Nutritional evaluation of cricket, *Gryllus bimaculatus*, meal as fish meal substitute for olive flounder, *Paralichthys olivaceus*, juveniles

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Abstract
An 8-week feeding trial was designed to evaluate the potential of cricket meal, *Gryllus bimaculatus*, as a locally available unconventional source of protein in a practical diet for olive flounder, *Paralichthys olivaceus*. Three hundred juvenile fish (initial weight, 33.5 ± 0.01 g) were randomly distributed into five dietary groups in triplicates (20 fish per tank) and each group was hand-fed one of the experimental diets containing graded level of a cricket meal (CR) replacing 0, 20, 40, 60, and 80% of fish meal (FM) protein (designated as CR0, CR20, CR40, CR60, and CR80). Although replacing more than 40% of FM protein resulted in lower growth rates and feed utilization efficiency, juvenile flounders fed the highest level of dietary CR (CR80) still performed as well as control group (CR0). Replacing more than 20% of FM with CR markedly reduced apparent nutrient digestibility in fish. Plasma total cholesterol concentration showed decreasing trend with increasing inclusion levels of CR in the diet. Whole body and fillet lipid content decreased with increasing levels of dietary CR. There was a remarkable enhancement in the levels of C18 fatty acids and reduction in the levels of n – 3 highly unsaturated fatty acids in the fillets of fish fed with the CR diets, which...
became more prominent with greater levels of dietary FM replacement. Antioxidant enzyme activities tended to increase with increasing dietary CR levels but gradually decreased when the replacement level exceeded 60%. Overall, the efficiency of CR as a promising substitute for FM in the flounder diets has been confirmed not only in relation to growth rates, but also in terms of immunopotency.

**KEYWORDS**

amino acids, fatty acids, *Gryllus bimaculatus*, insect meal, nonspecific immune activity, *Paralichthys olivaceus*

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1 | INTRODUCTION

It is well-recognized that the substitution of high value and unsustainable fish meal (FM) by less expensive and more readily available alternatives in aquafeeds is a priority for the sustainable development of the aquaculture sector. A variety of feedstuffs have been examined for their potential as alternative sources of protein with growing interest in insects mainly because of their nutritional and environmental benefits over their conventional plant- and animal-derived counterparts (van Huis, 2013). The chief advantages claimed for the insects, apart from their high nutritional values, are their greater feed conversion efficiency and extremely smaller environmental footprint compared to other types of livestock, which could significantly contribute to global food security, environmental protection, and economic growth (van Huis & Oonincx, 2017). Indeed, growing empirical evidence has suggested that partially replacing FM with a wide variety of insect meals is highly feasible for most marine and freshwater fish species (Barroso et al., 2014; Henry, Gasco, Piccolo, & Fountoulaki, 2015). Nevertheless, the extent to which FM can be spared without consequence is very much dependent on several factors including fish/insect species and life stage, diet composition, feeding duration, and rearing condition (Dossey, Morales-Ramos, & Rojas, 2016).

Among a wide variety of insects, crickets are probably one of the most popular farmed insects with highly advanced mass production methods today (Cortes Ortiz et al., 2016). High protein quality and quantity, great feed conversion efficiency, prolific breeding habits, short life-cycle, and rapid growth are among many reasons why crickets have long been recognized as promising candidates to produce inexpensive and sustainable source of protein for human food and animal feed. Indeed, although the literature on this subject is still very limited, encouraging results were obtained when crickets were incorporated in animal feed such as broilers (Makkar, Tran, Heuzé, & Ankers, 2014; Wang et al., 2005). With most studies available in the literature so far mainly focused on few well-known insects, that is, mealworms, soldier fly, and silk worm, very little is known about the suitability of crickets as a feed ingredient for farmed fish. In a study with African catfish, *Clarias gariepinus*, Taufek et al. (2016) discovered that total substitution of FM with a cricket meal, *Gryllus bimaculatus*, resulted in improved growth performance and feed efficiency, even, in comparison to FM-fed fish.

Olive flounder, *Paralichthys olivaceus*, is one of the most important marine finfish species cultured in Northeast Asian countries (Seong et al., 2018), particularly South Korea where its production alone (41,207 ton) comprised almost 48% of the total marine finfish aquaculture production (86,015) in 2017 (FAO, 2019). Despite its great popularity as an excellent candidate for profitable aquaculture, however, no studies have yet examined the feasibility and/or safety of replacing FM by insect meal, of any kind, in olive flounder feed. Therefore, this study was designed...
to evaluate the potential impact of replacing FM protein with a locally available and affordable cricket meal on overall performance and health status of juvenile olive flounder.

2 | MATERIALS AND METHODS

2.1 | Diet preparation

The cricket meal (CR) used in this study was kindly supplied by Korean Beneficial Insects Lab. Co. (Gokseong-gun, South Korea). The CR whose composition is given in Table 1 was tested by the Korea Feed Ingredients Association and found to have no detectable levels of heavy metals (arsenic, lead, cadmium, and mercury) and pesticide residues. For this experiment, experimental protocols followed the guidelines approved by the Animal Care and Use Committee of GWNU (GWNU-2019-14). A practical FM-based diet was designated with 55% crude protein and 10% fat, using steam-dried anchovy FM (TASA, Lima, Peru) and sardine FM (Orizon S.A., Santiago, Chile) as the main sources of protein (CR0). Four other experimental diets were formulated to contain 13, 26, 39, and 52% CR, with the CR replacing 20, 40, 60, and 80% of the FM protein (designated as CR20, CR40, CR60, and CR80). Ingredients and nutrient contents of the experimental diets are given in Table 2. In preparing each experimental diet, all the finely ground dry ingredients were thoroughly blended with soybean oil and double-distilled water in a 60-L upright DAEGUNG mixer (NVM-18, Daeyung Bakery Machinery Co. Ltd., Seoul, South Korea). The moist dough was then forced through a meat grinder (SMC-32, SL Co., Incheon, South Korea) fitted with a 3 mm die hole plate. The resulting moist strands were then crushed into desirable particle sizes, collected on aluminum trays, dried at 25°C in a forced air oven (SI-2400, SIN IL Drying Machine Co. Ltd., Daegu, South Korea) 48 hr, and stored at −43°C until used.

2.2 | Experimental fish and feeding conditions

Juvenile olive flounders were purchased from a private hatchery (Gangwon-do, South Korea) and on-grown in a 5,000-L fiberglass tank, connected to a flow-through system, at the Marine Biology Center of the GWNU in ambient ocean water temperature (21.6 ± 0.1°C; Mean ± SE) until the beginning of the experiment. Over the next 6 weeks, fish were acclimated to the experimental facilities and condition, feeding on a commercial feed (Daehan Feed Co. Ltd., Incheon, South Korea; 55% crude protein and 8% lipid). Following the 6-week acclimation period, 300 fish (initial mean body weight, 33.5 ± 0.01 g) were randomly captured and distributed into 15 fiberglass circular tanks of 150 L capacity at the density of 20 fish per tank supplied with filtered seawater at a flow rate of 2.5 L/min to provide one complete turnover of water exchange in each tank every hour. Experimental tanks were also supplied with supplemental aeration to ensure adequate dissolved oxygen levels. Fish were kept at ambient temperature (22.7 ± 1.8°C) and provided with simulated natural photoperiod (14 L:10 D) during the feeding trial. Salinity, dissolved oxygen, and pH levels of the water were monitored every 2 weeks (33.2 ± 0.7 ppt, 7.2 ± 0.5 mg/L, and 7.5 ± 0.4, respectively). Each diet was assigned to three tanks in a completely randomized design. Fish were hand-fed to apparent satiation, twice daily (9:00 a.m. and 17:00 p.m.), for a period of 8 weeks. The uneaten diets were collected, dried, and weighed to determine the amount of feed consumed on a daily basis. Fish were deprived of feed for 16 hr prior to sampling to reduce handling stress.

2.3 | Sample collection

At the end of the 8-week feeding trial, all surviving fish in each tank were counted, individually weighed, and total length measured to the nearest 0.01 g and 0.1 mm for calculation of the survival rate, growth performance, feed
|          | Proximate composition (%DM) | Essential amino acid (mg 100 g\(^{-1}\) DM) | Fatty acid (mg 100 g\(^{-1}\) DM) |
|----------|-----------------------------|---------------------------------------------|----------------------------------|
|          | DM  | CP  | CL  | Ash | Chitin | Arg | His | Ile | Leu | Lys | Met | Phe | Thr | Val | C12:0 | C14:0 | C16:0 | C18:0 | C16:1~7 | C18:1~9 | C18:2~6 | C20:5~3 | C22:5~3 | C22:6~3 |
| CR       | 87.2 | 64.9 | 17.4 | 4.6 | 7.2 | 2,198.3 | 659.4 | 488.0 | 1,852.7 | 1,713.6 | 1,188.5 | 1,032.8 | 1,441.6 | 42.4 | 166.8 | 3,725.4 | 1,115.4 | 411.7 | 4,748.0 | 4,960.9 | 265.9 | ND | ND |
| AFM      | 91.9 | 72.3 | 9.6  | 17.3 | ND | 1,838.6 | 1,047.4 | 476.4 | 1,817.7 | 2,080.7 | 545.7 | 1,214.2 | 1,092.4 | 766.5 | ND | 387.8 | 1,321.4 | 311.2 | 335.8 | 675.7 | 141.1 | 55.6 | 711.4 | 521.5 |
| SFM      | 90.9 | 71.4 | 7.2  | 14.7 | ND | 2,359.7 | 1,123.6 | 613.7 | 2,347.6 | 2,589.8 | 830.9 | 1,564.2 | 1,369.5 | 900.3 | ND | 502.2 | 1,752.0 | 445.5 | 469.3 | 612.4 | 143.1 | 26.9 | 838.8 | 912.3 |

