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RESEARCH PAPER

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Replacement of Fishmeal by Three Cricket Meals (*Acheta domesticus*, *Gryllus bimaculatus*, *Teleogryllus mitratus) in* Swordtail (*Xiphophorus helleri*) Fry Feed: Effect of Growth, Stress Tolerance, Pigmentation and Histopathological Alterations

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Abstract

Fishmeal (FM) has become one of the significant determinants of aquaculture due to its higher nutritional quality attributes. However, unsustainability, food security issues and soaring prices force the aqua feed industry to find alternatives to FM. Insect meals are becoming a sustainable alternative to FM due to their comparable nutritional content and environmental friendliness. A ten-week experiment was performed using the red velvet strain of swordtail (SF) (*Xiphophorus helleri*) fry. Twelve fiberglass tanks with four treatments and three replicates were used, placing fifteen fish in each tank. FM of the control (FM100) was replaced by house cricket (*Acheta domesticus*) meal (HC100), field cricket (*Gryllus bimaculatus*) meal (FC100), and ground cricket (*Teleogryllus mitratus*) meal (GC100). The growth performance and stress tolerance were measured at the end of the experiment. Further, color intensity and hepatic cells were analyzed. Results confirmed that the applicable three cricket meals can replace FM without affecting growth performances, pigmentation, and stress tolerance ($P<0.05$). In contrast, the alternations can be observed in all the treatments in liver cells. Thus, all the researched cricket meals are suitable to replace FM in SF fry diets. However, further research is required to minimize the damage to the hepatic cells.

Introduction

FM plays a prominent role in the conventional aqua feed industry as a significant source of protein owing to the availability of balanced nutrition concentration of proteins, minerals, essential amino acids and essential fatty acids (Zlaugotne et al., 2022). Further, FAO (2022) mentions that 20.4 million tons of global fish production have been utilized for non-food usage, meaning FM and fish oil production are broadly embodied. Simultaneously, another source of fish feed is pelagic fish, and the stocks are depleted due to the El Nino effect and unsustainable overfishing (Bakun, 2003). Therefore, adverse impacts on the environment, loss of biodiversity (Zlaugotne et al., 2022), less availability of FM due to the steady decline in wild fish catch for feed formulation (Sampathkumar & Raja, 2019), and inability to ensure uninterrupted supply has made FM more unsustainable. Therefore, the UN development goals under food safety and sustainability do not promote incorporating FM and fish oil usage as aqua feed ingredients (Zlaugotne et al., 2022).

According to the forecasted data for 2025, aqua feed demand would be another 37.4 million tons (Hua et al., 2019). Consequently, several studies are being conducted to discover a sustainable, cost-effective, highly nutritious and eco-friendly FM alternative that enables fish farmers to increase the yield and reduce the cost of fish production (NAERLS 2002) by replacing unsustainable FM. (Glencross, 2020).

As far as FM alternatives are concerned, one of the most sustainable and economically justifiable alternatives is using insects in fish feed production (Zlaugotne et al., 2022). The Present study focuses on cricket meal, which has the potential to be produced locally abiding by the selection criteria of a FM alternative such as being highly nutritious (Ngonga et al. 2020), having a lower carbon footprint, non-pathogenic, non-invasive, non-vectors (Hua et al., 2019) and easy rearing with minimal growth requirements (Van Huis, 2013).

Over two thousand edible insect species are produced globally and 13% out of them are edible crickets (Musungu et al., 2023). Crickets can be reared using conventional and low-cost feeds such as weeds, wastes, and byproducts (Kuo and Fisher, 2022). Moreover, cricket farming does not require a big investment, thus small-scale farmers can engage in this business (Fuah et al., 2015). More than 370 billion to 430 billion crickets are raised annually for human food and animal feed, and the industry is rapidly enhancing (Rowe, 2020).

Ornamental aquaculture, one of the world's top-notch industries, earned USD 6 billion in 2019, with a 10% annual growth rate, and it has been forecasted to increase to USD 12 billion by 2025 (Biondo & Burki, 2020). As far as the ornamental fish industry is concerned, fish feed is one of the significant determinants of production costs. Owing to the issues with the nutritional quality of locally produced fish feed, commercial fish farmers go for imported quality fish feed, which is expensive and incurs a large amount of earned foreign exchange as an importation cost.

Swordtail(*Xiphophorus helleri*)(SF), which is one of the widespread freshwater ornamental fish species (Tamaru et al., 2001) with high global demand, is used in the study. The fin patterns, colour variations, hardiness, environmental tolerance, stability under water quality variations has attributed to attain a high popularity in the industry. Similarly, the maintenance is easy (Das, 2023); (Ghosh et al., 2008). This experiment was conducted replacing the FM with HC100- (*Acheta domesticus),* FC100 (*Gryllus bimaculatus),* and GC100 (*Teleogryllus mitratus)* as three sustainable alternative aquafeed ingredients to expensive and unsustainable fishmeal using the strain of red velvet of SF to explore the effect of growth, stress tolerence, color intensity, and liver histology.

