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Dietary fishmeal protein replacement with superworm (*Zophobas morio*) larvae meal: Effect on growth, feed utilization, survival, digestive enzyme activity, skin colour enhancement of juvenile goldfish, *Carassius auratus*

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Abstract

This study evaluated superworm (*Zophobas morio*) larvae meal as a novel protein and carotenoid source in diets of goldfish, *Carassius auratus*. A six-months feeding trial was carried out to estimate the effect of dietary replacement of fishmeal with superworm meal at graded percentages on growth performance, survival, digestive enzyme activity and carotenoid content in skin and muscle tissue of goldfish. Five iso-nitrogenous diets were formulated with 38% of crude protein, substituting fishmeal protein with superworm meal protein at the levels of 0% (D0, control), 25% (D25), 50% (D50), 75% (D75), and 100% (D100), containing 0%, 10.59%, 21.18%, 31.77% and 42.35% superworm meal respectively. Escalating the percentage of superworm meal inclusion led to a significant improvement in growth, feed utilization, digestive enzyme activity, survival and carotenoid deposition in skin and muscle tissue of goldfish. Thus, complete replacement of fishmeal protein with superworm meal protein with an inclusion level of 42.35% of diet would be recommended for goldfish.

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Introduction

Ornamental fish keeping is the second most favourable hobby throughout world as ornamental fishes are one of the nature's most beautiful creations (Sharma, 2020).

Aquariculture, an important aquaculture section, have an excellent commercial value, fulfilling the aesthetic demands (Sharma, 2020). For several countries, the trade of ornamental fishes is a significant foreign exchange earner (Kamalii *et al.*, 2022). Aquariculture industry is an ever-expanding booming field, worthed around US$15 billion (Rhyne *et al.*, 2017).

In aquariculture, goldfish (*Carassius auratus*), that belongs to the family Cyprinidae, popularly domesticated in aquaria, is a wealthy option for trading and exportation because of its high demand (Sharma, 2020) due to its colouration, shape and size of tail and finnages, and also of its wide range of tolerance (Jia *et al.*, 2019). Its skin colouration is the pivotal quality that decides its market value and consumer acceptability (Gouveia and Rema, 2005).

Its vibrant reddish-orange colour is due to carotenoid deposition in its skin and muscle tissue.

Carotenoid not only improves skin colouration but also hastens growth performance and aid the fish to tolerate variable environmental conditions (Maiti *et al.*, 2017). It is a freshwater omnivorous fish, needs a good quality of protein rich feed for their proper growth and enhanced skin colouration as fishes are unable to synthesize colour producing pigments carotenoids (Jorjani *et al.*, 2019), meet their requirement from dietary sources (Duru, 2014).

In recent times, aquariculture industries include synthetic carotenoid such as astaxanthin (red) and canthaxanthin (orange-red) in fish feed through dietary supplementation (Maiti *et al.*, 2017). Generally, synthetic carotenoids are high-priced and bump up the feed cost by 10% to 20% (Bano *et al.*, 2020). In this context, there is a need of natural carotenoid sources for the current commercial aquariculture industries. Earlier study reports stated that skin colouration was improved when fishes were fed with natural carotenoid supplemented diet (Kaur and Shah, 2017). Hence, natural carotenoid sources either from plant-based or animal-based sources are cost-effective as well as alternative to synthetic carotenoid compounds for fish feed producers.

Among animal sources, some arthropods like shrimp (Pattanaik *et al.*, 2021), artemia (Seidgar *et al.*, 2019), krill (Roncarati *et al.*, 2016), lobster (Bell *et al.*, 2019), copepod (Gümüş *et al.*, 2022), mealworm (Das, 2023c), grasshopper, cricket, and black soldier fly larvae (Das, 2023d) were documented as an excellent source of carotenoid, able to deposit magnificent amount of carotenoid in fish skin and muscle tissue (Seidgar *et al.*, 2019; Bell *et al.*, 2016, Duru, 2014).

In aquaculture as well as aquariculture, the principal integrant is fish feed in which fishmeal is the commonly accepted primary protein source due to its nutritional profile, protein quality and amino acid composition (Tacon and Metian, 2008). Over the last few years, the high price and ever rising demand of fishmeal, have triggered a desperate search for non-conventional alternative cost-effective protein source which would be comparable to fishmeal in respect to protein quality and quantity. In the scientific research area of aqua feed, there are various earlier reports where cost-effective alternative plant origin protein sources were used as fishmeal replacement in aqua diets (Aas *et al.*, 2019; Ytrestoyl *et al.*, 2015; Das, 2023e).

But the results are not satisfying as the fishmeal-free formulated diets incorporating plant-based ingredients, have different anti-nutritional factors (Thakur *et al.*, 2019) and also have trouble with improper amino-acid profiles and low palatability. Moreover, most plant-based protein sources compete with livestock-animal and human diet. Nowadays, insects are used as an alternative ingredient for substituting conventional animal protein source, fishmeal in livestock (Huis and Gasco, 2023) as well as in aqua feed (Bera *et al*., 2022). Insect meals have awakened the interest of researchers due to their nutritional quality, sustainability, and recent authorisation for use in aquafeed (Alfiko *et al*., 2022). In contrast to plant by-products, insect meals are rich in important amino acids, particularly lysine, methionine, and leucine and with negligible quantity of anti-nutritional factors (Spranghers *et al*., 2017). In some western countries, people are also using insect in their daily meal (Orsi *et al*., 2019). Insect meal such as mealworms, crickets, grasshoppers, black soldier fly larvae were successfully used as a substitute of fishmeal in the diets of various food fish like rainbow trout, African catfish, Asian swamp eel, Jian carp and Atlantic salmon, and ornamental fish like common goldfish, swordtail, platy fish, guppy (Hu *et al*., 2020; Das, 2023a; Perera and Bhujel, 2022; Belghit *et al*., 2018).

Superworms, *Zoophobas morio*, the larval form of the darkling beetle commonly grown as feed for birds and reptiles due to their high nutritional value (Rumbos and Athanassiou, 2021). In some earlier studies, superworms were documented as an appreciable fishmeal replacement in the diet of Nile tilapia (Jabir *et al*., 2012), juvenile rainbow trout (Shekarabi *et al*., 2021), Asian sea bass (Prachom *et al*., 2021), sea trout (Mikołajczak *et al*., 2020).

Additionally, dietary supplementation of superworm meal was reported to increase the amount of WBC in blood of gilthead bream and also this insect meal has proven to have an antibacterial property (Henry *et al*., 2022). But there is insufficient documentation on superworm meal as a fishmeal replacement for the ornamental fish species.

