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Research Article

House cricket (*Acheta domesticus*) as a modulator of gene expression in digestion and oxidative stress in the redhead cichlid (*Vieja melanura*) (Günther, 1862)

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ABSTRACT. Cricket meal (*Acheta domesticus*), an accepted protein source for fish feed, is an excellent nutritional alternative to fishmeal in aquaculture. This study evaluated the effects of cricket meal inclusion in the diet of the redhead cichlid (*Vieja melanura*) on growth and the expression of genes related to protein digestion and oxidative stress. Five experimental diets were formulated with 0, 25, 50, 75, and 100% cricket protein inclusion as a substitute for fish meal (FM). Two hundred twenty-five fish (0.17 ± 0.01 g) were fed for 45 days at a rate of 5% of their biomass under controlled conditions. Biometric measurements were taken every 15 days. At the end of the experiment, final biometric data were recorded, and organs and tissues were collected to evaluate gene expression by qPCR using the elongation factor 1 (*ef-1*) gene as a reference. Results showed higher absolute weight gain at T_{25} and higher specific growth rate at T_{25} and T_{50}, while feed conversion ratio and protein efficiency ratio were negatively affected at T_{100}, compared with T_0. Pepsin was overexpressed in the T_{25}, T_{50}, and T_{75} groups; trypsin in the T_{25} group; and chymotrypsin in the T_{25} and T_{50} groups. Regarding oxidative stress, the liver showed the highest antioxidant gene expression, with superoxide dismutase overexpressed from T_{50} onward, glutathione peroxidase only at T_{50}, glutathione S-transferase at T_{25}, T_{50}, and T_{75}, and catalase overexpressed at T_{100}. We conclude that the optimal FM replacement level with house cricket meal in *V. melanura* is 50%. Higher inclusion levels compromise its growth, health, and well-being.

Keywords: cricket meal; gene expression; cricket meal (*Acheta domesticus*); redhead cichlid (*Vieja melanura*)

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INTRODUCTION

The redhead cichlid (*Vieja melanura*) is a neotropical endemic cichlid of commercial importance in Tabasco, Mexico, prized for its flavor and meat quality. It is distributed from the Grijalva-Usumacinta river system and neighboring watersheds in Mexico to Belize and Guatemala on the Atlantic slope of Central America, inhabiting lagoons, cenotes, streams, and rivers (McMahan et al. 2011, 2017, Mendoza-Carranza et al. 2018). As a mid-trophic-level species, it feeds on benthic detritus of plant matter and small crustaceans (Conkel 1993), which can potentially reduce production costs by 18 to 25% compared to high-trophic-level species (Ramírez-Ochoa et al. 2025), leading to proposals for its inclusion in aquaculture for restocking and human consumption, which requires the design of specific diets to ensure successful production. Currently, a major challenge in aquaculture is finding an alternative protein source to fish protein for fish feed that is highly digestible and bioavailable, high quality and nutritional value, cost-effective, palatable, and capable of improving feed conversion ratios and fish growth while reducing production costs. It must also be accessible, sustainable, environmentally friendly, and allow partial or total replacement of fish meal (FM) (Akter et al. 2025). Therefore, the ingredient selection process must evaluate its effects on fish growth and health (Guerreiro et al. 2022). The most studied protein sources are plant-based and animal by-products, but they often lack essential amino acids (EAAs) and contain antinutritional factors that reduce amino acid digestibility and assimilation, which can cause health problems (Sawant & Salin 2020, Calo et al. 2024). Zlaugotne et al. (2022) indicate that, while soybean meal is economical, it lacks the sustainability of insect meal, which is rich in protein and digestible fats, as well as fiber, minerals, vitamins, chitin, and other macronutrients that act as beneficial immunostimulants (Sawant & Salin 2020, Ojha et al. 2021, Islam et al. 2024). These flours typically have a lower fiber content and a more balanced amino acid profile, with limiting factors including low carbohydrate levels (less than 20%), a high chitin content, and deficiencies in methionine, lysine, and arginine, which can act as nutrients or antinutrients (Tran et al. 2015, Eggink et al. 2022, 2025). However, EU legislation has authorized their use in aquatic animal feed since 2017 (Regulation EC 1069/2009) (Sawant & Salin 2020).

Optimal inclusion in aquaculture has improved fish growth and health. For example, in snakehead fish (*Channa argus*), a 30% inclusion of cricket meal (*Gryllus testaceus*) improved antioxidant activity, the hepatic inflammatory response, and beneficial microbiota such as proteobacteria (Liu et al. 2023). The African catfish (*Clarias gariepinus*) also improved its crude protein content and hepatic antioxidant activity when fed a diet consisting entirely of cricket meal (*Gryllus bimaculatus*). In yellow catfish (*Pelteobagrus fulvidraco*), a 60% inclusion increased serum antioxidant capacity, serum glucose, and EAAs (arginine and valine) in muscle, while decreasing total cholesterol in juveniles (Fan et al. 2023, Huang et al. 2025).

A meta-analysis indicates that replacing 40% of FM with insect meal improves the immune response in fish, as dietary lipids modulate the expression of immune-related genes (Chen et al. 2024). However, inclusion levels exceeding the optimal range may negatively affect fish health and growth. To date, the highest inclusion levels of insect meal as a FM substitute have been achieved with cricket meals (A. *domesticus*, *G. bimaculatus*, and *Teleogryllus mitratus*), reaching 100% in guppies (*Poecilia reticulata*), *C. gariepinus*, *Oreochromis niloticus*, and *Xiphophorus helleri* (Perera & Bhujel 2022, Fan et al. 2023, Perera et al. 2023, 2025, Akle et al. 2025). However, suboptimal inclusions reduce digestive capacity, as reported for *O. niloticus* and rainbow trout (*Oncorhynchus mykiss*) fed black soldier fly (BSF), and for black bass (*Micropterus salmoides*) fed cricket meal (*Gryllodes sigillatus*) (Eggink et al. 2022, Wang et al. 2022). The main digestive proteases are: a) pepsin, which is a gastric protease that acts in the stomach at a pH of 2, and b) trypsin and chymotrypsin, which are alkaline or intestinal proteases that act at pH 7 and 11, respectively; these enzymes can be affected by the dietary regimen, which can modulate the activity and secretion of these digestive enzymes and antioxidant enzymes in fish through gene expression (Murashita et al. 2015, Fan et al. 2023, Liu et al. 2023, Huang et al. 2025), because certain nutrients bind directly or indirectly affect transcription factors, which regulate the expression of specific genes (Salazar et al. 2013).

