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Consumption of expanded polystyrene by *Tenebrio molitor* and *Zophobas atratus*, and use of their meal as feed for *Piaractus brachypomus*

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Abstract. Expanded polystyrene (PS) provides a great challenge for environmental management due to its high levels of production and insufficient waste management. However, recent studies have demonstrated the capacity of *Tenebrio molitor* and *Zophobas atratus* to biodegrade PS, as well as the possibility of using the meal of these species to feed fish. The objective of this study was to evaluate the effect of including insect meal as a substitute for fishmeal in diets for *Piaractus brachypomus* fingerlings. In one experiment, the effect of 5 different levels of PS and wheat bran (WB) was evaluated (100 % PS:0 % WB, 75% PS:25 % WB, 50 % PS:50 % WB, 25 % PS:75 % WB, and 0 % PS:100 % WB) on growth and composition of *T. molitor* and *Z. atratus* larvae. In a second experiment, 10 different diets for *Piaractus brachypomus* fingerlings were evaluated involving five levels (100, 75, 50, 25, and 0 %) of substitution of the fish meal of a conventional dietary formulation with meal of *T. molitor* or *Z. atratus* previously fed with PS. In the first experiment, the best treatment for both species was 25 % PS:75 % WB, resulting in the highest growth rate and consumption of PS. In the second experiment, no significant differences were found among treatments for any of the variables evaluated for productive performance for the fish. We conclude that up to 100 % of the fish meal in the diet of *Piaractus brachypomus* fingerlings may be replaced with meal of *T. molitor* or *Z. atratus* fed with PS, although there is a need for further studies regarding the long-term health effects on the fish and the humans that consume them.

Key words: cachama fish, circular economy, kingworm, mealworm, plastics

Consumo de poliestireno expandido por *Tenebrio molitor* y *Zophobas atratus*, y uso de su harina como alimento para *Piaractus brachypomus*

Resúmen. El poliestireno expandido (PS) representa un gran desafío para la gestión ambiental debido a sus altos niveles de producción e insuficiente manejo de residuos. Sin embargo, estudios recientes han demostrado la capacidad de *Tenebrio molitor* y *Zophobas atratus* para biodegradar el PS, así como la posibilidad de utilizar la harina de estas especies para alimentar peces. El objetivo de este estudio fue evaluar el efecto de la inclusión de la harina de estos insectos como sustituto de la harina de pescado dietas para alevines de *Piaractus brachypomus*. En un experimento, se evaluó el efecto de 5 niveles diferentes de PS y salvado de trigo (WB) (100 % PS: 0 % WB, 75 % PS: 25 % WB, 50 % PS: 50 % WB, 25 % PS: 75 % WB y 0 % PS: 100 % WB) en el crecimiento y composición de las larvas de *T. molitor* y *Z. atratus.* En un segundo experimento, se evaluaron 10 dietas diferentes para los alevines de *Piaractus brachypomus*, se emplearon cinco niveles (100, 75, 50, 25 y 0 %) de sustitución de la harina de pescado de una formulación dietética convencional con harina de *T. molitor* o *Z. atratus* previamente alimentadas con PS. En el primer experimento, el mejor tratamiento para ambas especies fue 25 % PS: 75 % WB, lo que resultó en la tasa de crecimiento más alta y consumo de PS. En el segundo experimento, no se encontraron diferencias significativas ($p > 0.05$) entre los tratamientos para ninguna de las variables evaluadas en el rendimiento productivo de los peces. Se concluye que hasta un 100 % de la harina de pescado en la dieta de los alevines de *Piaractus brachypomus* puede ser reemplazada con harina de *T. molitor* o *Z. atratus* alimentadas con PS, aunque se necesitan más estudios sobre los efectos a largo plazo en la salud de los peces y de los humanos que los consumen.

Palabras clave: Cachama, economía circular, gusano rey, gusano de la harina, plásticos

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Consumo de poliestireno expandido por *Tenebrio molitor* e *Zophobas atratus* e utilização de seu farelo como ração para *Piaractus brachypomus*

Resumo. O poliestireno expandido (PS) representa um grande desafio para o gerenciamento ambiental devido aos seus altos níveis de produção e ao gerenciamento insuficiente de resíduos. Entretanto, estudos recentes têm demonstrado a capacidade do *Tenebrio molitor* e *Zophobas atratus* de biodegradar o PS, bem como a possibilidade de utilizar a farinha dessas espécies para alimentar peixes. O objetivo deste estudo foi avaliar o efeito da inclusão de farinha de insetos como substituto da farinha de peixe em dietas para alevinos de *Piaractus brachypomus*. O primeiro experimento avaliou o efeito de 5 níveis de PS e farelo de trigo (WB) (100 % PS:0 % WB, 75 % PS:25 % WB, 50 % PS:50 % WB, 25 % PS:75 % WB e 0 % PS:100 % WB) no crescimento e na composição das larvas de *T. molitor* e *Z. atratus*. No segundo experimento foram avaliadas 10 dietas para alevinos de *Piaractus brachypomus*, sendo cinco níveis (100, 75, 50, 25 e 0 %) de substituição da farinha de peixe de uma formulação dietética convencional por farinha de *T. molitor* ou *Z. atratus* previamente alimentados com PS. No primeiro experimento, o melhor tratamento para ambas as espécies foi 25 % PS:75 % WB, resultando na maior taxa de crescimento e consumo de PS. No segundo experimento, não foram encontradas diferenças significativas entre os tratamentos para nenhuma das variáveis avaliadas para o desempenho produtivo dos peixes. Concluímos que até 100 % da farinha de peixe na dieta de alevinos de *Piaractus brachypomus* pode ser substituída por farinha de *T. molitor* ou *Z. atratus* alimentados com PS. Não entanto, são necessários mais estudos sobre os efeitos ao longo prazo na saúde dos peixes e nos seres humanos que os consomem.