In this scenario, the present feeding trial was undertaken to evaluate the effect of dietary replacement of fishmeal protein with superworm meal protein on growth performance, food utilization, survival, digestive enzyme activities and total carotenoid content in skin and muscle tissue of juvenile goldfish, *C. auratus*.

Materials and methods

Experimental fish and feeding trial

A six-month feeding trial was conducted in the Fish biology and aquaculture laboratory, Department of Zoology, Vidyasagar College, West Bengal, India. A total of 400 juveniles of goldfish were procured from local ornamental fish market, Kolkata, West Bengal, India. Then healthy seeds were acclimated for 15 days in a glass aquarium (120cm×60cm×20cm) by feeding them with commercial diet. For the feeding trial, 300 juveniles of an average initial weight of 1.66±0.03 gm, were selected randomly and distributed in 15 experimental glass aquaria (60cm×30cm×20cm) in triplicate group of five sets at a number of twenty fishes per aquarium. Satiation feeding was executed twice daily (09:00 and 18:00) and food consumption of each day was recorded by taking the weight of extra uneaten feed particles. Water exchange was done at 20% in every two days interval. Continuous aeration was provided throughout the feeding trial by air blower. During the feeding trial, water quality parameters were estimated (APHA, 2005) twice a week and the mean values were given as follows: water temperature at 30.67±3.70˚C, pH at 7.37±0.10, dissolved oxygen at 8.18±0.15 mg/L, alkalinity at 328±4.98 ppm, hardness at 625±6.32 ppm, free carbon dioxide at 1.75±0.18 ppm and total dissolved solids (TDS) at 996.5±13.41 ppm.

Experimental diets

In laboratory, superworms, *Z. morio* were purchased from a local commercial pet shop and then oven-dried for 24 hours at 40°C, grinded into a fine powder form and stored in an air-tight glass container until further utilise in making experimental diets for feeding trial and for the proximate analysis. Five different fishmeal- and superworm meal-based iso-proteic experimental diets were formulated containing 38% of crude protein. Here fishmeal protein was replaced with superworm meal protein at the levels of 0% (D0, control), 25% (D25), 50% (D50), 75% (D75), and 100% (D100) and thereby the formulated diets contained 0%, 10.59%, 21.18%, 31.77% and 42.35% superworm meal respectively. The oven-dried dietary ingredients were finely ground, properly mixed and blended with water into dough to prepare pellets by pelletizer with 2 mm die. Pellets were dried at room temperature and then were crushed into preferable particle size; airtight glass containers were used to store the experimental diets at room temperature.

Proximate composition analysis

The proximate composition of superworm, fishmeal, and experimental diets were analysed in accordance with the AOAC - Association of Official Analytical Chemists - technique (AOAC, 2006) for the evaluation of crude protein, crude lipid, ash and moisture.

Survival and growth study

After six-months of feeding trial, the number of fishes in each aquarium were recorded to calculate their survival and then they were starved for 24 hours. All the fishes of each aquarium were individually weighed and then final length of the respective fishes were taken to estimate the growth performance parameters like weight gain, specific growth rate (SGR), etc. Food utilization parameters in terms of total food consumption and feed conversion ratio (FCR) were calculated.

Estimation of carotenoid

Carotenoid content of experimental diets was estimated by following the method of Cyanotech (2002). At the end of the feeding trial, coloured region of skin and muscle tissue of fishes from five experimental sets were collected to determine the total carotenoid content by Tiewsoh *et al.* (2019) method.

Digestive enzyme analysis

At the end of feeding trial, fishes were fasted for 24 hours before sampling. Four fishes from each dietary set were randomly selected and dissected out on chilled condition to collect their intestines. Excess food particles were removed from the intestine and were rinsed with normal saline solution. Cold phosphate-buffered (pH 7.5) was used to homogenize the intestines and then homogenized samples were centrifuged at 10,000 rpm at 4°C for 20 mins. Supernatants were then used for the analysis of digestive enzymes such as amylase, protease and lipase using the protocols of Bernfeld (1955), Walter (1984) and Bier (1955) respectively.

Statistical analysis

The experimental data were presented in tables and figures as mean ± standard error (SE) of three replications. One-way analysis of variance (ANOVA), followed by Duncan's multiple range tests (DMRT) for multiple comparisons at the significance level of 0.05 was used to compare the differences among the five dietary treatments.

Results

Nutrient composition of experimental meals and their ingredients

Proximate composition in terms of crude protein, crude lipid, ash and moisture of superworm meal and fishmeal were listed in Table 1. Crude protein percentage of superworm meal was comparable with fishmeal protein percentage. It was found that the estimated value for superworm meal was slightly lower than the value of fishmeal. Crude lipid percentage on dry weight basis was found significantly higher ($P<0.05$; DMRT) in superworm meal, whereas ash and moisture content in fishmeal.

Table 1. Proximate composition of superworm meal and fishmeal (%, dry weight basis)

Nutritional composition (%)	Superworm meal	Fish meal
Crude protein	$51.00±0.96^a$	$54.00±1.26^b$
Crude lipid	$40.00±0.48^b$	$21.82±1.04^a$
Crude ash	$3.54±0.03^a$	$12.75±1.11^b$
Moisture	$5.90±0.32^a$	$8.94±0.41^b$

Values are presented as mean ± SE. Values with different superscript letters in a row denote significant differences as determined by DMRT after one way ANOVA ($P<0.05$)

Proportion of dietary ingredients and proximate composition of all the experimental diets were given in Table 2. Crude protein and moisture content of all the experimental diets of goldfish did not vary significantly ($P>0.05$) and its percentage on dry weight basis was ranged from 39.71% to 40.03%. On dry weight basis the percentage of crude lipid and ash were as expected as the level of replacement of fishmeal with superworm meal in the formulated diets.

