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Dietary inclusion of mealworm (*Tenebrio molitor*) meal as an alternative protein source in practical diets for rainbow trout (*Oncorhynchus mykiss*) fry

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Abstract

Background: An 8-week feeding trial was designed to evaluate the potential of yellow mealworm (MW; *Tenebrio molitor*) as a locally available nutrient-rich feedstuff for rainbow trout fry (*Oncorhynchus mykiss*).

Methods: Triplicate groups of fish (mean ± SE; 1.11 ± 0.01 g) were assigned to each of the five isonitrogenous and isocaloric practical diets containing graded level of a full fat MW (0, 7, 14, 21, and 28%) at the expense of fish meal (designated as MW0, MW7, MW14, MW21, and MW28, respectively).

Results: Fish growth performance in terms of weight gain and specific growth rate significantly increased with increasing dietary MW level up to 14% and then declined when dietary MW levels further increased to 28%. Significantly higher protein efficiency ratio and lower feed conversion ratio were found in fish fed with diets containing MW compared to fish fed the control MW0. Myeloperoxidase activity was significantly higher in fish fed MW7 diet compared to fish fed the MW0 diet. Fish fed the MW14 and MW28 diets had significantly higher lysozyme activities than those fed the MW0 diet.

Conclusions: Overall, the efficacy of MW as promising alternative to fish meal in practical diets for rainbow trout fry has been proved not only in relation to growth rates and feed utilization, but also from the viewpoint of immunopotentiation effects.

Keywords: *Tenebrio molitor*, *Oncorhynchus mykiss*, Essential amino acids, Hematological parameters, Non-specific innate immunity

Background

Much progress has been made in the development of intensive aquaculture through replacing fish meal (FM) with less expensive alternative protein sources. However, there are drawbacks, since dietary utilization may be limited, or fish have difficulty adapting to the plant and rendered animal-derived feedstuffs as substitutes for FM—which is a particular issue for carnivorous fish species (Oliva-Teles et al. 2015). The growth of the aquafeed industry signals that demand for alternative protein sources will continue to rise; therefore, research has been focused on identifying and developing novel products from locally available and acceptable feed ingredients that can satisfy both economic and environmental concerns.

Worms and insect larvae always have been considered as natural fish diets. Larvae of darkling beetle (*Tenebrio molitor* L.), known as yellow mealworm (MW), is one of the most promising candidate as alternative source of protein for use in aquafeed, primarily due to its high nutritional value and the economic feasibility of its large-scale production (see Sánchez-Muros et al. 2014;

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Henry et al. 2015 for review). Indeed, it has been demonstrated that meals derived from MW could successfully substitute part of dietary FM in feed for several commercially important marine and freshwater farmed finfish species including African catfish (*Clarias gariepinus*) (Ng et al. 2001), rainbow trout (*Oncorhynchus mykiss*) (Belforti et al. 2015), tilapia (*Oreochromis niloticus*) (Sánchez-Muros et al. 2016), European sea bass (*Dicentrarchus labrax* L.) (Gasco et al. 2016), gilthead sea bream (*Sparus aurata*) (Piccolo et al. 2017), and blackspot sea bream (*Pagellus bogaraveo*) (Iaconisi et al. 2017). Overall, the maximum amount of MW that can be incorporated into feed without depressing fish performance, apparently, depends on the quality of the MW meal itself, which varies according to its diet composition and rearing condition, and the nutritional requirements of a given fish species. Low level of methionine, inadequate fatty acid composition particularly absence of n-3 highly unsaturated fatty acids (HUFAs), and high proportions of non-starch polysaccharides, like chitin, are among the factors limiting the use of MW as a sustainable source of protein in aquafeed.

Rainbow trout (*Oncorhynchus mykiss*) is a commonly raised fish species in aquaculture. In addition to the fish raised for consumption, millions of rainbow trout are raised for stocking into ponds, lakes, streams, and rivers to provide additional sport fishing opportunities where few may otherwise exist. Together, rainbow trout cultured directly for human consumption, and those cultured to enhance or provide sport fishing opportunities, account for a measurable proportion of global aquaculture production, with a total production exceeding 800, 000 tons in 2016 (FAO FishStatJ 2018). To date, many studies have focused on evaluating a variety of different plant and animal protein ingredients to meet the rainbow trout's nutritional needs.