Materials and Methods

This research was conducted at the indoor hatchery of the Regional Research Center, Panapitiya, of the National Aquatic Resources Research and Development Agency (NARA), Sri Lanka, from December 2023 to February 2024.

Experimental Setup

Twelve fiberglass tanks (120 cm (length)x90 cm (width)×60 cm (height)) were prepared to accommodate SF fry for the study filling the tanks up to a depth of 15 cm with conditioned water and aerated by an RESUN GF-750 air compressor (Guangzhou Puyangli Aquarium & Accessories Co. Ltd, Guangdong, China). The study was comprised of four treatments, including the control and three replicates per treatment, thus the experimental design was completely randomized design. Then, same-sized (0.03±0.01g) fish were sorted by visual observation, and 180 randomly selected fries were selected for the experiment.

Proximate analysis of the Ingredients

The proximate analysis of the FM, cricket meals were tested from the Aqua Testing Laboratory of The Ceylon Grain Elevators, Colombo 15, Sri Lanka. The energy values of the ingredients and test feeds were tested utilizing IKA C7000 Oxygen Bomb Calorimeter (Digital data system, Staufen, Germany) at the Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Sri Lanka. The results are shown in Table 1.

Table 1. Nutritional information (%, dry weight basis) and energy values of the FM, and cricket meals

Parameter	FM	HC	FC	GC	P value
Dry matter content	94.3±0.8^{a}	92.8±2.3^{a}	91.3±3.1^{a}	93.5±2.2^{a}	0.29
Protein	67.1±0.9^{c}	48.4±1.4^{a}	58.0±2.8^{b}	50.1±1.3^{a}	0.00
Lipid	8.4±0.4^{a}	12.4±0.5^{a}	8.2±0.4^{a}	10.2±0.6^{a}	0.11
Moisture	5.7±0.2^{a}	7.2±0.6^{a}	8.7±0.4^{a}	6.5±0.4^{a}	0.34
Ash	14.7±0.0^{b}	6.3±0.1^{a}	4.8±0.2^{a}	12.7±0.4^{b}	0.01
Fiber	1.4±0.1^{a}	7.7±0.0^{b}	9.2±0.3^{b}	8.6±0.1^{b}	0.00
NFE	2.6±0.2^{a}	18.0±0.4^{d}	6.1±0.3^{b}	11.9±0.6^{c}	0.00
Energy (kj/g)	19.7±1.2^{a}	26.5±2.2^{c}	24.4±1.8^{b}	23.9±0.8^{b}	0.00

*FM; Fishmeal, HC; House cricket meal, FC; Field cricket meal, GC; Ground cricket meal. Mean±SD in each row, superscripted with different lowercase letters, are significantly different (P<0.05)

Preparation of Experimental Diets

HC, FC, and GC meals were purchased from Cricket Fit Limited, Thailand. The protein content of the control diet (FM100) was100% replaced by the respective cricket meals in HC100, FC100, and GC100. The test diet formulas and proximate composition are depicted in Table 2 and Table 3 respectively.

Rearing and Feeding the Fish

The initial weights of the fish were measured using Sartorius BSA822-CW electrical balance (Sartorius Lab Instruments GmbH and KG, Gottingen, Germany) before accommodating them in fish tanks separately. Then fifteen swordtail fries were stocked in each tank and the sample weighing was practiced weekly. Fish were fed to maximum satiation level twice daily at 9.00 h and 15.00 h. The final body weights of each fish were measured separately using the same balance after ten weeks (70 days).

Growth Performance Analysis

The growth of the fish was analyzed according to survival rate, weight gain, daily weight gain, relative weight gain and specific growth rate.

$$\text{Survival rate (\%)} = \frac{\text{Final fish number}}{\text{Initial fish number}} \times 100$$

$$\text{WG (g)} = \frac{\text{Final weight (g)}}{\text{Initial weight (g)}}$$

$$\text{DWG (g/day)} = \frac{\text{WG (g)}}{\text{Experiment period (days)}}$$

$$\text{RWG} = \frac{\text{WG (g)}}{\text{Initial weight (g)}} \times 100$$

$$\text{SGR (\%/day)} = \frac{\text{Ln (FW)}-\text{Ln (IW)}}{\text{Number of days}} \times 100$$

Where, WG: Weight gain (g), DWG: Daily weight gain (g/day), RWG: Relative weight gain, SGR: Specific growth rate, FW: Final weight, IW: Initial weight.

