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Comparative analysis of using housefly maggot, silkworm pupae and earthworm meal-based diets in rohu, *Labeo rohita* (Hamilton, 1822)

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ABSTRACT: A study was conducted in rohu fish, Labeo rohita (Hamilton, 1822) to assess the potential of silkworm pupae, housefly maggot, and earthworm meals as a replacement for soybean meal. In this context, four isonitrogenous and isolipidic diets were prepared, viz., control – SM (30% soybean meal inclusion level), SPM (30% silkworm pupae meal inclusion), HMM (30% housefly maggot meal inclusion level) and EWM (30% earthworm meal inclusion level). The rohu fingerlings (initial average body weight: 5.07 ± 0.01 g) were fed twice daily with the respective experimental diets to reach satiation levels. Specific growth rate (SGR), final body weight (FBW), per cent weight gain (WG), protein efficiency ratio (PER) and feed conversion ratio (FCR) were significantly affected among the experimental groups. SPM and HMM groups had significantly higher FBW, SGR, WG and PER values than the control - SM and EM groups. However, there was no significant difference between the control and EWM groups, and between SPM and HMM groups. FCR values showed a significant reverse trend in respect to WG. Incorporation of SPM and HMM in rohu diets gave greater growth performance and feed utilisation efficiency than SM and EWM-based diets. © 2024 Association for Advancement of Entomology

KEY WORDS: Fish meal, isonitrogenous and isolipidic diets, growth performance

INTRODUCTION

India's thriving aquaculture and fisheries sectors are not only crucial for feeding the nation but also generate substantial export earnings and provide livelihoods for around 14 million people (FAO, 2020). The growth rate of capture-based fisheries in the world has been relatively static since the late 1980s and worldwide fish production reached its maximum potential (approximately 178 million tons) in the year 2020 (FAO, 2012, 2022). Under these circumstances, the aquaculture sector playing a pivotal role in nutritional security requires lower production costs, efficient production processes, and eco-friendly measures. Fish feed, one of the costliest inputs (accounting for 60 to 70%) of the operating costs in semi-intensive and intensive aquaculture (Singh *et al.,* 2006), needs to be cost-effective, eco-friendly, and nutritionally rich. High fish feed costs due to ingredient shortages threaten to stall fish production despite strong market demand. Replacing conventional protein sources

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with locally available, nutrient-rich options like silkworm pupae, maggot and earthworm meal offers a sustainable and affordable solution to bridge this gap and support future fish farming in line with UNO's Sustainable Development Goals (FAO, 2022). India's sericulture generates approximately 40,000 tons of silkworm pupae yearly, with research highlighting their potential as an aquafeed ingredient (Jayaram and Shetty, 1980). Gohl (1981) and Nandeesha *et al.* (1990, 2000) successfully included varying levels of silkworm meal in carp diets, achieving comparable growth and feed conversion to traditional fishmeal-based diets. Compared to plant-based protein, silkworm meal demonstrated superior performance in carps (Swamy and Devaraj, 1994). Borthakur *et al.* (1998) further supported this, finding similar digestibility of crude protein from silkworm meal compared to fishmeal. Housefly maggots, are emerging as a promising alternative protein source in aquaculture. Processed into "magmeal," these larvae boast high proteins (39 - 61.4% crude protein), lipids (12.5 - 21%), and essential nutrients like phosphorus, B-complex vitamins, and trace minerals (Teotia and Miller, 1973). Studies showed magmeal diets can match the growth performance of fishmeal in *Oreochromis niloticus* fingerlings, while costing significantly less (Fashina-Bombata and Balogun, 1997). This makes magmeal a competitive, sustainable, and potentially cost-effective alternative for fishmeal in aquafeed. Vermicompost, the product of earthworm digestion, serves as a direct organic fertilizer in fish ponds. Studies even suggested dried earthworms (*Eudrilus eugeniae*) as a viable fish meal substitute, boosting carp growth. Similarly, earthworm extract (vermiwash) significantly enhanced fish growth and survival, likely due to its rich vitamin content (provitamin D and B complex) and other beneficial metabolites (Chakrabarty, 2008). Moreover, worm biomass produced during vermicomposting can be directly in Moreover, worm biomass produced during vermicomposting can be directly incorporated into fish feed, further maximizing resource utilization (Tuan, 2010; Tacon *et al.*, 2011). Given the high cost of fish and soybean meal, this study analyzes alternative protein sources (*viz.*, silkworm pupae, housefly maggot, and earthworm meal) substituted fish feed replacing soybean meal on the growth performance of rohu fish *Labeo rohita* (Hamilton, 1822).

MATERIALS AND METHODS

The experiment was carried out at the M/S. Shah Ji Fish Farm (28.1705^{0} N, 77.3182^{o} E), Palwal, Haryana. Four rohu fish feed diets were prepared; defatted soybean meal-based diet as control (SM) and three diets replacing defatted soybean meal with silkworm pupae (SPM), housefly maggot (HMM) and earthworm (EWM) meal (at 30% inclusion levels) separately; and their effect on rohu's growth performance was assessed under a completely randomized design experiment with three replications.

