

The Effect of Some Food Sources on the Production of *Enchytraeus Buchholzi* (Grindal)

Adrian-Dan Rășinar, Tiberiu Polen, Octavian Sorin Voia, Cătălin Zoican, Dragoș Moraru

University of Life Sciences "King Mihai I" from Timișoara, Faculty of Bioengineering of Animal Resources, Calea Aradului no.119

Abstract

Grindal worms are an important food source in aquaculture and aquarism. They are obtained with a relatively low production cost and the possibility of contamination with different pathogens is avoided compared to harvesting from nature. The aim of the work is the use in the feed of Grindal worms of two recipes of wet feed based on milk 92% and wheat flour 8%, (Lots C1-C5) compared to milk 84%, egg 10% and flour 6% (Lots E1- E5) on the development of Grindal biomass grown on a substrate represented by ground moss in a volume of 400 cm³ with 84% humidity. Feeding and harvesting were performed once a day. The weighing was carried out with a precision of 0.01 g. Following the research, we found that groups E2-E5 achieved a significantly higher biomass production ($p \leq 0.05$) by 1.53 grams per day, compared to groups C2-C5, with the exception of group E1, where an insignificant increase was found ($p \geq 0.05$) compared to C1. Feed conversion was superior in the experimental groups compared to the control groups by 10.2 %. The production of Grindal biomass per cm³ of substrate in groups E1-E5 was significantly higher (0.232 g, compared to control groups C1-C5, which had an average of 0.209 g/cm³).

Keywords: biomass, food sources, Grindal worms (*Enchytraeus buchholzi*)

1. Introduction

Enchytraeus buchholzi or popularly known Grindal worm belongs to the family *Enchytraeidae* of the order *Enchytraeida*. Together with earthworms and leeches, Grindal worms are a group of oligochaete invertebrates. Small, thin, segmented bodies are characteristic of the *Enchytraeidae* family, which are adapted for living in moist soils and organic detritus [1,2]. Most of the time, Grindal worms are associated with environments rich in organic matter, for example agricultural soils and compost. *Enchytraeus buchholzi* are also often found in places where there is decaying plant matter due to providing a nutrient-rich habitat [3].

The studies undertaken by Zhao et al., (2022) [4] but also by other researchers [5,6] give us a concrete description of the anatomy and

morphology of *Enchytraeus buchholzi* worms. The body consists of a prostomium, a peristomium, approximately 27-30 segments and a pygidium. The body segments are similar to each other and each segment has a set of lateral and ventral setae except the first segment which has no setae. Setae are chitinous structures that aid in movement and anchoring. The digestive system begins with the mouth located on the ventral side of the peristomium, followed by the oral cavity, a pharyngeal buffer, oesophagus, intestine and anus. The pharyngeal buffer is rich in muscle and aids in swallowing. It is followed by the oesophagus, which is surrounded by pharyngeal glands in segments IV-VI. The intestine extends over the rest of the body to the anus, located in the pygidium. The excretory system includes nephridia located in each segment, which are responsible for the

* Corresponding author: Adrian-Dan Rășinar,
adrian.rasinar@usvt.ro

elimination of metabolic waste. These are tubular structures that filter body fluids and excrete waste through the nephridial pores. *Enchytraeus buchholzi* is hermaphrodite, having both male and female reproductive organs. The reproductive system includes ovaries, testes, seminal vesicles, spermathecae, and penile bulbs. Sperm and eggs develop and mature in the worm's body, and fertilization is internal. Reproduction can occur either by direct copulation or by self-fertilization. The eggs are laid in gelatinous capsules and need moist conditions for development [4]. Larvae usually hatch after a few days and reach sexual maturity within a few weeks. In aquaculture and aquarism, Grindal worms are mainly used as live food due to their nutritional value. It is an excellent source of proteins and essential lipids for aquatic organisms and fish. Breeding is relatively easy, with low cost, making these worms a constant source of high-quality food.