Water Quality Management

Two-thirds of the water from each tank was removed and refilled with conditioned water up to the level of 15 cm weekly. Siphoning was performed every morning and excreta and leftover feed were removed and refilling was done. The essential water quality

Table 2. Feed formulas and nutritional information of the test diets (%, dry weight basis)

Ingredient	FM100	HC100	FC100	GC100
Fish meal	25.00	0	0	0
House cricket meal (HC)	0	35.33	0	0
Field cricket meal (FC)	0	0	28.93	0
Ground cricket meal (GC)	0	0	0	33.47
Soy meal	45.00	48.00	46.00	46.00
Rice bran	11.80	2.00	8.87	8.33
Corn	6.00	7.47	9.00	5.00
Fish oil	7.00	2.00	2.00	2.00
Vitamin premix	1.00	1.00	1.00	1.00
Mineral premix	1.00	1.00	1.00	1.00
DL-Methionine	0.10	0.10	0.10	0.10
L-Lysine	0.10	0.10	0.10	0.10
Choline chloride	0.10	0.10	0.10	0.10
Vitamin C	0.10	0.10	0.10	0.10
CMC[1]	2.00	2.00	2.00	2.00
DCP[2]	0.50	0.50	0.50	0.50
Yeast	0.30	0.30	0.30	0.30

*[1]CMC; Carboxyl methyl cellulose, [2]DCP; Dicalcium Phosphate FM100= 100% fishmeal-included diet as the animal protein source (control); HC100= Fishmeal totally replaced diet by house cricket meal; FC100= Fishmeal totally replaced diet by field cricket meal; GC100= Fishmeal totally replaced by ground cricket meal.

Table 3. Nutritional information (% dry weight basis) and energy values of the research diets

Parameter	FM100	HC100	FC100	GC100	P value
Dry Matter	91.8±3.2^{a}	92.1±4.3^{a}	91.6±3.8^{a}	92.0±4.8^{a}	0.41
Protein	40.5±0.9a	40.7±2.6^{a}	40.6±3.1^{a}	40.3±5.4^{a}	0.59
Lipid	6.7±0.3^{a}	7.6±0.4^{a}	6.5±0.1^{a}	7.3±0.8^{a}	0.06
Moisture	8.2±0.0^{a}	7.9±0.4^{a}	8.4±0.2^{a}	8.0±0.3^{a}	0.41
Ash	11.3±0.2^{a}	11.6±0.0^{a}	10.9±0.4^{a}	11.4±0.0^{a}	0.24
Fiber	2.0±0.3^{a}	1.9±0.1^{a}	2.6±0.0^{b}	2.4±0.2^{ab}	0.02
NFE	39.5±1.7^{b}	38.2±2.1^{a}	39.4±4.0^{b}	38.6±0.9^{ab}	0.03
Energy (kj/g)	19.7±4.4^{a}	20.0±1.1^{a}	20.1±0.5^{a}	20.2±1.3^{a}	0.43

*FM100= 100% fishmeal-included diet as the animal protein source (control); HC100= Fishmeal totally replaced diet by house cricket meal; FC100= Fishmeal totally replaced diet by field cricket meal; GC100= Fishmeal totally replaced by ground cricket meal.
Mean±SD in each row, superscripted with different lowercase letters, are significantly different ($p<0.05$).

parameters (pH, dissolved oxygen (DO), NH_3 concentration, and conductivity) were measured once a week, and these parameters were maintained among the optimum ranges (pH; 7.73±0.03, DO; 6.86±0.12, NH_3 concentration; 0.2±0.0 mg/L, 8.8±0.1 µs/cm at 25.4±0.4^{0}C). The pH was measured using the pH meter EUTECH PH6$^+$ pH meter (EUTECH Instruments Pte Ltd, Singapore). The DO was monitored using EZDO PDO-408 Dissolved Oxygen meter (Gon DO Electronic Co. Ltd, Taipe City, Taiwan). The NH_3 concentration was monitored using commercial test kits (NT Laboratories Ltd, Kent, TN 12 9QS, UK). Conductivity and water temperature were measured using WTW Cond 3310 Conductivity Meter (Xylem Analytics GmbH & Company, Weiheim, Germany). In addition to the water quality parameters, the light intensity was measured using the SMART SENSOR AS803 Digital Lux meter (ARCO Electronics Ltd, Dong Guan City, China) and maintained as 799±22 Lux at 29.7±0.0^{0}C. Further, the photoperiod was regulated as 12h/12h (dark/light) during the experiment.