Although the feasibility of replacing FM with MW has been proved in feed for rainbow trout at the late juvenile stage (over 100 g initial weight) (Belforti et al. 2015; Iaconisi et al. 2018), there is no available information on the consequences of using this potential feed ingredient in feed for the late larval or early juvenile stages of this species. Furthermore, since the concept of optimal inclusion rates of alternative protein sources, as substitutes to FM, in fish diets is associated not only with optimal growth and carcass composition traits but also with optimal health, we also explored the possibility of partial replacement of FM by MW in diets for rainbow trout fry in terms of selected plasma biochemical parameters and immune parameters.

Methods

Experimental diets

The mealworm larvae (MW) were kindly provided by Korea Rural Development Administration, Organization (Jeonju, South Korea). Upon arrival, MW were freeze-dried, grounded into a fine powder, and stored at − 40 °C until used. Proximate, essential amino acid and fatty acid compositions of the resultant MW are provided in Table 1.

A practical FM-based diet (MW0) was designed to satisfy the nutritional needs of rainbow trout (NRC 2011) with steam dried mackerel FM (FoodCorp S.A., Chile; crude protein 76.1%; crude lipid 7.8% DM) served as a main source of protein. Four other experimental diets were formulated to contain 7, 14, 21, and 28% MW replacing graded levels of dietary FM protein (designated as MW7, MW14, MW21, and MW28, respectively). Nutrient and essential amino acid compositions of the experimental diets are given in Table 2. All the isonitrogenous (48% crude protein) and isocaloric (22 MJ/kg) diets were produced following the procedure described by Lee et al. (2016). Briefly, all dried ingredients were well-mixed and, after addition of oil and double-distilled water, pelleted through a meat chopper machine. The pellets were then dried in a forced air oven at 25 °C for 48 h, crushed into desirable size, and stored at − 24 °C until used.

Fish and feeding trial

Rainbow trout fry were purchased from a private hatchery and acclimated to the laboratory conditions in a 5000-L capacity glass tank, connected to a freshwater recirculating system equipped with a chiller unit to help maintain the water temperature at 15.2 °C, feeding on a commercial diet (Woosung Feed Co., Daejeon, South Korea; 50% crude protein and 13% lipid). Following the 2-week acclimation period, 25 fish (initial mean body weight, 1.11 ± 0.01 g) were randomly stocked into each 60-L rectangular glass aquarium supplied with dechlorinated/filtered freshwater using a closed recirculating

Table 1 Proximate, essential amino acid and fatty acid compositions of the mealworm meal (% DM)

Proximate composition (% DM)					Essential amino acid (% protein)									Fatty acid (% total fatty acids)							
DM[1]	CP[2]	CL[3]	Ash	Chitin	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Val	C12:0	C14:0	C16:0	C18:0	C16:1n-7	C18:1n-9	C18:2n-6	C18:3n-3	C18:3n-6
96.4	52.5	34.1	3.5	5.4	5.2	3.2	3.8	6.5	6.0	0.7	3.9	4.1	6.4	0.3	3.1	13.7	6.4	1.4	29.1	42.7	1.5	0.3

[1]Dry matter
[2]Crude protein
[3]Crude lipid

Table 2 Ingredient and chemical compositions of experimental diets (% DM)

	Diets				
	MW0	MW7	MW14	MW21	MW28
Ingredients					
Mackerel fish meal	50.0	45.0	40.0	35.0	30.0
Mealworm meal	0.0	7.0	14.0	21.0	28.0
Wheat gluten meal	5.0	5.0	5.0	5.0	5.0
Wheat flour	30.0	30.0	30.0	30.0	30.0
Fish oil	5.0	4.5	4.0	3.5	3.0
Soybean oil	6.0	4.5	3.0	1.5	0.0
Vitamin premix[1]	2.0	2.0	2.0	2.0	2.0
Mineral premix[2]	2.0	2.0	2.0	2.0	2.0
Proximate composition					
Dry matter	95.3	95.9	96.4	93.0	94.4
Crude protein	48.5	48.5	49.8	49.3	48.9
Crude lipid	15.0	14.8	14.8	14.7	14.3
Ash	10.1	9.3	8.5	7.7	7.1
Energy (MJ/kg)	22.0	22.1	22.2	22.2	22.3
Essential amino acid (% protein)					
Arg	5.9	5.8	5.5	5.5	5.5
His	3.7	4.0	3.9	3.8	3.7
Ile	3.7	3.7	3.7	3.8	3.8
Leu	7.6	7.6	7.5	7.5	7.4
Lys	8.2	7.0	6.6	6.4	6.2
Met+Cys	4.0	3.3	3.2	3.1	3.1
Phe	4.2	4.2	4.1	4.1	4.2
Tyr	4.8	3.7	4.5	5.1	5.5
Thr	4.3	4.3	4.3	4.2	4.1
Val	5.1	4.9	5.4	5.6	5.6