Table 2. Proportion of dietary ingredients and proximate composition of experimental diets

Diet ingredients (gm/100gm)	Diets				
	D0	D25	D50	D75	D100
Fishmeal	40.00	30.00	20.00	10.00	00.00
Superworm	00.00	10.59	21.18	31.76	42.35
Soybean	26.50	26.50	26.50	26.50	26.50
Sattu	8.50	8.50	8.50	8.50	8.50
MOC- mustard oil cake	12.50	12.50	12.50	12.50	12.50
Rice bran	10.50	7.52	6.22	5.45	4.89
Wheat	0.40	2.79	3.50	3.68	3.66
Vitamin-mineral premix*	1.60	1.60	1.60	1.60	1.60
Total	100	100	100	100	100
Crude protein	40.03±0.34^{a}	39.91±0.37^{a}	39.84±0.20^{a}	39.77±0.39^{a}	39.71±0.48^{a}
Crude lipid	15.32±0.21^{a}	18.06±0.42^{b}	21.11±0.34^{c}	23.93±0.35^{d}	26.69±0.34^{e}
Ash	12.68±0.25^{e}	11.94±0.18^{d}	10.87±0.20^{c}	9.21±0.13^{b}	7.92±0.19^{a}
Moisture	6.68±0.49^{a}	6.46±0.36^{a}	6.71±0.47^{a}	6.57±0.34^{a}	6.87±0.27^{a}

*Vitamin-mineral premix (mg/kg diet): retinol-18,000 IU, Choleclaciferol-2000 IU, thiamine-15, menadione sodium bisulphate-10, riboflavin-25, pyridoxine-5, α-tocopherol-35, nicotinic acid-200, Ca-pantothenate-50, biotin-1.5, folic acid-10, cyanocobalamin-0.03, ascorbyl monophosphate-50, inositol-400, copper sulphate-20.2, dibasic calcium phosphate-5.9, sodium fluoride-2.21, potassium iodide-0.78, zinc oxide-37.5, ironsulphate-200, magnesium oxide-840, manganese oxide-26, cobalt sulphate-1.85, sodium selenite-0.65, potassium chloride-1.17, sodium chloride-0.45.

Values are presented as mean ± SE. Values with different superscript letters in a row denote significant differences as determined by DMRT after one way ANOVA ($P<0.05$)

Table 3. Growth parameters of goldfish, *Carassius auratus* fed with experimental diets in six months feeding trial

Growth parameters	Diets				
	D0	D25	D50	D75	D100
Initial weight (gm)	1.66±0.02^{a}	1.66±0.02^{a}	1.66±0.03^{a}	1.66±0.03^{a}	1.66±0.05^{a}
Final weight (gm)	3.19±0.06^{a}	3.68±0.11^{b}	4.33±0.17^{c}	4.73±0.06^{d}	6.88±0.38^{e}
Initial length (cm)	5.07±0.03^{a}	5.07±0.09^{a}	5.07±0.07^{a}	5.07±0.09^{a}	5.07±0.07^{a}
Final length (cm)	5.97±0.10^{a}	6.38±0.10^{b}	7.28±0.09^{c}	8.47±0.09^{d}	9.25±0.09^{d}
Weight gain (gm)	1.53±0.04^{a}	2.03±0.10^{b}	2.67±0.14^{c}	3.07±0.04^{d}	5.22±0.39^{e}
FCR	4.57±0.04^{e}	3.80±0.09^{d}	3.24±0.06^{c}	3.00±0.03^{b}	2.37±0.08^{a}
SGR (%)	0.72±0.01^{a}	0.89±0.02^{b}	1.06±0.02^{c}	1.16±0.01^{d}	1.58±0.07^{e}
Food consumption (gm)	6.99±0.33^{a}	7.69±0.41^{b}	8.63±0.37^{c}	9.21±0.53^{c}	12.29±1.54^{d}

Values are presented as mean ± SE. Values with different superscript letters in a row denote significant differences as determined by DMRT after one way ANOVA ($P<0.05$)

Growth parameters

Table 3 displayed the results of growth performances and feed utilization of goldfish after six-months of feeding trial. At the commencement of the feeding experiment, the average initial weight and length of the juveniles were recorded, varied from 1.60 gm to 1.75 gm and 4.90 cm to 5.20 cm respectively. At the end of feeding trial period, a significant difference was found in the growth performance and feed utilization parameters of experimental fishes fed with superworm supplemented diets and control diets. The growth performance parameters in terms of final weight and length of the fishes were considered, it was noticed that weight gain and specific growth rate (SGR) were significantly higher ($P<0.05$; DMRT) in the D100 diet fed set, followed by D75, D50, D25 and significantly lower ($P<0.05$; DMRT) in the control (D0). Similarly, best food conversion ratio (FCR) value ($P<0.05$; DMRT) was obtained from the D100 set, followed by the D75, D50, D25 and control (D0). On the other hand, total food consumption by the experimental fishes was noted to follow a opposite trend of the result of growth performance, that was significantly higher (P<0.05; DMRT) amount of food was consumed during the feeding trial by the fishes fed with control diet, followed by D25, D50, D75 and D100.

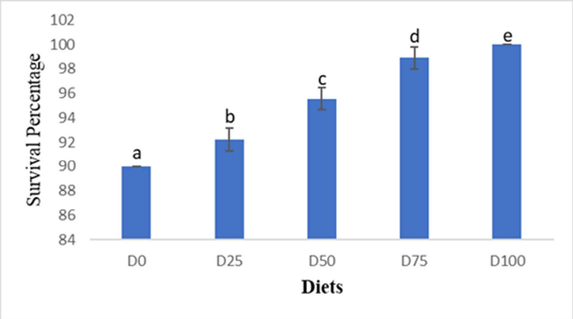

Fig. 1. Survival percent of *C. auratus* fed with five different experimental diets. Values are mean ± SE. Bars with different letters are significantly different (P<0.05) using DMRT after one way ANOVA

Survival percentage

Results of survival percentage after the six-months of feeding trial were presented in Fig. 1. Supplementation of fishmeal with superworm meal in the diet of juveniles exhibited a positive impact on their survival which was ranged from 90% to 100%. Survival percentage was found to be increased in a superior way with the increased inclusion level of superworm meal in the experimental diets. Here, 100% fishmeal replacement with superworm meal showed highest value (P<0.05; DMRT) of survival percentage and lowest value (P<0.05; DMRT) was obtained from the control (D0).

Activity of digestive enzymes

The findings of digestive enzyme activities were enlisted in Table 4. Dietary supplementation of superworm meal had significant effect (P<0.05; DMRT) on the intestinal digestive enzyme activities of experimental goldfishes. The amylase activity was observed significantly higher (P<0.05; DMRT) in fishes fed with D50 diet and the lowest value (P<0.05; DMRT) was noted from the fishes fed with control (D0) diet, while the remaining dietary treatments did not show any significant variations (DMRT). The protease activity and lipase activity were found to be significantly higher (P<0.05; DMRT) in the fishes fed with D100 diet, followed by D75, D50, D25 and significantly lower value (P<0.05; DMRT) was obtained from the fishes fed with control (D0) diet.