In this context, the intestine is the first organ that can be affected by diet, as it is the primary site for lipid degradation and absorption, as well as the major site for amino acid and sugar absorption, making it exposed to potential oxidative stress induced by insect meal (Concha-Frías et al. 2018, Wang et al. 2020, Mi et al. 2022), while the liver is the organ where antioxidant enzymes have the greatest activity. Finally, the muscle is the tissue where fish growth can be observed and where the final damage from reactive oxygen species (ROS) can be reflected. In these organs, nutrients such as amino acids and carbohydrates bind directly or

indirectly to DNA transcription factors, which regulate the expression of specific genes (Salazar et al. 2013), as observed in the Nrf-2 transcription factor, which governs the antioxidant response system (Sies 2015, Marrocco et al. 2017), positively or negatively affecting fish growth; therefore, many studies have focused on analyzing gene expression as a dietary effect. This alteration can increase oxidative stress, leading to an imbalance of ROS, including the superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical (•-OH) (Chowdhury & Saikia 2020), which under appropriate physiological conditions help to protect the organism against various diseases, meet cellular energy needs (Lozada & García 2009), prevent damage, and maintain intracellular balance, where enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione S-transferase (GST), and various antioxidant molecules such as ascorbic acid (vitamin C) and α-tocopherol (vitamin E) neutralize ROS. However, if ROS generation exceeds antioxidant defense capacity, it can damage DNA, proteins, and cell membrane lipids, causing immunosuppression from cellular damage and compromising fish health (Ríos de Molina 2003, Pamplona & Costantini 2011).

House cricket meal (HCM) is one of the seven insect species approved by the EU for aquaculture feed production; it belongs to the order Orthoptera and forms part of the natural diet of some fish (Sawant & Salin 2020). For example, in poeciliids, cricket meal can replace 100% of FM without affecting growth (Perera & Bhujel 2022). In contrast, efficient cultivation of *V. melanura* depends largely on designing specific diets adapted to its physiological needs. Therefore, this study aimed to evaluate the effect of HCM on the relative gene expression of *pepsin*, *trypsin*, *chymotrypsin*, *superoxide dismutase* (*sod*), *catalase* (*cat*), *glutathione S-transferase* (*gst*), and *glutathione peroxidase* (*gpx*), and its effect on growth in juvenile *V. melanura*, while controlling water physicochemical variables to prevent them from influencing gene expression.

MATERIALS AND METHODS

This study followed the Declaration of Helsinki and the protocol authorized by the Secretariat of Agriculture, Livestock, Rural Development, Fisheries, and Food (SAGARPA), Mexico (Norma Oficial Mexicana 2001), ensuring proper handling and treatment of the organisms and adherence to ethical guidelines for animal research.

Organisms and experimental design

Two hundred twenty-five juveniles of *V. melanura* with an average weight of 0.17 ± 0.01 g were used. The organisms were acclimated for 15 days in a 15,000-L pond at the Jalpa de Méndez Multidisciplinary Academic Center, Universidad Juárez Autónoma de Tabasco. Subsequently, they were randomly distributed into 15 experimental units at a density of 15 fish per unit, with a capacity of 80 L and a recirculation system providing 100% daily water exchange. The temperature was maintained at 28 ± 2°C, the pH at 7 ± 0.2, and the photoperiod at 12 h of light. Temperature was measured using a YSI 55 oximeter, and pH using a high-precision (0.01) Gomass digital pH meter. The experiment lasted 45 days.

Experimental diets

Five isoproteic diets (35% protein) were formulated with increasing levels of HCM inclusion at 0, 25, 50, 75, and 100%, designated as groups T_0, T_{25}, T_{50}, T_{75}, and T_{100}, respectively (Table 1). Table 2 shows the proximate analyses of the diets. The fish were fed four times a day (9:00, 11:30, 14:00, and 16:30 h) at 5% of their biomass. The treatments were conducted in triplicate.

Biological sampling

Biometric measurements were taken every 15 days until the bioassay concluded. The average weight of the organisms was obtained by weighing on a high-precision (0.01 g) CGOLDENWALL analytical balance, and measured length individually with an ichtyometer. At the end of the bioassay, we randomly selected five organisms per experimental unit and euthanized them by thermal shock after immersion in clove oil (1 mL L^{-1}). Because the organisms were small (<2 g), euthanasia was practically instantaneous (<8 s). Subsequently, tissues from the stomach, intestine, and liver were collected and preserved in RNA Later (Life Technologies) at room temperature for 24 h, and then stored at -20°C until analysis.

Determination of growth parameters

The following parameters were recorded: absolute weight gain (AWG), specific growth rate (SGR), daily growth coefficient (DGC), feed conversion ratio (FCR), protein consumption (PC), feed efficiency (FE), protein efficiency ratio (PER), daily weight gain (DWG), condition factor (K) and survival (P).

At the end of the bioassay, the fish were fasted for 48 h. Biometric parameters were determined at the end of the experiment according to Concha-Frías et al. (2018),

Table 1. Experimental diet formulations with different levels of cricket meal (*A. domesticus*) inclusion. Values are presented for 100 g preparation, and all ingredients were measured in grams. HCM: house cricket meal.