Different feed components (Table 1) were weighed and made into a dough by mixing all the previously dried ingredients (excluding oil and vitamin premix) using water. It was cooked for 30 minutes in a pressure cooker and cooled to room temperature. The calculated proportions of the vitamin premix and oil were then mixed. The dough was compressed through a pelletizer (S.B. Panchal and Company, Maharashtra, India) to obtain pellets of uniform size, allowed to air-dry, packed in zip lock pouches, sealed, and stored at -20 °C until further use. Water temperature and pH in all the experimental tubs were measured using a thermometer (MERCK, Germany) and a digital pH meter (LABINDIA). Dissolved oxygen, carbonate hardness, nitrite, nitrate, free carbon dioxide and ammonia were also analyzed (APHA, 1998).

Proximate composition analysis of the experimental diets *viz.*, estimation of moisture content using oven drying method, nitrogen concentration (crude protein - CP %) using semi-automated Kjeltec technique (Kjeltec Auto Distillation, Sweden), and fibre content using FibroTRON (Tulin equipments, India), and Total ash (TA) content using muffle furnace were done. Soxhlet apparatus was used to estimate crude fat or ether extract (EE) of experimental diets and ingredients using diethyl ether as the solvent.

The following formulae were used to calculate the digestible energy value (DE) and nitrogen free extract (NFE) ----

DE (kcal/100 g) = [{NFE (%) × 4} + {EE (%) × 9} + {CP (%) × 4}]

NFE (%) =100 – (CP % + CF % + TA % + EE %)

The growth parameters of the experimental feed fed fish as listed below were analysed using samples drawn out at fortnightly intervals. Before being weighed, the fish were fasted overnight.

$$\text{Weight gain (WG\%)} = \frac{\text{Final body weight (FBW) - Initial body Weight (IBW)}}{\text{Initial body weight(IBW)}} \times 100$$

$$\text{Specific growth rate (SGR)} = \frac{\text{Log}_e\text{(FBW) - Log}_e\text{(IBW)}}{\text{Experimental duration in days}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake}}{\text{Weight gain}} \times 100$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}} \times 100$$

The fatty acid content of fish muscle (one sample per tank) was assessed using an Agilent 7820a Series gas chromatograph (Agilent Tech., USA). The fatty acid methyl ester synthesis and gas chromatograph analysis were carried out using the procedures published previously (Tian *et al.*, 2014). Total lipids were extracted (Folch *et al.*, 1957) from 0.3-0.5g samples in chloroform/methanol (2:1,v/v), filtered, and methanol-extracted. Hexane and potassium hydroxide methanol (0.4 M) were added for methyl esterification, and the upper layer was analyzed using a gas chromatograph with a capillary column and flame-ionization detector. The data represented individual methyl esters as a proportion of total identified fatty acids

The experimental data were subjected to one-way analysis of variance (ANOVA) using SPSS software for Windows (Version 22.0). Duncan's Multiple Range Test was followed for post hoc mean ($p<0.05$) comparisons.

RESULTS AND DISCUSSION

Analysed proximate composition values of all the ingredients used to formulate the experimental diets. Notably, the crude protein contents of the SPM, HMM and EWM were 44.24, 54.13, and 52.48 per cent, respectively; while the crude fat content was 25.32, 22.45 and 3.55 per cent respectively. Digestible energy was 4.87, 4.93, and 4.82 kcal/ 100 g, respectively, for SPM, HMM and EWM (Table 2). The experimental diets were found isonitrogenous and isolipidic with crude protein (35%) and crude lipid (6%) (Table 3). No significant ($p>0.05$) difference was observed among the water quality parameters during the entire study period (Table 4).

At the end of the experiment, SGR, FBW, FCR, WG and PER were found significantly influenced by the diets. WG, FBW, PER and SGR were significantly higher in SPM and HMM fed groups compared to SM and EWM fed groups. However, no significant ($p>0.05$) difference was found between the SM and EWM fed groups and between SPM and HMM fed groups. FCR values showed a significantly inverse trend, higher in the SM and EWM fed groups than the SPM and EWM fed groups (Table 5). The crude lipid, moisture and total ash content of *L. rohita* were not significantly influenced by the experimental groups. However, feeding insect meal-based diets to *L. rohita* showed significant ($p<0.05$) changes in crude protein and total carbohydrate content. Among the insect meal-based diets, the highest body crude protein deposition was observed in the EWM based diet, followed by the SPM based diet. However, the control diet did not show significant difference from the SPM and EWM based diets. The total carbohydrate content was significantly higher in the SM and EM meal-based diets than in the SPM and HMM based diets (Table 6).

Fatty acid profile of the fish muscle: Among the muscle fatty acid compositions of different experimental diet fed fish, 16:00, 20:1 (n-9), 18:3 (n-3), and 22:6 (n-3) showed distinct differences. Besides, the sum of total saturated fatty acids was higher in the SM and SPM based diets than the EWM and HMM based diets. Furthermore, there wasn't any observable significant difference in the sum of mono-unsaturated and poly-unsaturated fatty acids (Table 7).