Palavras-chave: Peixe cachama, economia circular, larva de besouro (kingworm), larva de farinha (mealworm), plásticos

Introduction

Consumption of plastic has quadrupled over the past 30 years, and global production of plastic has doubled from 230 million tons in 2000 to 460 million tons in 2019 (European Bioplastics, 2018). Twenty years ago, it was calculated that plastics accounted for 60-80 % of marine trash (Derraik, 2002), and currently they account for 3.4 % of global greenhouse gas emissions (OECD, 2022). In 2013, the synthetic biopolymer polystyrene (PS), commonly known as styrofoam, accounted for approximately 7.1% (21 t/year) of all plastic consumed (Plastics Europe, 2015). Currently, no environmentally safe processes exist for efficiently degrading plastics. The most widely used option is chemical management, which may take several months, requiring nitric acid and other corrosive substances with great environmental impact (Gutiérrez, 2013).

On a global level, different technologies have been developed to depolymerize PS more easily and more naturally and rapidly achieve its final disposal through biodegradation, by which biological action by fungi, bacteria, and other microorganisms decompose the material, progressively reducing its molecular weight (Wenxiao *et al*., 2024; Tania & Anand, 2023; Kyrikou and Briassoulis, 2007). Currently, insect species are being used to degrade different types of waste (Khan *et al*., 2021). This is the case of the coleopteras mealworm (*Tenebrio molitor*, Linnaeus 1758) and kingworm (*Zophobas atratus*, Fabricius, 1775), whose larvae are capable of degrading different types of plastics, including PS. For example, Jordan (2015) and Yang *et al*. (2018) observed depolymerization of PS by larvae of *T. molitor*, resulting in reduction of its molecular weight, thereby facilitating its degradation. Furthermore, the bacteria *Exiguobacterium* sp. has been found to play an important role in biodegradation of PS by these larvae by contributing to its despolymerization (Yang *et al*., 2015a,b).

By 2050, the global human population is projected to reach 9 billion, necessitating a 60 increase in food production (van Dijk *et al*., 2021), ideally including sustainable production of high-quality animal protein (Biasato *et al*., 2018). Feed costs, which account for 60-70 % of production expenses, are a major limitation to increasing animal production (Khan *et al*., 2018; Prodhan & Khan, 2018; Coffey *et al*., 2016; Van Huis, 2013). The urgent need to find alternatives to conventional feed ingredients such as fishmeal and soybean meal has led to the exploration of insect protein as a non-conventional ingredient (Shah *et al*., 2022; Amza *et al*., 2021; Azagoh *et al*., 2016; Van Huis *et al*., 2013). Insects, with their rapid growth rate, efficient food conversion, and minimal resource requirements, have great potential for improving the sustainability of intensive animal production systems (Imathiu, 2020). They have been part of the natural diet of many species, such as fish, birds, and pigs, and their use in fish and poultry diets contributes to reducing feed costs, producing biofertilizer from insect frass, and promoting social inclusion (Barragán-Fonseca *et al*., 2020; Barragán-Fonseca *et al*., 2022; Smith and Barnes, 2015; Szendrő *et al*., 2020).

Several insect species have been demonstrated to provide a viable source of protein for use as animal feed. The meal of *T. molitor* and *Z. atratus* has a protein content of approximately 45-50 % DM. The larvae of *T. molitor* may be incorporated live or in the form of meal or oil in the feed of pets or livestock (Gasco *et al*., 2020; Henry *et al*., 2015; Sogari *et al*., 2019). Additionally, studies have established digestibility rates of 60–80 % for crude protein of *T. molitor* (Kovitvadhi *et al*., 2019; Mancini *et al*., 2021), and 77–92 % for *Z. atratus* (Bosch *et al*., 2016; Kovitvadhi *et al*., 2019).

Studies have documented the feasibility of substituting up to 25 % of fish meal with meal of *T. molitor* in aquaculture production (Gasco *et al*., 2019). Feed formulated for aquaculture species that contain *T. molitor* as a principal ingredient has been demonstrated to have beneficial effects on growth rates – and in turn yield - of a variety of commercially raised fish, including rainbow trout (*Oncorhynchus mykiss)* (Jeong *et al*., 2020; Melenchón *et al*., 2021), porgy (*Sparus aurata*) (Fabrikov *et al*., 2020; Piccolo *et al*., 2017), tench (Tinca tinca) (Fabrikov *et al*., 2020), European bass (*Dicentrarchus labrax*) (Gasco *et al*., 2016; Mastoraki *et al*., 2020), blackspot seabeam (*Pagellus bogaraveo)* (Iaconisi *et al*., 2017), Nile tilapia (*Oreochromis niloticus*) (Sánchez-Muros *et al*., 2016; Tubin *et al*., 2020), and Pacific whiteleg shrimp (*Litopenaeus vannamei*) (Motte *et al*., 2019; Panini *et al*., 2017). These studies have also indicated that: 1) *T. molitor* improves the quality of the meat; 2) insects in general are a potential source of protein in the diet of fish; and 3) their inclusion may contribute to developing circular economies (Moruzzo *et al*., 2021) by degrading waste while producing high quality protein at a low cost.

Using insects as alternative protein sources may also have a positive impact on production of tropical omnivorous freshwater species such as white cachama (*Piaractus brachypomus*), which has great commercial potential due to its adaptability to a variety of foods, resistance to low oxygen levels, high productive parameters, and high demand due to the colour and flavour of its meat (Chirinos *et al*., 2022; Mesa and Botero-Aguirre, 2007; Ribeiro *et al*., 2016). For these reasons, it is one of the most widely raised species in Latin America, with a total yield in 2021 of 31,200 tons, which continues to increase (Ministerio de Agricultura y Desarrollo Rural, 2021).

The objective of this study was to evaluate the effect of including insect meal as a substitute for fishmeal in diets for *Piaractus brachypomus* fingerlings. This would allow for contributing to development of circular economies in agriculture involving consumption and degradation of PS by insects to be used to feed fish for human consumption.

Materials and Methods

Experiment 1. Consumption of PS by *T. molitor* and *Z. atratus*

Experimental individuals

The larvae of *T. molitor* and *Z. atratus* were obtained from a colony maintained under constant conditions (LD 12:12 h photoperiod, 27±1 °C, 70 % relative humidity) in the Center for Terrestrial Arthropod Research of the National University of Colombia. The experiment was carried out in the breeding room of the Entomology Laboratory of the Institute of Biotechnology of the National University of Colombia, under environmental conditions controlled for ideal development of the species (LD 8:16 h photoperiod, 28±2 °C, 75 % relative humidity), like those reported in previous experiments (Yang *et al*., 2018).