2. Materials and methods

In order to carry out the experiment, we used 10 square glass trays (Figure 1) with sides of 20/20 cm in which we added the substrate for the culture of Grindal worms. The substrate was made of ground moss (*Polytrichum commune*), washed and dried beforehand, in an amount of 40 g, moistened with 200 ml of water (Figure 2).



Figure 1. Glass tray

Preparation of the culture medium: the substrate was placed evenly in the tray (Figure 3) in a 1-cm thick layer. A 1-cm dark green plastic mesh was placed over the moss to prevent it from adhering to the glass

coverslip, allowing for easier and impurity-free harvesting. 2 glass covers were placed over the plastic net. After that, for 24 hours, the trays were pressed to facilitate a better levelling of the muscle layer (Figure 4). The volume of the growth substrate, after pressing, was 400 cm³ (L=20 cm, H=1 cm) ensuring a humidity of 84%. 5 g of Grindal worms were introduced into each tray. The 10-culture media were divided into 2 batches (C vs E), of 5 trays each (C1-C5 and E1-E5 respectively).



Figure 2. Substrate



Figure 3. Laying the substrate

The trays were placed in two cabinets (Figure 5) to protect them from possible attacks by mites or black flies. In batch C, we used as feed a paste consisting of cow's milk with 1.5% fat to which wheat flour was added (Table 1). Milk and flour were mixed and boiled for 2 minutes. The obtained product provides 682 kcal/kg, 39 g of crude protein, 14.52 g of crude fat, 98.8 g of carbohydrates and 17.44 g of minerals.



Figure 4. Pressing the layer



Figure 5. Culture space

table 1. At an interval of 10 days, the trays were drained to remove excess moisture resulting from the administration of wet food and the pH of the substrate was also checked to keep it around 7. We note that from the experience of previous years, the decrease in pH values below the value of 6 leads to a decrease in crop productivity. We corrected the pH with the help of a 50-volume solution of 1% sodium carbonate added over the substrate for 5 minutes followed by the removal of excess moisture by free draining (Figure 7). This procedure was applied three times for all trays in batches C and E in order not to have different effects on the results obtained.



Figure 6. Weighing the worms

For batch E, we used a paste consisting of cow's milk with 1.5% fat, whole chicken egg and wheat flour (Table 2). Milk, eggs and flour were mixed and boiled for 2 minutes. The obtained product provides 702.2 kcal/kg, 46.1 g of crude protein, 26.1 g of crude fat, 82.1 g of carbohydrates and 17.7 g of minerals. During the pre-experimental period, for 2 weeks, we monitored the amount of food that could be consumed in total within 24 hours. Also, during this period, we followed the development of Grindal worm cultures. In order not to differentiate the cultures, we used only the food source distributed to group C. The temperature at which the cultures were kept was in the range of 20-24°C and the pH was neutral. After the mentioned interval, we started the differentiated feeding with the two food sources. The experimental period lasted for 30 days, during which we collected the Grindal worms daily, by scraping, and then we weighed them (Figure 6). After weighing, the worms were differentially administered 6 grams of food/day according to

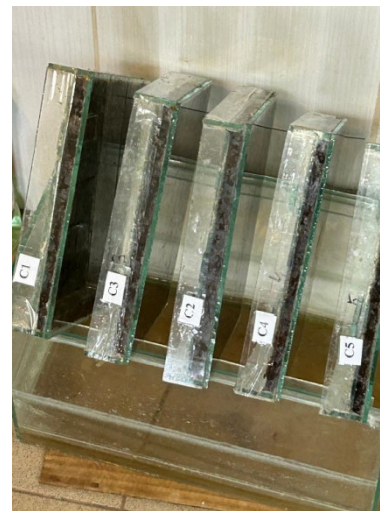


Figure 7. Removal of excess moisture

The scraped biomass from the 10 culture trays was weighed daily with a precision of 0.01g and the resulting data were statistically processed by applying the t-test with the Statistica 7 Program.