Table 1. Diet formulations for *Labeo rohita*

Ingredients (%)	SM	SPM	HMM	EM
Insect meal	0	30	30	30
Defatted soybean meal	30	0	0	0
Groundnut oil cake	30	18	30	27
Mustard oil cake	22	8	7	26
De-oiled rice bran	10	23	20	10
Wheat flour	4.5	19.5	12	6
Sunflower oil	2.5	0.5	0	0
Vitamin-mineral mix	1	1	1	1
Total	100	100	100	100

SM control (30% soybean meal inclusion level),
SPM (30% silkworm pupae meal inclusion),
HMM (30% housefly maggot meal inclusion level)
EM (30% earthworm meal inclusion level)

Dissolved oxygen, temperature, total hardness, nitrate, ammonia, pH, and nitrite were among the water quality indicators (Debnath *et al*., 2007; Mohapatra *et al*., 2012). The findings on these parameters implied that the water quality indicators had no effect on the assessed parameters across the treatment groups. The proximate composition values of all the feed ingredients assessed were found suitable for feed formulations and was supported by the previous studies (Aniebo *et al*., 2008; Salem *et al*., 2008; NRC, 2011; Mohanta *et al*., 2016). The nutritional contents of the diets were suitable for *L. rohita* and were prepared as per the nutritional requirement (Ngoc *et al*., 2016; Wang *et al*., 2017; Rahimnejad *et al*., 2019; Sahoo *et al*., 2020). In the present study, the overall growth performance of fish fed SPM based diet showed significantly better results than SM based diet, indicating that the SPM can be used as suitable alternative of the SM. Previously studies were mostly focused in common carp (Nandheesa *et al*., 1990, 2000) and rohu (Begum *et al*., 1994) fed with silkworm pupae meal (up to 50%) inclusion ratios instead of fish meal. According to Karthick Raja *et al*. (2019), WG in fish fed with diets replacing fishmeal with 40 or 50 per cent silkworm pupae was significantly lower than in fish fed with diets containing 30 per cent silkworm pupae. When the amount of silkworm pupae in the meal increased, the growth rate was considerably lowered. Similar results were reported by Salem *et al*. (2008) in Nile tilapia and Ji *et al*. (2015) in Jian carp. Further research on fish revealed that higher insect meal substitutes or replacements simultaneously raised chitin levels and impacted lipid and protein digestibility (Kroeckel *et al*., 2012; Longvah *et al*., 2011). In connection to fish fed a control diet, common carp (Gangadhar *et al*., 2017), catla (Umalatha *et al*., 2018), and *Labeo firmbriatus* (Jayaram and Shetty, 1980) demonstrated greater protein digestibility when fish meal was replaced with 30 per cent non-deoiled silkworm pupae. These observations also support the better growth performance of fish fed with SPM based diet in the present study. Salem *et al*. (2008) reported that silkworm pupae meal can be used profitably in Nile tilapia instead of fish meal up to 66.66 per cent due to its favorable impacts on growth, protein efficiency, feed conversion, and economic efficiency. Khatun *et al*. (2005) reported better fish growth when fish meal was replaced with silkworm pupae meal (@ 6 to 8%). However, 25 per cent fish meal protein replacement with *Bombyx mori* in *Clarias gariepinus*, produced best results (Kurbanov *et al.,* 2015). Enzymatic hydrolysates of defatted silkworm pupa can substitute 50 per cent of the fish meal in juvenile mirror carp without having a deleterious impact on growth, according to Xu *et al*. (2018). Shakoori *et al*. (2016) findings

Table 2. Proximate composition of different feed ingredients (% dry matter) in the diets

Composition	Silkworm pupa	Housefly maggot	Earth worm	DSBM	GNOC	MOC	DORB	Wheat flour
Dry matter	93.12 ±0.63[de]	94.45 ±0.47[e]	94.63 ±0.58[e]	90.99 ±0.73[bc]	92.4 ±0.57[cd]	91.61 ±0.48[cd]	89.54 ±0.42[ab]	88.76 ±0.79[a]
Crude protein	44.24 ±0.32[d]	54.13 ±0.4[g]	52.48 ±0.25[f]	48.59 ±0.65[e]	49.21 ±0.74[e]	39.86 ±0.71[c]	17.23 ±0.24[b]	11.7 ±0.16[a]
Crude fat	25.32 ±0.37[e]	22.45 ±0.45[d]	3.55 ±0.12[b]	1.23 ±0.10[a]	1.19 ±0.15[a]	7.47 ±0.27[c]	1.82 ±0.09[a]	1.16 ±0.05[a]
Nitrogen free extract	11.78 ±1.37[a]	10.26 ±1.36[a]	18.77 ±0.88[b]	40.75 ±1.11d	34.14 ±1.43[c]	34.03 ±1.71[c]	60.65 ±1.19[e]	85.33 ±0.36[f]
Crude fibre	4.56 ±0.17[c]	6.63 ±0.19[d]	13.84 ±0.09[h]	3.67 ±0.16[b]	9.62 ±0.23[f]	10.98 ±0.21[g]	8.43 ±0.25[e]	1.25 ±0.11[a]
Total ash	14.1 ±0.51[e]	6.53 ±0.32[bc]	11.36 ±0.42[d]	5.76 ±0.21[b]	5.84 ±0.31[b]	7.66 ±0.53[c]	11.87 ±0.61[d]	0.56 ±0.04[a]
Digestible energy	4.87 ±0.27[c]	4.93 ±0.39[c]	4.82 ±0.26[c]	4.81 ±0.22[c]	4.48 ±0.29b[c]	4.16 ±0.41[abc]	3.65 ±0.32[ab]	3.44 ±0.26[a]