Diets and experimental design

To evaluate consumption of PS, for each insect species five diets (treatments) were formulated, which varied in their ratio of polystyrene (PS) to wheat bran (WB): 100 % PS:0 % WB, 75 % PS:25 % WB, 50 % PS:50 % WB, 25 % PS:75 % WB, and 0 % PS:100 % WB. WB was selected as it is a source of B complex vitamins, protein, amino acids, and trace minerals; it has been shown to be a good substrate for breeding these species (Gibson and Hunter, 2010; Vega and Dowd, 2005); and it has been suggested that it could optimize biodegradation of PS by *T. molitor* larvae (Nukmal *et al*., 2018). For the trial with each insect species each treatment had six replicates (plastic recipient with 50 insect larvae) randomly assigned according to a completely randomized design. The plastic recipient of each experimental unit was 16 cm long by 11 cm wide by 12 cm high in the case of *T. molitor*, and 20 cm long by 15 cm wide by 12 cm high in the case of *Z. atratus*. The larvae used was selected after 12 days of development, in the case of *T. molitor* measuring 2.0 +/- 0.2 cm (instar 14), and for *Z. atratus* 3.0 +/- 0.2 cm (instar 12). A prior experiment showed that PS consumption by the larvae of both species was greater when it was placed in the form of 5 +/- 1 mm thick sheets and 0,5 weight, and therefore PS was provided in this manner. At the start of the experiment, the WB+PS was supplied for the duration of the experiment to *T. molitor* and *Z. atratus*, consisting of 100 % of the initial weight of the experimental unit of the 50 individuals (Latney *et al*., 2017). The experiment duration was 64 days for *T. molitor* and 32 days for *Z. atratus*, with the disparity due to the extended life cycle of each species, given that *T. molitor* has a longer larval stage but they are larvae of lower weight and size. while the *Z. atratus* larva develops more quickly, generating a greater animal biomass in less time. For each treatment, productive performance of the larvae was evaluated based on the following variables: 1) total final weight (g); 2) survival (# live larvae at end of experiment ÷ # initial larvae x 100); and 3) consumption of PS (weight in g of PS at beginning of experiment – weight at end of experiment).

Proximate analysis of meal of larvae of *T. molitor* and *Z. atratus*

The larvae of *T. molitor* and *Z. atratus* of the treatment with the best productive performance after consumption of PS +WB were ground into meal. A protocol of elaboration of meal from each of the two species was developed, which basically consisted of fasting the larvae for 24 hours, then screening, washing and sacrifice by freezing (< 18 °C 24h) were carried out, followed by thawing for 1 hour at room temperature, then blanching in water at 60 °C for 5 minutes. The larvae were dehydrated at 60°C for 24 hours, and ground until obtaining a fine and homogeneous mixture, which formed the insect flour of each species. Protocol adapted from Melgar-Lalanne *et al*. (2019) and Arévalo Arévalo *et al*. (2022). At the Laboratory of Animal Nutrition of the National University of Colombia, a proximate analysis was carried out of the meal of the larvae of each species, analysing the following: dry matter, crude protein, crude fiber, ether extract, gross energy, ash, calcium, and phosphorus (Scientific Standards & Methods - AOAC, 2023).

Experiment 2. Replacement of fish meal in the diet of white cachama (*Piaractus brachypomus*) with meal of *T. molitor* and *Z. atratus* previously fed with polystyrene

Experimental individuals

Fingerlings of white cachama (*Piaractus brachypomus*) employed in the experiments were obtained from the company Aquaprimavera Ltda (Meta, Colombia). The experimental phase was carried out in the bioterium of the Corporation of Veterinary Pathology, where the experimental individuals were submitted to a 15-day period of adaptation prior to the experiment in five 80 L plastic tanks, in accordance with maintenance parameters for the species (Vásquez-Torres *et al*., 2011).

Experimental diets

A total of 10 experimental diets were formulated, replacing 100, 75, 50, 25, or 0 % of the fish meal in their dietary formulation with meal of *T. molitor* or *Z. atratus* previously fed with PS, which corresponded to 10, 7.5, 5.0, 2.5, and 0 % of their total diet. The following requirements for white cachama fingerlings were fulfilled: crude protein 36 %, lipids 6 %, 4588 kcal/kg, lysine 2.22 %, methionine 0.78 %, total phosphorous 1.39 %, digestible phosphorous 0.76 %, calcium 1.14 %, linolenic acid (18:2n-6) 0.8 %, and linolenic acid (18:3n-3) 0.2 % (Abdel *et al*., 2010; Gao *et al*., 2011; Vásquez-Torres and Arias-Castellanos, 2012). The formulation was developed using the Linear Program Solver by Microsoft Excel©. The resulting diets were both isoproteinic and isoenergetic (Table 1).

Experimental design

For the second experiment, 180 white cachama fingerlings were used, with an average weight of 1.48 ± 0.14 g, distributed in 30 tanks (20L each; 6 fish/ tank; 3 tanks/ treatment). Each tank had a central aerator and heating with an individual thermostat. The parameters of water quality, temperature, pH, and dissolved oxygen were recorded daily, and the parameters NO_2, NO_3, and NH_4 were recorded weekly. Each tank was cleaned daily by siphoning, replacing water lost through cleaning or evaporation. Diets were randomly assigned to the tanks and feed was supplied three times daily (09:00 am, 11:30 am, and 3:00 pm) for 35 days, providing 10 % of their weight in feed, according to the formulation in Table 1. Total weight of the fish of each tank was recorded weekly and amount of feed adjusted correspondingly. Initial weight (IW, g), final weight (FW, g), and feed consumption (FC, g/fish) were determined weekly and used to calculate the following variables: weight gain (WG, g) = FW–IW; feed consumption per fish (FC, g/fish) = total consumption per tank / final # fish in tank; specific growth rate (SGR (% growth/day = (100*l [(lm FW) - (ln FW] / (days of experiment). The feed conversion rate was calculated using the following formula: FCR = FC /WG. Rate of survival (S) was calculated using the following formula: S (%) = (final # fish / initial # fish) x 100.