Table 4. Digestive enzyme activity of goldfish fed with experimental diets in six months feeding trial

Enzyme	D0	D25	D50	D75	D100
Amylase	$0.374±0.01^{a}$	$0.505±0.04^{b}$	$0.637±0.01^{c}$	$0.566±0.04^{b}$	$0.580±0.04^{b}$
Protease	$0.91±0.00^{a}$	$1.206±0.01^{b}$	$1.50±0.0^{c}$	$1.82±0.00^{d}$	$2.12±0.02^{e}$
Lipase	$0.82±0.01^{a}$	$1.24±0.01^{a}$	$1.77±0.00^{a}$	$2.08±0.01^{a}$	$2.36±0.01^{a}$

Values are presented in unit U/mg. Values are presented as mean ± SE. Values with different superscript letters in a row denote significant differences as determined by DMRT after one way ANOVA (P<0.05).

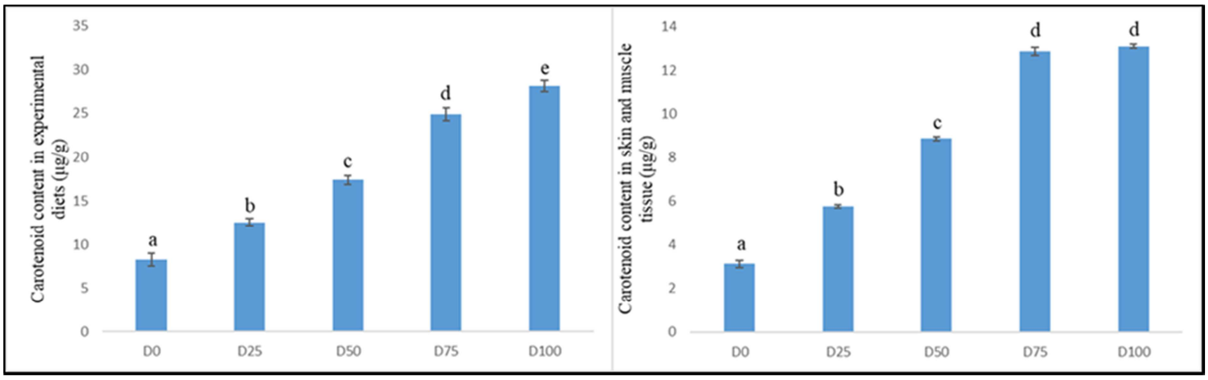

Fig. 2. Carotenoid content in experimental diets (µg/g) and fish skin and muscle tissue (µg/g). Values are mean ± SE. Bars with different letters are significantly different (P<0.05) using DMRT after one way ANOVA

Carotenoid concentration of experimental diets and of goldfish skin and muscle tissue

The spectrophotometric analysis of carotenoid content of experimental diets was shown in Fig. 2. The obtained value of total carotenoid content in the experimental diets revealed an increasing trend with the increased amount of superworm meal supplementation in the experimental diets which was ranged from 8.27 µg/g to 28.11 µg/g.

After six-months of feeding trial, carotenoid content in the skin and muscle tissue of goldfish from different dietary sets showed significant variations ($P<0.05$; DMRT) (Fig. 2). Skin colour improvement of the experimental fishes in terms of the carotenoid deposition in fish skin and muscle tissue showed an intensifying trend of their values from control (D0) to D75 dietary sets ($P<0.05$; DMRT), while the value of D75 and D100 did not vary significantly (DMRT).

Discussion

Over the past decade, the aquariculture industry has a mounting growth due to growing interest of aquarium fishes. At this situation, there needs urgent research to fill up the lacunae associated with the production of ornamental fishes and aquafeed (Calado *et al.*, 2017). For the sustainable growth of aquariculture, requires the exploration and introduction of novel protein source for the aquafeed industry (Gasco *et al.*, 2018). The new protein source for fish feed, should contain adequate amount of good quality protein and other nutrients that will enhance fish growth performance and ascertain sustainability and economic suitability (Gasco *et al.*, 2018). Insects might be the best alternative to fishmeal (Belghit *et al.*, 2018).

Hence, in the current study, superworm meal was used as a fishmeal replacement with the aim of reducing the fish feed cost and subsequently the cost of raising ornamental fish which will ultimately support the growth of aquariculture industry. To establish suitability of superworm meal as a fishmeal replacer, evaluation of fish growth performance and survival is crucial.

In the present investigation, it was observed that fishmeal substitute superworm meal had no deleterious effects on the growth performance, feed utilization and survival of juvenile goldfish. This finding was consistent with the observations of some earlier studies where reported that insect meal could partially or completely replace fishmeal without any negative effects on the growth efficiency, feed utilization and survival of ornamental fishes as well as food fishes (Fisher *et al.*, 2020; Kamalii *et al.*, 2022; Das, 2023a,b,c; Chemello *et al.*, 2020; Gasco *et al.*, 2016; Belghit *et al.*, 2018; Piccolo *et al.*, 2017; Li *et al.*, 2017; Devic *et al.*, 2018). In this connection, Li *et al.* (2017) reported that black soldier fly larvae had the potential to replace fishmeal totally in the diets of *Cyprinus carpio* without any negative impact on growth performance and feed utilization. Recently, Kamalii *et al.* (2022) evaluated the nutritional quality of black soldier fly larvae, used as fishmeal replacement in the diet for goldfish and obtained a favourable result on growth rates, feed utilization, digestive enzyme activity and haemato-biochemical indices, indicating good health status of the experimental fishes.

Moreover, for desirable growth of fish, the optimum incorporation level of nonconventional fishmeal substitute in the formulated aquafeed is crucial which depends on the nutrient quality of the novel fishmeal replacer. Here the goldfish fed with diet having 100% replacement of fishmeal with superworm meal showed the best results for growth performance and feed utilization. There were some earlier reports on replacing of fishmeal with superworm in the fish diet of consumable fish. When Nile tilapia (*Oreochromis niloticus*) juveniles were fed with diets having superworm meal replacing the fishmeal at different level, it was found that maximum weight gain and SGR was obtained from 25% and 50% fishmeal replaced diet fed fishes (Jabir *et al.*, 2012). An improved SGR was found in *Perca fluviatilis*, when fed with a mixture of superworm and house cricket meals (5% of each) replacing 25% of fishmeal (Tilami *et al.*, 2020). In this context, a few studies reported that 100% replacement of fishmeal with superworm had a negative impact on the growth performance of Guppy and Sea Trout (Kowalska *et al*., 2022; Mikołajczak *et al*., 2020). In support with the present study, Prachom *et al*. (2021) stated that replacing fishmeal with superworm meal at 80% had the highest growth efficiency and feed utilization of Asian sea bass, *Lates calcarifer*.

