year in man-made conditions with highly effective bioconversion of various solid organic wastes. Therefore, *H. illucens* can be grown in laboratory settings (Sheppard et al., 1994; Cickova et al., 2015; Dickerson et al., 2024).

The trophic specialization of the fly larva is related to practically all types of biowastes. Nutrients included in the diet of *H. illucens* concentrate in the larvae in the form of high-value protein that is used in food technologies as a bioadditive (Spranghers et al., 2017; He et al., 2024; Tognocchi et al., 2024). Moreover, insect protein has numerous advantages compared with protein from cattle. From the ecological perspective, raising flies does not require large land resources, and therefore can reduce the greenhouse gas emissions, as compared with agricultural animals. At the same time, the chemical composition of *H. illucens* includes a large amount of protein, over three times as much as calf meat (Cullere et al., 2017; Deguara et al., 2024; Fahmy et al., 2024).

The countries of the European Union are actively researching the adaptive abilities of flies (Carpentier et al., 2024; Lo et al., 2024; Morales-Ramos et al., 2024). At the same time, research is focused on advancing agricultural farming and the food industry, which will allow development of a number of directions in biotechnology and thus improve the ecological state of the environment (Babytskiy et al., 2020; Koyunoglu, 2024; Rodriguez-Rodriguez et al., 2024).

There is research which that partly demonstrates the possibility of using insects as a source of balanced nutrients for food technologies. Ultimately, the objective of our studies was determining the biochemical composition and dynamics of mass gain in the larvae of *H. illucens* during different periods of their development, depending on growth medium in specific conditions of species domestication.

Materials and methods

A complex of laboratory studies that included the technological methods of fly rearing was conducted during 2022–2024. The species were identified according to the morphological features. In particular, the length of imagoes varied 15–20 mm, the head position was prognathous, the licking-type oral apparatus was poorly expressed, the antennae were twice shorter than the head and had an elongated segment at the tip, and the legs were of running type, with the typical color (Fig. 1). The color of an adult individual was black, with smokey transparent wings. Sexual dimorphism was present in the form of colorless hairs on the lower part of the head.

Fig. 1. The imago stage of *H. illucens*

The larval stage was divided into several age groups that were identified according to the linear parameters of the biomaterial. At the same time, larvae at the initial stage, just after hatching, reached 5.12 ± 0.31 mm, at the first (I) stage lasting for six days the larvae were 10.23 ± 0.82 mm, at the second (II) stage lasting for 9 days their length was up to 15.25 ± 1.02 mm, at the third age stage lasting 12 days their length was up up to 18.14 ± 1.32 mm, and at the fourth (IV) stage lasting over 15 days the larvae measured up to 28.03 ± 1.87 mm (Fig. 2). At the final stage of development (pre-pupa) lasting 10 days their length was up to 19.15 ± 0.63 mm.

The experimental culture of the flies was raised under the hydrothermal conditions that are optimal for their development in a climate chamber (Memmert HPP260eco, Germany, 2019). The fly imagoes were held in special containers, where the devices for collecting eggs were placed. The embryonic development of the eggs and larvae occurred at the following parameters: the temperature of 27 ± 1.0 ºC, the relative humidity of 70%, and 14:10 L:D photoperiod. Prior to the experiment, the newly hatched larvae were put in plastic containers (40 x 30 x 10 cm) with a diet that was optimal for their laboratory maintenance (60% wheat bran and 40% fruit pieces) and held for four days. Then, the larvae were weighed at the initial mass, sorted for seven diets and held in a climate chamber. To record the studied parameters, we collected 100 specimens of larvae every three days (Scala et al., 2020).

The mean increment of biomass of the fly larvae during the certain time periods of their development was measured every three days of the experiment, separating the larvae from the residuals of the growth medium (Scala et al., 2020). At the same time, to quantitatively identify the biomass gain, we made records of their weight and determined the mean values by dividing the total mass of samples of larvae of a certain age (I–IV) by the number of analyzed samples.