Statistical analysis

Analysis of variance was carried out for data of both experiments; for experiment 1, analysis was carried out for each species using a completely random design, and for experiment 2, a completely random design with a 2 x 5 factorial arrangement was used, evaluating meal of larvae of each insect species for each of the five for each species using a completely random design, and for experiment 2, a completely random design with a 2 x 5 factorial arrangement was used, evaluating meal of larvae of each insect species for each of the five levels of inclusion of PS. In each analysis, assumptions of homogeneity of variances were verified through the Levene test, and normality of errors was verified through the Shapiro-Wilks test. For separation of means, the Tukey test was employed. For all statistical tests, the level of significance was 5 %. For experiment 1, regression models were used to describe degradation of PS in function of the level of inclusion of PS. All analyses were carried out using the R program (The R Project for Statistical Computing, 2021) .

Table 1. Experimental diets for white cachama (*Piaractus brachypo*mus), fully or partially replacing fish meal with meal of *T. molitor* (TM) and *Z. atratus* (ZA) fed with polystyrene[1]

Ingredients (%)	*Tenebrio molitor* Treatments					*Zophobas atratus* Treatments				
	100%TM	75%TM	50%TM	25%TM	0%TM	100%ZA	75%ZA	50%ZA	25%ZA	0%ZA
Tenebrio molitor meal	10	7.5	5	2.5	0	0	0	0	0	0
Zophobas atratus meal	0	0	0	0	0	10	7.5	5	2.5	0
Soybean meal, 45% CP, extracted with solvent	25	25	25	25	25	25	25	25	25	25
Soy, whole, extruded	10	10	10	10	10	10	10	10	10	10
Maize gluten meal, 60 % CP	14.4	14.4	14.4	14.4	14.4	14.4	14.4	14.4	14.4	14-4
Yellow maize	27.2	27.2	27.2	27.2	27.2	27.2	27.2	27.2	27.2	27.2
Tuna fish meal, manually extracted	0	2.5	5	7.5	10	0	2.5	5	7.5	10
Meat and bone meal 50%	10	10	10	10	10	10	10	10	10	10
Canola oil	2	2	2	2	2	2	2	2	2	2
Salt	.3	.3	.3	.3	.3	.3	.3	.3	.3	.3
Vitamin and mineral premix	.25	.25	.25	.25	.25	.25	.25	.25	.25	.25
Antioxidant	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013
Rovimix-stay-C 35, ascorbic monophosphate	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045
L-Lysine HCL	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61
Threonine	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Choline Chloride (60 %)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Parameters analyzed (%)										
Humidity	8.9	9.2	9.4	9.2	9.4	8.9	9.1	9.4	9.9	9.6
Dry matter	91.1	90.8	90.6	90.8	90.6	91.1	90.9	90.6	90.1	90.4
Crude protein (Nx6.25)	36.0	35.8	36.8	36.8	36.7	35.7	35.4	36.6	36.1	36.6
Crude fiber	1.5	1.3	1.4	1.4	1.3	1.3	1.2	1.2	1	1.4
Ether extract	7.7	8.6	7.8	9.3	7.8	10	9.6	8	8.3	7.4
Ash	5.4	5.6	5.9	6.3	6.6	5.4	5.6	6.0	6.3	6.9
Non-nitrogenated extract[2]	43.5	39.5	38.7	37.0	38.2	38.7	39.1	38.8	38.4	38.1
Calcium	1.6	1.7	1.7	1.8	1.9	1.5	1.4	1.7	1.7	2

[1]AOAC 1996. Official Methods of Analysis of the Association of Analytical Chemists

[2]Non-nitrogenated extract (%) = Dry matter – Crude protein – Crude fibre – Ether extract – Ash

Results

Experiment 1

PS consumption by *T. molitor* and *Z. atratus*

In the case of *T. molitor*, given varying initial larval weights, initial weight served as a covariate for weight-related analyses. Significance (P < 0.05) was observed only for final weight and total consumption, both showing positive covariate estimates, indicating higher values with increased initial weight at 60 days. The covariate persisted for all variables, and adjusted means were based on the mean initial weight (3.02 g/ 50 larvae). PS inclusion significantly (P < 0.05) affected total consumption and biodegradation percentage, generally resulting in lower values with increased PS levels (Table 2).

Table 2. Least squares method for the effect of different levels of PS inclusion on the performance of *Tenebrio molitor* larvae during a 32-day experiment (n=6). The initial weight was included as a covariate

Treatment	Initial weight (g/50 larvae)	Final weight (g/50 larvae)	Weight gain (g/50 larvae)	Total consumption (g/50larvae)	BD of PS (%)
100% PS:0%WB	3.13 a	4.37	1.35	0.45 e	17.67 b
75% PS:25% WB	3.05 ab	4.39	1.37	1.14 d	17.81 b
50% PS:50% WB	3.05 ab	4.46	1.50	1.90 c	26.34 b
25% PS:75% WB	3.05 ab	4.52	1.61	2.67 b	53.77 a
0% PS:100% WB	2.89 b	4.63	1.44	3.00 a	NA
EE	0.05	0.10	0.10	0.03	2.28
P-value	0.016	0.405	0.265	0.000	0.000
P-valor PI*		0.041	0.738	0.000	0.896

WB: wheat bran; PS: expanded polystyrene (styrofoam); SE: standard error of the means; BD: biodegradation; NA: not applicable. Different letters in the same column indicate significant differences as a result of the Tukey test (P<0.05). *P-value for the effect of the covariate initial weight.

The percentage of degradation of PS (Y) in function of level of inclusion of PS (X) was described using the Weibull function, resulting in the following equation: $Y = 100 - (83.11-83.10*\exp(-0.0076X^{1.448}))$ ($P > 0.05$; $R^2 = 0.98$; SE = 4). Quantity of PS degraded (QPSD), expressed as g / 100 g of substrate (PS + WB) was calculated by simulating QPSD = degradation PS (%)* Inclusion PS (%)/100, in function of the level of inclusion of PS. This indicates a growing tendency toward an initial maximum value of 14.1 g for a level of inclusion of 32 % with a level of degradation of PS of 44.05 % (14.1g); this level of degradation was achieved with inclusion of PS of 76 % and degradation of 18.6 % (14.1g) (Figure 1).