Abbreviations: CL, crude lipid; CP, crude protein; DM, dry matter; ND, not detected.
### TABLE 2  Ingredient and proximate composition of the experimental diets

| Ingredients (% DM) | CR0 | CR20 | CR40 | CR60 | CR80 |
|-------------------|-----|------|------|------|------|
| Anchovy fish meal | 32.5| 26.0 | 19.5 | 13.0 | 6.5  |
| Sardine fish meal | 32.5| 26.0 | 19.5 | 13.0 | 6.5  |
| Cricket meal      | 0.0 | 13.0 | 26.0 | 39.0 | 50.0 |
| Wheat flour       | 14.5| 16.0 | 17.0 | 18.0 | 19.0 |
| Fermented soybean meal | 12.0| 12.0 | 12.0 | 12.0 | 12.0 |
| Soybean oil       | 4.5 | 3.0  | 2.0  | 1.0  | 0.0  |
| Vitamin premixa   | 2.0 | 2.0  | 2.0  | 2.0  | 2.0  |
| Mineral premixb   | 2.0 | 2.0  | 2.0  | 2.0  | 2.0  |

| Proximate composition (% DM) | CR0 | CR20 | CR40 | CR60 | CR80 |
|-----------------------------|-----|------|------|------|------|
| Crude protein               | 56.7| 56.0 | 55.8 | 56.5 | 56.2 |
| Crude lipid                 | 11.5| 11.4 | 10.1 | 10.4 | 10.6 |
| Ash                         | 13.6| 10.8 | 11.4 | 10.6 | 9.4  |
| Gross energy (MJ/kg)        | 20.7| 21.0 | 21.3 | 21.5 | 21.9 |
| Energy/protein (MJ/kg protein) | 36.9| 37.4 | 38.2 | 38.5 | 39.1 |
| Estimated chitin (%)         | 0.0 | 0.9  | 1.9  | 2.8  | 3.7  |

| Essential amino acid (mg 100 g \(^{-1}\) DM) | CR0 | CR20 | CR40 | CR60 | CR80 |
|-----------------------------------------------|-----|------|------|------|------|
| Arg                                          | 2,359.0| 2,324.6| 2,558.7| 2,669.9| 2,761.1 |
| His                                          | 888.9 | 832.8 | 836.0 | 866.8 | 822.0 |
| Ile                                          | 1,484.5| 1,383.7| 1,628.7| 1,622.3| 1,732.1 |
| Leu                                          | 2,681.4| 2,591.5| 2,947.3| 3,041.1| 3,187.4 |
| Lys                                          | 2,551.1| 2,392.1| 2,529.9| 2,496.6| 2,495.9 |
| Met + Cys                                    | 158.7 | 171.6 | 180.7 | 182.6 | 179.7 |
| Phe                                          | 1,634.6| 1,509.4| 1,666.0| 1,680.5| 1,762.8 |
| Thr                                          | 1,475.9| 1,406.1| 1,558.2| 1,557.3| 1,550.0 |
| Val                                          | 1,940.5| 1,908.2| 2,284.2| 2,444.6| 2,566.8 |

| Fatty acid (mg 100 g \(^{-1}\) DM) | CR0 | CR20 | CR40 | CR60 | CR80 |
|-----------------------------------|-----|------|------|------|------|
| C14:0                             | 272.5| 245.0| 208.5| 165.4| 127.2 |
| C16:0                             | 1,346.2| 1,506.6| 1,651.8| 1,773.6| 1,884.3 |
| C18:0                             | 355.4 | 423.6 | 477.7 | 523.7 | 565.8 |
| C16:1n – 7                       | 314.3 | 309.5 | 298.2 | 278.6 | 275.7 |
| C18:1n – 9                       | 1,592.2| 1,754.8| 1,925.7| 2,108.3| 2,273.8 |
| C18:2n – 6                       | 2,154.4| 2,249.3| 2,368.2| 2,394.2| 2,436.1 |
| C18:3n – 3                       | 260.5 | 212.7 | 164.0 | 123.7 | 80.1  |
| C20:5n – 3 (EPA)                 | 617.5 | 517.2 | 397.4 | 262.6 | 129.7 |
| C22:6n – 3 (DHA)                 | 683.1 | 571.8 | 428.9 | 278.8 | 142.5 |

\(^a\)Vitamin premix contained the following amount which were diluted in cellulose (g/kg premix): L-ascorbic acid, 121.2; DL-(tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 18.8; D-biotin, 0.27; folic acid, 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003.

\(^b\)Mineral premix contained the following ingredients (g/kg premix); MgSO\(_4\) \(7\)H\(_2\)O, 80.0; NaH\(_2\)PO\(_4\) \(2\)H\(_2\)O, 370.0; KCl, 130.0; Ferric citrate, 40.0; ZnSO\(_4\) \(7\)H\(_2\)O, 20.0; Ca-lactate, 365.5; CuCl, 0.2; AlCl\(_3\) \(6\)H\(_2\)O, 0.15; KI, 0.15; Na\(_2\)Se\(_2\)O\(_3\), 0.01; MnSO\(_4\) \(2\)H\(_2\)O, 2.0; CoCl\(_2\) \(6\)H\(_2\)O, 1.0.
utilization efficiency, and morphological indices. A random sample of 10 and 3 fish (per tank) were selected at the beginning and the end of the trial, respectively, and kept at −43°C for subsequent proximate composition analyses.

Four randomly selected fish from each tank (12 fish/dietary treatment) were overdosed with 2-phenoxyethanol (200 mg/L), and their blood was drawn from the caudal vein via heparinized syringes. Blood samples were then centrifuged in a high-speed refrigerated microcentrifuge (Micro 17 TR; HanilBioMed Inc., Gwangju, South Korea) at 5,000 g for 15 min to obtain plasma, which was immediately stored at −80°C for subsequent biochemical analyses. Another set of blood samples was collected from the caudal veins of four anesthetized individuals per tank (12 fish/dietary treatment) using nonheparinized syringes to obtain serum for analyzing selected immunological parameters including myeloperoxidase (MPO) and lysozyme activities and total immunoglobulin (TIg) level or monitoring antioxidant enzyme activities such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities.

These fish were gutted (8 fish/tank; 24 fish/dietary treatment) immediately after blood sampling to obtain their visceral and liver weights for calculating the viscerosomatic (VSI) and hepatosomatic (HSI) indices, respectively. Gutted fish were then subjected to individual skin and fillet color assessment and subsequently filleted. Fillet samples were immediately frozen at −80°C for further proximate, amino acid and fatty acid analyses.

2.4 Analytical methods

Chemical composition of the CR, experimental diets, whole-body and fillet samples were analyzed according to the standard methods of the Association of Official Analytical Chemists (AOAC, 2003), which have been described in detail previously (Sankian, Khosravi, Kim, & Lee, 2018). The method described by Marono et al. (2015) was used to estimate chitin content in CR.

Total lipids in CR, diet, and fillet samples were extracted following the Folch, Lees, and Sloane Stanley (1957) method using a mixture of chloroform/methanol (2/1, vol/vol). The resulting extracts of total lipids were then analyzed for fatty acids by preparing fatty acid methyl esters by methanolysis and subjecting to gas chromatography analysis using a SP-2560 capillary column (100 m × 0.25 mm i.d., 0.2 μm film thickness; Supelco, Bellefonte, PA) equipped with a flame-ionization detector following the procedures described by Sankian et al. (2018). Fillet lipid quality was evaluated using different fatty acid ratios and nutritional quality indices such as atherogenic index (AI), thrombogenic index (TI), and hypocholesterolemic/hypercholesterolemic fatty acid ratio (h/H) according to Iaconisi et al. (2017) equations. The AI indicates the potential for stimulating platelet aggregation by considering the relationship between the sum of the main saturated (pro-atherogenic) and unsaturated (anti-atherogenic) fatty acids, thereby, the potential for preventing coronary disease. The TI relates the prothrombogenetic (saturated) and the antithrombogenetic (monounsaturated and polyunsaturated) fatty acids to indicate the potential for preventing thrombus in the vessels (Ulbricht & Southgate, 1991).

The CR, diet, and fillet samples were analyzed for essential amino acid composition, using an automatic amino acid analyzer after properly controlled acid hydrolysis with 6 N HCL reflux for 23 hr at 110°C (Hitachi, Tokyo, Japan).

An automated blood analyzer (DRI-CHEM NX500i, FUJIFILM Corporation Tokyo, Japan) was used to determine the total protein (TP), triglyceride (TG), total cholesterol (TCHO), high-density lipoprotein cholesterol (HDLc), glucose (GLU), aspartate aminotransferase (AST), alanine aminotransferase (ALT), Albumin (ALB), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and Total bilirubin (TBIL) concentrations in plasma samples using FUJIFILM kits (Tokyo, Japan).

Color assessments were performed by a Chroma Meter (CR400, Minolta Co. Ltd., Osaka, Japan) using the SpectraMagic NX software. Colors are presented as CIELab coordinates with L’, a’, and b’ representing the color lightness (from 0 = black to 100 = white), redness (from +60 = red to −60 = green), and yellowness (from +60 = yellow to −60 = blue), respectively (CIE, 1976). The hue angle and Chroma were then computed to indicate the visual sensation and the saturation or purity of the color.
The polyethylene glycol precipitation test was used to determine the TIg content of the serum samples as described by Anderson and Siwicki (1995). Enzymatic lysozyme activity was measured by a turbidimetric assay relying on the bactericidal lytic activity of serum lysozyme against Micrococcus lysodeikticus (Sigma, St. Louis, MO) as a substrate (Hultmark, Steiner, Rasmuson, & Boman, 1980). One lysozyme activity unit was defined as the loss of 0.001 absorbance units per minute at 450 nm. MPO activity was assayed according to Quade and Roth (1997) method as previously described in detail (Khosravi et al., 2015). A sigma assay kit (Sigma 19,160, St. Louis, MO) was used to estimate the superoxide anion radical scavenging activity of SOD based on the inhibition rate of WST-1 (2-(4-lodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) reduction by the superoxide anion generated from the xanthine oxidase reaction. One unit of SOD activity was defined as the amount of enzyme required to inhibit the rate of WST-1 reduction by 50%. GPx activity was measured by the glutathione reductase coupled oxidation of NADPH to NADP$^{+}$ using cumene hydroperoxide as substrate, following a BioVision assay kit (Biovision Inc., Milpitas, CA) protocol. One unit of GPx activity was defined as the amount of enzyme that caused the oxidation of 1.0 $\mu$mol of NADPH to NADP$^{+}$ per minute.

