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VALORIZATION OF BLACK SOLDIER FLY (*Hermetia illucens*) LARVAE MEAL IN GUPPY (*Poecilia reticulata*) FEED

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Abstract

The global trade in ornamental fish, feed, and aquarium equipment is an important component of the world economy. The majority of ornamental species are freshwater, fish of the Poeciliidae family are recognized as the most popular. Fishmeal is the most important ingredient in animal feed. The substantial reduction in oceanic fish stocks has imposed strategies to limit the use of fishmeal. Among these, the identification of alternative replacement sources is noteworthy. This study aimed to test a diet for guppy fish in which fishmeal was replaced by 60% insect meal. It also investigated whether the addition of enzymes promotes better availability and assimilation of this feed. The experiment lasted 75 days. The results showed no significant differences in growth between fish fed a commercial diet and those fed the insect-based diet. It was also found that feeding insect meal did not negatively influence the health of the fish and did not affect water quality parameters. It was also found that insect meal feed did not negatively affect the health of the fish or the water quality parameters.

Key words*: black soldier fly, guppy, protein larval meal.*

INTRODUCTION

The breeding of ornamental fish is in continuous evolution and is gaining increasing global interest (Olivier, 2001; Tolon, 2018). Due to current demand and environmental pressures, ornamental fish, particularly tropical live-bearing species, represent a true industry in certain countries (Sales & Janssens, 2003). Among the most popular species are the guppy (*Poecilia reticulata*), molly (*Poecilia sphenops*), swordtail (*Xiphophorus helleri*), and platy (*Xiphophorus maculatus*) (Alderton, 2012; Gavriloaie, 2023; Ghosh et al., 2007; Tolon, 2018). These species are also utilized in a wide range of biological and medical studies (Safian et al., 2021), serving as models for understanding invasive species, studying ecological consequences, and assessing the long-term effects of environmental pollutants (Boaru et al., 2021; Petrescu-Mag, 2023).

Native to South America, the guppy (*P. reticulata*) belongs to the family *Poeciliidae* (https://www.itis.gov/). It is a small fish with a broad tolerance for median environmental parameters (Gelsano & Demayo, 2022) and exhibits very pronounced sexual dimorphism (Petrescu-Mag & Bourne, 2008). Females give birth to 20-40 fry and have the capacity to store seminal material for subsequent fertilization (García & Giraldo-Gongora, 2023). Considered omnivorous like most poeciliids, the guppy's diet consists of zooplankton, insects, algae, and detritus (Arthington, 1989; Perera & Bhujel, 2022; Lawal et al., 2012; Tamaru et al., 2001; Yanong, 1999).

Fishmeal is one of the ingredients with the most complete properties utilized in feed formulations. In recent years, there has been a clear downward trend in fishmeal production due to the reduction of oceanic fish populations (FAO, 2016, 2018, 2020), coupled with an increased demand for feed production destined for aquaculture, livestock, and pets (Fontes et al., 2019; OCDE/FAO, 2021). Sustainability is now a mandatory requirement in all fields; therefore, the rearing and utilization of insects to substitute fishmeal in aquaculture and aquarium feeds is of high current relevance.

This study aimed to test a feed for guppies (*P. reticulata*) in which fishmeal was replaced by insect meal. Additionally, it investigated whether the addition of enzymes favors the availability and better assimilation of this feed.

MATERIALS AND METHODS

The experiment was conducted in 2023 at the Aquaculture Laboratory of the Faculty of Animal Science and Biotechnology, USAMV Cluj-Napoca. Sexually mature guppies (*P. reticulata* var. *Full Red Albino*), aged 2-3 months, were sourced from the Aquarium Complex of USAMV Cluj-Napoca. The insect meal feed was provided by the Laboratory of Aquaculture Rearing Technologies within the Faculty of Animal Science and Biotechnology.
Aquariums measuring 40 x 25 x 27 cm (L x l x h) with a useful water volume of 17 L were used. Each aquarium was equipped with a sponge filter (15 x 5 x 5 cm) powered by an air pump (Osaga MK-9510) at 10 W, with a maximum flow rate of 10 L/min. For lighting, a lamp (Resun DL-20R) with two neon tubes (OSRAM) at 18 W was used, and water temperature was maintained using heaters (Resun RH9000) at 25 W. Each aquarium was prepared with a maternity separator for the reproduction of live-bearing fish (Figure 1).
Tap water stored in containers was used; 35% of the water was replaced twice a week, and solid waste was vacuumed using a manual aquarium cleaner (Boyu model SC-002). Once every two weeks, filters were partially cleaned in water removed from the aquarium. The fish were brought into the laboratory and acclimated for 14 days, after which one male and one female (at first parturition) were released into the maternity separator of each aquarium (Figure 1), with the male being removed after 2-3 days. Following the expulsion of the fry, the female was also removed from the aquarium.