Stress Tolerance Test

At the end of the experimental period, stress tolerance test was performed following the adapted method of Lim et al. (2003). Initially prepared pre aerated saline water of 35 ppm was used to fill the twelve containers up to 500 ml volume each. Randomly selected ten swordtail fingerlings from each treatment were separately allocated in the above labelled containers. The number of dead fish were recorded in each three -minute intervals until 2 hours. The stress resistance of the fish was presented as the cumulative mortality index (CMI). CMI value was acquired by taking the sum of the 40 cumulative mortality readings recorded during the observation period.

Testing of Pigmentation Using Digital Photographs

Total five fish from each replicate were photographed using the Nikon D7500 DLSR camera (Nikon imaging Japan Inc., Tokyo, Japan) and YN 60 mm F2NE MF 1:1 macro lens (YONGNUO Photographic Equipment Co. Ltd, Shenzhen, China). The following camera settings and background setups were adjusted according to the recommendations of Stevens et al. (2007) and Straub et al. (2019). The shutter speed and aperture were 1/40 and 8 respectively while the ISO number was 320. A place with ample lighting conditions was selected, and a spotlight was used. The light intensity was maintained as 798±72 using the SMART SENSOR AS803 Digital Lux meter (ARCO Electronics Ltd, Dong Guan City, China). The camera was placed at a constant distance using a tripod.

Fish were anesthetized using 0.25 ml/l clove oil as per Jaiswal et al. protocol (2019). Then the fish was positioned on a white background and the photographs were taken within the shortest possible time.

The digital photographs of the fish were analyzed using MATLAB program, and the grey scale values were considered for analyzing. Specific values applicable to each photograph were obtained from the MATLAB program. Each pixel was allocated with a number from 0 to 255 where 0 indicates black while 255 indicates white. According to Uribe et al. 2018 "Values closer to zero denote a higher color intensity and values closer to 255 denote lower color intensity".

Histopathological Analysis

One fish from each tank/replicate was selected randomly. Selected fish from each tank were euthanized by immersing 250 mg/l of MS-222 for ten minutes (AVMA 2007). Then of the euthanized fish were dissected and preserved using 10% neutral buffered formalin (Mumford, 2004). The liver samples were kept in tagged vials and fixed for 24 hours. The analysis was done at the Department of veterinary Pathobiology, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka. The fixed liver tissue samples were washed with distilled water and deposited in the carrier of the automatic tissue processor SE 400. (Shandon Scientific Co. Ltd., England). A dehydration series was taken place in the tissue processor with 70%, 80%, 90% ethanol, and three absolute alcohol baths. Afterwards clearing was done and transferred to the wax bath of the tissue processor. The whole process in the tissue processor requires 22 hours.

Thereafter the embedding process was done in the TEC 2900 embedding machine (Histo-Line Laboratories, Italy) and cooled in the TEC 2900 cold chamber (Histo-Line Laboratories, Italy) for five minutes to enable the removal of the block from the mold. Then section cutting was done with the use of a 4 µm microtome (Histo-Line Laboratories, Italy). Then the staining was done through hydration followed by dehydration. Removal of wax is done using xylene while hydration was done using the descending sequence of alcohol. Hematoxylin and eosin staining was followed to conduct the histopathological analysis (NSH 2001).

Afterwards the dehydration process was done adhering to the ascending series of alcohol followed by clearing using xylene. Finally, the cover slipping and mounting was done by placing DPX mountant on slide and inverting the slide over the cover slip. The slides were prepared using livers of each representative fish from each replicate.

Statistical Analysis

Four treatments were repeated in triplicate. All data were presented in the text as mean±standard error (ER). Treatments were compared using one-way analysis of variance (ANOVA) followed by post hoc Tukey test with significance level $P<0.05$. and mean values were separated according to significance.

Results

Growth and Survival

The growth performance results and growth curves of *X. helleri* fry fed by different treatment diets have been mentioned in Table 4 and Figure 1 respectively.

Pigmentation

The summarized results of pigmentation using digital photographs are presented in Table 5.

A MATLAB program was prepared to analyze the photographs. Color intensity of each photograph was expressed as a numerical value. The grey-scale was used to identify the pigmentation status of the photographs.

Stress Tolerance of Swordtail Fish (*Xiphophorus helleri*) Fry

Stress tolerance of SF (*X. helleri*) was analyzed considering the stress index values and the results are presented in Table 6.

Histopathological Analysis of the Livers of *X. helleri*

The histological alterations of the livers of *X. helleri* were compared for all the treatments and the relevant results are furnished below.

Liver condition, in general, is an indicator of the general health condition of fish. The liver tissues obtained from all four treatments indicate a fatty liver condition. According to the visual observations the lowest fatty liver condition is observed in HC100 treatment.

