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THE GROWTH PERFORMANCE AND DIGESTIBILITY OF *TENEBRIO MOLITOR* FED WITH TWO DIFFERENT DIETS

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Abstract: *Tenebrio molitor* is an easily obtained highly nutritional alternative source of protein in animal feed. To get good results in growth performance and nutritional composition, it is necessary to know the diet digestibility and dietary needs of *T. molitor*. In the experimental condition, the *T. molitor* was fed with wheat bran and oat flakes, two easily accessible diets on the local market at an acceptable price. Both groups (four replicates each) of *T. molitor* were fed the same diet weekly (25 g of feed and water were supplied through agar). The production parameters such as feed conversion, intake of feed, and growth during the whole experimental period were observed. At the same time, the digestibility study was performed to obtain the coefficient of digestibility of the dry matter, organic matter, ash, crude protein, crude fat, neutral detergent fibre (NDF), acid detergent fibre (ADF), and hemicelluloses. For both diets, the growth parameters were relatively similar having been slightly better for the oat flakes. The feed conversion ratio for wheat bran and oat flakes was 2.16 and 2.00, with growth per unit average of 46.37 g and 50.30 g respectively. The digestibility trial showed much better digestibility of oat flakes with the average digestibility coefficient of dry matter of 0.78, organic matter 0.80, crude protein 0.46, and crude fat 0.85. The digestibility of wheat bran was for dry matter 0.50, organic matter 0.51, crude protein 0.33, and crude fat 0.88. Both diets can be successfully used for the nutritional needs of *T. molitor*, however, using the oat flakes gives better growth performance and digestibility results. The choice of the diet will depend on the costs at the moment.

Key words: mealworm beetle, feed conversion, coefficient of digestibility, wheat bran diet, oat flakes diet

INTRODUCTION

The protein component in the complete feed mixture intended for animal nutrition is usually the most expensive. Soybean as one of the most used protein feeds, has huge requirements for the vast surface of agricultural land which are limited especially in Europe. However, soybean is not a perfect source of protein in animal

nutrition, although the amino acid composition is favourable for animals it lacks in concentration of methionine and cysteine (Tomičić et al., 2020). Serbia is no exception, having limited agricultural land and an intention for huge animal production. Insects as alternative protein sources are becoming

increasingly acceptable in animal nutrition (Van Huis, 2013; De Marco et al. 2015; Pippinato, Gasco, Di Vita & Mancuso, 2020; Hong, Han, & Kim 2020). Up to date, four insect species are allowed in the EU as a source of protein in feed or food: *Tenebrio molitor* larvae (European Commission, 2022a), *Locusta migratoria* (European Commission, 2021), *Acheta domesticus* (European Commission, 2022b) and *Alphitobius diaperinus* larvae (European Commission, 2023). It is expected that the list of edible insects will increase in the future. Imported animal feed with insects as a source of protein is already available on the Serbian market, but there was no legislation regarding the production and use of insects in animal feed. In December 2023, a new legislation allowed the use of the processed protein of animal origin produced from farmed insects intended for the production of animal feed, and only from insects of the following species: *Hermetia illucens*, *Musca domestica*, *T. molitor*, *Alphitobius diaperinus*, *Acheta domesticus*, *Gryllodes sigillatus*, *Gryllus assimilis* and *Bombyx mori* (Pravilnik, 2023). In Europe, there is no tradition of using insects in the nutrition of humans or animals. The very idea of using insects as feed or food evokes strong feelings of discomfort and revulsion. However in some regions, mostly undeveloped regions of the world, there is a long tradition of consumption of insects in the human diet. The edible insects can be found in traditional nutrition mostly in Asia, followed by Africa and South America (Halloran, Roberto & Roos, 2018). However, there is no data regarding the marketing value of edible insects in this type of society.

In recent years, the market of edible insects in Europe has been on a constant rise. Currently, the market size of edible insects in Europe is USD 235.45 million, expected to increase to USD 1794.41 million by 2031, with a compound annual growth rate of 28.90% (DataBridge, 2023).