Fish feeding protocol

Differentiated feeding was initiated with the breeding stock, with two meals administered daily. The fry was fed *ad libitum* six days per week, with four daily feedings scheduled between 08:30 and 16:00. Based on the dietary treatments, the following experimental groups were established:

LGM: Consisted of a commercial control diet ("Hikari Fancy Guppy") specifically formulated for live-bearing fish. The nutritional profile, as specified by the manufacturer, included: Crude Protein (min. 50%), Fat (min. 8%), Fiber (max. 2%), Moisture (max. 10%), Ash (max. 18%), Phosphorus (min. 1%), Vitamin A (min. 19,000 IU/kg), Vitamin D3 (min. 2,800 IU/kg), Vitamin E (min. 850 IU/kg), and Ascorbic acid (min. 340 mg/kg).
LG60: Utilized a diet where 60% of the fishmeal was substituted with Black Soldier Fly (BSF) larvae meal (*Hermetia illucens*). This group was further divided into enzyme-supplemented (**60+**) and non-supplemented (**60-**) treatments. The calculated nutritional values for the BSF-based diet were: Crude Protein 44.12%, Crude Fat 3.26%, Cellulose 1.65%, Crude Ash 2.86%, Lysine 1.27%, Methionine 0.70%, and a Vitamin-Mineral Premix at 1.00%. For the supplemented group, the commercial preparation Hostazyme X (0.02%) was incorporated. Feed pellets were crushed into a coarse meal for adults, while particle size was calibrated according to the growth stage of the fry (Figure 2).

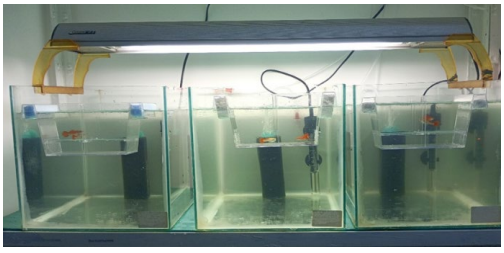

Figure 1. Experimental aquarium setup stocked with guppy (*Poecilia reticulata*) breeding pairs (♀ ♂) (original source)



Figure 2. Experimental diets used (from left to right: commercial control, LG60+, LG60-) (original source)

Water quality monitoring

Water temperature, electrical conductivity, and pH were recorded daily, while ammonia (NH_4) levels were determined on a weekly basis. The

monitoring was performed using a digital thermometer, a digital pH meter, a digital TDS meter, and a colorimetric titration kit (JBL ProAquaTest Combi Set Plus).

Growth parameters

To evaluate whether the substitution of fishmeal with insect meal influenced the growth performance and overall condition of the guppy fry, body weight (W), total length (Lt), and the condition factor (K) were assessed. The experimental period was divided into two evaluation stages: at 42 and 75 days post-parturition, respectively. At each interval, ten fish from each aquarium were weighed and measured. Body weight was recorded using an analytical balance (Ohaus Explorer Pro ep 114c) with a resolution of 0.1 mg. To determine the total length, the fish were photographed against a metric grid background; scale calibration was performed by measuring a standard 1 cm reference unit (Figure 3).



Figure 3. Biometric measurement and weighing of guppy fry (original source)

The following formula was used to calculate the condition factor (Gelsano & Demayo, 2022; Grozea, 2007):

$$K = \frac{(W \times 100)}{Lt^3}$$

where: K = condition factor/Fulton's index [%]; W = body weight [mg]; Lt = total length [cm].