2.5 Digestibility test

Apparent digestibility coefficient (ADC) of the experimental diets was estimated in vivo using new sets of olive flounders, with mean body weight of 40 g, housed in a fecal collection system designed according to Cho, Slinger, and Bayley (1982). Duplicate groups of 60 fish were fed one of the experimental diets containing 1% Chromic oxide (Cr$_2$O$_3$, Sigma) as an indigestible marker. Digestibility test was run in three periods of 10 days as previously described in detail (Khosravi et al., 2015). All feces samples of each tank were pooled in each period and frozen at $-80^\circ$C until analyzed for chromic oxide (AOAC, 2003) and proximate composition (Divakaran, Obaldo, & Forster, 2002). The apparent nutrient digestibility coefficient of the five experimental diets was calculated using the standard formula given below.

2.6 Formulae, calculations, and statistical analysis

Weight gain (WG, %) = (final body weight - initial body weight)/initial body weight) $\times$ 100.

Specific growth rate (SGR, %/day) = [(ln final body weight - ln initial body weight)/days of experiment] $\times$ 100.

Daily feed intake (DFI, %) = (feed intake $\times$ 100)/[(initial body weight + final body weight + dead fish weight) $\times$ days/2].

Feed efficiency (FE, %) = (wet weight gain/feed intake) $\times$ 100.

Protein efficiency ratio (PER) = wet weight gain/total protein given.

Feed conversion ratio (FCR) = feed intake/wet weight gain.

Protein or Lipid retention (PR or LR, %) = [(final body weight x final carcass protein or lipid proportion) - (initial body weight x initial carcass protein or lipid proportion)/protein or lipid intake] $\times$ 100.

Survival (%) = (final number of fish/initial number of fish) $\times$ 100.

Condition Factor (CF, %) = (wet weight of fish/[length of fish]$^3$) $\times$ 100.

Hepatosomatic index (HSI, %) = (wet weight of liver/wet weight of fish) $\times$ 100.

Viscerasomatic index (VSI, %) = (wet weight of viscera/wet weight of fish) $\times$ 100.

Apparent nutrient digestibility coefficient (%) = 1 - [(Cr$_2$O$_3$ in diet/Cr$_2$O$_3$ in feces)/(nutrient content of feces/nutrient content of diet)].

Atherogenic index (AI) = (C12:0 + [4 x C14:0] + C16:0)/(\Sigma n3 PUFA + \Sigma n6 PUFA + SUFA).

Thrombogenic index (TI) = (C14:0 + C16:0 + C18:0)/(0.5 x \Sigma n6 MUFA) + (0.5 x \Sigma n3 PUFA) + (3 x \Sigma n3 PUFA) + \Sigma n3 PUFA/\Sigma n6 PUFA).

Hypocholesterolemic/hypercholesterolemic fatty acid ratio (h/H) = (C18:1n9 + C18:2n6 + C20:4n6 + C18:3n3 + C20:5n3 + C22:5n3 + C22:6n3)/(C14:0 + C16:0).
Hue = \text{arctan} \left( \frac{b}{a} \right).
Chroma = \left( a^2 + b^2 \right)^{1/2}.

One-way analysis of variance (ANOVA), using SPSS program version 23 (SPSS Inc., Chicago, IL), followed by Tukey’s multiple range test was performed to determine if the observed responses were significantly \((p < .05)\) affected as FM was replaced by CR. In addition, orthogonal polynomial contrasts were used to further determine linear and quadratic effects of dietary treatments. Data were tested for normal distribution (Shapiro–Wilks’s test) and homogeneity of variance (Levene’s test). Data are presented as mean ± standard error (SE) of the triplicate groups.

3 | RESULTS

3.1 | Growth performance and biometry

Fish growth performance in terms of weight gain (WG) and specific growth rate (SGR) showed linear and quadratic responses to increasing CR inclusion (Table 3; \(p < .05\)). Substitution of FM at levels greater than 20% (CR40, CR60, and CR80) resulted in a linear decrease in growth rates. Fish that were offered a diet with the lowest CR level (CR20) grew significantly better than those fed with the CR80 diet. Linear and quadratic responses were also detected in the efficiency of feed utilization, as depicted by protein efficiency ratio (PER) and feed conversion ratio (FCR), from incremental substitution of CR for FM \((p < .05)\). The efficiency of feed utilization tended to improve with increasing percentage of dietary FM replacement up to 40%, but gradually decreased in higher replacement levels.

Protein retention (PR) followed a similar quadratic trend \((p < .05)\). Significant linear and quadratic trends were found between elevated dietary inclusion levels of CR and lipid retention (LR) \((p < .05)\), where the LR values generally tended to decline with increasing dietary CR inclusion, and fish fed with the CR80 diet had the lowest value. Dietary treatment had no remarkable effect on fish survival rate \((p > .05)\).

Regression analyses on biometric parameters revealed a significant quadratic tendency for condition factor (CF) \((p < .05)\), while no linear or quadratic trends were found for hepatosomatic index (HIS) \((p > .05)\). There were significant linear and quadratic trends for viscerosomatic index (VSI) to be generally higher in fish fed diets with reduced FM \((p < .05)\).

3.2 | Diet apparent digestibility

Significant linear and quadratic trends were noted for apparent digestibility coefficient (ADC) of dry matter, protein, and lipid as FM was replaced by CR (Table 3; \(p < .05\)). ADC of dry matter and protein enhanced with replacing 20% of the dietary FM and then tended to decline as the substitution of FM with CR further enhanced to 80%. Fish that were fed the diets in which more than 20% of FM protein replaced by CR had significantly lower dry matter digestibility compared to those fed with the CR20 diet. Significantly lower protein digestibility was recorded in CR40, CR60, and CR80 groups than CR0 and CR20 groups. ADC of lipid tended to decrease with increasing levels of CR in the diet, and notably lower values were observed in fish fed with CR60 and CR80 diets than those fed with other experimental diets (Table 3; \(p < .05\)).

3.3 | Plasma biochemistry

Plasma total protein content showed a significant negative linear response to increasing levels of dietary CR (Table 4; \(p < .05\)). There were significant linear and quadratic responses to dietary inclusion of CR in both total
cholesterol (TCHO) and high-density lipoprotein cholesterol (HDL-c) contents ($p < .05$). Plasma TCHO level generally showed decreasing trend with increasing inclusion levels of CR in the diet, and fish that were fed the diet containing the highest level of CR (CR80) had the lowest values compared to those fed with CR0 diet. Fish that were fed with CR80 diet also had significantly lower plasma HDL-c level compared to those fed with other experimental diets. However, neither a remarkable difference nor any obvious trend was noted in other analyzed biochemical parameters among dietary treatments ($p > .05$).

### 3.4 Whole-body and fillet proximate composition

With the exception of lipid content, which showed decreasing trends as the dietary CR level increased (Table 5; $p < .05$), no other changes were evident in the whole body and fillet proximate composition ($p > .05$). Fish that were fed the CR80 diet showed significantly lower whole body lipid content compared to those fed with CR0 and CR20 diets. Significantly lower lipid levels were found in the fillet of fish that were fed with CR-containing diets than those fed with CR0 diet.

### 3.5 Fillet essential amino acid and fatty acid compositions

Fillet essential amino acid composition did not alter when dietary CR replaced FM (Table 6; $p > .05$). Nevertheless, fillet fatty acid composition was notably changed by increasing CR in the experimental diets. C16:0 (palmitic acid), C18:1n-9 (oleic acid; OA), C18:2n-6 (linoleic acid; LA), and C22:6n-3 (docosahexaenoic acid; DHA) were the most abundant fatty acids found in juvenile flounder fillets. Fillet OA and monounsaturated fatty acid (MUFA) contents showed significant positive linear response to increasing levels of dietary CR ($p < .05$). Significant linear and quadratic trends were found between elevated dietary inclusion levels of CR and fillet LA and n-6 polyunsaturated fatty acids (PUFA) content, where the values generally tended to increase with increasing dietary CR inclusion ($p < .05$). As a result, dietary-induced alteration in the fillet fatty acid composition markedly affected nutritional quality of its lipid fraction. There were significant linear and quadratic trends toward decreased $\Sigma n3/\Sigma n6$ ratio in CR-fed fish and notably lower values were recorded in CR60 and CR80 groups than CR20 and CR0 groups ($p < .05$). Dietary treatment had significant linear and quadratic effects on both atherogenic (AI) and thrombogenic (TI) indices. The AI generally showed decreasing trend with increasing inclusion levels of CR in the diet while TI tended to increase as FM was replaced by CR ($p < .05$). A significant positive linear trend was observed for the fillet hypocholesterolemic/hypercholesterolemic fatty acid ratio as the level of dietary CR increased ($p < .05$).

### 3.6 Color quality of skin and fillet

Replacement of up to 80% of FM protein with CR caused no remarkable change in fish skin and fillet color parameters measured in the present study (Table 7; $p > .05$).