Under natural conditions, chicken nutrition partly involves the insects available in the surroundings. From the many published manuscripts on the production of broilers and the use of edible insects, the insects are incorporated into the complete feed mixture as a protein meal. Insect processing methods are utilized to prolong product shelf life, extract desired ingredients, or adapt insects into forms tailored for specific applications. Current insect process-

ing techniques rely on established technologies in the food and feed industries, including thermal treatments (blanching, boiling, drying, cooling, freezing, freeze-drying), mechanical processes (grinding, pressing, milling), and fractionation methods (extraction, purification, separation, centrifugation) (Ristić, Smetana & Heinz, 2023). The use of the insect's flour as a protein source gives results as good as the use of soybean or even better in animal nutrition regarding the production parameters of animals such as growth, mortality, weight gain, cetera (Veldkamp et al. 2012; Cullere et al. 2016; Biasato et al. 2017).

The drawbacks of insect-based protein feed include the high production costs and significant initial economic investment required. However, the benefits are reduced land usage and the ability to produce it year-round. If we consider that the life cycle of *T. molitor* in the larval stage is about 90 days, it is possible to grow more production cycles per year. The production of *T. molitor* takes place on several levels (vertical production, or vertical farms), which increases production area.

The development of insect breeding technology will increase the need for a better understanding of the nutritional requirements of insects. The digestibility is similar to the digestibility of monogastric animals with huge differences in the quality of used feed, in insect nutrition the low-quality feeds that are unsuitable for any other animals can be comfortably used with good results in production parameters such as weight gain, consumption, mortality... There is much information about insect feed, from commercially made feed to industrial waste used in various combinations.

Naturally, in each country, there are specifics regarding the representation of certain secondary products, depending on the processing industry, technology, and the like. Two diets were used in this work: wheat bran which is cheap and easily available, and oat flakes unsuitable for the human diet and available due to processing quality.

There are large differences in the design of insect trials. They can generally be divided into those dealing with production data, and to a lesser extent, those trials dealing with nutrient digestibility by larvae and additional production data.

Table 1.

The chemical composition of the materials used in insect diets

Feed material	Dry matter	Organic matter	Ash	Crude protein	Ether extract	ADF	NDF	Hemicelluloses
Wheat bran	90.88	85.86	5.02	16.73	4.1	11.63	36.62	25.02
Oat flakes	91.86	90.2	1.66	14.09	4.91	2.16	6.68	4.52

Despite the fact that trials were frequently conducted with various insect species, comparing results remains challenging even when the same species were tested. Some researchers decided on a certain number of individuals and observation of growth, where individual measurements are made (van Broekhoven, Oonincx, van Huis & van Loon, 2015; Montalbán et al., 2022; Montalbán, Martínez-Miró, Schiavone, Madrid & Hernández, 2023).

MATERIALS AND METHODS

Feed materials

Two feed ingredients were used as larvae diets: wheat bran and oat flakes, which were evaluated as a diet for the mealworm larvae. The feed materials were chosen based on their availability on the local market and price; also oat flakes were available due to the processing quality unsuitable for human consumption. Both ingredients were ground and homogenized. Table 1 shows the chemical composition of the materials used in insect diets.

Insects

Larvae of the *T. molitor* used for this study belonged to the standard entomological culture which was reared for several years in the Laboratory of Entomology, Department of Environment and Plant Protection, Faculty of Agriculture, University of Novi Sad, Serbia. The standard rearing substrate consisted of wheat bran (95%) and dried brewer's yeast (5%). Ten-day-old adults were put on fresh substrate in standard rearing trays (50x60x10cm) and left for 48 hours to copulate and lay eggs. Two days later, the adults were removed through sieving and the rearing trays were put in a climate-controlled chamber at 26 ± 1 °C, and $65 \pm 5\%$ Rh. Seven days after adult removal the fresh carrots were provided to newly emerged larvae. The fresh carrot pieces were provided every two days. For this study, rearing lasted 42 days, as the starting day was counted when neonate larvae were detected in a substrate. At the end of pre-experimental rearing, they were sieved

through the systems of vertical sieves whose cells ranged from 0.75 to 1.25 mm to separate them from frass and substrate which was not consumed. Pure larvae assay was transferred to a clean rearing tray and left for 24 hours to starve and excrete the remaining ingested substrate.

Experimental procedure

The trial design in this study was performed following Pascual et al. (2024) with some modifications. The preservative in agar gel and premix was not used in the trial. It was used sterilized agar gel. Also, the trials were 2 weeks shorter.

Larvae were weighed and transferred, after starvation and cleaning period, in experimental plastic trays (12.5 x 12.5 x 8 cm). One tray filled with a starting quantity of 50 g of larvae was considered as one replicate, with a density of 12.21 larvae/cm² and for set-up a total of four replicates per diet were used. The duration of the experimental period was 28 days.