The animal study protocol was approved by the Bioethics Committee of The University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca (decision no. 308 from 17 March 2022).

Statistical analysis

Raw data were statistically processed to determine mean values. For the monitored growth parameters, statistical significance was tested using one-way Analysis of Variance (ANOVA) with IBM SPSS Statistics 21 software.

RESULTS AND DISCUSSIONS

Water quality parameters

Mean water temperature values (Figure 4) were 26.28 ± 0.02°C (LGM), 26.06 ± 0.03°C (LG60+), and 26.10 ± 0.02°C (LG60-), falling within the ranges reported by other authors for guppy rearing (Perera & Bhujel, 2022; Shah et al., 2017).

Through daily pH monitoring (Figure 5), values of 7.76 ± 0.01 (LGM), 7.71 ± 0.01 (LG60+), and 7.77 ± 0.01 (LG60-) were recorded. These results indicate the alkaline nature of the water, which is specific to the growth requirements for this species (Bierbach et al., 2024; Serihollo et al., 2024). The consistency of the mean values obtained across all aquariums suggests that the administered feed did not influence water pH in any of the experimental groups.

Regarding electrical conductivity (Figure 6), the mean values obtained for each group were 165.25 ± 1.43 µS/cm (LGM), 154.11 ± 1.13 µS/cm (LG60+), and 158.94 ± 1.43 µS/cm (LG60-). These measurements fall within the recommended limits (100–4000 µS/cm) for guppy rearing (Bierbach et al., 2024).

Regarding ammonia (NH_4), recorded values were below the detection limit of the colorimetric assay (0.05 ppm) across all aquariums. Consequently, water quality relative to this parameter remained at an optimal level for the welfare of the ornamental fish (Bierbach et al., 2024; Shelton, 2010).

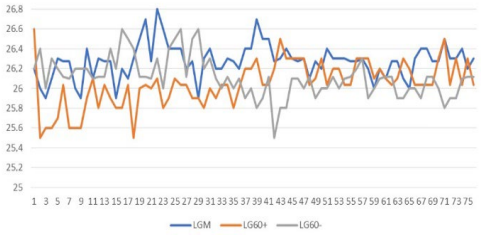

Figure 4. Water temperature monitoring results

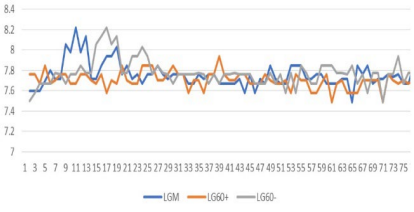

Figure 5. Water pH monitoring results

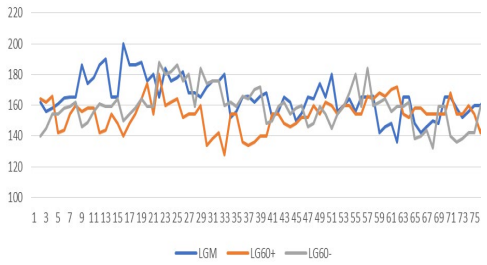

Figure 6. Water electrical conductivity monitoring results

Throughout the experimental period, fish behaviour was monitored, specifically focusing on feed acceptance or refusal. At the beginning of the experiment, females were less active; however, no differences in appetite were observed across any of the groups. During the first 2–3 days post-parturition, the fry exhibited low activity levels and remained concentrated in the central area of the aquarium floor. Subsequently, the fish began rising to the water surface to forage, with no feed refusal observed in any of the groups. A distinction was noted regarding the dispersion and physical stability of the feed in water. The commercial feed was drier and maintained its structural integrity in water longer, whereas the experimental feed dispersed significantly faster. Nevertheless, the water quality parameters indicated no significant differences between groups, confirming that the experimental diet did not adversely affect water quality.

Growth performance in relation to the experimental diets

The results obtained from the two evaluations are presented in tables, including the mean and standard error of the mean ($X \pm s_x$), the coefficient of variation (V %), and the variation limits (minimum-maximum) for each monitored trait.

The data obtained from the first evaluation are presented in Table 1.