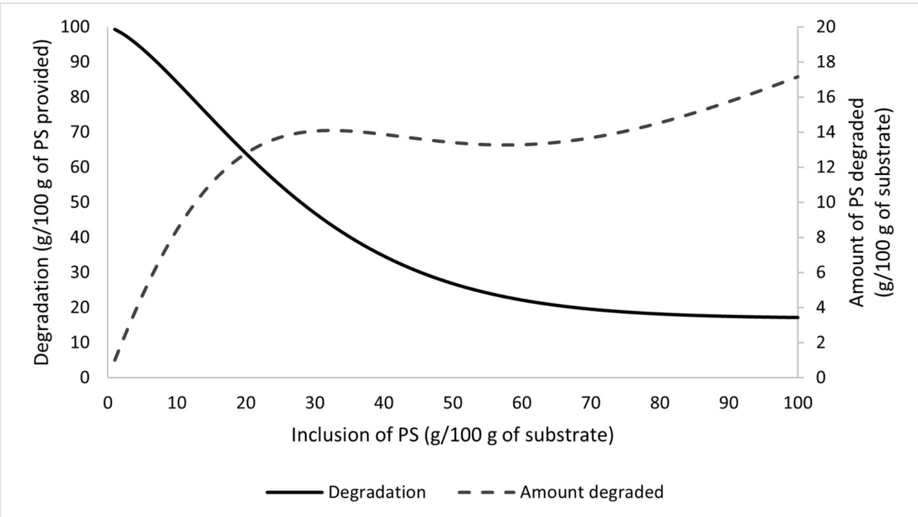

Figure 1. Regression model of the quantity of polystyrene (PS) biodegraded (g) by larvae of *T. molitor* in function of the level of inclusion of PS in their diet.

In the case of *Z. atratus*, increase in level of inclusion of PS reduced weight gain, total consumption of substrate (PS + WB), and degradation of PS ($P < 0.05$; Table 3).

Decrease in the percentage of degradation of PS (Y) in function of the level of inclusion of PS (X) was described through a Weibull third-degree polynomial, obtaining the following equation: $Y = 32.68-1.433*X+0.0246*X^2-0.000132*X^3$ ($P < 0.05$, $R^2 = 0.75$; SE = 1.49). The quantity of PS degraded expressed in g/100 g of substrate (PS + WB) was calculated as QPSD = Degradation PS (%) * Inclusion PS (%) /100 was simulated as function of the level of inclusion of PS. This indicates a growing tendency toward a maximum value of 6.4 g, with a level of inclusion of 48, and a level of degradation of 7.61 %. However, a peak value of 2.56 g was observed at an inclusion level of 21 %, accompanied by a 12.2 % degradation of PS (Figure 2).

Table 3. Effect of different levels of inclusion of polystyrene in the diet on productive performance of larvae of *Zophobas atratus* during a 30-day experiment

Treatment	Initial weight (g/50 larvae)	Final weight (g/50 larvae)	Weight gain (g/50 larvae)	Total consumption (g/50 larvae)	BD of PS (%)
100% PS: 0% WB	2.08	2.75 [b]	0.67 [bc]	0.08 [e]	3.76 [c]
75% PS:25% WB	2.08	2.70 [b]	0.63 [c]	0.65 [d]	8.05 [ab]
50% PS:50% WB	2.09	2.71 [b]	0.62 [c]	1.11 [c]	6.08 [bc]
25% PS:75% WB	2.12	2.92 [a]	0.80 [ab]	1.68 [b]	10.17 [a]
0% PS:100% WB	2.13	2.95 [a]	0.82 [a]	2.13 [a]	NA
SE	0.01	0.03	0.04	0.01	0.62
P value	0.062	0.000	0.001	0.000	0.000

WB: wheat bran; PS: expanded polystyrene (styrofoam); SE: standard error of the means; BD biodegradation; NA: not applicable. Different letters in the same column indicate significant differences as a result of the Tukey test ($P < 0.05$).

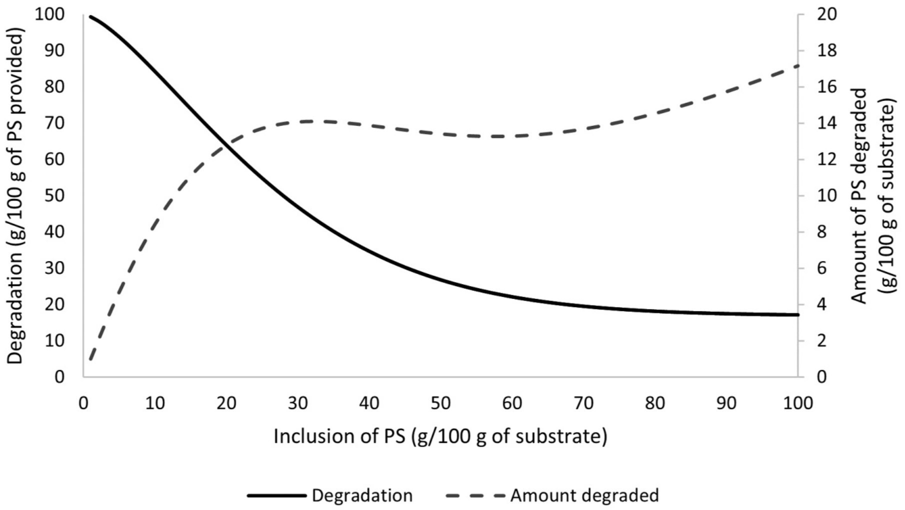

Figure 2. Regression model of the quantity of polystyrene (PS) biodegraded (g) by larvae of *Z. atratus* in function of the level of inclusion of PS in their diet.

Results allow for concluding that the best treatment for each of the two insect species was 25 % PS:75 % WB, which optimized growth and consumption of PS.

Proximate analysis of *T. molitor* and *Z. atratus meal*

Table 4 shows the results of the proximate analysis carried out of the meal of *T. molitor* and *Z. atratus* larvae fed with that treatment of experiment 1 which resulted in optimal insect growth and consumption of PS: 25 % PS:75 % WB.