All values are presented as mean of three. Abbreviation; DORB, Deoiled rice bran, MOC; Mustard oil cake, DSBM; Defatted soybean meal; GNOC, Groundnut oil cake. P= 0.01- 0.001; In a row means followed by different letters are significantly different by DMRT

on rainbow trout's flesh quality, growth, or survival revealed that the fish could be maintained for a period of 60 days while consuming silkworm pupae in proportions up to 10 per cent of fish meal. In *Litopenaeus vannamei*, a Pacific white prawn species, a diet replacing 75 per cent fish meal with silkworm pupae supported growth and immunological indices (Rahimnejad *et al*., 2019). While research on silkworm pupae is abundant, commercially produced and readily available housefly larval meal remains scarce, particularly in developing countries. This scarcity presents an opportunity to leverage the advantages of housefly larvae over earthworms in animal feed formulas, effectively addressing the limitations of the latter. In the present study, the housefly maggot meal-based diet showed a better growth performance than soybean meal and earthworm meal-based diet at 30 per cent inclusion level. This finding is in corroboration with the studies of Fasakin *et al.* (2003), and they observed no difference in growth or nutrient uptake for *C. gariepinus* fingerlings fed with diets containing either 32 per cent sun-dried or 27 per cent oven-dried housefly larvae meal. Similarly, Wang *et al.* (2017) suggested housefly larval meal as a favorable dietary component for Nile tilapia at levels up to 33 per cent. Ogunji *et al.* (2008) even reported no negative impact on growth or nutrient utilization in Nile tilapia fed with diets with up to 68 per cent housefly larval meal. Ngoc *et al.* (2016) found that common carp responded favorably to feeds containing earthworm meal as the predominant protein source, completely replacing fish meal in the diet. Popek *et al.* (1996) on *Carassius auratus* reached the lowest replacement level of 10 per cent, whereas Kostecka and Pczka (2006) on Guppy achieved the greatest replacement level of 100 per cent. However, Popek *et al.* (1996) found that 10 per cent *E. fetida* meal quadrupled the reproductive rate of *C. auratus*. Kostecka and Pczka (2006) discovered that replacing fish food with *E. fetida* meal resulted in enhanced survivability, improved reproduction, and increased biomass in aquarium fish, *P. reticulata*. Vodounnou *et al*. (2016) found that *Parachanna obscura* fingerlings experienced a high SGR of 2.11 g/day when fed with *E. fetida* meal. When *E. fetida* meals were present in amounts greater than 25 per cent, most studies found that fish growth was inhibited. These findings were attributed to the foul-smelling coelom fluid and indigestible chitin, which are known to impair palatability and

Table 3. Proximate composition of different experimental diets (n=6)

Composition	SM	SPM	HMM	EM
Moisture	9.76±0.27	8.84±0.45	9.25±0.19	9.63±0.23
Crude protein	35.17±0.35	35.21±0.29	35.48±0.33	35.24±0.36
Crude fat	6.04±0.19	6.37±0.13	6.22±0.2	6.30±0.18
Crude fibre	6.63±0.28	7.15±0.37	7.56±0.42	7.34±0.46
N free extract*	44.7±1.04	43.46±1.02	43.71±0.96	43.53±0.83
Total ash	7.46±0.14^{b}	7.81±0.19^{c}	7.03±0.09^{a}	7.59±0.17^{bc}

Nitrogen free extract; In a row means followed by different letters are significantly different by DMRT

Table 4. Quality parameters of water filled in experimental tanks for different diets

Parameters	SM	SPM	HMM	EM
Temperature (°C)	27.58±0.29	27.86±0.34	27.48±0.34	27.44±0.35
pH	7.5±0.05	7.52±0.07	7.43±0.07	7.42±0.07
Dissolved O_2 (mg/L)	6.83±0.07	6.82±0.06	6.76±0.08	6.39±0.42
Free CO_2 Hardness (mg/L)	ND 175.89±1.23	ND 176.03±0.68	ND 175.38±0.96	ND 175.19±1.29
Alkalinity (mg/L)	129.54±1.5	128.53±1.64	127.64±1.45	128.25±1.15
Nitrite (mg/L)	0.028±0.001	0.027±0.002	0.027±0.002	0.028±0.002
Ammonia (mg/L)	0.054±0.002	0.044±0.003	0.047±0.003	0.05±0.003
Nitrate (mg/L)	0.024±0.002	0.023±0.001	0.024±0.001	0.025±0.001

All the values are Mean ± SE (n=15). P=0.105-0.943; ND - Not detected;
In a row means followed by different letters are significantly different by DMRT

digestibility (Dedeke *et al*., 2013). Similarly, in the current study, at EW showed significantly lower growth performance in comparison with SPM and HMM based diet. De Chaves *et al*. (2015) found that when *E. fetida* meal given, instead of fish meal, Nile tilapia and common carp experienced slower SGR. Silkworm pupae protein is abundant in critical amino acids such as methionine, phenylalanine, and valine. The essential amino acid content of SWP protein was compatible with the dietary requirements of fish (FAO, 2007). In the present study, the carcass CP content of fish fed with SM and SPM based diets did not differ significantly. Despite this, Salem *et al*. (2008) found no significant differences in the dry matter, CP, EE, and TA of Nile tilapia fed with diets with or without silkworm pupae meal. These findings are comparable with those of Nandeesha *et al*. (2000).

The effectiveness of substituting fish meal in fish diets varies substantially depending on substituting ingredient's nutritional value. According to, the CP content of housefly meal ranged from 42.3 to 60.4 per cent. Protein concentration varies according to the processes employed for processing, drying, storage, and protein measurement, as well as the media components utilized to create housefly maggots (Ogunji *et al*., 2008; Makkar *et al*., 2014).