Regarding body weight, the mean values were 21.51 mg (LGM), 20.94 mg (LG60+), and 20.75 mg (LG60-), with ranges between 9.05 mg (LG60-) and 49.95 mg (LG60+). High variability was observed for this parameter both within and between groups. From this perspective, the LGM group showed the lowest coefficient of variation (27.64%), followed by the LG60- group (39.38%), while the highest value was recorded for the LG60+ group (54.25%).

Regarding body length, the mean values were 1.35 cm (LGM), 1.33 cm (LG60+), and 1.40 cm (LG60-), with ranges between 1.12 cm (LG60+) and 1.69 cm (LG60-). For this parameter, the calculated coefficients of variation showed similar values (9.42% for LGM, 10.90% for LG60+, and 13.19% for LG60-).

Regarding the condition factor, mean values were similar for the LGM (0.863) and LG60+ (0.826) groups, while a slightly lower value was recorded for LG60- (0.713). The coefficients of variation were 11.43% (LGM), 13.21% (LG60-), and 19.61% (LG60+).

The data obtained at the second evaluation are listed in Table 2.

Regarding body weight, the mean values obtained were 98.09 mg (LGM), 79.58 mg (LG60+), and 95.54 mg (LG60-). The calculated coefficients of variation indicate the same high variability both within and between groups; however, the lowest value (44.17%) was recorded for LG60+, followed by LG60- (57.22%), while the highest value was determined for LGM (73.33%).

Regarding body length, mean values of 1.99 cm were obtained for both LGM and LG60+, while the LG60- group recorded a mean of 2.06 cm. The calculated coefficients of variation were 13.04% (LG60+), 13.41% (LG60-), and 19.58% (LGM).

Regarding the condition factor, mean values were 1.031 (LGM), 0.961 (LG60+), and 1.011 (LG60-), with coefficients of variation of 18.74% (LGM), 15.58% (LG60+), and 16.53% (LG60-).

Table 1. Mean values and dispersion indices at 42 days

Parameter		$X \pm s_x$	V%	Variation limits
LGM	W	21.51±1.88	27.64	13.85-32.80
	L_t	1.34±0.04	9.42	1.16-1.52
	K	0.863±0.03	11.43	0.74-1.02
LG60+	W	20.94±3.59	54.25	10.85-49.95
	Lt	1.33±0.04	10.90	1.12-1.58
	K	0.826±0.05	19.61	0.68-1.27
LG60-	W	20.75±2.58	39.38	9.05-32.10
	Lt	1.40±0.06	13.19	1.17-1.69
	K	0.713±0.03	13.21	0.56-0.89

Table 2. Mean values and dispersion indices at 75 days

Parameter		$X \pm s_x$	V%	Variation limits
LGM	W	98.09±25.43	73.33	31.75-207.95
	L_t	1.99±0.13	19.58	1.60-2.57
	K	1.031±0.07	18.74	0.77-1.32
LG60+	W	79.58±12.42	44.17	39.35-159.8
	Lt	1.99±0.09	13.04	1.56-2.51
	K	0.961±0.02	15.58	0.87-1.04
LG60-	W	95.54±22.32	57.22	43.95-198.75
	Lt	2.06±0.11	13.41	1.80-2.51
	K	1.011±0.07	16.53	0.75-1.26

In both evaluations, the recorded limits and coefficients of variation revealed moderate variability for body length and the condition factor (V<20%), while more pronounced variability was observed for body weight (V>20%). Based on the mean values and the observed variability across the analysed traits, the biological material in all groups exhibited appropriate growth and development. These results fall within the ranges reported by other authors for this species (Harpaz et al., 2005).

For each evaluation stage, an analysis of variance was performed, and the results are presented in Table 3.

Statistical data obtained from both weighings did not indicate significant differences, suggesting that there are no functional distinctions between the commercial and experimental feeds regarding weight gain. Similar to body weight, the results for total length proved to be statistically non-significant (NS) in both evaluations. Regarding the condition factor (K), although differences between groups were observed at 42 days, these were not maintained by the 75-day evaluation.

Table 3. F-values and the significance of differences obtained in the two evaluations

Parameter	F statistic	Critical F (0.05 level)	Effect
W 42 days	0.02	3.35	Ns
L_t 42 days	0.61	3.35	Ns
K 42 days	4.05	3.35	S
W 75 days	0.25	3.52	Ns
Lt 75 days	0.09	3.52	Ns
K 75 days	0.46	3.52	Ns

Ns = Non-significant; S = Significant.