### 3.7 Serum immune parameters and antioxidant enzyme activities

Dietary CR level had no significant effect on serum total immunoglobulin level as well as myeloperoxidase and lysozyme activities (Table 8; $p > .05$). Although no notable trend was evident, significantly higher SOD activity was recorded in groups fed with CR60 diet than in those fed the other experimental diets. There was a significant linear
| Experimental diets | CR0 | CR20 | CR40 | CR60 | CR80 |
|--------------------|-----|------|------|------|------|
| **p Value**        |     |      |      |      |      |
| $L^1$ trend (Adj. $R^2$) | .027 (.271) | .017 (.318) | .193 (.059) | .032 (.254) | <.001 (.727) |
| $Q^2$ trend (Adj. $R^2$) | .005 (.510) | .006 (.503) | .391 (.002) | .025 (.370) | <.001 (.904) |

**Growth and feed utilization parameters**

- **WG**: Weight gain (%)
  - CR0: 361.3 ± 30.1<sup>ab</sup>
  - CR20: 386.0 ± 6.1<sup>a</sup>
  - CR40: 358.2 ± 10.4<sup>ab</sup>
  - CR60: 354.0 ± 7.2<sup>ab</sup>
  - CR80: 315.8 ± 9.0<sup>b</sup>
  - $p$ value: .027 (.271)

- **SGR**: Specific growth rate (%/day)
  - CR0: 2.72 ± 0.12<sup>ab</sup>
  - CR20: 2.82 ± 0.02<sup>a</sup>
  - CR40: 2.72 ± 0.04<sup>ab</sup>
  - CR60: 2.70 ± 0.03<sup>ab</sup>
  - CR80: 2.52 ± 0.04<sup>b</sup>
  - $p$ value: .017 (.318)

- **DFI**: Daily feed intake (%)
  - CR0: 1.95 ± 0.06
  - CR20: 2.01 ± 0.04
  - CR40: 2.03 ± 0.01
  - CR60: 1.88 ± 0.02
  - CR80: 1.83 ± 0.02
  - $p$ value: .193 (.059)

- **PER**: Protein efficiency ratio
  - CR0: 0.91 ± 0.03
  - CR20: 0.89 ± 0.02
  - CR40: 0.88 ± 0.01
  - CR60: 0.94 ± 0.01
  - CR80: 0.97 ± 0.01
  - $p$ value: .034 (.247)

- **FCR**: Feed conversion ratio
  - CR0: 1.95 ± 0.06
  - CR20: 2.01 ± 0.04
  - CR40: 2.03 ± 0.01
  - CR60: 1.88 ± 0.02
  - CR80: 1.83 ± 0.02
  - $p$ value: .032 (.254)

- **PR**: Protein or lipid retention (%)
  - CR0: 35.2 ± 1.0
  - CR20: 36.3 ± 0.7
  - CR40: 36.7 ± 0.2
  - CR60: 35.2 ± 0.4
  - CR80: 34.3 ± 0.3
  - $p$ value: .216 (.047)

- **LR**: Lipid retention (%)
  - CR0: 43.5 ± 0.9<sup>a</sup>
  - CR20: 41.5 ± 0.7<sup>a</sup>
  - CR40: 42.4 ± 0.4<sup>a</sup>
  - CR60: 38.5 ± 0.3<sup>b</sup>
  - CR80: 30.8 ± 0.3<sup>c</sup>
  - $p$ value: <.001 (.727)

- **Survival**: Survival (%)
  - CR0: 98.3 ± 1.7
  - CR20: 100.0 ± 0.0
  - CR40: 100.0 ± 0.0
  - CR60: 98.3 ± 1.7
  - CR80: 98.3 ± 1.7
  - $p$ value: .676 (.062)

**Morphological parameters (%)**

- **CF**: Condition Factor
  - CR0: 0.96 ± 0.02
  - CR20: 0.99 ± 0.01
  - CR40: 0.97 ± 0.02
  - CR60: 0.97 ± 0.02
  - CR80: 0.94 ± 0.01
  - $p$ value: .094 (.139)

- **HSI**: Hepatosomatic index
  - CR0: 1.97 ± 0.07
  - CR20: 1.99 ± 0.08
  - CR40: 1.88 ± 0.09
  - CR60: 1.82 ± 0.09
  - CR80: 1.83 ± 0.16
  - $p$ value: .496 (.038)

- **VSI**: Viscerasomatic index
  - CR0: 5.39 ± 0.09
  - CR20: 5.64 ± 0.11
  - CR40: 5.60 ± 0.14
  - CR60: 5.69 ± 0.13
  - CR80: 5.89 ± 0.41
  - $p$ value: .014 (.338)

**Apparent nutrient digestibility coefficient (%)**

- **Dry matter**
  - CR0: 71.1 ± 2.2<sup>ab</sup>
  - CR20: 74.5 ± 0.3<sup>a</sup>
  - CR40: 67.2 ± 0.6<sup>b</sup>
  - CR60: 67.0 ± 1.2<sup>b</sup>
  - CR80: 66.1 ± 1.5<sup>b</sup>
  - $p$ value: .002 (.502)

- **Protein**
  - CR0: 90.2 ± 0.9<sup>ab</sup>
  - CR20: 93.8 ± 1.3<sup>a</sup>
  - CR40: 87.9 ± 1.1<sup>c</sup>
  - CR60: 86.9 ± 0.4<sup>c</sup>
  - CR80: 86.4 ± 0.7<sup>c</sup>
  - $p$ value: .002 (.486)

- **Lipid**
  - CR0: 92.3 ± 1.6<sup>a</sup>
  - CR20: 88.8 ± 1.1<sup>b</sup>
  - CR40: 87.0 ± 1.6<sup>b</sup>
  - CR60: 83.5 ± 0.7<sup>c</sup>
  - CR80: 81.9 ± 0.2<sup>c</sup>
  - $p$ value: <.001 (.858)

**Note:** Values are mean of triplicate groups and presented as mean ± SE. Different superscripts in the same row indicate significant difference between means by ANOVA at $p < .05$. Orthogonal contrasts: $^1$L: linear; $^2$Adj. $R^2$: adjusted $R$ square; $^3$Q: quadratic; $^4$Weight gain (%); $^5$Specific growth rate (%/day); $^6$Daily feed intake (%); $^7$Protein efficiency ratio; $^8$Feed conversion ratio; $^9$Protein or lipid retention (%); $^{10}$Condition Factor; $^{11}$Hepatosomatic index; $^{12}$Viscerasomatic index.
### TABLE 4  Plasma biochemical parameters of olive flounder fed the five experimental diets for 8 weeks

| Experimental diets | CR0  | CR20 | CR40  | CR60  | CR80  | p Value | L1 trend (Adj. R²)² | Q2 trend (Adj. R²)² |
|--------------------|------|------|-------|-------|-------|---------|---------------------|---------------------|
| TP⁴                | 3.67 ± 0.35 | 3.77 ± 0.11 | 3.39 ± 0.17 | 3.36 ± 0.16 | 3.08 ± 0.06 | .017 (.317) | .055 (.281) |
| TG⁵                | 202.1 ± 19.3 | 231.8 ± 37.7 | 220.8 ± 12.7 | 225.7 ± 12.0 | 226.2 ± 4.4 | .492 (-.037) | .672 (-.092) |
| TCHO⁶              | 179.2 ± 32.8⁹ | 173.7 ± 8.2⁹ | 152.3 ± 18.1⁹ | 124.6 ± 6.5⁹ | 96.1 ± 4.0⁹ | .001 (555) | .003 (546) |
| HDL-c⁷             | 105.8 ± 2.7⁷ | 109.6 ± 0.4⁷ | 106.0 ± 4.0⁷ | 107.4 ± 1.4⁷ | 92.8 ± 1.1⁷ | .022 (291) | .002 (598) |
| GLU⁸               | 31.6 ± 11.1 | 17.9 ± 2.0 | 214 ± 1.6 | 21.3 ± 1.9 | 18.3 ± 2.3 | .195 (058) | .289 (051) |
| AST⁹               | 17.4 ± 2.1 | 16.2 ± 2.7 | 16.6 ± 1.2 | 15.1 ± 0.4 | 16.2 ± 0.4 | .442 (-.027) | .674 (-.092) |
| ALT¹⁰              | 8.33 ± 2.34 | 9.33 ± 2.03 | 7.22 ± 0.59 | 6.98 ± 0.38 | 6.84 ± 0.24 | .228 (.041) | .498 (.039) |
| ALB¹¹              | 0.70 ± 0.12 | 0.68 ± 0.04 | 0.64 ± 0.04 | 0.62 ± 0.05 | 0.56 ± 0.03 | .081 (156) | .219 (094) |
| LDH¹²              | 31.9 ± 3.6 | 30.2 ± 15.8 | 34.0 ± 8.0 | 34.4 ± 0.9 | 34.7 ± 9.4 | .705 (-.065) | .933 (-.153) |
| ALP¹³              | 138.8 ± 3.9 | 175.8 ± 23.5 | 138.9 ± 21.1 | 157.8 ± 13.9 | 135.7 ± 11.5 | .747 (-.068) | .654 (-.087) |
| TBIL¹⁴             | 0.24 ± 0.02 | 0.22 ± 0.09 | 0.28 ± 0.06 | 0.21 ± 0.03 | 0.22 ± 0.03 | .672 (-.062) | .862 (-.138) |