Table 1. Feed recipe for batches C1-C5

Specification	% participation	Dry matter	Metabolizable energy (kcal)	Crude protein (%)	Raw fat (%)	Carbohydrates (%)	Minerals (%)
Milk	92	10.12	405	2.9	1.38	4.14	1.656
Wheat flour	8	7.04	277	1	0.072	5.84	0.088
Total	100	17.16	682	3.9	1.452	9.98	1.744

Table 2. Feed recipe for batches E1-E5

Specification	% participation	Dry matter	Metabolizable energy (kcal)	Crude protein (%)	Raw fat (%)	Carbohydrates (%)	Minerals (%)
Milk	84	9.24	369.6	2.68	1.26	3.78	1.51
Egg	10	2.7	125	1.15	1.3	0.05	0.2
Wheat flour	6	5.28	207.6	0.78	0.05	4.38	0.06
Total	100	17.22	702.2	4.61	2.61	8.21	1.77

Table 3. Experimental organization scheme

Batch	Feed	Feed amount	No of trays	Experiment duration
Control batch (C1-C5)	Milk + flour	6g.	5	30 days
Experimental batch (E1-E5)	Milk + egg + flour	6g.	5	30 days

3. Results and discussion

Table 4 shows the average daily amounts of harvested biomass (g). In batch C1 compared to batch E1 we did not obtain a statistically significant difference because in C1 we recorded a high variability. In the rest of the batches, the differences between the two experimental variants were statistically significant. Analysing the total biomass harvested in the two experimental variants, a significant difference is found in favour of the experimental batch compared to the control batch. The superiority of the experimental batch consists in a difference of 1.53 g bigger than in the control batch. Table 5 shows the production of biomass harvested during the 30 days of the experiment in the 2 experimental variants, as well as the amount of food consumed. Based on the 2 values, we determined specific consumption for each experimental variant and compared the experimental batch with the control batch. The data presented in Table 5 proves that the control batches had a specific consumption between 2.12 and 2.18 higher compared to the experimental batch, where the specific consumption varied between 1.91 and 1.98. The experimental batches achieved a lower specific consumption, compared to the control batches, between -0.19 g and -0.25 g. The best results were obtained in batches E3, E4 and E5 where the specific consumption was between 1.91 and 1.93, compared to the control batches, where the feed conversion was

higher (C3, C4 and C5), with values between 2.12 and 2.18. The better utilization of food in the experimental batches, compared to the control batches, can be explained by a higher protein intake in the experimental batches, of 4.61%, compared to 3.9% in the control batches. Also, the use of egg in the recipe intended for the experimental batches also brought an intake of protein better balanced in amino acids. Based on the data in table 5, where we have highlighted the amounts of biomass of worms harvested from the 5 control trays and the 5 experimental trays, we calculated the amount of biomass of worms obtained on each tray taken into the study. Each tray had a total volume of 400 cm³ of substrate. By dividing the amount of biomass by the volume of the substrate, we obtained the amount of biomass harvested per cm³ of substrate, data that are presented in Table 6. In this table, we may observe that in the control batches, where we used milk and wheat flour as a food source, the biomass production per cm³ was between 0.206 g and 0.212 g. In the experimental batches where the worm food was made up of milk, egg and wheat flour according to the recipe, we obtained a production of Grindal biomass per cm³ of between 0.227 g and 0.236 g. All 5 experimental variants achieved a superior Grindal production compared to the control batches, this superiority being between 0.019 g and 0.028 g. The differences between batches E and C are statistically significant.

Table 4. Comparison between the Grindal biomass harvested in the 2 experimental variants (g/daily average)

Batch C			Batch E			Significance of differences
Code	Mean (g)	SD	Code	Mean (g)	SD	
C1	2.75	0.72	E1	3.02	0.42	0.27 ^{ns}
C2	2.78	0.46	E2	3.04	0.40	0.26*
C3	2.75	0.31	E3	3.11	0.46	0.36***
C4	2.80	0.39	E4	3.14	0.48	0.34**
C5	2.83	0.47	E5	3.12	0.51	0.29*
Total	13.91	0.73	Total	15.44	1.35	1.53***