This might explain why *L. rohita* CP levels were greater when fed with HMM based diet. Ogunji *et al*. (2008) and Idowu *et al*. (2003) found relatable results in *Oreochromis niloticus* and *Clarias gariepinus*, respectively. Wang *et al*. (2017) discovered that a dietary housefly meal had no influence on the muscle proximate composition of Nile tilapia.

Earthworms have a high nutritional value, including lipids and protein, and have been reported as a feasible aquafeed component (Sogbesan and Ugwumba, 2008). According to Dong *et al*. (2010) and Tacon and Metian (2009), the protein content of earthworm meal is equivalent to that of fishmeal. In the current study, the total body protein content of soybean and earthworm meal-based diets was shown to be statistically similar. Pucher *et al*. (2014) discovered a substantial increase in protein content in common carp fed on Em based diet in place of a plant protein. However, there was no significant influence of dietary earthworm meal on the common carp's body proximate composition (Ngoc *et al*., 2016). Fatty acids, or lipids, are the primary form of stored energy in the body, which is crucial during normal cellular metabolic functioning and

Table 5. Nutrient utilization and growth of *Labeo rohita* fed with different diets

Parameters	SM	SPM	HMM	EM
Initial weight (g)	5.06±0.02	5.06±0.02	5.08±0.03	5.1±0.01
Final weight (g) (FBW)	14.58±0.1[a]	17.21±0.66[b]	17.8±0.54[b]	14.4±0.13[a]
Feed conversion ratio (FCR)	2.11±0.03[b]	1.85±0.07[a]	1.85±0.06[a]	2.14±0.03[b]
Protein efficiency ratio (PER)	1.35±0.02[a]	1.54±0.06[b]	1.54±0.05[b]	1.33±0.02[a]
Feed conversion efficiency (FCE)	0.47±0.01[a]	0.54±0.02[b]	0.54±0.02[b]	0.47±0.01[a]
Specific growth rate (SGR)	1.76±0.02[a]	2.04±0.07[b]	2.09±0.04[b]	1.73±0.02[a]
Weight gain percentage (WG%)	188.38±3.18[a]	239.93±13.55[b]	250.45±9.24[b]	182.19±2.29[a]

In a row means followed by different letters are significantly different by DMRT

Table 6. Proximate composition of *Labeo rohita* fed with different diets

Variables	SM	SPM	HMM	EWM
Moisture	74.18±0.2	74.2±0.15	74.15±0.5	74.03±0.16
Crude protein	15.89±0.06[ab]	16.22±0.13[b]	16.78±0.15[c]	15.52±0.21[a]
Crude lipid	3.66±0.24	3.72±0.03	3.67±0.27	4.06±0.12
Total carbohydrate	3.54±0.11[b]	2.86±0.1[a]	2.56±0.29[a]	3.73±0.14[b]
Total ash	2.74±0.09	3.01±0.07	2.84±0.12	2.67±0.18

In a row means followed by different letters are significantly different by DMRT

Table 7. Muscle fatty acid composition of *Labeo rohita* fed with different diets

Fatty acids	SM	SPM	HMM	EM
Lauric acid (12:0)	5.35±0.15	5.57±0.21	4.95±0.14	5.19±0.04
Myristic acid (14:0)	6.27±0.12	5.58±0.44	5.92±0.12	6.19±0.09
Pentadecylic acid (15:0)	2.28±0.12	2.4±0.07	2.51±0.18	2.16±0.07
Palmitic acid (16:0)	11.34±0.15[c]	11.05±0.18[bc]	10.4±0.15[a]	10.49±0.23[ab]
Palmitoleic Acid (16:1)	5.02±0.05	4.9±0.12	5±0.03	5.3±0.24
Margaric acid (17:0)	1.18±0.05	1.47±0.2	1.2±0.08	1.2±0.11
Stearic acid (18:0)	9.97±0.19	10.18±0.09	10.44±0.17	9.66±0.3
Oleic acid (18:1, n-9)	6.09±0.13	6.23±0.14	6.63±0.22	6.19±0.04
Linoleic acid (18:2, n-6)	4.2±0.1	4.18±0.18	4.37±0.2	3.74±0.25
Linolenic Acid (18:3, n-3)	3.95±0.08[b]	3.87±0.06[b]	3.32±0.26[a]	3.07±0.05[a]
Arachidic acid (20:0)	8.77±0.25	8.67±0.11	8.16±0.06	8.43±0.16
Gondoic acid (20:1, n-9)	6.1±0.03[a]	6.66±0.13[b]	6.19±0.05[a]	6.21±0.18[a]
Eicosadienoic acid (20:2, n-6)	2.05±0.12	2.26±0.08	2.16±0.02	2.1±0.07
Dihomo-ã-linolenic acid (20:3, n-6)	3.29±0.18	2.93±0.24	3.11±0.26	3.01±0.15
Mead acid (20:3, n-3)	3.11±0.02	3.33±0.16	3.38±0.12	3.42±0.23
Arachidonic Acid (20:4, n-6)	1.27±0.06	1.79±0.13	1.34±0.05	1.68±0.29
Eicosapentaenoic acid (20:5, n-3)	3.05±0.09	2.83±0.2	2.38±0.27	2.22±0.1
Docosahexaenoic acid (22:6, n-3)	0.81±0.03[a]	0.85±0.05[a]	1.13±0.03[b]	1.02±0.08[b]
Nervonic acid (24:1, n-9)	4.14±0.06	4.22±0.15	4.36±0.18	4.47±0.24
∑FA	95.44±0.17	96.2±0.41	93.78±1.12	92.32±1.5
Other	7.19±0.03	7.25±0.35	7.3±0.1	7.07±0.08
∑Saturated FA	45.16±0.3[b]	44.92±0.57[b]	43.59±0.24[a]	43.31±0.43[a]
∑Monounsaturated FA	28.55±0.15	29.24±0.2	29±0.54	28.75±0.63
∑Polyunsaturated	21.73±0.3	22.04±0.48	21.19±0.48	20.26±0.48
∑n-3	7.87±0.09	8.04±0.17	7.82±0.26	7.51±0.22
∑n-6	13.86±0.25	14±0.34	13.36±0.34	12.75±0.28
∑n-3/n-6	0.57±0.01	0.57±0.01	0.59±0.02	0.59±0.01