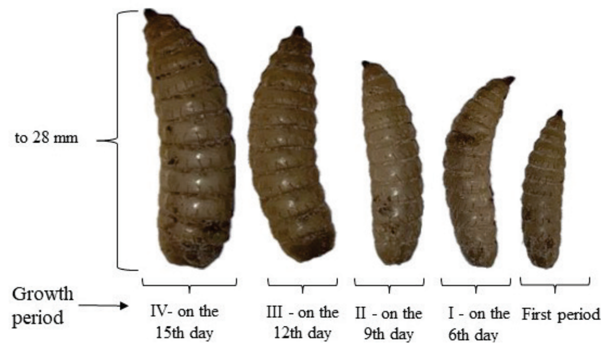

Fig. 2. The age structure of *H. illucens* larvae

The mean biomass gain in the larvae throughout the development period was determined as a difference between the end average mass of age IV larva and the mean initial mass of age I larvae (Scala et al., 2020).

The mass share of humidity was determined by drying sample of the product to the constant mass at a temperature of 100–105 ºC; mass ash share by the weighing method, after mineralization of the weighed amount of the product in a muffle furnace at a temperature of 500–600 °C; the mass share of lipids was determined by the Soxhlet's method, when fat is weighed after it is extracted by a solvent from a dry weighed amount in a Soxhlet's apparatus, based on determining changes in the mass of sample after extraction of fat with solvent; the mass share of protein was measured according to the general nitrogen using the Kjeldahl method. The ashing of the samples was conducted on a mineralizer (SOX 406, China, 2014) with a vacuum pump (JP, Italy, 2012). The pumping was carried out on the apparatus on a vapor exchange unit (Velp Scientifica UDK 129, Italy, 2022) (Slobodianiuk et al., 2018).

The statistical analysis of the results was conducted using ANOVA; the data in the tables are presented in the form of x ± SD (mean value ± standard deviation). The difference among the group was considered significant according to the Tukey Test ($P < 0.05$).

Results

Considering the broad array of compositions of growth media for insects and methods of their feeding, the peculiarities of feeding saprophage insects can be well illustrated on the example of larvae of the *H. illucens* fly. Important aspect for understanding of the metabolism pattern in insects is also the data on peculiarities of the structure of their digestive system. As is known, the digestive system is a regulator of homeostasis in the internal environment of the body. First and foremost, fly larvae adapt so as to live in relatively homeostatic feeding conditions, with stable influx of necessary energy and organic compounds into the body.

The composition of growth medium for feeding fly larvae is demonstrated in Table 1, in particular, the diet was divided into three groups: animal fodder (wheat bran, soybean meal), food waste (confectionary and pieces of vegetables, fruits, and greens) and waste of animal farming (bird guano, pig manure). The observations revealed that the composition and suitability of the main components of the substrates significantly affect the biological parameters of larvae. In particular, we assessed the mass gain in the larvae during different periods of development, and also their vitality, and the level of substrate reduction.

The dynamics of live biomass gain in the larvae ranged on average 5.19 g/100 specimens (waste of animal farming) to 10.76 g/100 specimens (animal fodders). Therefore, the group given food waste produced moderate results: the groups given pieces of vegetables and fruits and confectionery produced gains in mass per 100 individuals of around 9.20 ± 0.87 and 9.78 ± 1.96 g, respectively, while the group given meat waste showed a slightly lower result, equaling 8.78 ± 1.23 g. The mass of the larvae that consumed animal fodder was higher, wheat bran and soybean meal producing the values of 10.15 ± 1.20 and 10.76 ± 0.73 g,/100 specimens respectively. On the other hand, the group given animal farming waste displayed the lowest parameters of mass gain. Such differences among the variants indicate that the population is quite variable in different conditions of the development, having high adaptive properties.

Table 2 demonstrates the modified diets that affected the development of fly larvae. Ultimately, the diet containing wheat bran, soybean mill, food-grade yeasts, glucose, and vitamin B9 did increase the mass and reduced the period of larval development (Table 2). In particular, the mean mass gain per 100 specimens in the larvae consuming this diet accounted for 14.06 ± 1.18 g against the control variant with 10.91 ± 1.59 g. At the same time, the development period in the conditions of such diet equaled 12.52 ± 0.51 days.