Table 4. Growth performance of *X. helleri* fry during the research period

Parameter	FM100	HC100	FC100	GC100	P value
Initial weight (g)	0.02±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.14
Final weight (g)	0.58±0.07	0.72±0.07	0.60±0.05	0.61±0.06	0.63
WG (g/fish)	0.56±0.07	0.69±0.07	0.57±0.04	0.58±0.06	0.12
DWG (g/fish)	0.008±0.001	0.010±0.001	0.008±0.000	0.009±0.001	0.10
RWG	2335±393	2430±326	1852±58	2077±397	0.21
SGR	4.68±0.25	4.74±0.20	4.37±0.04	4.51±0.27	0.21
Survival rate (%)	100.0±0.0	100.0±0.0	97.8±3.9	97.8±3.9	0.52

*FM100= 100% fishmeal-included diet as the animal protein source (control); HC100= Fishmeal totally replaced diet by house cricket meal; FC100= Fishmeal totally replaced diet by field cricket meal; GC100= Fishmeal totally replaced by ground cricket meal.

Mean±SD in each row, superscripted with different lowercase letters, are significantly different ($p<0.05$); WG= Weight Gain; RWG= Relative Weight Gain; SGR= Specific Growth Rate

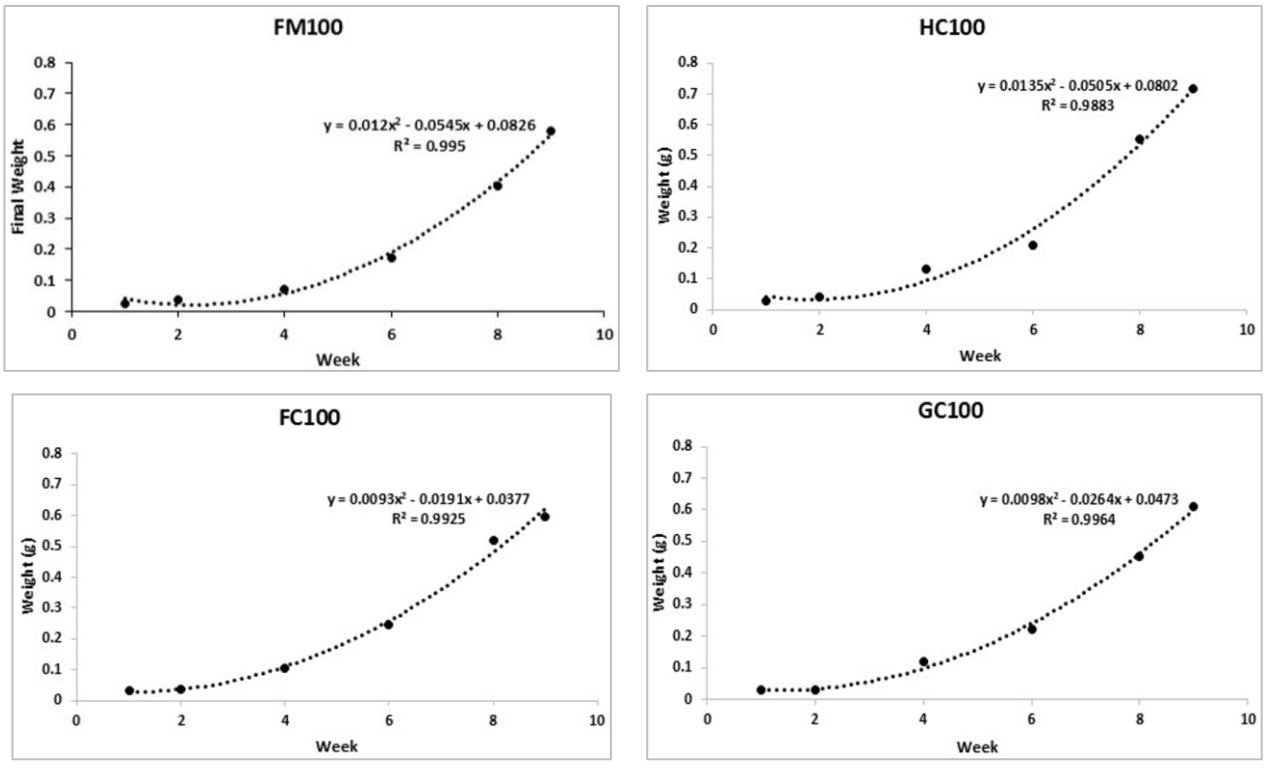

Figure 1. Growth curves of *X. helleri* fry fed with the experimental diets during the experimental period. FM100= 100% fishmeal-included diet as the animal protein source (control); HC100= Fishmeal totally replaced diet by house cricket meal; FC100= Fishmeal totally replaced diet by field cricket meal; GC100= Fishmeal totally replaced by ground cricket meal.