During four weeks, for five days each week (Monday to Friday), a daily amount of 5 g of diet was given per tray, summing in a total of 25 g per week. In the remaining two days in the week (Saturday and Sunday) larvae were fasting. Every Monday, at the same time, larvae were measured and frass were collected. Frass impurification with diets was minimized to obtain more accurate data regarding digestibility. To supply water, 20 g of agar gel based on agarose and agaropectin was used per day and tray. Agar gel was prepared by dissolving 1 g agar (AGAR, Torlak, Serbia) in 1 l distilled water, and then sterilized in an autoclave (Sutjeska, Belgrade, Serbia).

Trays with larvae were placed in a controlled environment under the temperature and humidity same as during the insect rearing (26 ± 1 °C, and $65 \pm 5\%$ RH). The microclimate environment, data was closely monitored and controlled during the experimental period. The frass was collected during the whole experimental

period per tray, and each week larvae were measured to obtain the production parameters as feed conversion ratio and growth of larvae per tray.

The apparent total tract digestibility coefficient (ATTD) of dry matter or other nutrients was determined as follows:

$$\text{ATTD (\%)} = ((\text{DM ingested} - \text{DM excreted}) / (\text{DM ingested}))$$

In addition to the digestibility, the performance parameters of larvae per tray were observed. Feed intake and the weight gain per tray were monitored to calculate the feed conversion ratio (FCR) according to the following formula:

$$\text{FCR} = \text{Feed intake (g DM)} / \text{Weight gained (g DM)}$$

The efficiency of conversion of ingested food (ECI) was calculated as follows:

$$\text{ECI (\%)} = \text{Weight gained (g DM)} / \text{Feed intake (g DM)} * 100$$

Chemical analysis

Dry matter content was determined after drying (AOAC Official Method 934.01). Crude protein was analyzed according to the standard Kjeldahl method (AOAC Official Method 2001.11) conversion factor of 6.25, while crude fat content (ether extract) was determined as petroleum ether extract (AOAC Official Method 991.36). Ash content was determined in the furnace at 600 °C (AOAC Official Method 942.05). NDF and ADF were determined on the ANKOM2000 fibre analyzer (ANKOM Technology, Macedon, NY, USA) by applying methods provided by the manufacturer (ADF Method, 2017; NDF Method, 2017). Hemicelluloses were calculated by subtracting ADF from NDF.

Statistical analysis

Statistical analysis was performed using the StatSoft Statistica software v. 14 (TIBCO® Data Science/STATISTICA™, StatSoft, Hamburg, Germany). Descriptive statistics was used to calculate the average digestibility coefficient, while one-way ANOVA was used to discern the differences between means using Tukey's (HSD) post-hoc test at 5% significance level.

RESULTS AND DISCUSSION

The production results are shown in Table 2. The larvae fed with oat flakes exhibited a higher average daily gain and overall gain throughout the entire period compared to those fed with wheat bran. Generally, all production parameters are better for the group of larvae fed with oat flakes. There is no standardized method for

measuring production parameters or digestibility in insects, making it challenging to compare our results with previous studies due to varying experimental designs. Typically, growth is expressed as weight gain from the initial value of larvae at a certain age or by using a specific number of individuals of average weight, then measured as average gain.

This variability makes result comparisons extremely difficult. Our goal in this research was to establish an experiment that allows us to predict the expected growth of larvae per unit area in real time. Therefore, we chose an experimental design closely resembling that of Pascual et al. (2024). Wheat bran is mostly used as a control group in studies with the *T. molitor* (Fasce et al., 2022; Pascual et al. 2024), however, oat flakes are rarely used in the larval diet. We chose to use oat flakes in our experiment because they were technologically unsuitable for human consumption and ready for disposal, making them highly accessible for our purposes. In the study of Pascual et al. (2024), the average daily gain of 50 g of *T. molitor* larvae fed with wheat bran was 2.16 g/day, while in our study, it was 1.66 g/day per tray.