[1]Vitamin premix contained the following amount which were diluted in cellulose (g/kg mix): DL-α-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, 181.8; D-biotin, 0.27; folic acid (98%), 0.68; p-aminobenzoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalciferol, 0.003; cyanocobalamin, 0.003

[2]Mineral premix contained the following ingredients (g/kg mix): $MgSO_4 \cdot 7H_2O$, 80.0; $NaH_2PO_4 \cdot 2H_2O$, 370.0; KCl, 130.0; ferric citrate, 40.0; $ZnSO_4 \cdot 7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3 \cdot 6H_2O$, 0.15; KI, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2.0; $CoCl_2 \cdot 6H_2O$, 1.0

system previously described (Sankian et al. 2018). Dissolved oxygen, pH, ammonia-N, and nitrite levels were measured twice a week (7.6 ± 0.2 mg/L, 7.8 ± 0.2, < 0.6 mg/L, and < 0.2 mg/ L), and photoperiod was left under natural conditions during the experiment. Each experimental diet was hand-fed to triplicate groups of fish to apparent satiation (twice daily at 09:00 and 17:00 h) for 8 weeks. The uneaten feed was siphoned out and weighed to determine net daily feed consumption. Fish were fasted for 18 h before sampling to minimize handling stress on fish.

Sample collection and analyses

At the end of the feeding trial, all the fish in each aquarium were counted and bulk weighed for calculation of growth performance, feed utilization efficiency indices, and survival rate. Total body length was measured to the nearest 0.1 mm. Three intact fish per aquarium were randomly selected and kept at − 43 °C for whole-body proximate composition analyses. Epaxial samples of white muscle were taken from three other fish per aquarium and kept at − 80 °C for proximate and essential amino acid composition analyses. Viscera and liver were separately dissected out from these fish and weighed for the determination of viscerosomatic index (VSI) and hepatosomatic index (HSI), respectively.

Three fish per aquarium (9 fish/dietary treatment) were anesthetized with 2-phenoxyethanol (200 mg/L), and blood samples were collected from their caudal vein with heparinized syringes. Then, plasma samples were separated by centrifugation at 5000*g* for 10 min using a high-speed refrigerated microcentrifuge (Micro 17 TR; Hanil Bio Med Inc., Gwangju, Korea) and stored at − 80 °C until biochemical analyses were performed using an automated blood analyzer (DRI-CHEM NX500i, FUJiFILM Corporation Tokyo, Japan).

Another set of blood samples (3 fish/aquarium, 9 fish/dietary treatment) were collected using non-heparinized syringes and allowed to clot at room temperature for 30 min. Then, serum samples were separated by centrifugation for 10 min at 5000*g* and stored at − 80 °C for the analysis of non-specific immune parameters including myeloperoxidase (MPO), lysozyme, superoxide dismutase (SOD), and glutathione peroxidase (GPx) activities as previously described in detail (Khosravi et al. 2015).

Proximate composition of the MW, experimental diets, whole-body, and muscle samples were determined following the standard methods of the Association of Official Analytical Chemists (AOAC 2003). Moisture content was determined by oven drying to a constant weight at 105 °C and ash content by combustion in a Thermolyne™ muffle furnace (Thermo Scientific, Asheville, NC, USA) at 600 °C for 4 h. Crude protein was estimated by the Kjeldahl method using an automatic Kjeldahl System (Buchi, Flawil, Switzerland). Crude lipid was measured by a petroleum ether extraction method, using a Soxhlet extractor (VELP Scientifica, Milano, Italy). Diet gross energy content was evaluated by an oxygen bomb calorimeter (PARR 6100, Moline, IL, USA).