Note: Values are mean of triplicate groups and presented as mean ± SE. Different superscripts in the same row indicate significant difference between means by ANOVA at p < .05. Orthogonal contrasts: ¹L: linear; ²Adj. R²: adjusted R square; ³Q: quadratic; ⁴Total protein (g/dl); ⁵Triglyceride (mg/dl); ⁶Total cholesterol (mg/dl); ⁷High-density lipoprotein cholesterol (mg/dl); ⁸Glucose (mg/dl); ⁹Aspartate aminotransferase activity (U/L); ¹⁰Alanine aminotransferase activity (U/L); ¹¹Albumin (g/dl); ¹²Lactate dehydrogenase (U/L); ¹³Alkaline phosphatase (U/L); ¹⁴Total bilirubin (mg/dl).
| Experimental diets | CR0   | CR20  | CR40  | CR60  | CR80  | p Value          | $L^1$ trend (Adj. $R^2$) | $Q^2$ trend (Adj. $R^2$) |
|--------------------|-------|-------|-------|-------|-------|------------------|--------------------------|--------------------------|
| Whole body         |       |       |       |       |       |                  |                          |                          |
| Moisture           | 75.0 ± 0.2 | 74.4 ± 0.2 | 74.3 ± 1.0 | 74.5 ± 1.6 | 75.1 ± 0.3 | .876 (.075) | .640 (.083) |
| Crude protein      | 17.3 ± 0.4 | 17.2 ± 0.3 | 17.3 ± 0.6 | 17.8 ± 0.4 | 17.7 ± 0.4 | .235 (.038) | .467 (.027) |
| Crude lipid        | 3.84 ± 0.52 | 3.74 ± 0.17 | 3.61 ± 0.41 | 3.01 ± 0.50 | 2.71 ± 0.43 | <.001 (.636) | .001 (.637) |
| Ash                | 3.60 ± 0.33 | 3.91 ± 0.16 | 3.73 ± 0.37 | 3.70 ± 0.58 | 3.18 ± 0.22 | .348 (.004) | .332 (.029) |
| Fillet             |       |       |       |       |       |                  |                          |                          |
| Moisture           | 76.3 ± 0.2 | 76.5 ± 0.7 | 76.2 ± 0.2 | 77.0 ± 0.4 | 75.0 ± 1.8 | .410 (.020) | .419 (.009) |
| Crude protein      | 21.8 ± 0.5 | 22.7 ± 0.5 | 22.7 ± 0.8 | 22.0 ± 1.7 | 21.4 ± 0.4 | .605 (.054) | .459 (.025) |
| Crude lipid        | 1.05 ± 0.09 | 0.70 ± 0.01 | 0.63 ± 0.07 | 0.52 ± 0.03 | 0.34 ± 0.04 | <.001 (.826) | <.001 (.836) |
| Ash                | 1.72 ± 0.14 | 1.69 ± 0.07 | 1.65 ± 0.08 | 1.71 ± 0.11 | 1.66 ± 0.13 | .765 (.069) | .979 (.157) |

Note: Values are mean of triplicate groups and presented as mean ± SE. Different superscripts in the same row indicate significant difference between means by ANOVA at $p < .05$. Orthogonal contrasts: $^1$L: linear; $^2$Adj. $R^2$: adjusted R square; $^3$Q: quadratic.
| Essential amino acid (mg 100 g⁻¹ DM) | CR0      | CR20     | CR40     | CR60     | CR80     |
|-------------------------------------|----------|----------|----------|----------|----------|
| Arg                                | 1,893.8 ± 1,171.5 | 3,143.2 ± 872.2 | 2,254.5 ± 1,811.5 | 3,162.7 ± 214.3 | 3,234.8 ± 160.4 |
| His                                | 561.5 ± 362.4  | 924.1 ± 263.0  | 629.1 ± 505.9  | 908.4 ± 57.0   | 923.3 ± 52.4   |
| Ile                                | 1,155.8 ± 705.2 | 1,876.0 ± 522.9 | 1,287.0 ± 1,009.1 | 1,870.1 ± 142.1 | 1,911.5 ± 55.8 |
| Leu                                | 2,161.7 ± 1,317.7 | 3,531.3 ± 982.4 | 2,401.5 ± 1,892.2 | 3,530.8 ± 278.4 | 3,563.8 ± 61.6 |
| Lys                                | 2,634.7 ± 1,606.1 | 4,318.2 ± 1,220.7 | 2,898.8 ± 2,292.9 | 4,290.8 ± 345.5 | 4,311.4 ± 58.6 |
| Met + Cys                           | 1,034.5 ± 608.8 | 1,782.1 ± 443.5 | 1,140.9 ± 840.2  | 1,706.0 ± 62.8  | 1,749.0 ± 24.7 |
| Phe                                | 1,148.2 ± 699.0 | 1,885.6 ± 540.0 | 1,282.2 ± 1,002.7 | 1,877.1 ± 162.6 | 1,906.1 ± 32.9 |
| Thr                                | 1,206.7 ± 762.6 | 2,005.9 ± 538.9 | 1,380.4 ± 1,090.4 | 1,988.8 ± 140.7 | 2,007.1 ± 70.0 |
| Val                                | 1,399.4 ± 861.0 | 2,304.6 ± 622.5 | 1,645.8 ± 1,308.8 | 2,267.5 ± 158.3 | 2,315.3 ± 90.7 |

| Fatty acids (mg 100 g⁻¹ DM) | CR0      | CR20     | CR40     | CR60     | CR80     |
|-----------------------------|----------|----------|----------|----------|----------|
| C14:0                       | 52.7 ± 9.2  | 35.3 ± 9.7  | 44.1 ± 8.2  | 53.6 ± 9.5  | 43.8 ± 7.0  |
| C16:0                       | 416.4 ± 56.6 | 325.3 ± 99.7 | 476.4 ± 43.1 | 534.6 ± 80.9 | 532.0 ± 80.7 |
| C18:0                       | 151.4 ± 13.9 | 129.1 ± 38.4 | 167.1 ± 11.4 | 177.5 ± 19.5 | 184.8 ± 17.8 |
| ΣSFA4                       | 697.7 ± 82.3 | 555.8 ± 166.2 | 765.2 ± 68.4 | 849.9 ± 121.4 | 859.6 ± 119.4 |
| C16:1n − 7                  | 70.0 ± 13.8  | 47.4 ± 15.7  | 66.8 ± 11.8  | 88.5 ± 14.2  | 80.5 ± 17.9  |
| C18:1n − 9                  | 361.9 ± 62.8 | 283.5 ± 83.4 | 440.9 ± 57.8 | 588.1 ± 118.7 | 605.5 ± 141.8 |
| ΣMUFA5                      | 481.1 ± 86.8 | 371.2 ± 113.5 | 562.5 ± 73.7 | 735.8 ± 135.8 | 750.4 ± 170.6 |
| C18:2n − 6                  | 461.1 ± 79.1 | 374.2 ± 116.5 | 610.3 ± 80.3 | 770.2 ± 144.2 | 854.8 ± 149.6 |
| Σn6 PUFA6                   | 503.9 ± 88.7 | 408.4 ± 156.7 | 659.9 ± 87.1 | 834.4 ± 160.1 | 923.8 ± 160.4 |
| C18:3n − 3                  | 42.2 ± 8.8   | 23.8 ± 7.0   | 30.2 ± 5.9   | 32.3 ± 5.5   | 24.0 ± 4.1   |
| C20:5n − 3                  | 151.7 ± 21.1 | 115.6 ± 35.0 | 146.8 ± 15.0 | 130.4 ± 14.4 | 97.5 ± 8.9   |
| C22:6n − 3                  | 459.1 ± 58.3 | 373.4 ± 117.1 | 451.6 ± 28.9 | 391.0 ± 53.4 | 356.3 ± 19.5 |
| Σn3 PUFA7                   | 657.3 ± 86.8 | 516.7 ± 161.1 | 632.2 ± 47.7 | 556.9 ± 68.1 | 481.6 ± 32.8 |

(Continues)
| Experimental diets | CR0  | CR20 | CR40 | CR60 | CR80 | p Value |
|--------------------|------|------|------|------|------|---------|
|                    |      |      |      |      |      | L trend (Adj. $R^2$) | Q trend (Adj. $R^2$) |
| Σn3/Σn6            | 1.34 ± 0.13<sup>a</sup> | 1.25 ± 0.09<sup>a</sup> | 0.97 ± 0.05<sup>ab</sup> | 0.71 ± 0.15<sup>b</sup> | 0.55 ± 0.10<sup>b</sup> | <.001 (.753) | <.001 (.735) |
| ΣPUFA/ΣSFA         | 1.65 ± 0.07 | 1.64 ± 0.04 | 1.68 ± 0.03 | 1.64 ± 0.01 | 1.65 ± 0.05 | .898 (−.076) | .929 (−.152) |
| AI<sup>8</sup>     | 0.39 ± 0.01<sup>a</sup> | 0.38 ± 0.01<sup>ab</sup> | 0.36 ± 0.01<sup>bc</sup> | 0.35 ± 0.01<sup>bc</sup> | 0.33 ± 0.01<sup>c</sup> | <.001 (.732) | <.001 (.709) |
| TI<sup>9</sup>     | 0.25 ± 0.01<sup>b</sup> | 0.25 ± 0.01<sup>b</sup> | 0.27 ± 0.01<sup>ab</sup> | 0.31 ± 0.02<sup>ab</sup> | 0.33 ± 0.02<sup>a</sup> | <.001 (.654) | .001 (.659) |
| h/H<sup>10</sup>   | 3.14 ± 0.09 | 3.25 ± 0.03 | 3.22 ± 0.04 | 3.25 ± 0.05 | 3.37 ± 0.06 | .017 (.319) | .061 (.269) |

Note: Values are mean of triplicate groups and presented as mean ± SE. Different superscripts in the same row indicate significant difference between means by ANOVA at $p < .05$. Orthogonal contrasts: <sup>1</sup>L: linear; <sup>2</sup>Adj. $R^2$: adjusted $R$ square; <sup>3</sup>Q: quadratic; <sup>4</sup>Saturated fatty acids; <sup>5</sup>Monounsaturated fatty acids; <sup>6</sup>6–polyunsaturated fatty acids; <sup>7</sup>3–polyunsaturated fatty acids; The fatty acids C12:0, C13:0, C14:1n−5, C15:0, C16:1n−9, C17:0, C17:1, C18:1n−7, C18:3n−6, C20:0, C20:1n−9, C20:2n−6, C20:3n−6, C20:3n−3, C20:4n−6, C22:0, C22:1n−9, C22:2n−6, C22:5n−3, C24:0, in percentage ≤1%, were also detected and used to calculate the fatty acid groups; <sup>8</sup>Atherogenic index = [C12:0 + (4 × C14:0) + C16:0]/(Σn3 PUFA + Σn6 PUFA + ΣMUFA); <sup>9</sup>Thrombogenic index = [(C14:0 + C16:0 + C18:0)/(0.5 × ΣMUFA) + (0.5 × Σn6 PUFA) + (3 × Σn3 PUFA)]/(Σn3 PUFA/Σn6 PUFA); <sup>10</sup>Hypocholesterolemic/hypercholesterolemic fatty acid ratio = (C18:1n9 + C18:2n6 + C20:4n6 + C18:3n3 + C20:5n3 + C22:5n3 + C22:6n3)/(C14:0 + C16:0).
### TABLE 7  Skin and fillet color parameters of olive flounder fed the five experimental diets for 8 weeks