Fatty liver condition of FM100 is high, and comparable to GC100. The fatty liver condition of FC100 and HC100 is lower than that of FM100 and GC100.

The fatty liver variation was as follows: FM100=GC100>FC100>HC100 (Figure 2).

Discussion

One of the most appropriate evidence to emphasize the suitability of insect meal as a fish feed is that, many freshwater fish species consume insects as their main diet (Ferrer Llagostera et al., 2019). Thus, the possibility of replacing fishmeal with insect meal in the fish feed industry has been recognized (Nogales-Mérida et al., 2019). More importantly, the gut analysis of guppy and swordtail has shown that they eat not only aquatic insects but also terrestrial insects along with some macro algae and phytoplankton (Tamaru et al., 2001).

Some research results have confirmed that fishmeal can be entirely replaced by different cricket meals in the diets of *Catla catla* (Perera et al., 2023a), *Ictalurus punctatus* (Fan et al., 2023), *Poecilia reticulata* (Perera and Bhujel, 2022), *Litopenaeus vannamei* (Peh et al., 2021), and *Clarias gariepinus* (Taufek et al., 2017). However, most of the research results indicate that FM can be replaced with cricket meals partially (Fontes et al., 2019; Perera and Bhujel, 2021; Perera et al., 2023b). However, cricket meal inclusion levels depend on the fish species and some internal and external factors. Swordtail is a live-bearing ornamental fish (Ghosh et al., 2008), and the results of the present study shows that FM can be successfully replaced by the researched cricket meals when the proper formulations are available in terms of growth, pigmentation, and stress tolerance.

These findings are supported by a previous study conducted for guppy fry by feeding field cricket meal and house cricket meal-included formulated diets by Perera and Bhujel (2022). On the contrary, the results obtained from a study done with *X. helleri* feeding four different insect meals have shown significantly lower final weight, WG and SGR in cricket meal-fed fish compared to grasshopper, mealworm and Black soldier fly larvae meals (Das, 2023). Those treatment fish were fed by solely insects that had been prepared by drying and crumbling without formulating with other ingredients. However, the present study has been done by 100% replacing the protein content of the fishmeal with the three cricket meals separately through proper formulations. Therefore, it could be assumed that the proper formulation is essential for successful fishmeal replacement. Generally, one of the key factors that is responsible for growth parameters is the protein content and that was balanced in this study preparing the iso-protein diets. Cricket meal is rich in some limiting amino acids compared with FM. Generally, cricket meal is higher in cysteine, methionine, lysine, tyrosine and histidine (Taufek et al., 2017). Nevertheless, at high inclusion levels of cricket meal, the growth is reduced due to the high fiber content (Hanan et al., 2022). Thus, the growth stage of the cricket that is used in the meal preparation and the level of incorporation are crucial factors. The present study was conducted with the meal obtained from the pre pupae stage of crickets where the fiber content is minimum. Contrary, the stage of growth of the crickets used to produce the meal has not been specifically indicated in the mentioned previous study. Therefore, this might be a one of the key reasons for the contradictory results of the present study and the referred study done with *X. helleri*. In addition, the nutrient composition of insects varies depending on the cultivated substrate (Tschirner and Kloas 2017). According to Bawa et al., (2020) high protein content of the growth substrate has resulted in high protein content in the body composition of the cricket. Therefore it can be concluded that, the protein and the mineral content of crickets can be influenced by the nutritional composition of the substrate (Bawa et al., 2020). Thus the protein content and amino acid content of the insect meal can be increased by enriching the insect-rearing substrate with fish offal (Ferrer Llagostera et al., 2019). Thus, the variation of the insect-rearing media might also have affected the final result of the two studies.

Similarly, the survival rates have also not been significantly (P>0.05) affected by the four treatments during the experimental period. Correspondingly, the results of the previous study done for guppy fry by Perera and Bhujel (2022) showed no significant difference (P>0.05) in survival among the two cricket meal treatments at different inclusion levels. In contrast, the survival rate of SF was lower than the

Table 5. Grey-scale values expressed by MATLAB values of *X. helleri* fry at the end of the experiment

Parameter	FM100	HC100	FC100	GC100	P value
MATLAB value	155.9±6.7	149.0±3.5	157.0±8.2	153.3±6.6	0.48

FM100= 100% fishmeal-included diet as the animal protein source (control); HC100= Fishmeal totally replaced diet by house cricket meal; FC100= Fishmeal totally replaced diet by field cricket meal; GC100= Fishmeal totally replaced by ground cricket meal.