In terms of nutritional value, the protein content of superworm meal, estimated in the current study, was found very close to the value of fishmeal. This finding was reinforced by the study where reported that the essential amino acid profile of superworm meal, is of a similar pattern compared to a standard fishmeal (Prachom *et al*., 2021). In agreement with this observation, Jabir *et al*. (2012) reported that all essential amino acid present in superworm meal was similar with the fishmeal except for methionine. In some cases, the protein content of superworm meal showed a significant variation (Ojewola *et al*., 2005) which might be due to several factors like different larval stage, nutritional ecology of larvae, conditions of production and also variation in the processing methods. Crude protein content of superworm, used in the present study was 51%, while in the study of Prachom *et al*. (2021), it was somewhat higher at 61.4% and of Jabir *et al*. (2012) it was almost equal as the present study (50.5.3%). Hence, superworm meal might be a potential fishmeal substitute as it contains a high quantity of good quality protein and do not have any identifiable anti-nutritional factors (Spranghers *et al*., 2017) and is also a good additional source of minerals and vitamins (Fisher *et al*., 2020).

Monitoring of digestive enzyme activities are widely used in feeding trials with new protein sources. The results of digestive enzyme activities reflect the health status, and the ability of fish to digest the nonconventional protein source-based diets. It could be effectively used to know the acceptance of feed by the fishes. The results of the present study revealed that dietary supplementation of superworm meal had a significant effect on the estimated digestive enzyme activities of goldfish. Protease and lipase activity were found to be increased in the fishes fed with diets having increased level of superworm meal inclusion. This observation confirmed that goldfish could efficiently utilize the protein and lipid of the experimental diets having superworm meal as a total replacement of fishmeal. The intestinal amylase activity was significantly higher in the D50 set. The fishes fed with control diet (D0), containing 0% superworm meal, exhibited low amylase, protease and lipase activity.

This finding was consistent with the study where black soldier fly larvae meal was used as fishmeal replacement in the diet of goldfish and *Monopterus albus* (Kamalii *et al*., 2022; Hu *et al*., 2020). In contrary, digestive enzyme activity was reported to be decreased when fishmeal replacement with mealworm meal and black soldier fly larvae meal was increased in the diet of *Argyrosomus regius* and *Danio rerio* respectively (Coutinho *et al*., 2021; Fronte *et al*., 2021). Additionally, it was found that digestive enzymes activity did not vary significantly, when *Litopenaeus vennami* and *Cyprinus carpio* were treated with different gradients of insect meal replacing the fishmeal (Wang *et al*., 2021; Hoffmann *et al*., 2020; Li *et al*., 2017).

Fishes cannot synthesize their skin and muscle pigments, carotenoids on their own (Jorjani *et al*., 2019). Fishes with vibrant colours such as orange, red and yellow absorb carotenoids from various dietary sources and store them in their skin and muscle tissue (Duru, 2014). So, the colour intensity of ornamental fishes could be improved by adding natural or synthetic carotenoids to their diets (Kaur and Shah, 2017; Maiti *et al*., 2017). The results of feeding trial depicted that the amount of carotenoid of skin and muscle tissue of experimental fishes were upsurged with the increased inclusion proportion of superworm meals in the diet.

Maximum amount of carotenoid deposition was found in the D75 and D100 diet fed set, where data insignificantly varied. This finding revealed that fishes are able to absorb and deposit the dietary carotenoid to their full potential up to a certain concentration in their diet which leads to the maximal accumulation of carotenoids and enhanced colouring of the muscle and fish skin. Beyond that fraction of carotenoid in fish feed did not effective for further carotenoid deposition in fish muscle and skin, rather provide a negative impact with faded fish skin (Jagadeesh *et al.*, 2015). So, for the maximum amount of carotenoid deposition in fish skin and muscle, needs a species-specific amount of carotenoid incorporation in fish feed (Ha *et al.*, 1993). Several plant-derived natural carotenoid sources have been effectively included in the diets of ornamental fishes. Still, a few studies were carried out on ornamental fishes fed with diet supplemented with natural carotenoid from animal sources. Few earlier reports revealed that cricket, grasshopper, black soldier fly larvae and mealworm had natural carotenoids and these insect meal supplemented diets showed a positive impact on skin colouration of ornamental fishes like *Xiphophorus helleri, Xiphophorus maculatus* (Das, 2023a; Das, 2023b). Previous investigations on goldfish portrayed that plant-derived (alfalfa, red yeast and spirulina) as well as insect-meal derived (mealworm) natural carotenoid supplemented diet had favourable effect on fish skin pigmentation (Yanar *et al.*, 2008; Xu *et al.*, 2006; Kiriratnikom *et al.*, 2005; Das, 2023c).

Furthermore, natural carotenoid had a growth promoting role (Maiti *et al.*, 2017). In the present study, it was found that the growth performance and feed utilization, and the carotenoid concentration in fish skin and muscle tissue, both were significantly higher in D100 set, followed by D75, D50, D25 and control (D0) set. This finding was supported by the study of Amar *et al.* (2001) who reported that carotenoid has a positive role in the intermediary metabolism of fish and it enhances food utilization and improves growth. This observation was also in line with the study of Jha *et al.* (2012), Ezhil *et al.* (2008) and Jagadeesh *et al.* (2015), where natural plant derived supplemented diets improved growth and feed utilization of *Barilius bendelisis,* red sword tail and rosy barb respectively. In this aspect, Pailan *et al.* (2012), Maiti *et al.* (2017), Jain *et al.* (2019), Bano *et al.* (2020) and Tiewsoh (2019) stated there was a correlation between the supplemented carotenoids of diet and growth performance of rosy barb, koi carp, gourami and goldfish respectively.

In the field of aquafeed research, very limited data are available regarding the use of superworm meal as fishmeal supplement for the aquafeed industry. While, sufficient scientific literatures are at hand concerning the use of black soldier fly larvae meal (Das, 2023d; Li *et al.*, 2017; Hu *et al.*, 2020; Belghit *et al.*, 2019), mealworm meal (Melenchón *et al.*, 2023), grasshopper meal (Olaleye and Asuquo, 2021; Tsado *et al.*, 2021; Das, 2023a, b, c), cricket meal (Jeong *et al.*, 2021; Ndione *et al.*, 2022; Das, 2023a,b), and many other insect meals in fish feed. Insect meals are acknowledged not only as a valuable nutritional source but also as a promising alternative to traditional fishmeal. In comparison to high cost, high demand, scarce and quality compromised fishmeal, insect meal would be a cost effective one as insects are protein rich, could be harvested or could be mass reared easily in insect farm.