Ingredients	0% HCM	25% HCM	50% HCM	75% HCM	100% HCM
Soft wheat grain	43.44	42.56	42.74	41.86	41.6
Fish meal (herring)	26.01	20	12.83	5.72	0
Soybean meal 44%	20	18.9	18	18	17.03
Cricket meal	0	8	16	24	3
Fish oil	3.05	4	4	4	3.86
Soy lecithin	3	2.05	1.93	1.92	2
Gelatin	2	2	2	2	2
Vit-min premix	1	1	1	1	1
dehydrated fish solubles	1	1	1	1	1
Vitamin C	0.5	0.5	0.5	0	0
Nutrients	As fed	As fed	As fed	As fed	As fed
MEt energy (m)	1355.36	1654.59	1987.15	2286.38	2564.29
Protein	35	35	35	35	35
Total fat	10	10	10	10	10
Fiber	2.89	3.54	4.1	4.75	5.27
Ashes	5.07	4.31	3.68	2.92	2.31

Table 2. Comparison of amino acid profiles of fish meal and house cricket meal used in the experimental diets (mg 100 g^{-1}, dry weight basis). N: nitrogen, C: carbon, Cal: calories.

Treatment	Protein (%)	Approx. sample weight	% N	% C	Humidity	Ashes	Total lipids	Cal g^{-1}
T_0	0	1,074	6.57	45.31	11.460	6.598	3.771	4573.192
T_{25}	25	1,049	6.82	46.05	9.703	6.613	6.048	4678.849
T_{50}	50	1.032	6.81	45.71	11.796	6.556	4.217	4868.103
T_{75}	75	0.949	6.75	46.89	10.079	4.862	7.702	4419.706
T_{100}	100	1.118	7.10	46.42	10.129	5.420	5.986	5125.193

Huang et al. (2025), and De la Cruz-Alvarado et al. (2025), using the following formulas: AWG (g $fish^{-1}$) = Wf (g) - W_i (g); SGR % = 100 (Ln W_f - Ln W_i) / T, where W_i and W_f are the initial and final weights of the fish, respectively, and T is the total number of days of the experiment.

DGC (mg) = ($Wf^{1/3}$ - $Wi^{1/3}$) / T (d); PC = ((feed consumption (g d^{-1}) × (dietary protein level)) / 100; FCR = dry feed delivered (g) / wet weight gain (g); PER = wet weight gain (g) / protein delivered (g); DWG (g d^{-1}) = WG (mg) /T (d); K = ((Wf (g) / total length $(cm)^3$ × 100 and FE = (Wf - Wi) / dry feed delivered; P (%) = final fish / initial fish × 100.

RNA extraction and cDNA synthesis

We analyzed the mRNA expression of oxidative stress zymogens in these three organs to evaluate the effect of ROS, from food digestion and absorption to cell proliferation. Total RNA for the expression studies of *pepsin* (stomach), *trypsin* and *chymotrypsin* (intestine), and oxidative stress zymogens (intestine, liver, and muscle) was extracted using TRIzol reagent (Invitrogen); the product obtained was rehydrated in ultrapure distilled water and was subsequently quantified at 260/280 to identify the purity and concentration of the samples. We performed cDNA synthesis using 1 µg of RNA and a random primer with the iScriptTM Select kit (Cat. No. 170-8896, Bio-Rad, Hercules, California, USA) following the manufacturer's instructions. We performed RT reactions in a thermal cycler (Mastercycler nexus GSX1, Eppendorf AG, Hamburg, Germany), primarily following De la Cruz-Alvarado et al. (2025). The standard RT program used was as follows: cDNA synthesis with oligo(dT)18 or a gene-specific primer. Incubation was performed for 60 min at 42°C. For the synthesis with a random hexameric primer, incubation was performed for 5 min at 25°C and subsequently for 60 min at 42°C. To terminate the reaction, the sample was heated to 70°C for 5 min.

Table 3. Sequences, sizes, and efficiency of oligonucleotides used for *Vieja melanura*.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Size (pb)	Amplification efficiency (%)
Pepsin	GGCTGCATGACTGGTTTTGG	TCTAGTGGATCTCTCAGTGGATA	154	96.30
Trypsin	GAACTCCGGCTACCACTTCT	TAGGAGCTGTAGTTGGGGTG	183	97.54
Chymotrypsin	AACGAGAACTGGGTGGTGAC	ATTGTGTGGGGGTTCCAGTA	158	97.50
Superoxide dismutase (*sod*)	CCCGTTTGATTGCCTCCATT	GCAAAGGGAGACGTGACAAC	151	96.50
Catalase (*cat*)	CTTGGGTCACGTTGTCATCG	CCCGTGTGGCAAACTATCAG	186	97.23
Glutathione-s-transfe-rase (*gst*)	TGAAACGCCTGGATTTTGGG	AACCGGCACACCTCAATACT	199	97.30
Glutathione peroxi-dase (*gpox*)	GCACATGAAACAGTCCTCGG	GAGCCTCCTTCGTCTTCACT	192	97.12
ef-1	GAGGAAATCACCAAGGAAGTGA	CTTGAACCAGCTCATCTTGGAG	154	99.82

Gene expression analysis (qPCR)

Oligonucleotide primers specific for *pepsin* (EC 3.4.23.1), *trypsin* (EC 3.4.21.4), *chymotrypsin* (EC 3.4.21.1), *sod* (EC 1.15.1.1), *cat* (EC 1.11.1.6), *gst* (EC 2.5.1.18), and *gpx* (EC 1.11.1.9) (Table 3), using data from NCBI and Primer3Plus. The qPCR reactions were performed on a CFX96 real-time PCR system (Bio-Rad) using Luminaris Color HRM master mix (Thermo Scientific). The total reaction volume was 10 µL. The RT-qPCR program included an initial denaturation step at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 45 s. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method, with elongation factor 1 (*ef*-1) as the reference gene (Pfaffl 2001).

Statistical analysis

Growth parameters were analyzed using one-way ANOVA and Duncan's test, and gene expression data using Nemenyi's *post-hoc* tests, after verifying normality. Differences were considered statistically significant at a *P*-value < 0.05. All analyses were performed using STATISTICA™ software version 7.0 (Statsoft, Tulsa, OK), and graphs were generated using SigmaPlot version 12.0.