The difference in growth can come from the genetics of larvae or the feed quality. The quality of wheat bran can vary due to differences in the technological processing of wheat grains and natural biological variability. Feed conversion, as a measure of feed efficiency, is easier to compare because it quantifies the amount of feed used to gain body weight. In our study, it was 2.16 for wheat bran and 2.00 for oat flakes. The conversion values differ a lot among studies. Previous studies indicate the feed conversion of 1.65 (Pascual et al., 2024), and 3.44 (Fasce et al. 2022) in *T. molitor* fed with wheat bran. However, some researchers used the complete feeding mixture in the experiments for the determination of the feed conversion and reported feed conversion efficiency in the range from 2.62 to 6.05 (van Broekhoven et al. 2015), as well as from 1.4 to 2.1 (Montalbán et al. 2023).

Digestibility is much easier to compare regardless of the experiment design, whether it is measured on 50 individuals or at 50 g of larvae because it is calculated as a digestibility coefficient or as a percentage by multiplying the coefficient by 100.

Table 2.Growth performance of *T. molitor* during the digestibility trial (28 days)

Diet	Average daily gain (g/day and tray)	The average gain for the whole period (g) (28 days)	Feed conversion ratio	Efficiency of conversion (%)
Wheat bran	1.66	46.37	2.16	46.37
Oat flakes	1.80	50.30	2.00	50.30
p-value	0.17	0.16	0.18	0.16

Table 3.The apparent fecal digestibility coefficients, expressed per gram of nutrient ingested, of main nutrients for the experimental diets in *T. molitor* larvae from 42 to 70 d post-hatch

Apparent digestibility coefficients	Diet		p-value
	Wheat bran	Oat flakes	
Dry matter	0.50 ^a ±0.005	0.78 ^b ±0.006	<0.001
Ashes	0.27 ^a ±0.013	0.36 ^b ±0.036	<0.001
Organic matter	0.51 ^a ±0.004	0.80 ^b ±0.017	<0.001
Crude protein	0.33 ^a ±0.024	0.46 ^b ±0.028	<0.001
Ether extract	0.88±0.004	0.85±0.015	0.0200
NDF	0.36 ^a ±0.025	0.15 ^b ±0.041	<0.001
ADF	0.16 ^a ±0.012	0.24 ^b ±0.082	<0.001
Hemicelluloses	0.45±0.034	0.34±0.054	0.0100

^{a,b} Means within the same row followed by different letters differ significantly ($P < 0.05$)

Most researchers calculated the digestibility of dry matter as the basic indicator of digestibility (Fasce et al. 2022; Montalbán et al., 2022 Pascual et al. 2024). Besides, dry matter, the digestibility of organic matter, ether extract, fibre, and crude protein can be calculated to carefully observe the differences in digestibility between the investigated diets. However, comparing results is challenging because digestibility is rarely estimated using a single ingredient. Typically, it involves a mixture of several different ingredients. Additionally, the source of moisture in larval nutrition varies across studies. Agar was used as a water source in only one experiment (Pascual et al. 2024), while other experiments utilized apples, carrots, jellified water, or sprayed water (van Broekhoven et al. 2015; Montalbán et al. 2023). These differences can impact the digestibility results.

The digestibility of dry matter of wheat bran in previous studies was from 40.7% (Pascual et al. 2024) to 39% (Fasce et al., 2022). In our research, the digestibility of wheat bran was 50%, while that of oat flakes was 78%. Oat flakes are much more usable for insects due to the low level of fiber which is less available for insects.

In the case of using a feed mixture the digestibility of dry matter was from 38.03 to 77.66% depending on the inclusion level of some of the nutrients (Montalbán et al., 2023).

The digestibility of almost all feed components as dry matter, crude protein, NDF, ether extract, and organic matter, was much better in larvae fed with oat flakes, as reflected in production traits. The best explanation is that the two feeds are very different in chemical composition. Oat flakes have less fibre and are thermally treated which affects the chemical bonds among their constituents (mostly starch), due to which oat flakes are more easily utilized by larvae. The occurrence of negative values for the digestibility of the ADF fraction in larvae fed with oat flakes is challenging to explain. Interpreting the results is difficult due to a lack of research on the digestibility of fibres in insects. Several possibilities arise. First of all, the amount of ADF from diets was low and amounted to 2.16%. The larvae consumed 100 g of feed, which contained 2.16 g of ADF. Based on frass analysis, 2.7 g ADF was obtained, which indicated that the ADF amount was increased by 24% in frass. Laboratory error is possible, because small quantities and minor errors in the

analysis may cause great variability in the calculation. The fibre content in the whole oat is in the range of 200-370 g/kg, however, it reduces significantly during processing (Peterson, 1992), so oat flakes have a relatively small amount of fibre. ADF fraction consists of lignin and cellulose, where lignin is almost completely inert and cannot be digested, it is even used as an inert marker in the digestibility experiments. On the other hand, certain gram-negative bacteria that are normal inhabitants of the gastrointestinal tract of insects can produce cellulose (Zogaj, Bokranz, Nimtz & Römling et al., 2003).