Conclusion

The alternative protein source, superworm meals could be one of the best choices as fish meal replacement in aquafeed for the ornamental fishes in India and beyond. Notably, this investigation revealed that fishmeal could be completely substituted by superworm meal in the juvenile goldfish diets using up to 42.35% which support maximum growth performance, skin colouration, digestive enzyme activity and survival. Further research is recommended to determine the efficiency of superworm meal as alternative to traditional fishmeal in the diets of some other ornamental fishes and consumable fishes.

References

Aas TS, Ytrestoyl T, Asgard T. 2019. Utilization of feed resources in the production of Atlantic salmon (*Salmo salar*) in Norway: An update for 2016. Aquaculture Reports **15**, 1–10.

Alfiko Y, Xie D, Astuti T, Wong J, Wang L. 2022. Insects as a feed ingredient for fish culture: Status and trends. Aquaculture and Fisheries **7**(2), 166–178.

Amar EC, Kiron V, Satoh S, Watanabe T. 2001. Influence of various dietary synthetic carotenoids on bio-defence mechanisms in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture Research **32**(1), 162–163.

AOAC. 2006. Official methods of analysis. 18th ed. Revised. Association of Official Analytical Chemists, Washington, DC, USA.

APHA. 2005. Standard methods for the examination of water and wastewater. 21st ed. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC.

Bano F, Kashyap A, Serajuddin M. 2020. Effects of different dietary supplementation of plant carotenoids on growth, coloration and behaviour of giant gourami, *Trichogaster fasciata* (Bloch and Schneider, 1801). Iranian Journal of Fisheries Sciences **19**(6), 2770–2789.

Belghit I, Liland NS, Gjesdal P, Biancarosa I, Menchetti E, Li Y, Waagbø R, Krogdahl A, Lock EJ. 2019. Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (*Salmo salar*). Aquaculture **503**, 609–619.

Bell NA, Jeffrey S, MacIsaac JL, Colombo SM. 2019. Aquaculture **13**(100187), 1–5.

Bera P, Dhara H, Sau SK. 2022. Insects as alternative protein sources for aquaculture: A review. Environment and Ecology **40**(4A), 2156–2162.

Bernfeld P. 1955. Amylase [alpha] and [beta]. In: Kolowick SP, Kaplan NO, Ed. Methods of enzymology. Academic Press, New York, NY, USA, p. 149–150.

Bier BM. 1955. Lipases. In: Colowick SP, Kaplan NO, Ed. Methods in enzymology. Academic Press, New York, p. 627–642.

Calado R, Olivotto I, Oliver MP, Holt J. (Eds.). 2017. Marine ornamental species aquaculture. Vol. 712. Wiley Blackwell.

Chemello G, Renna M, Caimi C, Guerreiro I, Oliva-Teles A, Enes P, Biasato I, Schiavone A, Gai F, Gasco L. 2020. Partially defatted *Tenebrio molitor* larva meal in diets for grow-out rainbow trout, *Oncorhynchus mykiss* (Walbaum): Effects on growth performance, diet digestibility and metabolic responses. Animals **10**(2), 229.

Coutinho F, Castro C, Guerriro I. 2021. Mealworm larvae meal in diets for meagre juveniles: Growth, nutrient digestibility and digestive enzymes activity. Aquaculture **535**, 736362–736762.

Cyanotech. 2002. Analysis of beta-carotene and total carotenoids from *Spirulina* (Spectrophotometric method). *Spirulina pacifica*. Technical Bulletin, 003b. Cyanotech Corporation.

Das M. 2023a. Effect of four insect meals on the growth performance, survival, total carotenoid content and body composition of an ornamental fish *Xiphophorus helleri* (Poeciliidae). International Journal of Entomology Research **8**(6), 23–29.

Das M. 2023b. Effect of insect meals on the growth efficiency, survival and total carotenoid content of an ornamental fish *Xiphophorus maculatus* (Gunther, 1866). Asian Journal of Fisheries and Aquatic Research **22**(3), 1–9.

Das M. 2023c. Effect of fishmeal replacement with mealworm (*Tenebrio molitor*) meal on growth performance and survival of the black molly (*Poecilia sphenops*). Environment and Ecology **42**(2B), 902–909.

Das M. 2023d. Effect of black soldier fly (*Hermetia illucens*) larvae meal as a fishmeal protein replacement on the growth, feed utilization, survival and whole-body composition of black molly (*Poecilia sphenops*). International Journal of Biosciences **22**(5), 168–181.

Das M. 2023e. Effects of dietary supplementation of carrot meal on growth, survival, whole body composition and total carotenoid content of zebrafish, *Danio rerio*. International Journal of Zoological Investigations **9**(2), 575–585.

Devic E, Leschen W, Murray F, Little DC. 2018. Growth performance, feed utilization and body composition of advanced nursing Nile tilapia (*Oreochromis niloticus*) fed diets containing black soldier fly (*Hermetia illucens*) larvae meal. Aquaculture Nutrition **24**(1), 1–8.

Duru M. 2014. Effects of *Spirulina platensis* added to different amounts of feed on coloring and growth performance of goldfish (*Carassius auratus*). M.Sc. Thesis, Graduate School of Natural and Applied Sciences, Mersin University, p. 68.

Ezhil J, Jeyanthi C, Narayan M. 2008. Marigold as a carotenoid source on pigmentation and growth of red swordtail, *Xiphophorus helleri*. Turkish Journal of Fisheries and Aquatic Sciences **8**, 99–102.

Fisher HJ, Collins SA, Hanson C, Mason B, Colombo SM, Anderson DM. 2020. Black soldier fly larvae meal as a protein source in low fish meal diets for Atlantic salmon (*Salmo salar*). Aquaculture **521**, 734978.

Fronte B, Licitra R, Bibbiani C, Casini L, De Zoysa M, Miragliotta V, Sagona S, Coppola F, Brogi L, Abramo F. 2021. Fishmeal replacement with *Hermetia illucens* meal in aquafeeds: Effects on zebrafish growth performances, intestinal morphometry, and enzymology. Fishes **6**, 28.

Gasco L, Finke M, van-Huis A. 2018. Can diets containing insects promote animal health? Journal of Insects as Food and Feed **4**(1), 1–4.

Gasco L, Henry M, Piccolo G, Marono S, Gai F, Renna M, Lussiana C, Antonopoulou E, Mola P, Chatzifotis S. 2016. *Tenebrio molitor* meal in diets for European sea bass (*Dicentrarchus labrax* L.) juveniles: Growth performance, whole-body composition and in vivo apparent digestibility. Animal Feed Science and Technology **220**, 34–45.

Gouveia L, Rema P. 2005. Effect of microalgal biomass concentration and temperature on ornamental goldfish (*Carassius auratus*) skin pigmentation. Aquaculture Nutrition **11**, 19–23.