Table 1
Analysis of accumulation of mass in the larvae of *H. illucens* depending on the composition of growth medium (x ± SD, n = 8)

Groups of growth media for feeding the fly larvae		Mass of newly hatched larvae during different development period, g/100 specimens					
		mass of the larvae at the beginning of experiment	6 days	9 days	12 days	15 days	mean mass increment during the entire period of consuming the diet, g/100 specimens
Animal fodder	wheat bran	6.47 ± 0.73^{a}	9.32 ± 0.55^{a}	12.46 ± 0.85^{a}	16.32 ± 0.81^{a}	19.43 ± 1.16^{a}	10.15 ± 1.20^{a}
	soybean meal	6.81 ± 0.46^{a}	9.86 ± 0.64^{a}	13.65 ± 0.46^{a}	18.41 ± 0.66^{a}	20.62 ± 0.71^{a}	10.76 ± 0.73^{a}
Food waste	confectionary	5.89 ± 0.91^{a}	6.33 ± 0.73^{b}	9.44 ± 1.12^{b}	12.06 ± 0.89^{b}	16.37 ± 1.63^{b}	9.78 ± 1.96^{ab}
	pieces of fruits and vegetables	6.43 ± 0.83^{a}	8.56 ± 0.96^{ab}	12.93 ± 0.74^{a}	15.31 ± 0.80^{a}	17.75 ± 1.37^{ab}	9.20 ± 0.87^{ab}
	meat waste	5.51 ± 0.64^{a}	9.32 ± 0.49^{a}	11.07 ± 0.68^{ab}	14.34 ± 0.52^{ab}	16.09 ± 0.97^{b}	8.78 ± 1.23^{b}
Waste of animal farming	poultry guano	5.64 ± 0.89^{a}	6.47 ± 0.81^{b}	8.79 ± 0.86^{b}	9.36 ± 1.01^{c}	12.97 ± 1.84^{c}	6.50 ± 1.32^{c}
	pig manure	5.09 ± 0.58^{a}	6.22 ± 1.06^{b}	7.93 ± 0.94^{b}	8.78 ± 1.28^{c}	11.13 ± 2.05^{c}	5.19 ± 1.83^{c}

Table 2
Optimization of growth and development of *H. illucens* by using modified growth media (x ± SD, n = 8)

Variants of growth media	Analyzed parameters					
	mass of the larvae at the beginning of experiment, g/100 specimens	mass of age I larvae, g/100 specimens	end mass of age IV larvae, g/100 specimens	mean mass gain in the larvae over the entire development period (age I–age IV), g/100 specimens	development period of the larval stage, days	share of pupated larvae, %
Wheat bran (control)	6.24 ± 1.64^{a}	9.11 ± 0.82^{b}	20.02 ± 1.41^{b}	10.91 ± 1.59^{a}	17.25 ± 0.43^{a}	88.87 ± 9.44^{b}
Wheat bran (50%) + soybean meal	5.62 ± 0.97^{a}	10.81 ± 1.38^{ab}	23.74 ± 0.91^{ab}	12.93 ± 1.85^{ab}	14.51 ± 0.87^{b}	90.03 ± 7.21^{ab}
Wheat bran (55%) + soybean meal (35%) + dietary supplements: yeasts (4%), glucose in a concentrate of 50-60% (1%), vitamin B_9(1%)	6.02 ± 1.02^{a}	12.14 ± 0.63^{a}	26.46 ± 1.57^{a}	14.06 ± 1.18^{a}	12.25 ± 0.51^{c}	92.94 ± 4.35^{a}

Note: different letters indicate values that are statistically reliably different one from another within one column of the table (Tukey test, P < 0.05).

The types of substrates on which the larvae were grown also affected their biochemical composition. Our observations revealed that the larvae grown on wheat bran and soybean meal accumulated a higher content of proteins and lipids than the larvae that consumed the standard diet (Table 3). In the control substrate, the dynamics of biomass growth slowed, in particular, the growth and development, which in turn resulted in a low percentage of accumulation of proteins and lipids, which accounted for 12.2 ± 0.55% and 14.4 ± 0.61%, respectively. Despite such variability, all the values indicated that the fly is a good source of proteins and fats. To be specific, in an optimal substrate they on average accumulated 16.8 ± 2.14% of protein and 20.3 ± 1.19% of fat, suggesting a positive effect of the additional component on the protein metabolism and storage of energy reserves. According to content of water, we observed different values in the larvae that exerted such a tendency, produced by the feeding substrates. In particular, the larvae had the lowest water content at the final stage (55.8 ± 4.38%), indicating more intensive use of water during growth. The positive dynamics manifested in the ash content, which varied 4.7–6.9%, indicating high content of mineral compounds in live biomass of the larvae. The yielded data state that the fly larvae, among other insects, are a quite good alternative source of nutrients in the food technology.