In a row means followed by different letters are significantly different by DMRT starvation. In this experiment, the total saturated fatty acid content of SM and SPM diets was substantially greater than that of HMM and EWM. However, there are limited number of studies on the effect of feeding these insect- meal in rohu carp. Moreover, the studies of Cheng *et al*. (2017) and Feng *et al*. (2021) in silkworm pupae-based diets; Lin and Mui (2017) and Hashizume *et al*. (2019) in HMM; and Gunya *et al*. (2016) in EWM revealed that the fatty acid composition and their ratio greatly varies depending upon the ingredients that used to grow the insects. In the present study, insect meals were used in the replacement of SM which contains high amount of saturated fatty acid than fish meal (NRC, 2011). SPM and HMM in rohu diets gave superior performance in terms of growth and feed consumption efficacy and can be used in preparing cost-effective feed for rohu.

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REFERENCES

Aniebo A.O., Erondu E.S. and Owen O.J. (2008) Proximate composition of housefly larvae (*Musca domestica*) meal generated from mixture of cattle blood and wheat bran. Livestock Research for Rural Development 20(12): 1–5.

APHA (1998) Standard Methods for the Examination of Water and Waste Water. American Public Health Association, Washington, D.C.

Begum N.N., Chakraborty S.C., Zaher M., Abdul M.M. and Gupta M.V. (1994) Replacement of fishmeal by low-cost animal protein as a quality fish feed ingredient for Indian major carp, *Labeo rohita*, fingerlings. Journal of the Science of Food and Agriculture 64(2): 191–197.

Borthakur S. and Sarma K. (1998) Protein and fat digestibility of some non-conventional fish meal replacers in diets of fish *Clarius batrachus* (Linnaeus, 1758) Environment and Ecology 16(2): 368–371.

Chakrabarty D. (2008) Vermicompost and organic Pisciculture. AkshayKrishiVikash Publisher, West Bengal, India. 16pp.

Cheng L., Huang H., Chen S., Wang W., Dai F. and Zhao H. (2017) Characterization of silkworm larvae growth and properties of silk fibres after direct feeding of copper or silver nanoparticles. Materials and Design 129: 125–134.

De Chaves R.C., Paula R.Q., Gücker B., Marriel I.E., Teixeira A.O. and Boëchat I.G. (2015) An alternative fish feed based on earthworm and fruit meals for tilapia and carp postlarvae. Brazilian Journal of Biosciences 13(1): 15–24.

Debnath D., Pal A.K., Sahu N.P., Yengkokpam S., Baruah K., Choudhury D. and Venkateshwarlu G. (2007) Digestive enzymes and metabolic profile of *Labeo rohita* fingerlings fed diets with different crude protein levels. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 146: 107–114.

Dedeke G.A., Owa S.O., Olurin K.B., Akinfe A.O. and Awotedu O.O. (2013) Partial replacement of fish meal by earthworm meal (*Libyodrilus violaceus*) in diets for African catfish, *Clarias gariepinus*. International Journal of Fisheries and Aquaculture 5: 229–233.

Dong X.H., Guo Y.X., Ji-Dan Y., Song W.D., Huang X.H. *et al.* (2010) Apparent digestibility of selected feed ingredients in diets for juvenile hybrid tilapia, *Oreochromis niloticus* x *Oreochromis aureus*. Aquacult Research 41: 1356–1364.

FAO (2007) The State of World Fisheries and Aquaculture. FAO Fisheries and Aquaculture Department, Rome.

FAO (2012) The State of World Fisheries and Aquaculture Sustainability in action, Rome.

FAO (2020) The State of World Fisheries and Aquaculture. Sustainability in action, Rome.

FAO (2022) The State of World Fisheries and Aquaculture Sustainability in action, Rome.

Fasakin E.A., Balogun A.M. and Ajayi O.O. (2003) Evaluation of full-fat and defatted maggot meals in the feeding of clariid catfish *Clarias gariepinus* fingerlings. Aquaculture Research 34: 733–738.

Fashina-Bombata H.A. and Balogun O. (1997) The effect of partial or total replacement of fish meal with maggot meal in the diet of tilapia (*Oreochromis niloticus*) fry. Journal of prospects in science 1(1): 178–181.

Feng Q.F., Wang S.W., Huang X.Z., Li H., Liu C.J., Lu H., Chen Y.J., Lin S.M. and Luo L. (2021) Effects of oxidized silkworm (*Bombyx mori* L.) pupae on growth performance, and intestine, liver and muscle histology and function of Gif Tilapia (*Oreochromis niloticus*). Aquaculture Research 52(9): 4127–4137. doi:10.1111/are.15251.