RESULTS

Growth Performance

In terms of growth parameters and gene expression, the T_{25}, T_{50}, T_{75}, and T_{100} treatments were compared to T_0, the control diet with 0% HCM inclusion. Animals fed the T_{25} diet had higher final weight (1.66 ± 0.21 g) and length (4.56 ± 0.05 cm), followed by T_0 (1.63 ± 0.06 g and 4.31 ± 0.12 cm, respectively). AWG and SGR (Table 4) also showed the highest T_{25} and T_0 values, respectively, with lower values in the remaining treatments. Completely replacing FM with HCM increased FCR values. The lowest FCR was observed in T_{50} and T_{75}, while T_0, T_{25}, and T_{100} showed no statistically significant differences among the groups. Likewise, the T_{100} group showed lower PER and FE and differed significantly from the other treatments. Although T_0, T_{25}, T_{50}, and T_{75} did not differ significantly, a decreasing trend was observed as HCM inclusion increased. No treatments negatively affected P.

Relative expression of digestive genes

Digestive gene expression showed a pattern modulated by the proportion of the alternative ingredient. Fish fed T_{25}, T_{50}, and T_{75} showed overexpression of the *pepsin* gene in the gastric tract compared to T_0. In contrast, fish in the T_0 and T_{100} treatments showed no statistically significant differences between them (Fig. 1a). On the other hand, *trypsin* gene overexpression was observed in the intestine of fish fed T_{25}, which decreased progressively with increasing HCM inclusion (Fig. 1b). C*hymotrypsin* gene expression showed a pattern similar to that of *trypsin*, with overexpression in the T_{25} treatment, followed by T_{50}, and under expression in T_{75} and T_{100}, respectively (Fig. 1c).

Relative expression of oxidative stress genes

Regarding the effect of cricket meal inclusion levels on the gene expression of enzymes involved in oxidative stress, the bioassay revealed that the expression of *sod* and *cat* in the intestine was lower than or equal to T_0 at inclusion levels up to 75% but showed overexpression when the fish were fed a diet with 100% inclusion. Furthermore, increasing cricket meal level increased *sod* and *cat* expression starting at T_{50}, while downreg-

Table 4. Biometric data of *Vieja melanura* fed diets with different inclusion levels of *Acheta domesticus*. The values in the table represent the means ± standard deviation. Means with different superscripts differ significantly at $P < 0.05$. Wi: initial weight; Wf: final weight; LF: final length; AWG: absolute weight gain; FCR: feed conversion ratio; PC: protein consumption; PER: protein efficiency ratio; SGR: specific growth rate; FE feed efficiency; and P: survival.

Control	T_0	T_{25}	T_{50}	T_{75}	T_{100}
Wi (g)	0.18 ± 0.0	0.17 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.17 ± 0.01
Wf (g)	1.63 ± 0.06^{b}	1.66 ± 0.21^{b}	1.26 ± 0.16^{a}	1.07 ± 0.02^{a}	0.97 ± 0.05^{a}
LF (cm)	4.31 ± 0.12	4.56 ± 0.05	4.17 ± 0.15	4.37 ± 0.15	4.12 ± 0.35
AWG (g)	1.45 ± 0.06^{c}	1.49 ± 0.22^{c}	1.1 ± 0.16^{b}	0.9 ± 0.02^{al}	0.8 ± 0.06^{a}
FCR	1.65 ± 0.07^{a}	1.67 ± 0.12^{a}	1.93 ± 0.23^{a}	2.03 ± 0.1^{ab}	3.14 ± 0.93^{b}
PC (%)	1.10 ± 0.01^{abc}	1.13 ± 0.09^{bc}	0.96 ± 0.03^{ab}	0.84 ± 0.02^{a}	1.3 ± 0.31^{c}
PER	1.32 ± 0.06^{b}	1.31 ± 0.09^{b}	1.14 ± 0.14^{b}	1.08 ± 0.05^{b}	0.65 ± 0.23^{a}
SGR (%)	4.95 ± 0.09^{b}	5.12 ± 0.38^{b}	4.65 ± 0.34^{b}	4.11 ± 0.09^{a}	3.86 ± 0.23^{a}
FE (g)	0.61 ± 0.03^{b}	0.60 ± 0.04^{b}	0.52 ± 0.07^{b}	0.49 ± 0.02^{al}	0.34 ± 0.12^{a}
P (%)	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0

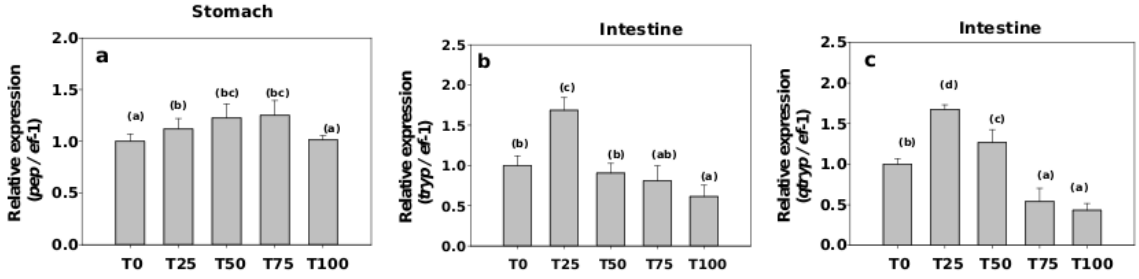


Figure 1. a) Relative expression of the *pepsin* (*pep*) gene in the stomach, b) relative expression of the *trypsin* (*tryp*) gene, and c) relative expression of the *chymotrypsin* (*qtryp*) gene in the intestine of *Vieja melanura* fed diets with different inclusion levels of house cricket meal s a protein source to replace fish meal. Data represent the mean ± standard deviation. Different letters indicate significant differences among treatments (ANOVA, $P < 0.05$). *ef*-1: elongation factor 1.

ulation persisted at T_{25} and T_{50} (Figs. 2a-b). In contrast, intestinal *gpx* was downregulated in all treatments compared with T_0 (Fig. 2c). *gst* was overexpressed at T_{25}; however, higher inclusion levels showed a pattern of under expression (Fig. 2d).