Table 3
The chemical composition of live *H. illucens* larvae at different development stages subject to different growth media (x ± SD, n = 8)

Groups of nutrient media for feeding the fly larvae	The parameters monitored at different times of the larvae's development, %							
	water content		protein		fat		ash	
	6 days	15 days	6 days	15 days	6 days	15 days	6 days	15 days
Wheat bran (55%) +soybean meal (35%) + dietary supplements: yeasts (4%), glucose in the concentration of 50-60% (1 %), vitamin B_9(1%)	63.87 ± 3.01^{b}	55.82 ± 4.38^{b}	14.06 ± 0.81^{a}	16.83 ± 2.14^{a}	16.08 ± 0.72^{a}	20.38 ± 1.19^{a}	5.67 ± 0.22^{a}	6.89 ± 0.62^{a}
Wheat bran (50%) + soybean meal (50%)	69.24 ± 2.75^{ab}	60.19 ± 2.98^{ab}	11.82 ± 0.59^{b}	14.23 ± 0.84^{b}	15.42 ± 1.54^{ab}	19.45 ± 0.91^{a}	3.54 ± 0.12^{b}	6.15 ± 0.40^{ab}
Wheat bran (control)	75.30 ± 7.36^{a}	68.81 ± 5.41^{a}	9.06 ± 0.64^{c}	12.15 ± 0.55^{c}	12.11 ± 0.77^{b}	14.56 ± 0.61^{b}	2.55 ± 0.34^{c}	4.68 ± 0.43^{b}

Note: different letters indicate the values that are significantly different one from another within one column of the table (Tukey test, P < 0.05).

Discussion

The scientific literature provides a broad spectrum of data on a number of insects and their use in food (Huis et al., 2020). For example, the EU countries have officially allowed four insects to be used for this purpose, and thus they have been successfully integrated into the food industry. Those are larvae of yellow mealworm beetle *Tenebrio molitor* L., migratory locust *Locusta migratoria* L., house cricket *Acheta domesticus* L., and larvae of lesser mealworm *Alphitobius diaperinus* Panz. However, *H. illucens* remains outside the scope of studies specifically in food technologies (Wang & Shelomi, 2017; Cunha et al., 2023). The fly *H. illucens* is one of almost 2,000 insects that can potentially be consumed in dietary and gerodietetic nutrition of humans (Meyer-Rochow & Chakravorty, 2013; Megido et al., 2014). Despite the plethora of the literature data pertaining to insects, the existing research still lacks information on their use on an industrial scale. As of now, there are numerous studies of using biomass of larvae as a food ingredient for feeding animals (Veldkamp, 2015; Shelomi, 2016; Wang & Shelomi, 2017). However, our studies revealed a strong possibility of laboratory cultivation of population of the *H. illucens* fly for the purposes of accumulation of valuable protein in fly larvae to utilize them in food technologies for mankind. From the perspective of the nutrition value of larval biomass, our tests revealed that *H. illucens* can be valuable for the food industry, and marketing as a healthy alternative to animal meat. Moreover, there is potential for a partial substitution of animal meat. The greatest advantage of such raw material in the food industry is its lower impact on the environment, for example, production of 1 kg of beef uses 8–10 kg of feeding substrate (Tomberlin et al., 2015; Hannah et al., 2019; Awasthi et al., 2022), while growing 1 kg of fly larvae in our studies used 2–3 kg of fodder. Such an alternative protein raw material would lead to reduction of greenhouse gas emissions and reduce the excessive use of natural resources such as land, water, and others (Smetana et al., 2015; Smetana, 2016; Wang & Shelomi, 2017; Huis et al., 2020).