Chitin content of the MW was estimated following the method described by Marono et al. (2015).

The amino acid composition of the MW, experimental diets, and muscle samples were determined using an automatic amino acids analyzer after acid hydrolysis

with 6 N HCL (reflux for 23 h at 110 °C) (Hitachi, Tokyo, Japan).

Formulae, calculations, and statistical analysis

Weight gain (%) = [(final body weight − initial body weight)/initial body weight] × 100. Specific growth rate (%/day) = [(ln final body weight − ln initial body weight)/days] × 100.

Daily feed intake (%) = (feed intake × 100)/[(initial body weight + final body weight + dead fish weight) × days/2].

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

Feed conversion ratio = feed intake/wet weight gain.

Condition factor (%) = (weight of fish/(length of fish)^3) × 100.

Hepatosomatic index (%) = (weight of liver/weight of fish) × 100.

Viscerosomatic index (%) = (weight of viscera/weight of fish) × 100.

All dietary treatments were assigned by a completely randomized design. Data were subjected to one-way analysis of variance (ANOVA) in SPSS version 21.0 (SPSS Inc., Chicago, IL, USA). Tukey's multiple range test was used to identify statistically significant differences among groups at $p < 0.05$. Data are presented as mean ± SE.

Result and discussion

The results of the present study showed that MW can be a potential source of protein for rainbow trout fry, of about 1 g, significantly improving fish performance and feed utilization at dietary inclusion level of 14% as compared to those fish fed the control MW-free diet (Table 3). Although fish performance was slightly impaired by a further increase of dietary MW to 28%, fish offered MW28 diet were still able to perform better than the control group. In a recent study with a larger rainbow trout, Iaconisi et al. (2018) concluded that MW can be used at an inclusion level of up to 50% without any significant loss in fish performance, confirming the trend also observed in the previous study by Belforti et al. (2015) with the similar fish size of about 115 g. Even though no statistical differences were observed, fish growth performance in the present study was numerically lower in fish fed diet containing more than 14% MW. The discrepancy between the results in the present study and those in the previous ones regarding the maximum recommended levels of MW inclusion as a potential alternative protein source in practical fish feeds could be attributed to the several methodological differences among these studies including diet composition and experimental condition. Moreover, it could also be suggested that rainbow trout fry could tolerate lower levels of dietary MW in comparison with larger juveniles. Impaired growth performance and poor feed utilization efficiency in fish whenever dietary inclusion levels of MW exceeds a certain level has been associated with several factors including low level of methionine, inadequate fatty acid composition particularly absence of n-3 HUFAs, and high proportions of fat and non-starch polysaccharides, like chitin. In the present study, the efficiency of feed utilization represented by PER and FCR values started to decrease when the dietary inclusion level of MW exceeded 14%, which could perhaps be the

Table 3 Growth performance, feed utilization efficiency, and morphological parameters of rainbow trout fed the five experimental diets for 8 weeks

	MW0	MW7	MW14	MW21	MW28
WG[1]	943 ± 24^{b}	1073 ± 34^{ab}	1115 ± 14^{a}	1052 ± 56^{ab}	1053 ± 19^{ab}
SGR[2]	4.69 ± 0.05^{b}	4.92 ± 0.06^{ab}	4.99 ± 0.02^{a}	4.88 ± 0.10^{ab}	4.89 ± 0.03^{ab}
DFI[3]	3.37 ± 0.12^{a}	2.98 ± 0.01^{b}	2.82 ± 0.10^{b}	2.92 ± 0.04^{b}	2.98 ± 0.02^{b}
PER[4]	2.03 ± 0.07^{b}	2.34 ± 0.01^{a}	2.42 ± 0.10^{a}	2.33 ± 0.04^{a}	2.32 ± 0.03^{a}
FCR[5]	1.02 ± 0.04^{a}	0.88 ± 0.01^{b}	0.83 ± 0.03^{b}	0.87 ± 0.01^{b}	0.88 ± 0.01^{b}
Survival (%)	100.0 ± 0.0	100.0 ± 0.0	96.7 ± 1.7	98.3 ± 1.7	96.7 ± 1.7
CF[6]	1.25 ± 0.03	1.26 ± 0.03	1.27 ± 0.02	1.25 ± 0.01	1.23 ± 0.02
HSI[7]	1.68 ± 0.05	1.45 ± 0.06	1.43 ± 0.14	1.61 ± 0.06	1.54 ± 0.09
VSI[8]	14.5 ± 0.7	12.9 ± 1.2	13.5 ± 0.4	14.2 ± 0.1	13.7 ± 0.1