Gumus E, Gurler C, Gulle I. 2022. Effect of dietary supplementation of copepod meal in goldfish *Carassius auratus* (Linnaeus, 1758) on growth performance, fatty acid profile and colouration. Indian Journal of Fisheries **69**(1), 121.

Ha BS, Kang DS, Kim JH, Choi OS, Ryu HY. 1993. Metabolism of dietary carotenoids and effects to improve the body color of cultured flounder and red seabream. Bulletin of the Korean Fisheries Society **38**, 331–338.

Henry MA, Golomazou E, Asimaki A, Psofakis P, Fountoulaki E, Mente E, Rumbos CI, Athanassiou CG, Karapanagiotidis IT. 2022. Partial dietary fishmeal replacement with full-fat or defatted superworm (*Zophobas morio*) larvae meals modulates the innate immune system of gilthead seabream, *Sparus aurata*. Aquaculture Reports **27**, 101347.

Hoffmann L, Rawski M, Nogales-Merida S, Mazurkiewicz J. 2020. Dietary inclusion of *Tenebrio molitor* meal in sea trout larvae rearing: Effects on fish growth performance, survival, condition, and gut and liver enzymatic activity. Annals of Animal Science **20**, 579–598.

Hu Y, Huang Y, Tang T, Zhong L, Chu W, Dai Z, Hu Y. 2020. Effect of partial black soldier fly (*Hermetia illucens* L.) larvae meal replacement of fish meal in practical diets on the growth, digestive enzyme and related gene expression for rice field eel (*Monopterus albus*). Aquaculture Reports **17**, 100345.

Huis AV, Gasco L. 2023. Insects as feed for livestock production. Science **379**(6628), 138–139.

Jabir MAR, Jabir SAR, Vikineswary S. 2012. Nutritive potential and utilization of super worm (*Zophobas morio*) meal in the diet of Nile tilapia (*Oreochromis niloticus*) juvenile. African Journal of Biotechnology **11**(24), 6592–6598.

Jagadeesh TD, Murthy HS, Surendranath SV, Panikkar P, Manjappa N, Mahesh V. 2015. Effect of supplementation of marigold (*Tagetes erecta*) oleoresin on growth, survival and pigmentation of rosy barb, *Puntius conchonius* (Hamilton). The Bioscan **10**(3), 1431–1435.

Jain A, Kaur VI, Hollyappa SA. 2019. Effect of dietary supplementation of carrot meal on survival, growth and pigmentation of freshwater ornamental fish, Koi Carp, *Cyprinus carpio* (L.). Indian Journal of Animal Nutrition **36**(4), 405–413.

Jeong SM, Khosravi S, Mauliasari IR, Lee BJ, You SG, Lee SM. 2021. Nutritional evaluation of cricket, *Gryllus bimaculatus*, meal as fish meal substitute for olive flounder, *Paralichthys olivaceus*, juveniles. The Journal of the World Aquaculture Society **52**, 859–880.

Jha GN, Sarma D, Qureshi TA. 2012. Effect of spirulina (*Spirulina platensis*) and marigold (*Tagetes erecta*) fortified diets on growth, body composition and total carotenoid content of *Barilius bendelisis*. Indian Journal of Animal Sciences **82**(3), 336–340.

Jia Y, Liu Y, Chen K, Sun H, Chen Y. 2019. Climate, habitat and human disturbance driving the variation of life-history traits of the invasive goldfish *Carassius auratus* (Linnaeus, 1758) in a Tibetan Plateau River. Aquatic Invasions **14**, 724–737.

Jorjani M, Sharifrohani M, Mirhashemi Rostami A, Tan S. 2019. Effects of *Hibiscus rosa-sinensis* as a natural carotenoid on growth performance, body composition, pigmentation and carotenoid in blood plasma of blue gourami, *Trichogaster trichopterus* at different stocking densities. Iranian Journal of Fisheries Sciences **18**(4), 619–634.

Kamalii A, Antony C, Ahilan B, Uma A, Prabu E. 2022. Dietary protein replacement of fish meal with black soldier fly larvae meal: Effects on growth, whole-body composition, digestive enzyme activity, muscle-growth-related gene expression and haemato-biochemical responses of juvenile goldfish, *Carassius auratus*. Turkish Journal of Fisheries and Aquatic Sciences **23**(2), 1–11.

Kaur R, Shah TK. 2017. Role of feed additives in pigmentation of ornamental fishes. International Journal of Fisheries and Aquatic Studies **5**, 684–686.

Kiriratnikom S, Zaau R, Suwanpugdee A. 2005. Effects of various levels of spirulina on growth performance and pigmentation in goldfish *Carassius auratus*. Songklanakarin Journal of Science and Technology **27**, 133–139.

Kowalska J, Rawski M, Homska N, Mikołajczak Z, Kierończyk B, Świątkiewicz S, Wachowiak R, Hetmańczyk K, Mazurkiewicz J. 2022. The first insight into full-fat super worm (*Zophobas morio*) meal in guppy (*Poecilia reticulata*) diets: a study on multiple-choice feeding preferences and growth performance. Annals of Animal Science **22**, 371–384.

Li S, Ji H, Zhang B, Zhou J, Yu H. 2017. Defatted black soldier fly (*Hermetia illucens*) larvae meal in diets for juvenile Jian carp (*Cyprinus carpio* var. Jian): Growth performance, antioxidant enzyme activities, digestive enzyme activities, intestine and hepatopancreas histological structure. Aquaculture **477**, 62–70.

Maiti M, Bora D, Nandeesha TL, Sahoo S, Adarsh BK, Kumar S. 2017. Effect of dietary natural carotenoid sources on colour enhancement of koi carp, *Cyprinus carpio* L. International journal of fisheries and aquatic studies **5**(4), 340–345.

Melenchón F, Larrán AM, Sanz M-A, Rico D, Fabrikov D, Barroso FG, Galafat A, Alarcón FJ, Morales AE, Hidalgo MC, Lourenço HM, Pessoa M-F, Tomás-Almenar C. 2023. Different diets based on yellow mealworm (*Tenebrio molitor*)—Part A: Facing the decrease in omega–3 fatty acids in fillets of rainbow trout (*Oncorhynchus mykiss*). Fishes **8**(6), 286–295.

Mikołajczak Z, Rawski M, Mazurkiewicz J, Kierończyk B, Józefiak D. 2020. The effect of hydrolyzed insect meals in sea trout fingerling (*Salmo trutta m. trutta*) diets on growth performance, microbiota and biochemical blood parameters. Animals **10**(6), 1031.