| Experimental diets | CR0  | CR20 | CR40  | CR60  | CR80  | p Value | L¹ trend (Adj. $R^2$)² | Q² trend (Adj. $R^2$)² |
|--------------------|------|------|-------|-------|-------|---------|------------------|------------------|
| **Skin dorsal region** |      |      |       |       |       |         |                  |                  |
| $L'$               | 30.1 ± 0.9 | 28.4 ± 0.7 | 28.1 ± 1.0 | 29.7 ± 0.5 | 30.0 ± 0.1 | .512 (.040) | .359 (.027) |
| $a'$               | −2.30 ± 0.19 | −1.68 ± 0.17 | −1.70 ± 0.28 | −2.27 ± 0.12 | −2.29 ± 0.13 | .473 (.033) | .077 (240) |
| $b'$               | 2.60 ± 0.29  | 2.54 ± 0.15  | 2.57 ± 0.10  | 2.75 ± 0.04  | 2.86 ± 0.12  | .141 (.094) | .244 (078) |
| Chroma$^4$         | 3.47 ± 0.34 | 3.06 ± 0.11 | 3.09 ± 0.24 | 3.57 ± 0.10 | 3.67 ± 0.10 | .225 (.043) | .094 (213) |
| Hue$^5$            | 131.7 ± 0.8 | 123.6 ± 3.8 | 123.5 ± 3.2 | 129.5 ± 1.2 | 128.7 ± 2.2 | .997 (.077) | .139 (160) |
| **Fillet epaxial region** |      |      |       |       |       |         |                  |                  |
| $L'$               | 44.7 ± 0.3  | 45.7 ± 0.3  | 44.2 ± 0.3  | 44.4 ± 0.2  | 43.5 ± 0.4  | .348 (.021) | .409 (017) |
| $a'$               | −1.30 ± 0.16 | −1.18 ± 0.09 | −1.29 ± 0.03 | −1.14 ± 0.05 | −1.10 ± 0.08 | .136 (.098) | .334 (028) |
| $b'$               | −3.44 ± 0.68 | −3.75 ± 0.43 | −3.28 ± 0.16 | −3.73 ± 0.31 | −3.20 ± 0.27 | .686 (.063) | .814 (0127) |
| Chroma             | 3.72 ± 0.58 | 3.95 ± 0.38 | 3.52 ± 0.14 | 3.90 ± 0.30 | 3.39 ± 0.27 | .522 (.042) | .721 (.105) |
| Hue                | 247.4 ± 6.1 | 251.9 ± 3.3 | 248.5 ± 1.4 | 252.8 ± 1.2 | 250.9 ± 1.1 | .425 (.023) | .671 (.092) |

Note: Values are mean of triplicate groups and presented as mean ± SE. Orthogonal contrasts: $^1$L: linear; $^2$Adj. $R^2$: adjusted $R$ square; $^3$Q: quadratic; $^4$Chroma = $(a'^2 + b'^2)^{1/2}$; $^5$Hue = arctan ($b'/a'$).
TABLE 8  Selected immune parameters and antioxidant enzyme activities of olive flounder fed the five experimental diets for 8 weeks

| Experimental diets | MPO⁴ | Lysozyme⁵ | Tlg⁶ | SOD⁷ | GPx⁸ |
|--------------------|------|-----------|------|------|------|
| CR0 | 0.40 ± 0.07 | 484.9 ± 99.5 | 13.6 ± 1.9 | 61.6 ± 5.9b | 147.4 ± 15.3b |
| CR20 | 0.80 ± 0.31 | 528.1 ± 29.7 | 11.8 ± 14 | 63.9 ± 2.7b | 176.2 ± 30.2ab |
| CR40 | 0.62 ± 0.20 | 581.9 ± 56.2 | 12.8 ± 1.5 | 65.7 ± 2.9b | 201.3 ± 45.7ab |
| CR60 | 0.59 ± 0.14 | 661.9 ± 21.5 | 15.9 ± 1.4 | 85.8 ± 4.7a | 257.4 ± 31.0a |
| CR80 | 1.00 ± 0.34 | 549.6 ± 19.2 | 14.5 ± 1.9 | 62.1 ± 2.5b | 229.3 ± 20.4ab |

*p Value*  
$L^1$ trend (Adj. $R^2$) | .158 (.081) | .188 (.062)  
$Q^2$ trend (Adj. $R^2$) | .151 (.148) | .427 (.012)  

Note: Values are mean of triplicate groups and presented as mean ± SE. Different superscripts in the same row indicate significant difference between means by ANOVA at $p < .05$. Orthogonal contrasts: $^1$L: linear; $^2$Adj. $R^2$: adjusted $R$ square; $^3$Q: quadratic; $^4$Myeloperoxidase; $^5$Lysozyme (U/ml); $^6$Total immunoglobulin (mg/ml); $^7$Superoxide dismutase (% inhibition); $^8$Glutathione peroxidase (mU/ml).
trend for serum GPx activity as the level of dietary CR increased \((p < .05)\), exhibiting significantly higher values in CR60 group compared to the CR0 group.

4 | DISCUSSION

To the best of our knowledge, this study represents one of the very first attempts to assess the feasibility of using CR, or insect meal in general, to partially replace FM in diet formulations for olive flounder, *Paralichthys olivaceus* juveniles. Although growth performance showed a significant negative correlation with substitution of FM protein at levels greater than 20%, flounders still showed comparable performance with those of the control group even at 80% substitution. Replacing dietary FM protein at an appropriate rate appears to be effective in promoting growth performance and feed efficiency, as has been indicated in other fish species fed diets supplemented with insect meals (Iaconisi et al., 2017; Ng, Liew, Ang, & Wong, 2001). Apart from their nutritional value, insect meals contain chitinous materials (Finke, 2007), which are frequently attributed with functional properties (Shavandi, Hou, Carne, McConnell, & Bekhit, 2019). These chitinous materials have proven to exert beneficial effects on nutrient utilization by positively modulating gastrointestinal microbiota, and consequently promoting growth at low dietary inclusion levels (Makkar et al., 2014). Nevertheless, chitin utilization is conditioned by its source and dietary inclusion, and may interfere in the digestion of other nutrients, particularly lipid, if included at high dietary levels (Kroeckel et al., 2012), leading to impaired growth performance. This remark seems to be further supported by the present results, where increasing the levels of CR reduced apparent digestibility of lipid. Regarding apparent digestibility of dry matter and protein, the results indicated a reduction when the replacement level of FM protein exceeded 20%. It seems therefore reasonable to assume that poor nutrient digestibility observed at high dietary inclusion levels of CR, in the present study, may at least partly explain the reduction in feed utilization efficiency, which can in turn lead to slower growth. Previous studies with other marine finfish species on FM substitution by various insect meals had also reported lower feed efficiency accompanied by poor growth when insect meal incorporation level exceeded 20–30% level of diet dry matter (Sánchez-Muros et al., 2016; Sánchez-Muros, Barroso, & de Haro, 2016). In contrast with these findings, however, the only available data regarding the potential impact of using CR in fish feed indicated that total replacement of FM protein by CR was not only possible but significantly improved African catfish performance and feed efficiency (Taufek et al., 2016). Besides the general methodological approach, there is one major difference between the present study and that of Taufek et al. (2016) that could potentially account for the different findings. This distinct discrepancy between the two studies could be the result of basic differences in dietary nutrient requirement of the two studied species and/or their ability to utilize dietary nutrient, particularly carbohydrates. Olive flounder is a typical marine fish, which are known to have a limited ability for digestion and metabolism of carbohydrate and an absolute requirement for LC-PUFA, to be supplied by feed (Bell & Koppe, 2011; Gatlin III et al., 2007). In the present study, dietary \(n - 3\) LC-PUFA had a decreasing trend with the increasing levels of CR in the diets, and C80 diet contained the lowest values of these \(n - 3\) essential fatty acids, which might partly be a reason for lower performances of flounders. This has also been suggested by other authors that, in addition to high chitin content, low dietary LC-PUFA was another cause leading to inferior performance of fish that were fed diets rich in insect meal (Gasco et al., 2016).

Monitoring hematological indices has long been considered a reliable tool for assessing fish health and nutritional status. In the present study, plasma TCHO concentration declined with the dietary CR inclusion in a dose-dependent manner. A similar reduction in blood lipid parameters has also been reported in Jian carp, *Cyprinus carpio* var. Jian (Ji, Zhang, Huang, Cheng, & Liu, 2015), and European sea bass, *Dicentrarchus labrax* (Magalhães et al., 2017) fed diets with FM were replaced by silkworm pupae and black soldier fly, *Hermetia illucens*, pre-pupae meals, respectively. The observed reduction in blood lipid indices following the addition of insect meal to the diet has been attributed to the increased content of chitin in those diets (Li, Ji, Zhang, Zhou, & Yu, 2017; Magalhães et al., 2017). Chitin, a primary component of the insect’s exoskeletons, is suggested to exert its hypolipidemic effect by disrupting the normal processes of lipid digestion/assimilation, disturbing the bile acid enterohepatic circulation, and interrupting
enterohepatic fatty acid synthesis (Koide, 1998; Magalhães et al., 2017; Xia, Liu, Zhang, & Chen, 2010). Indeed, a marked reduction in the carcass and fillet lipid content of fish that were fed with the diet with the highest inclusion of CR may yet provide further evidence of their abnormal fat uptake and metabolism. Despite the pronounced hypolipidemic effects of dietary CR, however, the absence of any other remarkable changes in the carcass/fillet proximate composition and/or other plasma biochemical parameters may suggest that there were no noticeable adverse effects on the health status of flounders even at the highest CR inclusion level.