Values are mean of triplicate groups and presented as mean ± SE. Values with different superscript letters in the same row are significantly different ($p < 0.05$). The lack of superscript letter indicates no significant differences among treatments

[1]Weight gain (%) = [(final body weight − initial body weight)/initial body weight] × 100

[2]Specific growth rate (%/day) = [(ln final body weight − ln initial body weight)/days] × 100

[3]Daily feed intake (%) = (feed intake × 100)/[(initial body weight + final body weight + dead fish weight) × days/2]

[4]Protein efficiency ratio = wet weight gain/total protein given

[5]Feed conversion ratio = feed intake/wet weight gain

[6]Condition factor (%) = (weight of fish/(length of fish)^3) × 100

[7]Hepatosomatic index (%) = (weight of liver/weight of fish) × 100

[8]Viscerosomatic index (%) = (weight of viscera/weight of fish) × 100

Table 4 Whole body proximate composition of rainbow trout fed the five experimental diets for 8 weeks (% wet weight)

	MW0	MW7	MW14	MW21	MW28
Moisture	72.1 ± 0.2	71.9 ± 0.6	71.6 ± 0.3	72.3 ± 0.6	72.1 ± 0.1
Crude protein	14.7 ± 0.2	14.2 ± 2.2	15.3 ± 1.9	15.1 ± 3.1	14.5 ± 1.7
Crude lipid	9.93 ± 4.91	9.67 ± 0.34	9.85 ± 1.20	9.69 ± 1.82	9.01 ± 0.5
Ash	1.98 ± 0.72	1.74 ± 1.15	2.01 ± 1.43	2.18 ± 0.21	2.20 ± 1.40

Values are mean of triplicate groups and presented as mean ± SE. The lack of superscript letter indicates no significant differences among treatments

reason to cause the slight depression in growth rates at higher inclusion levels of MW. Such a reduction in fish performance has also been pointed out by previous studies when the inclusion of MW was increased above a certain level (Ng et al. 2001; Belforti et al. 2015). This phenomenon may be partly related to the remarkable fat content or the presence of poorly digestible carbohydrates, particularly chitin, in the MW (Ng et al. 2001) which might impair nutrient digestibility and/or disturb their absorption and uptake (Alegbeleye et al. 2012; Kroeckel et al. 2012) in fish fed diets containing MW.

The results of the present study revealed that up to 28% MW in rainbow trout diet had no significant effect on the fish whole body and fillet proximate composition compared to the control group (Tables 4 and 5). The constant nutrient composition of fish at the end of the present feeding trial is a substantial achievement, as several earlier studies have shown remarkable alterations in fish whole body and/or fillet chemical composition when insect meal exceeded a certain level in the diet (Ng et al. 2001; Kroeckel et al. 2012; Alegbeleye et al. 2012; Belforti et al. 2015; Gasco et al. 2016). In fact, there was a marked tendency towards higher protein and lower lipid and dry matter content in rainbow trout fillet with increasing dietary inclusion of MW up to 50% (Belforti et al. 2015). In accordance with the present study, however, no significant differences were recorded by Iaconisi et al. (2018) in the raw and/or cooked fillet proximate composition of juvenile rainbow trout fed MW as a FM replacer. This discrepancy could be attributed to a number of factors such as age/size of the fish, composition and nutrient content of the diet, source or quality of the MW, experimental conditions, and water temperature. Indeed, these enormous variations among the studies make it problematic to compare the results from different studies. Furthermore, the lack of significant effects on fish composition may also suggest that the MW product used in the present study had high nutritive value for rainbow trout fry and diets containing MW were equal or superior to FM-based diet in supplying adequate nutrient and energy for normal gains.