In the liver, *sod* was overexpressed at T_{50}, T_{75}, and T_{100}, with no significant differences among these three treatments compared with T_0 and T_{25} (Fig. 3a). In contrast, *cat* showed overexpression only when FM was completely replaced (Fig. 3b). In fish fed T_{25}, *gpx* expression was downregulated. In the remaining treatments, expression remained unchanged with no significant differences compared to T_0 (Fig. 3c). Similarly, *gst* was overexpressed in T_{25}, T_{50}, and T_{75} compared to T_0, while T_{100} showed no significant differences compared to T_0 (Fig. 3d).

In muscle tissue, sod increased gradually with dietary HCM, showing overexpression in T_{100} and under expression in T_{25}. At the same time, fish fed T_{75} showed no statistically significant differences compared to T_0 (Fig. 4a). In contrast, *cat*, *gpx*, and *gst* showed downregulation in all treatments containing HCM compared to the control (T_0) (Figs. 4b-d).

DISCUSSION

Insect meal has attracted research attention as a potential substitute for FM in aquaculture feeds for species such as *O. mykiss* and Siberian sturgeon (*Acipenser baerii*) fed BSF meal and *Tenebrio molitor* (TM) (Melenchón et al. 2020, Rawski et al. 2020); golden pomfret (*Trachinotus ovatus*) fed BSF meal (Yu et al. 2023); and *C. gariepinus*, and *O. niloticus* fed *G. bimaculatus* and HCM (Fan et al. 2023, Akle et al. 2025), among others. Insect meal inclusion levels depend on the insect and fish species because of the insect's nutritional composition and the fish's nutritional requirements and digestive capacity. Gebremichael et al.

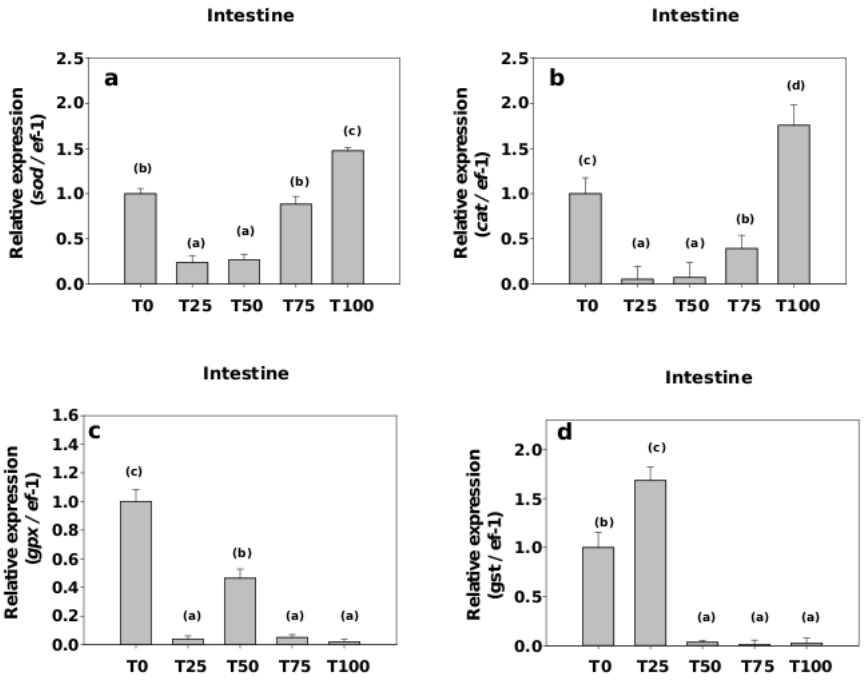


Figure 2. a) Relative expression of the superoxide dismutase (*sod*) gene, b) relative expression of the catalase (*cat*) gene, c) relative expression of the glutathione peroxidase (*gpx*) gene, and d) relative expression of the glutathione S-transferase (*gst*) gene in the intestine of *Vieja melanura* fed diets with different inclusion levels of house cricket meal as a protein source to replace fish meal. Data represent the mean ± standard deviation. Different letters indicate significant differences among treatments (ANOVA, $P < 0.05$).). *ef*-1: elongation factor 1.

(2023) reported that *C. gariepinus* utilized BSF meal more efficiently than TM meal.

In our bioassay, fish fed diets T_0 and T_{25} showed a significantly higher AWG than fish fed T_{50}, T_{75}, and T_{100}. This finding is consistent with Yu et al. (2023), who found that including 25% TM meal in the diets of *T. ovatus* improved feed intake, hepatic lipid deposition, and colonization by beneficial bacteria, thereby enhancing digestion, growth, and fish health. Similarly, Rawski et al. (2020) reported that replacing 30% of FM with BSF improved FCR, SGR, and PER in *A. baerii*. In our study, fish with the highest FCR and lowest PER were observed in T_{100}, while T_0, T_{25}, and T_{50} showed the best PER values; higher substitutions affected growth. Comparable inclusion levels for BSF and TM meals have been reported in *O. mykiss*, leading to improved growth, PER, and intestinal histological changes that enhanced nutrient utilization (Melenchón et al. 2022). Gebremichael et al. (2023) also observed improved growth and metabolic responses in *C. gariepinus* fed BSF meal.

Higher PER and/or FE can lead to higher SGR, as reported for *T. ovatus* (Yu et al. 2023), while a lower PER and/or FE may result in a higher FCR, as observed in *C. gariepinus* fed partial and complete replacements with BSF and TM meals (Gebremichael et al. 2023). We found that inclusion levels above 50% affect SGR, a pattern consistent with reports for *O. niloticus*, *O. mykiss*, and *C. gariepinus* (Eggink et al. 2022, Gebremichael et al. 2023). This decrease likely reflects the diet's chitin content. Moderate amounts of chitin (e.g. 3%) have been shown to improve growth factors such as SGR and FCR. In contrast, higher levels (e.g. 4.5%) can decrease levels of cholesterol, triglycerides, total protein, albumin, and aspartate aminotransferase in *O. mykiss* (Pascon et al. 2024), as it reduces diet palatability and SGR, consequently increasing the FCR (Naveed et al. 2023, Zhao et al. 2023, Zulhisyam et al. 2023).