ns-non-significant; *-p ≤ 0.05; **-p ≤ 0.01; ***- p ≤ 0.001

Table 5. Food conversion in Grindal biomass

Batch C				Batch E				Differences	
Code	Feed consumed (g)	Total biomass (g)	FCR	Code	Feed consumed (g)	Total biomass (g)	FCR	g (E-C)	% (E/C)
C1		82.5	2.18	E1		90.7	1.98	-0.2	90.83
C2		83.4	2.16	E2		91.3	1.97	-0.19	91.2
C3	180	82.4	2.18	E3	180	93.4	1.93	-0.25	88.53
C4		84.1	2.14	E4		94.2	1.91	-0.23	89.25
C5		84.8	2.12	E5		91.6	1.92	-0.2	90.57
Total	900	417,2	2,16	Total	900	463,2	1.94	-0.22	89.8

FCR – feed conversion ratio

Table 6. The amount of Grindal worm biomass obtained per cm³ of culture in the 30 days

Batch C			Batch E			Significance of differences
Code	Mean (g/cm ³)	SD	Code	Mean (g/cm ³)	SD	
C1	0.206	0.5	E1	0.227	0.45	0.021*
C2	0.209	0.48	E2	0.228	0.41	0.019*
C3	0.206	0.33	E3	0.234	0.42	0.028***
C4	0.210	0.37	E4	0.236	0.45	0.026***
C5	0.212	0.45	E5	0.234	0.50	0.022**
Average	0.209	0.27	Average	0.232	0,24	0.023***

ns-non-significant; *-p ≤ 0.05; **-p ≤ 0.01; ***- p ≤ 0.001

4. Conclusions

Maintenance of Grindal worms in glass trays on moss medium ensures stability in the growth of worms, making the cultures easy to maintain. Keeping trays in cabinets avoids contamination of cultures with possible pests (mites, flies). The experimental batches that were fed with milk, egg and flour compared to the batches fed with milk and flour achieved a superior biomass production, of 1.53 g. The experimental batches also achieved 10.2% better feed conversion rates than the control batches. In the experimental batches (E) fed with the recipe containing milk, egg and flour we obtained, after the experiment, a higher production of Grindal worm biomass, related to the culture

volume, 11% higher compared to the control batches (C), which were fed milk and flour. The use of egg in the food structure, in the experimental batches, compared to the control batches, brought an increase in the production of worm biomass, a decrease in specific consumption and an increase in the amount of worm biomass related to the culture volume. Following the experiment, we recommend the growth and use of Grindal worm cultures in aquaculture and aquarism, because they provide a natural food, rich in nutritional principles, with a low production and maintenance cost, being able to provide live food throughout the year and avoiding the risks of accidental contamination with pathogens. In the following, we consider it is necessary to deepen the studies regarding other

sources of food used, other culture substrates, the control over the pH, as well as the quality of the biomass obtained.

References

1. Christensen, B., Glenner, H., Molecular phylogeny of Enchytraeidae (Oligochaeta) indicates separate invasions of the terrestrial environment. *Journal of Zoological Systematics and Evolutionary Research*, 48(3), 2010, 208-212. <https://doi.org/10.1111/j.1439-0469.2009.00558.x>
2. Coates K.A., Phylogeny and origins of Enchytraeidae. *Hydrobiologia* 180: 17–33, 1989
3. Dózsa-Farkas, K., The description of three new species and some data to the enchytraeid fauna of the Baradla Cave, Hungary. (*Biospeologica Hungarica*, XXXIV). *Opusc. zool.*, Bpest, 1970, 10 (2): 241–251.
4. Zhao, L., Xie, X., Chen, D., Ma, G., Sun, Y., Microscopic Observations on Form and Structure of the Worm *Enchytraeus buchholzi* (Clitellata: Enchytraeidae). *BMC Zoology*, 7(1), 2022, 31. <https://doi.org/10.1186/s40850-022-00123-y>
5. Rota E. Enchytraeidae (Oligochaeta) of western Anatolia: Taxonomy and faunistics. *Bolletino di zoologia*. 1994; 61(3):241–60.
6. Nielsen CO, Christensen B., The Enchytraeidae: critical revision and taxonomy of European species (Studies on Enchytraeidae VII). *Natura Jutlandica*. 1959; 8–9:93–4.