Hematological parameters are considered to be reliable biological indicators of fish health status and their physiological response to the dietary manipulation. In

Table 5 Muscle proximate and essential amino acid compositions of rainbow trout fed the five experimental diets for 8 weeks

	MW0	MW7	MW14	MW21	MW28
Proximate composition (% wet weight)					
Moisture	78.0 ± 1.6	76.3 ± 1.6	77.9 ± 0.8	76.7 ± 0.3	77.6 ± 0.7
Crude protein	19.9 ± 1.3	18.6 ± 1.1	18.3 ± 3.7	17.9 ± 0.7	18.8 ± 2.6
Crude lipid	2.72 ± 0.47	2.55 ± 0.42	2.12 ± 0.64	3.04 ± 0.44	2.51 ± 0.43
Ash	1.3 ± 0.01	1.46 ± 0.05	1.27 ± 0.05	1.34 ± 0.02	1.30 ± 0.1
Essential amino acids (% protein)					
Arg	6.2 ± 0.1	6.1 ± 0.0	6.0 ± 0.1	6.0 ± 0.1	6.1 ± 0.0
His	3.4 ± 0.1	3.5 ± 0.1	3.6 ± 0.1	3.7 ± 0.2	3.6 ± 0.1
Ile	4.3 ± 0.0	4.3 ± 0.1	4.2 ± 0.0	4.2 ± 0.1	4.2 ± 0.1
Leu	8.5 ± 0.0	8.4 ± 0.1	8.4 ± 0.1	8.4 ± 0.1	8.4 ± 0.0
Lys	9.8 ± 0.0	9.7 ± 0.0	9.7 ± 0.0	9.8 ± 0.0	9.7 ± 0.0
Met + Cys	3.9 ± 0.0	4.0 ± 0.1	4.1 ± 0.1	4.0 ± 0.1	4.0 ± 0.1
Phe	4.8 ± 0.0	4.8 ± 0.0	4.9 ± 0.0	5.1 ± 0.1	4.9 ± 0.0
Tyr	3.9 ± 0.1	3.9 ± 0.0	3.8 ± 0.1	3.9 ± 0.0	3.9 ± 0.1
Thr	4.7 ± 0.0	4.7 ± 0.0	4.7 ± 0.0	4.6 ± 0.0	4.7 ± 0.0
Val	5.0 ± 0.0	5.0 ± 0.1	5.0 ± 0.0	4.9 ± 0.1	4.9 ± 0.1

Values are mean of triplicate groups and presented as mean ± SE. The lack of superscript letter indicates no significant differences among treatments

Table 6 Hematological parameters of rainbow trout fed the five experimental diets for 8 weeks

	MW0	MW7	MW14	MW21	MW28
AST[1]	377.3 ± 34.2	294.0 ± 6.0	347.7 ± 60.7	344.0 ± 37.6	318.7 ± 9.7
ALT[2]	22.3 ± 3.7	15.3 ± 2.2	17.3 ± 3.5	19.3 ± 4.5	17.3 ± 0.3
ALP[3]	911.7 ± 175.1	1034.7 ± 21.7	953.3 ± 49.8	1007.0 ± 39.3	942.3 ± 28.8
ALB[4]	1.1 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.0	0.8 ± 0.1
TCHO[5]	361.7 ± 12.7	361.3 ± 33.6	365.3 ± 32.9	335.3 ± 11.1	363.0 ± 22.7
TP[6]	4.1 ± 0.2	3.7 ± 0.1	4.0 ± 0.3	3.9 ± 0.2	3.6 ± 0.1
TBIL[7]	1.3 ± 0.4	0.6 ± 0.2	0.9 ± 0.3	0.6 ± 0.2	0.4 ± 0.1

Values are mean of triplicate groups and presented as mean ± SE. The lack of superscript letter indicates no significant differences among treatments
[1]Aspartate aminotransferase activity (U/L)
[2]Alanine aminotransferase activity (U/L)
[3]Alkaline phosphatase (U/L)
[4]Albumin (g/dL)
[5]Total cholesterol (mg/dL)
[6]Total protein (g/dL)
[7]Total bilirubin (mg/dL)

the present study, dietary inclusion of MW in place of FM had no significant effect on selected hematological parameters of rainbow trout fry (Table 6), suggesting that fish were generally in good health conditions. This is in accordance with earlier findings where hematological parameters remained unchanged as the inclusion of house fly, *Musca domestica*, and black soldier fly, *Hermetia illucens*, increased in diets for Nile tilapia, *Oreochromis niloticus*, and Jian carp, *Cyprinus carpio* var. Jian, respectively (Ogunji et al. 2008; Li et al. 2016).