While their specific fatty acid profiles can vary depending on the species, stage of growth, and nutritional history, insects are usually rich in n-6 PUFA and devoid of n-3 LC-PUFAs (Barroso et al., 2014; Makkar et al., 2014), the latter being of particular importance for human well-being and health (Zárate, el Jaber-Vazdekis, Tejera, Pérez, & Rodríguez, 2017). Although essential amino acid composition of the fillets remained unchanged, in the present study, juvenile flounders that were fed diets containing increasing amounts of CR at the expense of FM become increasingly enriched with CR-associated fatty acids, such as C18:1n-9 and C18:2n-6, at the expense of beneficial n-3 LC-PUFAs, which may have unfavorable impacts on consumer health. The results obtained in the present study are in line with previous studies showing that replacing high levels of FM with insect meal negatively affects the composition and nutritional value of the fillet lipid fraction in various freshwater and marine fish species (Belforti et al., 2015; Iaconisi et al., 2017; Renna et al., 2017; Sánchez-Muros, Barroso, & de Haro, 2016; Sánchez-Muros, de Haro, et al., 2016; Sankian et al., 2018). However, given the insect’s dietary flexibility, such drawback can easily be addressed by feeding the insects with n-3 LC-PUFA-rich feed stuff, like fish offal, prior to being offered to the fish (Dossey et al., 2016; Sealey et al., 2011). In addition, although inclusion of CR, up to 80% of dietary FM protein, significantly reduced n-3/n-6 PUFA ratio in flounder fillets, fillet DHA concentrations were still remarkably higher than diet concentrations. This condition was suggested to be caused by an active biosynthesis and/or selective accumulation of the LC-PUFA (Turchini, Ng, & Tocher, 2010); nevertheless, the latter is more likely in consideration of the absence of the endogenous LC-PUFA biosynthetic capacity in olive flounder (Kabeya, Chiba, Haga, Satoh, & Yoshizaki, 2017). The selective deposition and retention of DHA were also observed in other fish species that were fed a diet with low n-3 LC-PUFAs, denoting the significant structural and functional importance of this fatty acid to fish (Eröldoğan et al., 2012; Turchini et al., 2010).

Antioxidant enzymes play a crucial role in maintaining oxidative balance, which, in turn, is essential for optimal cellular functions and efficient operation of the animal’s immune system. Antioxidant enzyme activities have been therefore commonly used as indicators of fish physical health and response to external feed-related stimuli (Lee, Mohammadi Azarm, & Chang, 2016; Zheng, Wen, Han, Li, & Xie, 2012). In the present study, dietary inclusion of the CR in juvenile flounder feed notably enhanced SOD and GPx activities, which are the main enzyme components of antioxidant defense system. Similar observations were also reported by Taufek et al. (2016) who demonstrated that feeding a CR-based diet to African catfish juveniles could boost their antioxidant status. Indeed, there has been increasing evidence to support the concept that dietary inclusion of insect meals may improve both antioxidant status and immune response of the fish (Henry, Gasco, Chatzifotis, & Piccolo, 2018; Ido et al., 2015; Ming et al., 2013; Ogunji, Nimptsch, Wiegand, Schulz, & Rennert, 2011; Su et al., 2017). The possible beneficial health effects of diets rich in insect meals have been attributed, at least in part, to their high chitin contents, which are proved to possess antimicrobial (Rinaudo, 2006) and antioxidant properties (Ngo & Kim, 2014). This viewpoint is further supported by previous studies revealing that chitin-supplemented diets exerted immunostimulatory effects in fish (Esteban, Cuesta, Ortuno, & Marigómez, 2001; Gopalakannan & Arul, 2006; Harikrishnan, Kim, Balasundaram, & Heo, 2012). However, further investigation is needed to ascertain whether enhanced antioxidant enzyme activities, observed in the present study, could confer greater oxidative stress resistance in juvenile flounders.

**CONCLUSION**

In general, the efficiency of CR as a promising substitute for FM in the flounder diets has been confirmed not only in relation to growth rates, but also in terms of immunopotency. Our findings provide further evidence that the use of
CR by aquafeed industry should be strongly encouraged not only to reduce their dependence on high-priced and finite feed ingredients, but also to enhance production performance and health status of farmed seafood. However, although flounder juveniles are shown to tolerate up to 80% substitution of FM protein with CR, fillet $n-3/n-6$ LC-PUFA ratio was remarkably reduced at the highest levels of CR inclusion, which is not an ideal trait from the consumer’s perspective. Thus, further research is necessary to develop a strategy for maintaining a balanced and healthier fatty acid profile for the human consumer, particularly at the highest CR inclusion levels.

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**CONFLICT OF INTEREST**
The authors declare that they have no conflict of interest.

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**REFERENCES**
Anderson, D. P., & Siwicki, A. K. (1995). Basic hematology and serology for fish health programs. In M. Shariff, J. R. Arthur, & R. P. Subasinghe (Eds.), Diseases in Asian aquaculture II (pp. 185–202). Manila, Philippines: Fish Health Section, Asian Fisheries Society.

AOAC. (2003). Official methods of analysis of the Association of Official Analytical Chemists International (17th ed.). Washington, DC: Association of Official Analytical Chemists. Inc.

Barroso, F. G., de Haro, C., Sánchez-Muros, M. J., Venegas, E., Martínez-Sánchez, A., & Pérez-Bañón, C. (2014). The potential of various insect species for use as food for fish. Aquaculture, 422-423, 193–201. https://doi.org/10.1016/j.aquaculture.2013.12.024

Belforti, M., Gai, F., Lussiana, C., Renna, M., Malfatto, V., Rotolo, L., ... Gasco, L. (2015). Tenebrio molitor meal in rainbow trout (Oncorhynchus mykiss) diets: Effects on animal performance, nutrient digestibility and chemical composition of fillets. Italian Journal of Animal Science, 14(4), 670–675. https://doi.org/10.4081/ijas.2015.4170

Bell, J. G., & Koppe, W. (2011). Lipids in aquafeeds. In G. M. Turchini, W. K. Ng, & D. R. Tocher (Eds.), Fish oil replacement and alternative lipid sources in aquaculture feeds (pp. 21–59). Florida, USA: CRC Press.

Cho, C. Y., Slinger, S. J., & Bayley, H. S. (1982). Bioenergetics of salmonid fishes: Energy intake, expenditure and productivity. Comparative Biochemistry & Physiology B, 73(1), 25–41. https://doi.org/10.1016/0305-0491(82)90198-5

CIE (Commission Internationale de L’Eclairage). (1976). Colorimetry. Vienna, Austria: Bureau Central de la CIE.

Cortes Ortiz, J. A., Ruiz, A. T., Morales-Ramos, J. A., Thomas, M., Rojas, M. G., Tomberlin, J. K., ... Jullien, R. L. (2016). Insect mass production technologies. In A. T. Dossey, J. A. Morales-Ramos, & M. G. Rojas (Eds.), Insects as sustainable food ingredients production, processing and food applications (pp. 153–201). San Diego, CA: Academic Press.

Divakaran, S., Obaldo, L. G., & Forster, I. P. (2002). Note on the methods for determination of chromic oxide in shrimp feeds. Journal of Agriculture & Food Chemistry, 50(3), 464–467. https://doi.org/10.1021/jf011112s

Dossey, A. T., Morales-Ramos, J. A., & Rojas, M. G. (2016). Insects as sustainable food ingredients production, processing and food applications. San Diego, CA: Academic Press.

Erdoğán, T., Turchini, G. M., Yılmaz, H. A., Taşbozan, O., Engin, K., Ölcülü, A., ... Mumoğluurlarda, P. (2012). Potential of cottonseed oil as fish oil replacer in European sea bass feed formulation. Turkish Journal of Fisheries & Aquatic Sciences, 12, 787–797. http://doi.org/10.4194/1303-2712-v12_4_07

Esteban, M. A., Cuesta, A., Ortuño, J., & Meseguer, J. (2001). Immunomodulatory effects of dietary intake of chitin on gilthead seabream (Sparus aurata L.) innate immune system. Fish & Shellfish Immunology, 11(4), 303–315. https://doi.org/10.1006/fsim.2000.0315

FAO. (2019). FishStatJ-Software for Fishery and Aquaculture Statistical Time Series. Retrieved from June 2019 www.fao.org/fishery/statistics/software/fishstatj/en.

Finke, M. D. (2007). Estimate of chitin in raw whole insects. Zoo Biology, 26(2), 105–115. https://doi.org/10.1002/zoo.20123
Iaconisi, V., Marono, S., Parisi, G., Gasco, L., Genovese, L., Maricchiolo, G., Hultmark, D., Steiner, H., Rasmuson, T., & Boman, H. G. (1980). Insect immunity: Purification and properties of three inducible bactericidal proteins from hemolymph of immunized pupae of Dicentrarchus labrax L. juveniles: Growth performance, whole-body composition and in vivo apparent digestibility. Animal Feed Science & Technology, 220, 34–45. https://doi.org/10.1016/j.anifeedsci.2016.07.003

Gatlin, D. M., III, Barrows, F. T., Brown, P., Dabrowski, K., Gaylord, T. G., Hardy, R. W., ... Wurtele, E. (2007). Expanding the utilization of sustainable plant products in aquafeeds: A review. Aquaculture Research, 38(6), 551–579. https://doi.org/10.1111/j.1365-2109.2007.01704.x

Gopalan, A., & Arul, V. (2006). Immunomodulatory effects of dietary intake of chitin, chitosan and levamisole on the immune system of Cyprinus carpio and control of Aeromonas hydrophila infection in ponds. Aquaculture, 255(1–4), 179–187. https://doi.org/10.1016/j.aquaculture.2006.01.012

Harikrishnan, R., Kim, J. S., Balasundaram, C., & Heo, M. S. (2012). Dietary supplementation with chitin and chitosan on haematology and innate immune response in Epinephelus bruneus against Philasterides dicentrarchi. Experimental Parasitology, 131(1), 116–124. https://doi.org/10.1016/j.exppara.2012.03.020