Expression of digestive proteases

Pepsin is an acid protease with optimal activity at low pH, and its expression is influenced by the type and quality of dietary protein (Lazo et al. 2007). Regarding the effect of CM inclusion levels on *pepsin* gene expression, our results showed overexpression in T_{25}, T_{50}, and T_{75}, with T_{100} values similar to the control (T_0). No published studies were found on the effects of *H.*

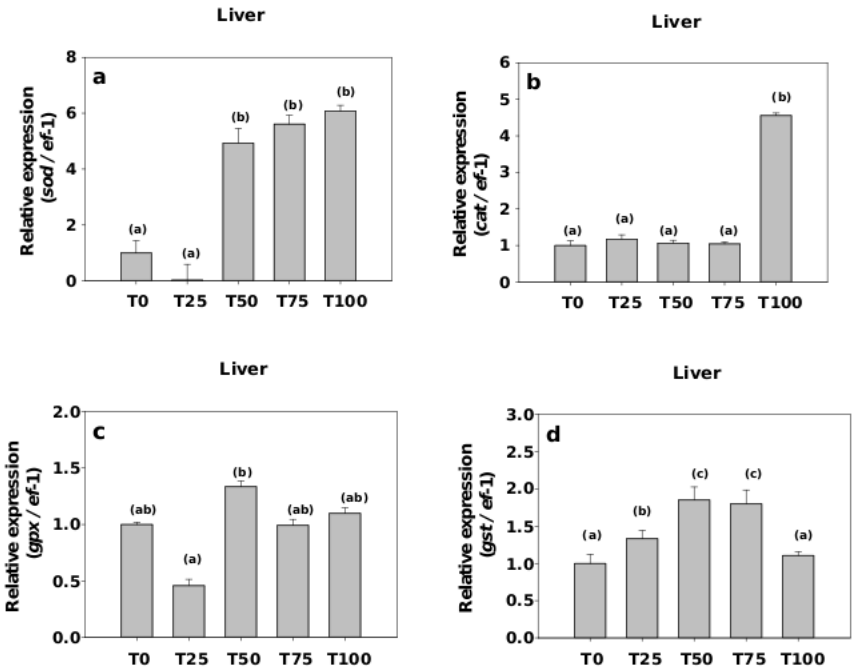


Figure 3. a) Relative expression of the superoxide dismutase (*sod*) gene, b) relative expression of the catalase (*cat*) gene, c) relative expression of the glutathione peroxidase (*gpx*) gene, and d) relative expression of the glutathione S-transferase (*gst*) gene in the liver of *Vieja melanura* fed diets with different inclusion levels of house cricket meal as a protein source to replace fish meal. Data represent the mean ± standard deviation. Different letters indicate significant differences among treatments (ANOVA, $P < 0.05$). *ef*-1: elongation factor 1.

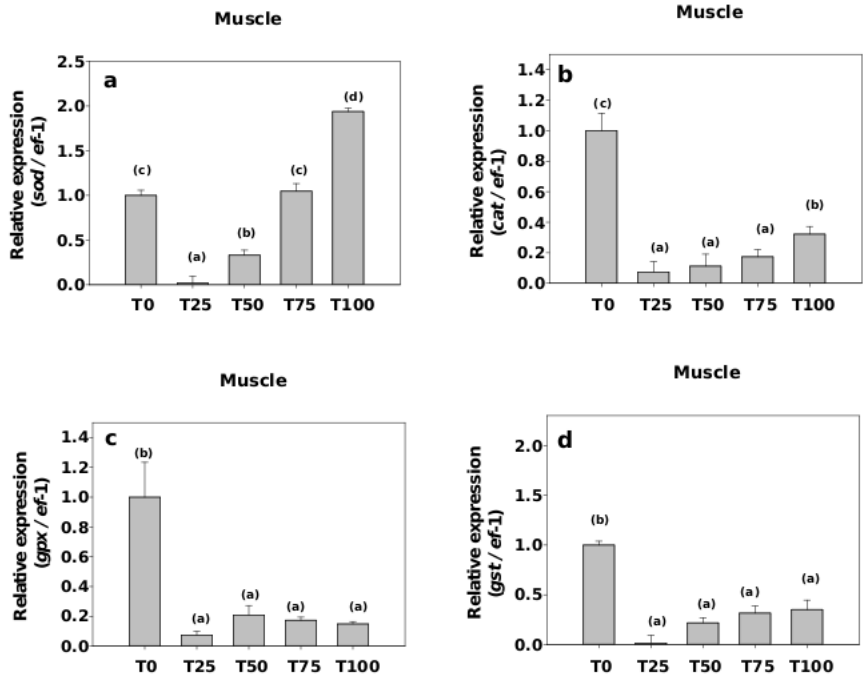


Figure 4. a) Relative expression of the superoxide dismutase (*sod*) gene, b) relative expression of the catalase (*cat*) gene, c) relative expression of the glutathione peroxidase (*gpx*) gene, and d) relative expression of the glutathione S-transferase (*gst*) gene in the muscle of *Vieja melanura* fed diets with different inclusion levels of house cricket meal as a protein source to replace fish meal. Data represent the mean ± standard deviation. Different letters indicate significant differences among treatments (ANOVA, $P < 0.05$). *ef*-1: elongation factor 1.

illucens and insect flours on protease zymogen expression and oxidative stress enzymes; however, effects on their activities have been reported. Among these publications is the work by Melenchón et al. (2020), who observed an increase in gastric protease activity in *O. mykiss* when 30% *H. illucens* meal was used as a substitute for FM, and a decrease in the activity of these proteases when the fish were fed 30% TM meal. They also observed a decrease when 50% of the corn meal was replaced with these same two insect meals in *O. mykiss*, indicating that substitutions exceeding 30% with these meals negatively affect gastric protease activity (Melenchón et al. 2022). The increase in enzymatic activity at partial corn meal replacement levels may be due to moderate inclusion of corn meal, which enhances immunomodulatory effects (Lin et al. 2012) by promoting bacterial growth factors in the gut microbiota that facilitate fish digestion, because the chitin present in insect meal can act as a substrate that increases the proliferation of chitinolytic bacteria, such as *Actinomyces* and *Bacillus*, which are potential chitin degraders, and this increases the activity of bacterial chitinase and reduces the level of chitin substrates available to endogenous intestinal chitinases (Holen et al. 2023, 2024, Zhao et al. 2023, Mes et al. 2024, Libisch et al. 2025). These changes may be a compensatory response to improve the digestion of a less conventional protein, as indicated by Munilla-Morán & Saborido-Rey (1996) and Morimoto-Kofuji et al. (2006). Benítez-Hernández (2018) reported similar patterns of increased *pepsin* expression in response to less palatable diets, suggesting a digestive adaptation in fish. In the present study, intermediate HCM levels appear to stimulate this adaptation. Still, levels above T_{75} negatively affected expression, likely due to digestive limitations associated with chitin content, as reported by Chiranjiv-Pradhan (2021).