In the present study, the interesting results were that dietary inclusion of MW could markedly enhance the selected non-specific immune responses of rainbow trout fry (Table 7). Results showed that dietary inclusion of 7% MW led to a significant increase in MPO activity of rainbow trout after an 8-week feeding trial which have been linked to the anti-inflammatory activity (Dorward et al. 2012; Loria et al. 2008). In the present study, fish fed the MW14 and MW28 diets exhibited significantly higher lysozyme activity which is known to play a critical role in mediating protection against microbial invasion by cleaving the 1-4-β-linkages between *N*-acetylmuramic acid and *N*-acetylglucosamine in the bacteria cell wall (Saurabh and Sahoo 2008). Enhanced immune function has also been reported for other fish reared on diets containing low to moderate levels of insect meals (Henry et al. 2018; Ido et al. 2015; Ming et al. 2013; Su et al. 2017). The immunostimulating effect of dietary insects was hypothesized to be either direct through the secretion of antimicrobial peptides by the insect (Fu et al. 2009; Hou et al. 2007; Schuhmann et al. 2003) or indirect through the stimulation of the fish immune system by chitin (Lee et al. 2008). Indeed, growing evidence in fish supports the notion that chitin, a primary component of insect exoskeletons, has an immunopotentiating effect on immune response of fish (Esteban et al. 2001) and can protect these fish against bacterial challenges (Ido et al. 2015).

Conclusion

In summary, our research findings suggested that at least 28% MW could be included as a protein source in place of FM in a practical diet for rainbow trout fry without compromising growth performance, nutrient

Table 7 Non-specific immune responses of rainbow trout fed the five experimental diets for 8 weeks

	MW0	MW7	MW14	MW21	MW28
MPO[1]	0.75 ± 0.02^{b}	1.23 ± 0.24^{a}	1.09 ± 0.17^{ab}	1.03 ± 0.31^{ab}	1.01 ± 0.15^{ab}
Lysozyme[2]	88.3 ± 12.4^{b}	140.4 ± 18.9^{ab}	160.3 ± 15.7^{a}	145.6 ± 27.5^{ab}	162.4 ± 18.6^{a}
SOD[3]	49.8 ± 5.0	53.3 ± 2.0	52.1 ± 6.5	48.4 ± 1.7	54.0 ± 5.0
GPx[4]	32.1 ± 3.2	38.7 ± 5.6	39.3 ± 1.7	38.6 ± 3.2	39.6 ± 2.9

Values are mean of triplicate groups and presented as mean ± SE. Values with different superscript letters in the same row are significantly different ($p < 0.05$). The lack of superscript letter indicates no significant differences among treatments
[1]Myeloperoxidase
[2]Lysozyme (U/mL)
[3]Superoxide dismutase (% inhibition)
[4]Glutathione peroxidase (mU/mL)

composition, and health status of fish. The best performance of fish was achieved with a diet containing 14% MW, which was significantly better than fish offered FM-based control diet. Further investigation, however, is needed to determine the maximum dietary inclusion level of the tested MW product in feeds for rainbow trout fry.

Abbreviations

FM: Fish meal; MW: Yellow mealworm; HUFAs: Highly unsaturated fatty acids; WG: Weight gain; SGR: Specific growth rate; DFI: Daily feed intake; PER: Protein efficiency ratio; FCR: Feed conversion ratio; CF: Condition factor; HSI: Hepatosomatic index; VSI: Viscerosomatic index; AST: Aspartate aminotransferase activity; ALT: Alanine aminotransferase activity; ALP: Alkaline phosphatase; ALB: Albumin; TCHO: Total cholesterol; TP: Total protein; TBIL: Total bilirubin; MPO: Myeloperoxidase; SOD: Superoxide dismutase; GPx: Glutathione peroxidase

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Authors' contributions

SMJ and IRM manufactured the experimental feed and drafted the manuscript. SK conducted the feeding trial and performed the analyses. SML conceived and designed the study and experimental facility and also revised the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

All datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Experimental protocols followed the guidelines of the Animal Care and Use Committee of Gangneung-Wonju National University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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