Henry, M., Gasco, L., Piccolo, G., & Fountoulaki, E. (2015). Review on the use of insects in the diet of farmed fish: Past and future. Animal Feed Science & Technology, 203, 1–22. https://doi.org/10.1016/j.anifeedsci.2015.03.001

Henry, M. A., Gasco, L., Chatzifotis, S., & Piccolo, G. (2018). Does dietary insect meal affect the fish immune system? The case of mealworm, Tenebrio molitor on European sea bass, Dicentrarchus labrax. Developmental & Comparative Immunology, 81, 204–209. https://doi.org/10.1016/j.dci.2017.12.002

Hultmark, D., Steiner, H., Rasmuson, T., & Boman, H. G. (1980). Insect immunity: Purification and properties of three inducible bactericidal proteins from hemolymph of pupae of Hyalophora cecropia. European Journal of Biochemistry, 106(1), 7–16. https://doi.org/10.1111/j.1432-1033.1980.tb05991.x

Iaconisi, V., Marono, S., Parisi, G., Gasco, L., Genovese, L., Maricchiolo, G., ... Piccolo, G. (2017). Dietary inclusion of Tenebrio molitor larvae meal: Effects on growth performance and final quality treats of blackspot sea bream (Pagellus bogaraveo). Aquaculture, 476, 49–58. https://doi.org/10.1016/j.aquaculture.2017.04.007

Ido, A., Iwu, T., Ito, K., Ohta, T., Mizushige, T., Kishida, T., ... Miura, T. (2015). Dietary effects of housefly (Musca domestica) (Diptera: Muscidae) pupae on the growth performance and the resistance against bacterial pathogen in red sea bream (Pagrus major) (Perciformes: Sparidae). Applied Entomology and Zoology, 50(2), 213–221. https://doi.org/10.1007/s13355-015-0325-z

Ji, H., Zhang, J. L., Huang, J. Q., Cheng, X. F., & Liu, C. (2015). Effect of replacement of dietary fish meal with silkworm pupae meal on growth performance, body composition, intestinal protease activity and health status in juvenile Jian carp (Cyprinus carpio var. Jian). Aquaculture Research, 46(5), 1209–1221. https://doi.org/10.1111/are.12276

Kabeya, N., Chiba, M., Haga, Y., Sato, S., & Yoshizaki, G. (2017). Cloning and functional characterization of fads2 desaturase and elo1v5 elongase from Japanese flounder Paralichthys olivaceus. Comparative Biochemistry & Physiology B, 214, 36–46. https://doi.org/10.1016/j.cbpb.2017.09.002

Khosravi, S., Rahimnejad, S., Herault, M., Fournier, V., Lee, C. R., Bui, H. T. D., ... Lee, K. J. (2015). Effects of protein hydrolysates supplementation in low fish meal diets on growth performance, innate immunity and disease resistance of red sea bream Pagrus major. Fish & Shellfish Immunology, 45(2), 858–868. https://doi.org/10.1016/j.fsi.2015.05.039

Koide, S. S. (1998). Chitin-chitosan: Properties, benefits and risks. Nutrition Research, 18(6), 1091–1101. https://doi.org/10.1016/S0271-5317(98)00091-8

Kroecel, S., Harjes, A. G. E., Roth, I., Katz, H., Wuerz, S., Susenbeth, A., & Schulz, C. (2012). When a turbot catches a fly: Evaluation of a pre-pupae meal of the black soldier fly (Hermetia illucens) as fishmeal substitute—Growth performance and chitin degradation in juvenile turbot (Psetta maxima). Aquaculture, 364-365, 345–352. https://doi.org/10.1016/j.aquaculture.2012.08.041

Lee, S. M., Mohammadi Azarm, H., & Chang, K. H. (2016). Effects of dietary inclusion of fermented soybean meal on growth, body composition, antioxidant enzyme activity and disease resistance of rockfish (Sebastes schlegeli). Aquaculture, 459, 110–116. https://doi.org/10.1016/j.aquaculture.2016.03.036

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. https://doi.org/10.1016/j.aquaculture.2017.04.015

Magalhães, R., Sánchez-López, A., Leal, R. S., Martinez-Llorens, S., Oliva-Teles, A., & Peres, H. (2017). Black soldier fly (Hermetia illucens) pre-pupae meal as a fish meal replacement in diets for European sea bass (Dicentrarchus labrax). Aquaculture, 476, 79–85. https://doi.org/10.1016/j.aquaculture.2017.04.021

Makkar, P. S. H., Tran, G., Heuzé, V., & Ankers, P. (2014). State-of-the-art on use of insects as animal feed. Animal Feed Science & Technology, 197, 1–33. https://doi.org/10.1016/j.anifeedsci.2014.07.008
Marono, S., Piccolo, G., Loponte, R., Di Meo, C., Attia, Y. A., Nizza, A., & Bovera, F. (2015). In vitro crude protein digestibility of Tenebrio molitor and Hermetia illucens insect meals and its correlation with chemical composition traits. *Italian Journal of Animal Science*, 14(3), 338–349. https://doi.org/10.4081/ijas.2015.3889

Ming, J., Ye, J., Zhang, Y., Yang, X., Wu, C., Shao, X., & Liu, P. (2013). The influence of maggot meal and L-carnitine on growth, immunity, antioxidant indices and disease resistance of black carp (*Mylopharyngodon piceus*). *Journal of Chinese Cereals & Oils Association*, 28(2), 80–86.

Ng, W. K., Liew, F. L., Ang, L. P., & Wong, K. W. (2001). Potential of mealworm (*Tenebrio molitor*) as an alternative protein source in practical diets for African catfish, *Clarias gariepinus*. *Aquaculture Research*, 32(s1), 273–280. https://doi.org/10.1046/j.1357-9779.2001.00024.x

Quade, M. J., & Roth, J. A. (1997). A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. *Veterinary Immunology & Immunopathology*, 58(3–4), 239–248. https://doi.org/10.1016/S0165-2427(97)00048-2

Renna, M., Schiavone, A., Gai, F., Dabbou, S., Lussiana, C., Malfatto, V., S.Ngo, D. H., & Kim, S. K. (2014). Antioxidant effects of chitin, chitosan, and their derivatives. *Advances in Food & Nutrition Research*, 73, 15–31. https://doi.org/10.1016/B978-0-12-800268-1.00002-0

Rinaudo, M. (2006). Chitin and chitosan: Properties and applications. *Progress in Polymer Science*, 31(7), 603–632. https://doi.org/10.1016/j.progpolymsci.2006.06.001

Sánchez-Muros, M. J., Barroso, F. G., & de Haro, C. (2016). Brief summary of insect usage as an industrial animal feed/food ingredient. In A. T. Dossey, J. A. Morales-Ramos, & M. G. Rojas (Eds.), *Insects as sustainable food ingredients production, processing and food applications* (pp. 273–309), San Diego, CA: Academic Press.

Sealey, W., Gaylord, T., Barrows, F., Tomberlin, J., McGuire, M., Ross, C., & St-Hilaire, S., 2011. Sensory analysis of rainbow trout (*Oncorhynchus mykiss*) diets. *Journal of Animal Science & Biotechnology*, 8, 57. https://doi.org/10.1186/s40104-017-0191-3

Shavandi, A., Hou, Y., Carne, A., McConnell, M., & Bekhit, A. A. (2019). Marine waste utilization as a source of functional and health compounds. In F. Toldrá (Ed.), *Advances in food and nutrition research* (Vol. 87), MA, USA: Academic Press.

Su, J., Gong, Y., Cao, S., Lu, F., Han, D., Liu, H., ... Xie, S. (2017). Effects of dietary *Tenebrio molitor* meal on the growth performance, immune response and disease resistance of yellow catfish (*Peleobagrus fulvidraco*). *Fish & Shellfish Immunology*, 69, 59–66. https://doi.org/10.1016/j.fsi.2017.08.008

Taufek, N. M., Aspiani, F., Muin, H., Raji, A. A., Razak, S. A., & Alias, Z. (2016). The effect of dietary cricket meal (*Gryllus bimaculatus*) on growth performance, antioxidant enzyme activities, and haematological response of African catfish (*Clarias gariepinus*). *Fish Physiology & Biochemistry*, 42(4), 1143–1155.

Ullbrich, T. L. V., & Southgate, D. A. T. (1991). Coronary heart disease: Seven dietary factors. *Lancet*, 338, 985–992.

van Huis, A. (2013). Potential of insects as food and feed in assuring food security. *Annual Review of Entomology*, 58, 563–583. https://doi.org/10.1146/annurev-ento-120811-153704

van Huis, A., & Oonincx, D. G. A. B. (2017). The environmental sustainability of insects as food and feed: A review. *Agronomy for Sustainable Development*, 37, 34. https://doi.org/10.1007/s13593-017-0452-8

Wang, D., Zhai, S. W., Zhang, C. X., Bai, Y. Y., An, S. H., & Xu, Y. N. (2005). Evaluation on nutritional value of field crickets as a poultry feedstuff. *Asian Australian Journal of Animal Sciences*, 18(5), 667–670. https://doi.org/10.5713/ajas.2005.667

Xia, W., Liu, P., Zhang, J., & Chen, J. (2010). Biological activities of chitosan and chitooligosaccharides. *Food Hydrocolloids*, 25(2), 170–179. https://doi.org/10.1016/j.foodhyd.2010.03.003
Zárate, R., el Jaber-Vazdekis, N., Tejera, N., Pérez, J. A., & Rodríguez, C. (2017). Significance of long chain polyunsaturated fatty acids in human health. *Clinical & Translational Medicine, 6*, 25. https://doi.org/10.1186/s40169-017-0153-6

Zheng, Q., Wen, X., Han, C., Li, H., & Xie, X. (2012). Effect of replacing soybean meal with cottonseed meal on growth, hematology, antioxidant enzymes activity and expression for juvenile grass carp, *Ctenopharyngodon idellus*. *Fish Physiology and Biochemistry, 38*(4), 1059–1069. https://doi.org/10.1007/s10695-011-9590-0

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