Ndione A, Fall J, Mbaye S, Ndour PM, Fall SKL, Jatta S, Loum A, Sagne M, Diouf A, Ndong D, Diouf M, Ba CT, Chien A. 2022. Effects of replacement of fishmeal by cricket meal on the growth performance, feed efficiency, survival and body composition of Nile tilapia (*Oreochromis niloticus*) fry. Journal of the Fisheries Society of Taiwan **49**(3), 183–191.

Ojewola GS, Okoye FC, Ukoha OA. 2005. Comparative utilization of three animal protein sources by broiler chickens. International Journal of Poultry Science **4**, 462–467.

Olaleye IG, Asuquo PE. 2021. Proximate composition and mineral content of grasshopper meal as an alternative to fishmeal for fish feed production. Current Journal of Applied Science and Technology **40**(19), 72–75.

Orsi L, Voege LL, Stranieri S. 2019. Eating edible insects as sustainable food? Exploring the determinants of consumer acceptance in Germany. Food Research International **125**, 108573.

Pailan GH, Archana S, Mrinal K. 2012. Rose petals meal as natural carotenoid source in pigmentation and growth of rosy barb (*Puntius conchonius*). Indian Journal of Animal Nutrition **29**, 291–296.

Pattanaik SS, Sawant PB, Xavier MKA, Srivastava PP, Dube K, Sawant BT, Chadha NK. 2021. Dietary carotenoprotein extracted from shrimp shell waste augments growth, feed utilization, physio-metabolic responses and coloration in Oscar, *Astronotus ocellatus* (Agassiz, 1831). Aquaculture **534**, 736303e.

Perera GSC, Bhujel RC. 2022. Replacement of fishmeal by house cricket (*Acheta domesticus*) and field cricket (*Gryllus bimaculatus*) meals: effect for growth, pigmentation and breeding performances of Guppy (*Poecilia reticulata*). Aquaculture Reports **25**, 101260.

Piccolo G, Iaconisi V, Marono S, Gasco L, Loponte R, Nizza S, Bovera F, Parisi G. 2017. Effect of *Tenebrio molitor* larvae meal on growth performance, in vivo nutrients digestibility, somatic and marketable indexes of gilthead sea bream (*Sparus aurata*). Animal Feed Science and Technology **226**, 12–20.

Prachom N, Boonyoung S, Hassaan MS, El-Haroun E, Davies SJ. 2021. Preliminary evaluation of Superworm (*Zophobas morio*) larval meal as a partial protein source in experimental diets for juvenile Asian sea bass, *Lates calcarifer*. Aquaculture Nutrition, 1–11.

Rhyne AL, Tlusty MF, Szczebak JT, Holmberg RJ. 2017. Expanding our understanding of the trade in marine aquarium animals. PeerJ **5**, e2949.

Roncarati A, Sirri F, Felici A, Stocchi L, Melotti P, Meluzzi A. 2016. Effects of dietary supplementation with krill meal on pigmentation and quality of flesh of rainbow trout (*Oncorhynchus mykiss*). Italian Journal of Animal Science **10**(2), e27.

Rumbos CI, Athanassiou CG. 2021. The superworm, *Zophobas morio* (Coleoptera: Tenebrionidae): a 'Sleeping Giant' in nutrient sources. Journal of Insect Science **21**(2), 3–17.

Seidgar M, Mohebbi F, Nekuiefard A, Hafezieh M, Dadgar S, Anbi AA, Shiri A. 2019. The effect of *Artemia urmiana*, earthworm, cow heart and concentrate as supplementary diets on skin color and pigmentation of Oscar fish (*Astronotus ocellatus*). International Journal of Aquatic Science **10**(2), 88–93.

Sharma M. 2020. Ornamental fish rearing and breeding—a new dimension to aquaculture entrepreneurship in Himachal Pradesh. International Journal of Fisheries and Aquatic Studies **8**, 157–162.

Shekarabi SPH, Mostafavi ZS, Mehrgan MS, Islami HR. 2021. Dietary supplementation with dandelion (*Taraxacum officinale*) flower extract provides immunostimulation and resistance against *Streptococcus iniae* infection in rainbow trout (*Oncorhynchus mykiss*). Fish & Shellfish Immunology **118**, 180–187.

Spranghers T, Ottoboni M, Klootwijk C, Ovyn A, Deboosere S, De-Meulenaer B, Michiels J, Eeckhout M, De-Clercq P, De-Smet S. 2017. Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste. Journal of the Science of Food and Agriculture **97**, 2594–2600.

Tacon AGJ, Metian M. 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture **285**(1–4), 146–158.

Thakur A, Sharma V, Thakur A. 2019. An overview of anti-nutritional factors in food. International Journal of Chemical Studies **7**(1), 2472–2479.

Tiewsoh W, Singh E, Nath R, Surnar SR, Priyadarshini A. 2019. Effect of carotenoid in growth and colour enhancement in goldfish, *Carassius auratus* (L.). Journal of Experimental Zoology, India **22**(2), 765–771.

Tilami SK, Turek J, Červený D, Lepič P, Kozák P, Burkina V, Sakalli S, Tomčala Sampels S, Mráz J. 2020. Insect meal as a partial replacement for fish meal in a formulated diet for perch (*Perca fluviatilis*). Turkish Journal of Fisheries and Aquatic Sciences **20**(12), 867–878.

Tsado AN, Okoli RN, Jiya GA, Gana D, Zubairu R, Alhassan EH, Salihu ZI. 2021. Effect of supplementing fish diet with grasshopper meal on growth parameters of African catfish (*Clarias gariepinus*). AROC in Agriculture **1**(1), 01–05.

Walter HE. 1984. Methods of enzymatic analysis. Verlag Chemie, Weinheim, Germany.

Wang P, Chen S, Wei C, Yan Q, Sun Y-Z, Yi G, Li D, Fu W. 2021. *Monascus purpureus* M-32 improves growth performance, immune response, intestinal morphology, microbiota and disease resistance in *Litopenaeus vannamei*. Aquaculture **530**(2), 735947.

Xu J, Tao N, Liu Q, Deng X. 2006. Presence of diverse ratios of lycopene/ß-carotene in five pink or red-fleshed citrus cultivars. Scientia Horticulturae **108**(2), 81–184.

Yanar M, Erçen Z, Hunt AO, Büyükçapar HM. 2008. The use of alfalfa as a natural carotenoid source in diets of goldfish, *Carassius auratus*. Aquaculture **284**, 196–200.

Ytrestøyl T, Aas TS, Åsgård T. 2015. Utilisation of feed resources in production of Atlantic salmon (*Salmo salar*) in Norway. Aquaculture **448**(1), 365–374.