Folch J.M.L. and Lees M.P. and Stanley G.H.A. (1957) A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues. The Journal of biological chemistry 226: 497–509. doi:10.1016/S0021-9258(18)64849-5.

Gangadhar B., Umalatha H., Ganedpish H., Saurabh S. and Sridhar N. (2017) Digestibility of dry matter and nutrients from three ingredients by the carps, *Labeo fimbriatus* (Bloch, 1795) and *Cyprinus carpio* Linnaeus, 1758 with a note on digestive enzyme activity. Indian Journal of Fisheries 64(3): 75–84.

Gohl B. (1981) Tropical Feeds. Food and Agricultural Organization of the United Nations, Rome. 528pp.

Gunya B., Masika P.J., Hugo A., Muchenjen V. (2016) Nutrient composition and fatty acid profiles of oven-dried and freeze-dried earthworm *Eisenia foetida*. Journal of Food and Nutrition Research 4 (6): 343–348.

Hashizume A., Ido A., Ohta, T., Thiaw S.T., Morita R., Nishikawa M., Takahashi T., Miura C. and Miura T. (2019) Housefly (*Musca domestica*) larvae preparations after removing the hydrophobic fraction are effective alternatives to fish meal in Aquaculture Feed for Red Seabream (*Pagrus major*). Fishes 4: 38.

Idowu A.B., Amusan A.A.S. and Oyediran A.G. (2003) The response of *Clarias gariepinus* fingerlings (Burchell 1822) to the diet containing housefly maggot (*Musca domestica*) (L). Nigerian Journal of Animal Production 30(1): 139–144.

Jayaram M.C. and Shetty H.P.C. (1980). Studies on the growth rates of catla, rohu and common carp fed on different formulated feeds. Mysore journal of agricultural sciences 14(4): 598–606.

Ji H., Zhang J.L., Huang J.Q., Cheng X.F. and 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.

Karthick Raja P. (2019) Effect of silkworm pupae (*Bombyx mori*) on the growth and maturation of Rainbow shark, (*Epalzeorhynchos frenatum*) (Fowler, 1934) under captive rearing", M.F.Sc. Thesis (unpublished), Dr. J. Jayalalitha Fisheries University, Tamil Nadu.

Khatun R., Azmal S.A., Sarker M.S.K., Rashid M.A., Hussain M.A. and Miah M.Y. (2005) Effect of Silkworm pupae on the growth and egg production performance of Rhode Island Red (RIR) pure line. International Journal of Poultry Science 4: 718–720.

Kostecka J. and P¹czka G. (2006) Possible use of earthworm *Eisenia fetida* (Sav.) biomass for breeding aquarium fish. European Journal of Soil Biology 42: 231–233.

Kroeckel S., Harjes A.G., Roth I., Katz H., Wuertz S., Susenbeth A. and Schulz C. (2012) When a turbot catches a fly: Evaluation of a pre-pupae meal of the black soldier fly (*Hermetia illucens*) as fish meal substitute – Growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). Aquaculture 364: 345–352.

Kurbanov A.R., Milusheva R.Y., Rashidova S.S. and Kamilov B.G. (2015) Effect of replacement of fish meal with silkworm (*Bombyx mori*) pupa protein on the growth of *Clarias gariepinus* fingerling. International Journal of Fisheries and Aquatic Studies 2(6): 25–27.

Lin Y.-H. and Mui J.-J. (2017) Evaluation of dietary inclusion of housefly maggot (*Musca domestica*) meal on growth, fillet composition and physiological responses for barramundi, *Lates calcarifer*. Aquaculture Research 48: 2478–2485.

Longvah T., Mangthya K. and Ramulu P. (2011) Nutrient composition and protein quality evaluation of eri silkworm (*Samia ricinii*) prepupae and pupae. Food Chemistry 128(2): 400–403. doi:10.1016/j.foodchem.2011.03.041.

Makkar H.P., Tran G., Heuzé V. and Ankers P. (2014) State-of-the-art on use of insects as animal feed. Animal feed science and technology 197: 1-33. doi: 10.1016/j.anifeedsci.2014.07.008.

Mohanta K.N., Subramanian S. and Korikanthimath V.S. (2016) Potential of earthworm (*Eisenia foetida*) as dietary protein source for rohu (*Labeo rohita*) advanced fry. Cogent Food and Agriculture 2(1): 1138594. doi: 10.1080/23311932. 2016.1138594.

Mohapatra S., Chakraborty T., Prusty A.K., Das P., Paniprasad K. and Mohanta K.N. (2012) Use of different microbial probiotics in the diet of rohu, *Labeo rohita* fingerlings: Effects on growth, nutrient digestibility and retention, digestive enzyme activities and intestinal microflora. Aquaculture Nutrition 18: 1–11.

Nandeesha M.C., Srikanth G.K., Keshavanath P., Varghese T.J., Basavaraja N. and Das S.K. (1990) Effects of non-defatted silkworm-pupae in diets on the growth of common carp, *Cyprinus carpio*. Biological Wastes 33(1): 17–23.

Nandeesha M.C., Gangadhara B., Varghese T.J. and Keshavanath P. (2000) Growth response and flesh quality of common carp, *Cyprinus carpio* fed with high levels of nondefatted silkworm pupae. Asian Fisheries Science 13(3): 235–242.

Ngoc T., Pucher J., Becker K. and Focken U. (2016) Earthworm powder as an alternative protein source in diets for common carp (*Cyprinus carpio* L.). Aquaculture Research 47: 2917–2927.