Reports have proposed myriad examples of growth media for laboratory maintenance of populations of the fly *H. illucens* (Makkar et al., 2014; Scala et al., 2020; Awasthi et al., 2022). At the same time, populations of the fly were evaluated at the larval stage, determining the mass of larvae and accumulation of energy resources after consuming different food diets. However, the available studies utilized various wastes that are unsuitable for dietary nutrition of humans. We adapted the saprophage specifically to broadly known substrates that are suitable for most regions for laboratory rearing of flies and evaluated the parameters of mass gain and the biochemical composition of the larvae.

Our studies revealed the general patterns of metabolism of nutrients in the fly population, since the biochemical composition of larvae varies greatly and depends not only on their diet, but also the physiological condition of the culture. Indeed, 92.9% of the saprophage larvae completed their development and pupated on the substrates provided. Also, we observed changes in the duration of development and accumulation of mass in the larvae compared with the balanced substrate. Therefore, the greatest biomass gain was produced by the substrate of bran, soybean mill, and dietary supplements, being 3.15 g/100 specimens higher than in those that fed on the standard diet. Perhaps, such an effect was caused by combined content of protein and water.

We should emphasize that the results of our studies had statistical differences in the parameters of biochemical composition of the fly larvae (Araujo et al., 2016; Elhag et al., 2016; Bisconsin-Junior et al., 2022). In particular, the observations revealed that *H. illucens* has a high level of protein, and also several macro- and microelements that are important for the development of animals and human nutrition. The differences with our studies indicate that the types of diets for the larvae which we provided did not lead to negative effects such as loss of energy resources and promoted the accumulation of live biomass with high content of nutrients.

We can state that the most favorable diets for growing larvae of *H. illucens* are animal fodders, which can be used as a basis for growth medium. The peculiarity of such substrates is the high content of cellulose that promotes accumulation of the larvaes' biomass. The vitality of the larvae was enhanced by the substrate structure, specifically the water content and aeration. The data produced in our study indicated that the diet comprising wheat bran and soybean meal created optimal conditions for the larvae, promoting their high metabolic activity, which provided unobstructed molting and pupation, with subsequent hatching of the imago. Based on those studies, the qualitative and quantitative characteristics of fly populations were optimized using modified growth media, making the grown biomaterial potentially suitable in the sphere of food industry.

However, the safety of food products worldwide is regulated by the legal norms. For example, EU countries have more rigid norms regarding insects as new food resources. According to their regulations, raw material must be studied in all aspects and only then can an insect be used in a food product for of humans (Vos, 2000; Knowles et al., 2007; Siegrist, 2008). In the future, our studies will be oriented at in-detail and in-depth analysis of the safety and risks regarding consumption of larval biomass as a dietary supplement in human diet. In particular, we are planning to analyze the sanitary parameters (mesophilous aerobic and facultative-anaerobic organisms), conditionally pathogenic, pathogenic, mold fungi and yeasts, organisms that provoke food deterioration, and also organoleptic parameters and others. Microbiological analysis would allow assessment of the quality of the biomaterial as raw material in food technologies, and also detect microbiological factors that can negatively impact on human health.

Conclusion

In this study, we demonstrated that laboratory culture of *H. illucens* successfully adapts and can be reared in countries with a cold climate, thus highlighting its potential as a source of protein in human food technologies. In different periods of their development, the fly larvae were observed to have varying properties of the biochemical composition and mass gain in general, which significantly depended on diet. In particular, the diet that consisted of wheat bran combined with soybean meal and additional nutrients approximated the parameters of chemical composition of larval biomass to the parameters of meat products. This would allow the flies to be used as a dietary supplement. Despite the fact that the levels of using this raw material in human diet are not established, our studies have been aiming at solving this particular problem, because the agrarian sector has significantly grown over the two recent decades, as have land use and also food production. Ultimately, the searches for alternative types of raw material should be oriented considering the economic and ecological roles in the environment.

Larvae of *H. illucens* are promising food ingredients, because they contain valuable nutrients, but also have additional components that are beneficial for the health of people compared with the regular sources of organic compounds. Despite the fact that production of protein from insects is at the starting stage, the experimental studies have produced significant results.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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