The negative values for the digestibility of the ADF fraction are known in chickens and rabbits, but antiperistaltic movements and accumulation usually explain this phenomenon, however, this explanation cannot be used in this study (Glamočić, Polovinski Horvatić, Ivković, Beuković & Bjedov, 2011; Jamroz, Jakobsen, Orda, Skorupinska & Wiliczekiewicz, 2001).

Although we did not find literature data on the appearance of a negative ADF balance in insects, it is known that the microflora of the digestive tract is very rich, it depends on the diet, and in addition, frass is a mixture of faeces and insect cuticles, so the increased amount of ADF may come from chitin. Obviously, there is a need for further research in this field.

CONCLUSIONS

A comparison of the digestibilities and production traits of the *T. molitor* larvae fed with two nutrients, wheat bran, and oat flakes, showed that from the nutritional side, oat flakes are better for growth and yield. The production and digestibility results support this observation.

The digestibility study's design effectively determined the nutritional value of a specific feed for larvae. Utilizing this type of test will enable a more efficient application of *T. molitor* as an alternative protein source, primarily by identifying their nutritional requirements. By properly balancing the feed ratio, the desired growth and chemical composition of the larvae can be achieved at an optimal cost. The availability of feed materials varies by country and region, depending on industrial development. There are still numerous opportunities and adjustments to be made through research to

achieve optimal costs and insect growth potential.

AUTHOR CONTRIBUTIONS

Conceptualization, M.P.H.; Methodology, I.J., S.K., N.L., M.P. and M.V.; Investigation, formal analysis, validation, writing-original draft preparation, M.P.H., S.K., and D.B; Writing-review and editing, M.P.H and S.K; Supervision, A.P and I.J.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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PROIZVODNI POKAZATELJI I SVARLJIVOST BRAŠNARA (*TENEBRIO MOLITOR*) HRANJENIH SA DVE VRSTE HRANIVA

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Sažetak: Brašnar (*Tenebrio molitor*) je zanimljiv alternativni izvor proteina u animalnoj ishrani. Da bi se dobili dobri proizvodni rezultati i povoljan hemijski sastav potrebno je da se poznaju nutritivne potrebe i svarljivost brašnara. U eksperimentalnim uslovima, brašnar je hranjen sa pšeničnim mekinjama i ovsenim pahuljama, dva lokalno lako dostupna hraniva dostupna po pristupačnoj ceni. Obe grupe (svaka sa po četiri ponavljanja) *T. molitor* hranjene su nedeljno istom količinom hraniva (25 g hrane i voda je obezbeđena preko agara). Proizvodni pokazatelji kao što su konverzija, konzumacija i rast tokom eksperimentalnog perioda su praćeni tokom oglada. U isto vreme ogled svarljivosti je sproveden da bi se dobili koeficijenti svarljivostu suve materije, organske materije, pepela, sirovih protein, sirovih masti, NDF, ADF i hemiceluloze. Proizvodni parametri za oba hraniva su bila relativno slična sa malo boljim rezultatima kod larvi hranjenih ovsenim pahuljicama. Konverzija za pšenične mekinje i ovsene pahulje bila je 2,16 i 2,00, sa prirastom po kutiji sa insektima od 46,37 g i 50,30 g, respektivno. Ogled svarljivosti je ukazao na mnogo bolju svarljivost ovsenih pahuljica sa prosečnim koeficijentom svarljivosti suve materije 0,78, organske materije 0,80, sirovih proteina 0,46, i sirove masti 0,85. Svarljivost pšeničnih mekinja za suhu metriju je bila 0,50, organsku materiju 0,51, sirovih proteina 0,33, i sirove masti 0,88. Oba hraniva se mogu uspešno koristiti za podmirenje hranljivih potreba brašnara, međutim upotreba ovsenih pahuljica daje bolje proizvodne pokazatelje i svarljivost. Izbor hraniva će zavisiti od cene hraniva na tržištu.

Ključne reči: brašnar, konverzija hraniva, koeficijent svarljivosti, ishrana pšeničnim mekinjama, ishrana ovsenim pahuljicama

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