Table 3. Effect of different levels of inclusion of polystyrene in the diet on productive performance of larvae of *Zophobas atratus* during a 30-day experiment

Parameter	*T. molitor*	*Z. atratus*
Moisture (%)	11.1	9.9
Dry matter (%)	88.9	90.1
Crude protein (Nx6.25)[1]	51.0	42.6
Crude fiber [1] (%)	6.1	4.6
Ether extract [1] (%)	25.6	36.2
Ash [1] (%)	3.5	2.2
Non-nitrogenated extract (%)	2.8	4.5
Calcium[1] (%)	0.06	0.05

[1]Scientific Standards & Methods - AOAC, 1996

Experiment 2

Replacement of fish meal with meal of *T. molitor* and *Z. atratus* fed with PS in diets of white cachama (*Piaractus brachypomus*)

In terms of productive parameters, no significant differences were observed ($p > 0.05$) among treatments for all the studied variables; rather, *P. brachypomus* fed with ten diets with different levels of inclusion of *T. molitor* and *Z. atratus* in turn fed with PS showed equal productive performance. Table 5 shows average values and standard deviation calculated for performance of *P. brachypomus* fingerlings. Survival of 100% of the fish was recorded for all treatments.

Table 5. Average values (±SD) of productive variables* for white cachama (*Piaractus brachypomus*) fed with diets including meal of *T. molitor* (TM) or *Z. atratus* (ZA), in turn fed with polystyrene

Experimental Diet	IW (g/fish)	FW (g/fish)	WG (g/fish)	SGR (%/day)	FC (g/fish)	FCR
100% TM	1.54 (0.08)	9.77 (1.68)	8.23 (1.76)	525 (064)	12.83 (0.93)	1.59 (0.22)
75% TM	1.46 (0.07)	9.53 (0.52)	8.06 (0.58)	5.35 (0.28)	13.09 (0.52)	1.63 (0.12)
50% TM	1.49 (0.16)	10.24 (2.15)	8.75 (2.23)	5.48 (0.77)	12.87 (0.84)	1.52 (0.27)
25% TM	1.54 (0.20)	11.19 (0.91)	9.64 (0.93)	5.67 (0.45)	13.84 (0.90)	1.44 (0.09)
0% TM	1.55 (0.08)	10.33 (0.74)	8.79 (0.79)	5.43 (0.33)	13.63 (0.66)	1.56 (0.18)
100% ZA	1.36 (0.03)	9.10 (0.71)	7.74 (0.71)	5.42 (0.23)	12.32 (0.65)	1.59 (0.07)
75% ZA	1.49 (0.04)	11.20 (1.03)	9.71 (1.00)	5.75 (0.22)	13.98 (0.57)	1.45 (0.15)
50% ZA	1.48 (0.04)	10.42 (0.92)	8.94 (0.94)	5.57 (0.30)	13.82 (0.30)	1.56 (0.14)
25% ZA	1.56 (0.09)	10.91 (0.80)	9.34 (0.90)	5.55 (0.38)	13.87 (0.09)	1.49 (0.15)
0% ZA	1.47 (0.14)	10.22 (2.18)	8.75 (2.05)	5.52 (0.35)	13.83 (2.08)	1.60 (0.13)
P-Value						
Inclusion of insect meal	0.5969 ^{ns}	0.3561 ^{ns}	0.4285 ^{ns}	0.8338 ^{ns}	0.1566 ^{ns}	0.6940 ^{ns}
Species	0.2973 ^{ns}	0.7437 ^{ns}	0.6762 ^{ns}	0.4285 ^{ns}	0.3582 ^{ns}	0.8944 ^{ns}
Inclusion of Insect meal x species	0.4293 ^{ns}	0.5874 ^{ns}	0.6491 ^{ns}	0.8844 ^{ns}	0.6074 ^{ns}	0.6941 ^{ns}

^{ns} non-significant differences (P > 0.05)

Productive variables measured: Initial weight (IW), Final weight (FW); Weight gain (WG) = FW – IW; Specific growth rate (SGR) = 100 [(ln FW) - (ln FW)] / (days of experiment); Feed consumption per fish (FC) = total consumption per tank / final number of fish in tank; Feed conversion rate (FCR) = FC / WG

Discussion

Consumption of PS by *T. molitor* and *Z. atratus*

The larvae of both *T. molitor* and *Z. atratus* were observed to be capable of consuming PS; for both species, in each treatment in which sheets of PS were placed, the larvae consumed the PS, as evidenced by orifices and loss of volume of PS. This was corroborated by data obtained for weight of PS remaining at the end of the experiment, as well as weight gain of individuals upon consumption of different proportions of PS and WB. These results indicate that larvae of *T. molitor* may reduce the volume of PS supplied by 10 to 65 % in 64 days, and larvae of *Z. atratus* may reduce PS by 3 to 12 % in 32 days (in accordance with the different life cycles of the two species). Larvae fed with a combination of PS and WB consumed more PS than those fed only with PS. For both insect species, the diet of 25 % PS:75 % WB resulted in the greatest level of PS consumption - and thus the greatest percentage of biodegradation of PS, which may indicate that provision of WB contributed to increased consumption – and optimized biodegradation – of PS by the larvae, as has been suggested for *T. molitor* (Yang *et al.*, 2018). Additionally, as a nutritional adjuvant, WB contributed to weight gain of individuals, principally for *Z. atratus.*

Previous studies have found greater increase in weight of the larvae - and proportionally the process of biodegradation - in treatments containing both plastic and natural feed such as wheat bran (Brandon *et al.*, 2018; Nukmal *et al.*, 2018; Yang *et al.*, 2018).This concords with the findings of the present study, which indicated that the best treatments were those containing 25 % PS and 75 % WB, the latter of which provides nutrients and has been proven to be a good substrate for breeding the species included in the present study and to foment biodegradation of PS by *Z. atratus* larvae (Nukmal *et al.*, 2018). Similarly, use of wheat bran was found to contribute to biodegradation of PS by the larvae in the present study. Due to their greater size, the larvae of *Z. atratus* showed greater consumption of PS than those of *T. molitor*. The differences between these species could also be associated with differences in their genetic traits and their intestinal microbial communities. Additional studies are necessary to address the mechanisms of degradation of PS in relation to the insects` digestive enzymes, the insect and microbial genes, and the natural dietary behaviors of *T. molitor* and *Z. atratus*.