The condition factor (K) is an indicator of the physiological state of fish and feed availability, with higher values reflecting a robust state of development (Grozea, 2007; Savin et al., 2022). From this perspective, the indices obtained in both evaluations reveal a good nutritional status for the fish in all groups, falling within optimal limits. Furthermore, at the end of the experiment, the fish condition factor values (1.031, 0.961, and 1.011) were consistent with those obtained by Abdul Khader & Altaff (2021) for guppy fry fed four different diets over 90 days (0.950, 0.962, 0.969, and 1.039). This highlights the healthy status of the fish and an isometric growth pattern. The analysis of variance reveals that no significant differences exist between the group fed with commercial feed-containing approximately 50% crude protein and based on fishmeal, and the groups fed with the experimental diet, where the crude protein content was approximately 44.12% and fishmeal was substituted at a 60% rate with BSF larvae meal. Furthermore, it can be stated that for this species, the addition of enzymes to BSF larvae meal-based feed is not necessary, as no significant differences were observed between the LG60+ and LG60- groups. This hypothesis is further supported by the fact that previous studies on *P. reticulata* have shown that insect meal, including Black Soldier Fly (BSF), acts as an attractant and increases feed palatability. Research conducted by Kowalska et al. (2021) demonstrated that guppies exhibit a significant interest in insects and even prefer diets based on insect meal over those containing fishmeal.

The effects of replacing fishmeal with insect meal derived from two species (*Acheta domesticus* and *Gryllus bimaculatus*) at varying inclusion levels (50%, 75%, and 100%) on the growth and reproduction of *P. reticulata* were also investigated by Perera & Bhujel (2022). No differences were recorded between the experimental groups and the control group regarding the growth performance of guppy fry. Furthermore, as the fry continued to grow, their reproductive performance was tested, revealing that insect meal had no influence on the number of offspring obtained or their dimensions. The conclusions and results of this study confirmed that meal from both insect species can replace fishmeal at a 100% substitution rate in guppy feed. In line with these findings, research conducted by other authors recommends the use of insects in aquarium fish diets to achieve optimal growth and reproductive performance (Adil et al., 2014; Ahmed et al., 2022). BSF larvae meal has been successfully utilized to replace fishmeal in the diets of other ornamental

species as well. Kamalii et al. (2023) reported that goldfish (*Carassius auratus*) fry fed with BSF larvae meal achieved superior growth performance and demonstrated enhanced feed utilization. The conclusions drawn indicate that a 60% substitution rate of fishmeal with BSF larvae meal is optimal for goldfish nutrition. Furthermore, the results of the study conducted by Linh et al. (2024) showed that replacing both fishmeal and fish oil with BSF larvae meal has significant implications for ornamental fish nutrition and may represent a novel and sustainable strategy for enhancing disease resistance. Through the improvement of captive breeding techniques, marine ornamental aquaculture continues to expand (King, 2019; Sinha et al., 2023), and addressing feeding-related challenges represents a current trend. Research is particularly focused on identifying high-value nutritional sources with minimal environmental impact. From this perspective, insects are being extensively studied, and the black soldier fly (*H. illucens*) shows significant potential. The partial inclusion of BSF meal in the diet of clownfish (*Amphiprion ocellaris*) fry was first reported by Vargas-Abúndez et al. in 2019. Experimental results demonstrated that no negative effects were recorded on fish growth, survival, or stress response; however, further studies are necessary.

CONCLUSIONS

By utilizing *P. reticulata*, a highly popular species in the aquarium hobby and a recognized model organism, and analysing the data obtained in this research, it was found that no differences in growth and development occurred between fish fed a fishmeal-based diet and those fed an insect meal-based diet. Furthermore, BSF larvae meal can be administered in the diet of these fish without adversely affecting water quality parameters. Given the unique anatomical and physiological specificity of each species, additional testing is recommended prior to the broad implementation of such diets. In the context of declining fishmeal production and the increasing trend of reserving marine fish strictly for human consumption, the use of insect meal in ornamental fish diets is highly opportune.

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