Trypsin, an alkaline protease, is key to protein digestion in the fish intestine, and its expression has been used as a biomarker of digestive adaptation (García-Carreño et al. 2002). We observed overexpression in fish fed T_{25} compared to T_0, while replacements exceeding 50% gradually reduced *trypsin* expression. Studies in Atlantic salmon (*Salmo salar*) have demonstrated that diets containing plant-based meals or alternative proteins affect *trypsin* expression and activity (Einar-Lilleeng et al. 2007). Pascon et al. (2024) indicate that a high chitin content in the diet can induce a decrease in transcriptional activity, while Mohanta et al. (2008) highlight that this effect may also be due to a deficiency of EAAs such as lysine. Finally, Rungruangsak-Torrissen et al. (2006) mention that a saturation of adaptive digestive mechanisms may also cause this response. Likewise, chymotrypsin acts under alkaline conditions and is another important enzyme in protein digestion, just like trypsin, and its expression is modulated by the quality of the dietary protein (Sunde et al. 2004). The reduction observed in fish fed diets with inclusions greater than T_{50} suggests that as HCM inclusion increased, it coincided with reports in juveniles of other species, such as *Pseudoplatystoma punctifer*, where abrupt changes in protein composition altered digestive enzyme activity (Castro-Ruiz et al. 2021). Melenchón et al. (2022) reported that alkaline protease activity in *O. mykiss* increased upon exposure to 30 and 50% BSF meal and TM, respectively. Furthermore, Yu et al. (2012) indicates that feed type, feeding habits, and the presence of antinutritional factors directly influence fish digestive activity and affect the enzymatic profile that facilitates feed absorption and assimilation.

Expression of antioxidant enzymes

Insect meal can improve fish immune status by modulating the mucosal immune response and antioxidant activity (Islam et al. 2024).

Antioxidant enzyme expression in the intestine showed that fish fed T_{25} and T_{50} exhibited gene downregulation, suggesting reduced oxidative stress, while those fed T_{75} showed no significant effect compared to T_0. A similar pattern was observed for *cat*, except that for this gene, downregulation extended to T_{75}. However, completely replacing FM in the fish diet increased expression of these two genes. Unlike what was observed for *gpx* -where the presence of HCM downregulated expression- *gst* was the only gene that showed overexpression in fish fed T_{25}, with downregulation in diets containing over 25% replacement compared to T_0. This pattern is similar to that reported for tench fed up with 10% BSF and TM meal (Hidalgo et al. 2022), black bass (*Micropterus salmoides*) fed 24.5% TM meal (Gu et al. 2022), and corvina (*Argyrosomus regius*) fed up with 30% BSF meal (Guerreiro et al. 2022). At the 25% inclusion level (T_{25}), *gst* was overexpressed, while higher inclusions caused underexpression. Comparable findings were reported in the intestine of red seabass (*A. regius*), where CAT activity increased, and GPX activity decreased with 10, 20, and 30% TM meal inclusion in the diet (Guerreiro et al. 2023). In contrast, when fed BSF meal at 10, 20, and 30% inclusion levels, SOD and CAT activity decreased as the meal level increased, while GPX activity started low and stabilized as the

replacement percentage increased (Guerreiro et al. 2022). Similarly, Melenchón et al. (2022) reported increased SOD and CAT activity in *O. mykiss* when 50% of the FM was replaced with BSF and TM meal (with higher activity for TM), while GPX activity decreased in both treatments. In contrast to the findings in *O. mykiss*, Guerreiro et al. (2022) reported reduced SOD and CAT activity with a 60% TM meal (Feidanthsis et al. 2024).

The overexpression of *sod* and *cat* observed in fish fed T_{100} compared to T_0 may be because these enzymes constitute the first line of defense against oxidative stress, such that high expression levels may indicate elevated levels of free radicals (Guerreiro et al. 2022), while the downregulation of *gpx* and *gst* may indicate that the conversion of ROS into water and oxygen is carried out solely by SOD and CAT in the intestine. We currently know that *sod* initiates the response to oxygen radicals by catalyzing the dismutation of the superoxide anion ($O_2^{\bullet-}$) into molecular oxygen and H_2O_2, thereby preventing cascade cellular damage. Subsequently, CAT or GPX reduce H_2O_2 to molecular oxygen and water (Halliwell & Gutteridge 2015), thereby neutralizing the stress caused by ROS generation from its onset (Taufek et al. 2016), suggesting that the gradual increase in the expression of *sod* and CAT with higher levels of HCM inclusion could be due to an imbalance in the intestinal redox state or perhaps to histomorphological alterations in the small intestine (Couto et al. 2022). The liver has the greatest detoxification capacity and high antioxidant activity, which helps fish cope with oxidative stress in their environment (Ansaldo 2001, Ruiz-Picos & López-López 2012). In this organ, *sod* expression increased from T_{50} onward compared to the control. At the same time, *gpx* showed downregulation in fish fed T_{25} compared to T_0, unlike what was reported in the liver of *A. regius* by Guerreiro et al. (2023), where the activity of these two enzymes, CAT and GPX, decreased with the inclusion of 30% TM. The same author reported high GPX activity in the same species fed a 20% inclusion level, with a decrease at 30% inclusion; a similar result was found for CAT, while SOD activity remained stable (Guerreiro et al. 2022). In contrast, Fan et al. (2023) observed similar effects on GPX activity in *C. gariepinus* fed a diet with a 75% inclusion of *G. bimaculatus* meal.