It is noteworthy that although the rates of consumption of PS and weight gain of *T. molitor* and *Z. atratus* in those treatments with 100 % PS were lower than those fed with treatments including WB, the individuals increased in size as well as weight. This indicates that the larvae did assimilate some energy upon biodegrading PS. Thus, for all five treatments evaluated, the larvae of both species increased in weight while reducing the weight of PS, and survival was high in all treatments, which concords with Wang *et al.* (2022), who reported that these species may survive with solely plastic diets, although a decrease occurred in weight of the individuals after 20 days fed with this diet. Effectively, while larvae of *T. molitor* and *Z. atratus* may degrade these materials, they do not provide them with an efficient energy source to complete their life cycle, but rather merely allow them to survive (Kooijman, 2009; Wu *et al*., 2019).

Results of the nutritional analysis of the meals obtained from the *T. molitor* and *Z. atratus* larvae fed with the diet that resulted in the highest yield (25 % PS:75 % WB) were similar to those reported by other authors for these species (Basto *et al*., 2020; Benzertiha *et al*., 2019; Fontes *et al*., 2019; Gasco *et al*., 2016; Kulma *et al*., 2020; Soares *et al*., 2019; Zielińska *et al*., 2021). Protein is a principal nutritional component of insects used as animal feed, although protein content varies among species. Adults and larvae of the order Coleoptera have a protein content of 20-71 % (Sánchez-Muros *et al*., 2014). In the present study, the crude protein content obtained for *T. molitor* and *Z. atratus* fed with PS was 51 and 42.6 %, respectively, which is similar to those reported by other authors for meals of these species. This demonstrates their high protein content and their potential value as non-conventional sources of protein for feeding fish and livestock (De Marco *et al*., 2015). The present study obtained a crude fat content of 25.6 % for *T. molitor* and 36.2 % for *Z. atratus*, similar to those reported by other authors, indicating that the larvae of these insect species possess a high level of fat compared to other potential ingredients in animal feed. *Tenebrio* larvae have a high level of unsaturated fatty acids, corresponding to close to 80 % of their total fat, principally consisting of monounsaturated oleic acid and polyunsaturated linoleic acid (Valdez and Untiveros, 2010). This high fat content directly affects production of meal; compared to ingredients typically used in feed, *Tenebrio* meal has a greater level of clumping, is courser, and has a shorter shelf life, suggesting that oxidation of the lipid fraction of insects may be responsible for it easily becoming rancid (Ribeiro *et al*., 2019). This high fat content may provide energy when used in feed, but may also be a limiting factor as it may become rancid when using the live or complete larvae. The Ca content in larvae - and in insects in general - is relatively low due to the fact that the majority of insects do not possess an internal skeleton (Nowak *et al*., 2016). In the meals of the larvae of *T. molitor* and *Z. atratus*, Ca as well as P are low and should be taken into account upon balancing the rations for species that require high levels of Ca.

The experiment corroborated that the larvae of *T. molitor* and *Z. atratus* may provide an option for biodegrading PS, as they may consume PS and gain weight, independently of the nutritional adjuvant. However, it is clear that an adjuvant such as wheat bran may improve the efficiency of the biodegradation process, which may facilitate standardization of PS degradation on a medium or large scale. There is a need for additional studies of different adjuvants, including organic waste, that may contribute to efficiency of biodegradation in industrial insect production. With respect to the nutritional content of meals obtained from *T. molitor* and *Z. atratus*, this study showed values of dry matter, crude protein, and crude fibre similar to those reported by other authors, demonstrating that the larvae of these insect species, aside from being capable of biodegrading PS, may be a viable food source for animals and humans. Nevertheless, it is necessary to evaluate their safety for animal and human consumption, especially regarding presence of microplastics and toxic residues resulting from biodegradation of plastics, which may accumulate in the insects` biomass.

Replacement of fish meal with *T. molitor* and *Z. atratus* meal in the diet of white cachama (*Piaractus brachypomus*)

The results of the present study for productive performance of *P. brachypomus* fingerlings fed with the 10 experimental diets indicate that up to 100 % of the fish meal in dietary formulations for commercial fish production – or 10 % of the total diet - may be replaced with full fat meal of larvae of *T. molitor* or *Z. atratus* without negatively affecting their productive performance or survival. This allows for formulating isonitrogenated diets without having to modify ingredients of conventional dietary formulations other than fish meal. Thus, variations observed in content of ether extract and raw energy among the different treatments as a result of the proportion of these nutrients in the total insect meal provided did not affect the parameters studied.

P. brachypomus is omnivorous, and in its natural environment its feeding habits vary according to the food resources available in the rivers it inhabits. Although the majority of studies have been carried out on carnivorous species, principally with protein of *T. molitor*, a recent study (Couto *et al*., 2021) evaluated substitution of 10, 20, and 30 % of commercial feed concentrate with *T. molitor* meal in the diet of *P. brachypomus* fingerlings, finding the parameters of weight increase, total average growth, survival rate (57 %), feed conversion index, and the total productivity index similar to those of the control only in the case of 10 % replacement, while for 20 and 30 % replacement, yield of the fish decreased. A tendency toward weight loss in fish fed with diets with a high lipid content has been observed for *P. brachypomus* as well as other species. For example, for the red drum (*Sciaenops ocellatus*), upon comparing diets with 4, 7, 14, and 21 % of lipids, less growth was observed in those fed diets with the higher lipid contents (14 and 21 %) (Craig *et al*., 1999).