NRC (2011) Nutrient Requirements of Fish and Shrimp. National Academies Press, Washington, DC. pp57–92.

Ogunji J., Schulz C. and Kloas W. (2008) Growth performance, nutrient utilization of Nile tilapia *Oreochromis niloticus* fed housefly maggot meal (magmeal) diets. Turkish Journal of Fisheries and Aquatic Sciences 8(1): 141–147.

Popek W., £uszczek E. and Roœciszewska M. (1996) Influence of supplemental feeding of earthworm (*Eisenia fetida*) (Annelida, Lumbricidae) on growth, gonad maturation and spawning in goldfish (*Carassius auratus*). Agriculture Academy 310: 45–53.

Pucher J., Mayrhofer R., El-Matbouli M. and Focken U. (2014) Effects of modified pond management on limnological parameters in small scale aquaculture ponds in mountainous Northern Vietnam. Aquaculture Research 47(1): 1-15. doi:10.1111/are.12465

Rahimnejad S., Hu S., Song K., Wang L., Lu K., Wu R. and Zhang C. (2019) Replacement of fish meal with defatted silkworm (*Bombyx mor*i L.) pupae meal in diets for Pacific white shrimp (*Litopenaeus vannamei*). Aquaculture 510: 150–159.

Sahoo S., Jain K.K., Sahu N.P., Deo A.D., Shamna N., Patro D. and Maiti M.K. (2020) Dietary optimisation of black gram (*Vigna mungo*) leaf meal as substitute for deoiled rice bran in the diet of *Labeo rohita* (Hamilton, 1822) fingerlings. Indian Journal of Fisheries 67(4): 71–79.

Salem M.F., Khalafalla M.M.E., Saad I.A.I. and El-Hais A.M.A. (2008) Replacement of fish meal by silkworm *Bombyx mori* pupae meal, in Nile tilapia, *Oreochromis niloticus* diets. Egyptian Journal of Nutrition and Feeds 11(3): 611–624.

Shakoori M., Gholipour H., Naseri S. and Khara H. (2016) Growth, survival, and body composition of rainbow trout, *Oncorhynchus mykiss*, when dietary fish meal is replaced with silkworm (*Bombyx mori*) pupae. Archives of Polish Fisheries 24(1): 53–57.

Singh P.K., Gaur S.R. and Chari M.S. (2006) Effect of Varying Protein Levels on the Growth of Indian Major Carp Rohu, *Labeo rohita* (Hamilton). International Journal of Zoological Research 2(2): 186–191. doi: 10.3923/ijzr.2006.186.191.

Sogbesan A.O. and Ugwumba A.A.A. (2008) Nutritional Values of Some Non-Conventional Animal Protein Feedstuffs Used as Fishmeal Supplement in Aquaculture Practices in Nigeria. Turkish Journal of Fisheries and Aquatic Sciences 8: 159–164.

Swamy H.V. and Devaraj K.V. (1994) Nutrient utilization by common carp (*Cyprinus carpio* Linn) fed protein from leaf meal and silkworm pupae meal based diets. Indian Journal of Animal Nutrition 11(2): 67–71.

Tacon A.G.J. and Metian M. (2009) Fishing for aquaculture: Non-food use of small pelagic forage fish-a global perspective. Reviews in Fisheries Science 17: 305–317.

Tacon A.G.J., Hasan M.R. and Metian M. (2011) Demand and supply of feed ingredients for farmed fish and crustaceans: trends and prospects. FAO Fisheries and Aquaculture technical paper 564: 87pp.

Teotia J.S. and Miller B.F. (1973) Fly pupae as a dietary ingredient for starting chicks. Poultry Science 52: 1830–1835.

Tian F., Zhou J., Sun Z., Cai Z., Xu N, An M. and Duan S. (2014) Inhibitory effects of Chinese traditional herbs and herb-modifies clays on the growth of harmful algae *Phaeocystis globosa* and *Prorocentrum donghaiense*. Harmful Algae 37: 153 –159.

Tuan N.N. (2010) Development of supplemental diets for carp in Vietnamese upland ponds based on locally available resources. PhD thesis in Agriculture Sciences, Stuttgart, University of Hohenheim.

Umalatha H., Gangadhar B., Hegde G. and Sridhar N.

(2018) Digestibility of three feed ingredients by *Catla catla* (Hamilton, 1822). Oceanography and Fisheries Open Access Journal 5: 555–672.

Vodounnou D.S., Juste V., Kpogue D.N.S., Apollinaire M.G. and Didier F.E. (2016) Culture of earthworm (*Eisenia fetida*), production, nutritive value and utilization of its meal in diet for *Parachanna obscura* fingerlings reared in captivity. International Journal of Fisheries and Aquatic Studies 4(5): 01–05.

Wang L., Li J., Jin J.N., Zhu F., Roffeis M. and Zhang X.Z. (2017) A comprehensive evaluation of replacing fishmeal with housefly (*Musca domestica*) maggot meal in the diet of Nile tilapia (*Oreochromis niloticus*): growth performance, flesh quality, innate immunity and water environment. Aquaculture Nutrition 23(5): 983–993.

Xu X., Ji H., Yu H. and Zhou J. (2018) Influence of replacing fish meal with enzymatic hydrolysates of defatted silkworm pupa (*Bombyx mori* L.) on growth performance, body composition and nonspecific immunity of juvenile mirror carp (*Cyprinus carpio* var. specularis). Aquaculture research 49(4): 1480–1490.

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