Regarding CAT, this gene maintained stable expression levels and exhibited overexpression only at 100% replacement (T_{100}). Fan et al. (2023) reported an increase in CAT activity with inclusions of 50% and above in *C. gariepinus*, while Naveed et al. (2023) indicated that in *O. niloticus*, SOD and CAT activities decreased as levels of larval and BSF meal increased, while *gpx* activities remained stable. These discrepancies may arise because, in some cases, ribosomes can read a single mRNA molecule multiple times, increasing enzymatic activity without affecting expression levels (Krebs et al. 2018). Insect meal possesses antioxidant properties related to its chitin or antimicrobial peptide content. Its inclusion at optimal levels enhances immunostimulatory effects, improving gut health and immunity (Motte et al. 2019, D'Antonioet al. 2021, Guerreiro et al. 2022); however, levels above the optimum values affect the expression of genes involved in digestion and antioxidant activity, resulting in poor growth in fish, as can be seen in our results.

However, the negative effects on the growth of fish fed T_{75} and the overexpression of digestive enzymes and antioxidants in fish fed T_{100} compared with T_0 may be due to inhibition of superoxide formation, leading to high levels of nitric oxide and subsequent oxidative stress, which is likely attributable to excess chitin in the diet, as chitin can bind to selenocysteine in the catalytic center of selenium-dependent glutathione peroxidase (SeGPX), inactivating it and inhibiting superoxide formation, thereby causing elevated nitric oxide levels and oxidative stress (Hu et al. 2017, Li et al. 2017); this may result in liver damage, growth retardation, and compromised health (Xia et al. 2011, Sankian et al. 2018), and may explain the overexpression of *sod* in fish fed diets containing more than 25% inclusions, and of *cat* in fish fed T_{100}. The presence of HCM in the diet resulted in overexpression the *gst* in all treatments except T_{100}, which could be due to adaptation, inhibition of the transcription factor for this zymogen, or liver damage.

In muscle tissue, the final recipient of nutrient metabolism in the diet, sod expression decreased in fish fed T_{25} and T_{50} compared with T_0, whose expression was equal to that shown by T_{75}. As HCM inclusion increased, expression also increased, reaching overexpression in T_{100}. CAT expression decreased in all treatments containing HCM. Feidantsis et al. (2024) reported an increase in SOD and CAT activity in muscle of *O. mykiss* with the inclusion of TM meal. These differences in gene expression between species may relate to feeding habits in early life stages and to the insect species, since these organisms are part of the diet of some fish species, such as *V. melanura*, and their biochemical composition varies among them. Above the optimal inclusion level, the results are detrimental to fish growth and health, as observed in our study. *Trypsin* expression was reduced in fish fed T_{100}, while

chymotrypsin expression decreased when 75% of FM was replaced, compared to T_0, a finding consistent with FCR, SGR, PER, and FE. However, *sod, gpx, and gst* were overexpressed in the liver, possibly due to increased oxidative stress markers (Hu et al. 2017, Li et al. 2017, Elia et al. 2018), which may be due to deficiencies in methionine, lysine, and arginine, which are common in insect meals and can affect selenoperoxidase activity, nitric oxide synthesis, and protein and lipid metabolism (Xia et al. 2011, Tran et al. 2015, Elia et al. 2018). Therefore, including high levels of these flours in the diet compromises growth and digestibility (Guerreiro et al. 2020, 2021, Couto et al. 2022).

Furthermore, high concentrations of chitin reduce diet palatability and digestibility because certain proteins bind to it, hindering absorption (Kouřimská & Adámková 2016), which in turn may affect the expression of proteolytic enzyme genes, resulting in poor hydrolysis of nutritional components (Guerreiro et al. 2021). The downregulation observed in *sod* and *cat* in fish fed T_{25} and T_{50} may be because insect meals, at their dietary proportions, tend to enhance antioxidant capacity in fish (Fan et al. 2023, Liu et al. 2023, Huang et al. 2025).

CONCLUSION

This study demonstrates that the highest AWG in *V. melanura* was achieved with a 50% inclusion of cricket meal (T_{50}). Similarly, pepsin, trypsin, and chymotrypsin expression levels in T_{50} were higher than or equal to those in T_0, indicating efficient utilization of dietary nutrients without adverse effects on growth. Nevertheless, hepatic antioxidant gene expression at T_{50} showed overexpression of *sod* and *gst*, while *cat* was overexpressed only at the 100% inclusion level (T_{100}). This pattern signifies elevated antioxidant activity, likely reflecting a compensatory response to oxidative stress. This imbalance may lead to ROS accumulation and potential cellular and molecular (DNA) damage. Such stress may stem from antinutritional factors or deficiencies in EAAs, such as lysine and methionine, which directly and indirectly influence the expression and activity of digestive and physiological enzymes.

We conclude that a 50% inclusion level is optimal for *V. melanura*. Higher inclusions compromise growth, health, and welfare. To potentially increase the inclusion level of cricket meal in diets for this species, future formulations should consider supplementing limiting amino acids (lysine and methionine) and partially removing chitin from the meal. These findings underscore the necessity for further molecular studies on the digestive and physiological adaptations of *V. melanura* to insect-based diets.

Credit author contribution

J.N. Almeida-De la Cruz: writing-original draft preparation, editing, methodology, formal analysis, investigation and resources; B. Concha-Frías: conceptualization, methodology, investigation, writing-original draft preparation and project administration; F.J. De la Cruz-Alvarado: methodology, formal analysis, project administration; writing-review and editing; C.A. Álvarez-González: conceptualization, software, supervision, writing-review and editing; G. Gaxiola-Cortes: conceptualization, validation, supervisión and funding acquisition; J.P. Alcantar-Vázquez: software, formal analysis and validation; A. Ferreira-da Silva: validation, data curation and supervisión; M.F. Martínez-Segovia: methodology, investigation, resources and supervisión; J.L. Arias-Moscoso: supervision, data curation and visualization; L.D. Jiménez-Martínez: supervisión, data curation and validation. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no potential conflict of interest in this manuscript.

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