Table 6. Stress Index values *X. helleri* fry at the end of the experiment

Parameter	FM100	HC100	FC100	GC100	P value
Stress Index	221.7±2.3	229.7±1.2	224.3±1.8	226.3±1.5	0.52

*FM100= 100% fishmeal-included diet as the animal protein source (control); HC100= Fishmeal totally replaced diet by house cricket meal; FC100= Fishmeal totally replaced diet by field cricket meal; GC100= Fishmeal totally replaced by ground cricket meal.

control treatment in comparison to the solely cricket meal-included diets in the study done by Das (2023). Another study done for guppy using solely insect-included meals by Kowalska et al. (2022) has shown that it does not affect the survival of guppy. Therefore, the present study confirms that proper formulations could achieve a higher survival.

The market price of the ornamental fish is determined by the colouration and diversity of colour patterns (Kaur and Shah, 2017). Previous research results confirmed that specific feed ingredients have a potential to enhance the pigmentation in fish (Sefc et al., 2014; Sathyaruban et al., 2021; Perera et al., 2024a; Perera et al., 2024b; Sathyaruban et al., 2024). The summarized results of MATLAB program confirmed that the body colour of the fish could be obtained by using cricket meals similar to the FM-included diet. Contrary to the present study MATLAB analysis results of a previous study done with guppy fry showed significantly higher ($P<0.05$) values for the 100% field cricket meal-included diet compared with the control (Perera & Bhujel, 2022).

As per the spectrophotometric analysis done for SF by a previous study (Das, 2023) has shown that the pigmentation of fish in the insect meal-treated tanks was significantly high compared to the control treatment and the highest value was obtained by black soldier fly larvae meal-fed fish. Control treatment contained a commercial diet composed of fishmeal. Generally, fish are unable to synthesize pigments by themselves (Higuera-Ciapara et al., 2006). So as the commercial diet which is made of FM lacks pigments and the level of pigments transferred to ornamental fish through diet is low. That might be the reason for the significantly lower colour intensity in the control diet-fed fish. On the other hand, according to the observations of Perera and Bhujel (2022) when the cricket meal inclusion level has been increased, it has resulted in high colour intensity in guppy fry owing to the high inclusion level of carotenoid in the growth substrate. This concept further confirms by the study done by Sukarman el al. (2023) for Asian Arowana (*Scleropages formosus*) which is a freshwater ornamental fish to evaluate the effect of crickets enriched with sources of carotenoids. The results showed that the fish fed with crickets enriched with a combination of 50% fish feed, 48% sand crab meal and 2% marigold petal meal has increased the yellowness incompared to the other treatments while the crickets enriched with synthetic astaxanthin has increased redness, whereas the control treatmen or the crickets fed without enrichment showed poor color enhancement. Therefore it confirms that enriched crickets affected the colour of the Arowana juveniles. Similarly, there are several natural colour enhancers such as marigold petals, red yeast, seaweed, microalgae, crustacean by products (Sathyaruban et al., 2021) which can be used as growth substrates for crickets.

The findings from previous studies confirm that the carotenoid content in *X. helleri* was increased with the increased level of carotenoid content in the growth substrates of the crickets. Those previous studies revealed that, the expensive synthetic colour enhancers incorporated to ornamental fish feed can be replaced by researched cricket meals which enables to reduce the cost of production (Lippolis et al., 2019).