In their natural environment, *P. brachypomus* consumes seeds and fruits with high levels of carbohydrates, and uses carbohydrates as an energy source with greater efficiency than it does lipids (Vásquez-Torres *et al*. 2011). For example, a study of juvenile *P. brachypomus* evaluating nine commercial type isoproteinic diets (all with a protein content of 32 %) with different levels of carbohydrates (20, 28, and 36 %) and lipids (4, 8, and 12 %) indicated a clear tendency of reduction in weight gain of fish as the levels of lipids increased (Vásquez-Torrres and Arias-Castellanos, 2012). It appears that reduction in lipids in diets with a high carbohydrate content increases weight gain of fish. Similar results have been reported for fingerlings of Nile tilapia (*Oreochromis niloticus*), in which replacement of up to 50 % of fish meal with *T. molitor* meal did not affect the quantity of feed consumed, but negatively affected growth of the fish (Sánchez-Muros *et al*., 2016).

Such results of replacement of *T. molitor* meal may adversely affect productive parameters, whether due to the high fat content or the content of chitin, a polymer found in the exoskeleton of insects. For example, a study of tilapia (*Oreochromis niloticus*) suggests that consumption of chitin may reduce the efficiency of enzymes that break down nutrients in food, impeding full absorption of proteins and lipids by the gastrointestinal tract (Fontes, 2018). However, Henry *et al.* (2015) suggest that low productive indices in fish consuming insects may be attributed to a lack of certain amino acids in the protein of some insect species consumed, which could be avoided by combining different protein sources or supplementing with amino acids.

It has also been reported that different levels of inclusion of *T. molitor* meal may have positive effects on several species de fish. For example, for juvenile rainbow trout (*Oncorhynchus mykiss*), upon partially replacing (0, 33 and 66 %) of fish meal in dietary formulations with *T. molitor* meal (0, 25, and 50 % of the total diet), there were no differences in weight gain of the fish, or in the physical characteristics of the raw or cooked fillets (Iaconisi *et al*., 2018). Furthermore, replacement of 0, 25, 50, and 75 % of fishmeal with *T. molitor* meal in dietary formulations of yellow catfish (Pelteobagrus fulvidraco; 0, 9, 18, and 27 % of the total diet) did not result in significant differences in feeding rate, specific growth rate, or feed conversion efficiency (Su *et al*., 2017). Similarly, upon replacing 0, 35, and 71 % of fish meal with *T. molitor* meal in diets for gilthead seabeam (*Sparus aurata*; 0, 25, and 50 % of the total diet), it was found that up to 35 % of fish meal may be replaced by *T. molitor* meal without negative effects on weight gain (Piccolo *et al*., 2017). For fingerlings of the hybrid giant tiger grouper (*Epinephelus lanceolatus* x *Epinephelus fuscoguttatus*), Song *et al*. (2018) studied six diets, all of which were isonitrogenated and isolipidic, replacing 0, 6.25, 12.5, 18.75, 25, and 31.25 % of fish meal with *T. molitor* meal, finding the greatest growth rate with 12.5 % (4.92 % of the total diet) replacement of fish meal.

Several authors have evaluated inclusion of full fat, degreased, and hydrolyzed *Z. atratus* meal in diets for diverse species de fish. Nevertheless, no records exist of its use to feed *P. brachypomus*. For lubina (*Dicentrarchus labrax*) in aqua-entoponic systems using insects as feed, Stathopoulou *et al*., (2022) recently evaluated substitution of 10 % and 20 % of fish meal with defatted *Zophobas* meal, and Prachom *et al*. (2021) 11.1 to 44.4 % of fish meal with degreased meal of larvae of *Z. atratus* in a study feeding barramundi fish (*Lates carcarifer*). Both studies established that no significant differences exist among different rates of replacement of fish meal with insect meal in volume consumed, weight gain, final weight, feed conversion index, specific growth weight, or survival of individuals. Thus, we conclude that 10 % (Stathopoulou *et al*., 2022) to 12 % (Prachom *et al*., 2021) of the total diet may consist of degreased *Z. atratus* meal without negative effects on the productive performance of the fish.

Similar studies with hydrolyzed *Z. atratus* or *T. molitor* meal as a substitute for 40 % of fish meal in diets of juvenile brown trout (*Salmo trutta*) found no differences in parameters of growth, volume of feed consumed, or intestinal histomorphology (Mikołajczak *et al*., 2020). In a study by Doğankaya (2016) of rainbow trout (*Oncorhynchus mykiss*) in which 0, 25, 50, and 100 % of fish meal was replaced with *Z. atratus* meal, the best performance was found replacing 25 %, which was similar to the control treatment using commercial feed. Another study established replacing up to 25 % of fish meal in the diet of juvenile Nile tilapia (*Oreochromis niloticus*) with *Z. atratus* meal did not present any adverse effect on the productive parameters or body composition of the fish (Jabir *et al*., 2012). Similar results were found in the diets of cobia (*Rachycentron canadum*) upon replacing up to 30 % of fish meal with *Z. atratus* meal, without significant effects on growth among treatments, final average weight of the fish, or the feed conversion index (Chainark *et al*., 2022).

Conclusions

As observed in this study, in accordance with the results of other studies using these insect species as feed for different freshwater fish, it is possible to partially replace fish meal with *T. molitor* or *Z. atratus* meal without affecting the productive performance of the fish (Abdel *et al*., 2010; Henry *et al*., 2018). There is a need to evaluate replacement of greater levels of fish meal with other foods with a nutritional content similar to these insect meals, such as whole soybeans, soybean meal, and other protein sources used in the animal feed industry; to evaluate differences in productive performance upon using whole, degreased, vs. hydrolyzed insect meal; and to evaluate diets free of fish meal. Although this study found no differences among the various treatments of insect meal and fish meal on productive performance of the fish, it is important to evaluate use of different percentages of inclusion of the meal of insects that have consumed PS or other types of plastic, including over a greater time in the productive cycle of the fish, in order to observe any possible changes over time in productive performance or health problems. Evaluation of the hepatosomatic index (HSI) and viscerosomatic index (VSI) is necessary to determine the effect of insect meal on the fish`s metabolism, for example with respect to synthesis and secretion of digestive enzymes, digestion and absorption of food, and metabolism of carbohydrates (Gümüş and İkiz, 2009).

Authors' Ethical Animal Welfare Declaration: The authors declare that the experimental procedures were executed in accordance with the accepted principles of animal welfare in experimental science. These procedures were conducted only after the experimental protocols received approval from the Bioethics Committee of the Faculty of Veterinary Medicine and Animal Sciences at Universidad Nacional de Colombia.

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