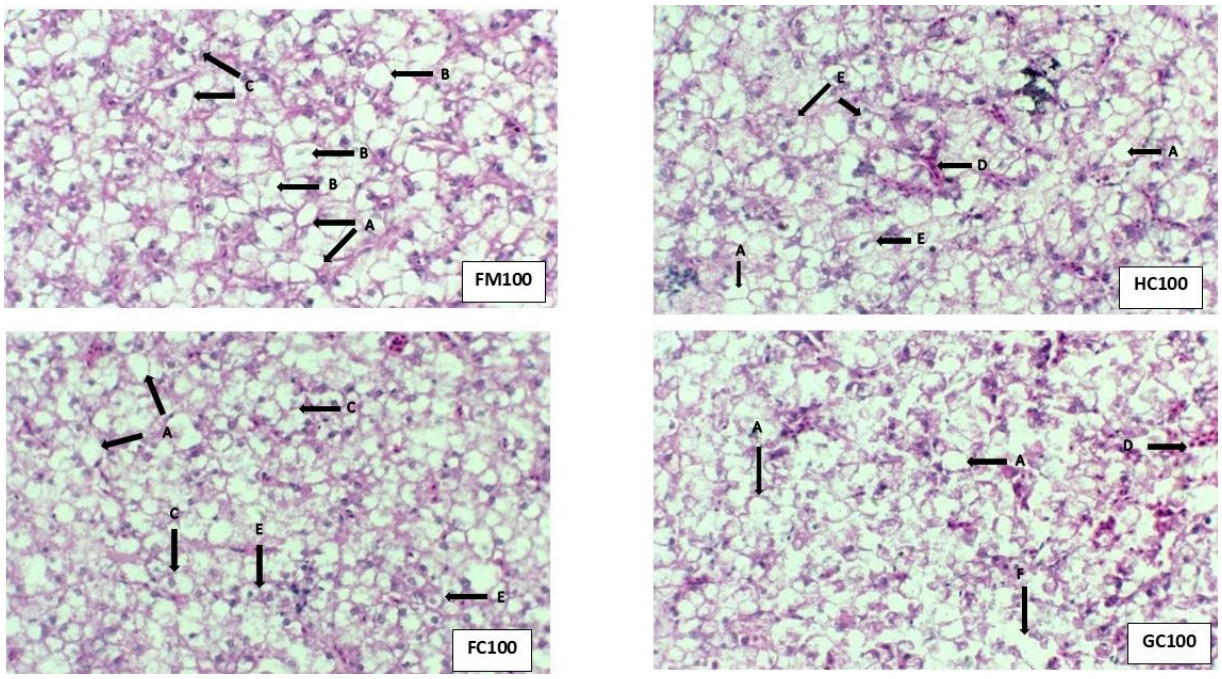

Figure 2. Histological structure of the hepatocytes of *X. helleri* (Magnification= ×10×40)**.***FM100) Hepatocytes filled with large fat globules(A), Nuclear degeneration (B), Decentralized nucleus(C), HC100) Fat globules(A), Erythrocyte infiltration into blood sinusoids (D), Central and round nucleus(E), FC100) fat globules(A), Decentralized nucleus(C), Central nucleus (E), GC100) Fat globules(A), Erythrocyte infiltration into blood sinusoids (D), Vacuolation(F).

However, according to the present study there is no significant effect from cricket meals to the colouration. According to Finke (2015), crickets, mealworms and superworms contain β- carotene. It suggests that the carotenoid content of the live insects can be altered by the diet given to the insects (Finke, 2015). Therefore, the reason for the variation in results in compared with the previous studies can be the less content of carotenoids in the growth substrate.

There was no evidence of previous salinity stress tests done for SF fish fed with insect meal. The stress involves to neural, endocrine, and immune systems (Verrier et al., 2011). The results suggested that inclusion of the cricket meals did not affect adversely on functions on the above regulatory systems.

One of the widely used biomarkers that is used to evaluate the health of fish is histopathological changes (Wester and Canton, 1991; Thophon et al*.,* 2003; Camargo and Martinez, 2007).These histopathological studies examines some target organs such as liver, kidney and gills which are responsible for major body functions including biotransformation of xenobiotics of the fish, accumulation, excretion and respiration. (Gernhofer et al., 2001). Thus, in the present study the liver histopathology is used as a health indicator. One of the reasons for the development of fatty liver condition in fish is indirect ingestion of pollutants through feed and get combined in the tissues (Mohamed, 2009). Besides, the excess proportion of insect meal results an increase in the fat globules in hepatocytes (Melenchón et al., 2022). Similarly, the high carbohydrate contents in feed might also contribute to the fatty liver conditions (Huang et al., 2022). Therefore, the exact reason/s for the alterations in hepatic tissues cannot be judged. Even though the defatted insect powders were used, fatty liver symptom could be happened due to the adverse effect of the other ingredients. However, further research is required to reduce the damage to the hepatic cells and the formulations should be revised to gain the optimum liver functions in fish.

Conclusion and Recommendations

Acheta domesticus, *Gryllus bimaculatus*, *and Teleogryllus mitratus* meals could be used to replace the fishmeal in *X. helleri* fry diet in terms of growth, stress tolerance and colour intensity. However, further research and suitable formulas are required to reduce the adverse effects on the hepatic cells and to explore the technical knowledge of how to produce cost-effective cricket meals to the market.

Ethical Statement

Methodology, and protocols were approved by the Proposal Presentation Examination Committee of the Department of Animal Science, University of Peradeniya, Sri Lanka. Moreover, this study was conducted as an approved project of NARA, Sri Lanka under the project No: 10.0 (2024).

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Author Contribution

G.S.C.P: Conceptualization, designing and performing the experiment, formal analyzing and original draft writing.

S.A.D.N.S: Developing the methodology, original draft writing, analyzing the histopathological slides and performing the experiment.

D.R.S.: Performing the experiment, and analyzing

M.C.K.S.S.: Performing the experiment, and analyzing

A.D.W.R.R.: Histopathological slide analysis, and editing the original draft.

S.A.: Editing the original draft and lab testing

Conflict of Interest

The authors reported no conflict of interest.

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