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Nutritional evaluation of a short-horned grasshopper, *Oxya hyla hyla* (Serville) meal as a substitute of fishmeal in the compound diets of rohu, *Labeo rohita* (Hamilton)

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Abstract

Background: Aqua feed demand is rising with the growth of intensive aquaculture. Fishmeal is the major conventional protein source of fish feed but its production cannot be increased due to resource limitation. Thus, suitable fishmeal (FM) substitute is a need to sustain the growth of aquaculture. Use of edible insects in animal and fish feed is gaining interest. Grasshoppers are edible insect and contain considerably high percentage of crude proteins and other nutrients. In the present study, the nutritional efficiency of *Oxya hyla hyla* meal as replacer of FM was evaluated by feeding *Labeo rohita* fingerlings with seven iso-nitrogenous compounded diets (33% crude protein) where FM was substituted by *Oxya* meal (OM) at 0%, 17%, 33%, 50%, 67%, 83% and 100% levels in diet 1 (D1) to diet 7 (D7) respectively. Feeding trail was continued for 100 days.

Results: Feeding experiments showed that OM-incorporated diets were acceptable to the fish and feeding on the diets they grew significantly after 100 days feeding trial in wet weight and length. Results revealed that up to 50% replacement of FM (up to diet 4) did not affect the growth performances, condition factor of fish, apparent protein digestibility, nitrogen metabolism, food conversion ratio (FCR) and protein efficiency ratio (PER) with respect to reference diet (D1, without OM). FCR and PER were found inferior in the diets D5, D6 and D7 where more than 50% FM was replaced. Moreover, diets regardless of FM replacement level showed no negative impact on hepato-somatic index, digesto-somatic index, relative length of gut, retention of protein, lipid and ash and on carcass compositions of the fish.

Conclusion: The results revealed that the grasshopper species, *O. hyla hyla*, might be a new protein-rich ingredient for aqua feed formulation and up to 50% replacement of FM by OM did not affect the growth performances, feed utilisation parameters and flesh quality of the fish *L. rohita* fingerlings. Therefore, this study suggested that OM meal could be incorporated at least as partial substitute of FM in the compounded carp diet.

Keywords: Grasshopper meal, Protein source, Aqua feed, *Labeo rohita*

Background

Aquaculture is now a rapid growing sector in India, and to maintain its sustainable growth and productivity, proper supplementary feeding management is an essential need. Fishmeal has been used conventionally as a protein-rich ingredient in aqua feed formulation because of its compatibility in protein and amino acid requirements of fish (Alam, Maughan, & Matter, 1996; Watanabe, Verakunpiriya, Watanabe, Kiron, & Satoh, 1997). However, due to constrain of its uncertainty and inadequate availability and increasing cost, partial or complete replacement of fishmeal by other protein-rich feed staffs is becoming necessary. Numerous considerable research efforts have been undertaken to find out suitable substitutes of fishmeal from plant as well as animal sources (Gur, 1997; Kaushik, Coves, Dutto, & Blanc, 2004; Middleton, Ferket, Boyd, Daniels, & Gallagher, 2001; Mukhopadhyay & Ray, 1999; Ojha et al., 2014;

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Rangacharyulu et al., 2003; Subhadra, Lochmann, Rawles, & Chen, 2006; Subramanian & Balasubramanian, 2014; Turker, Yigit, Ergun, Karaali, & Erteken, 2005; Webster, Goodgame-Tiu, & Tidwell, 1995; Webster, Yancey, & Tidwell, 1992; Yang et al., 2004; Yoshitomi, Aoki, & Oshima, 2007). Plant ingredients are cheap source of protein. However, inclusion of plant ingredients in high level or complete substitution of animal protein by plant materials may result in poor growth and feed efficiency in fish (Dabrowski, Kok, & Takashima, 1986; Lim, 1992) due to a number of factors including the presence of anti-nutritional factors and improper amino acid balance. Many animal protein sources viz., meat and bone meal, feather meal, poultry by product meal and silkworm pupae meal have been tried as an alternative of fishmeal (Hassan & Khan, 1999; Rangacharyulu, Paul, Nandi, Sarkar, & Mukhopadhyay, 2000; Singh et al., 2005).

Many workers have suggested the use of grasshopper meal in animal and fish feed formulation (Alegbeleye, Obasa, Olude, Otubu, & Jimoh, 2012; Anand, Ganguly, & Haldar, 2008; DeFoliart, 1999; Finke, 2013; Rumpold & Schlüter, 2013; Ueckert, Yang, & Albin, 1972; Wang, Shao-Wei, Chuan-XI, Zhang, & Chen, 2007). Studies revealed that grasshoppers are rich in protein, essential amino acids and other nutrients (Ganguly et al., 2013; Wang et al., 2007). Several reports suggested that dietary protein of fishmeal could be partially substituted in the fish diets with different grasshopper meal without compromising their growth, reproduction and feed utilisation efficiency (Balogun, 2011; Emehinaiye, 2012; Ganguly et al., 2014; Johri, Singh, & Johri, 2010). *Oxya hyla hyla* (Serville) is a multivoltine grasshopper distributed throughout the Indian subcontinent, and this species can be farmed for biomass production (Ghosh, Haldar, & Mandal, 2014, 2015). Moreover, the dry matter of this insect contains about 64% crude protein, good amount of essential amino acids, fat, minerals and vitamins and negligible amount of anti-nutritional factors (Ghosh, Haldar, & Mandal, 2016). *Labeo rohita* is a major carp, excellent table fish and an essential candidate in aquaculture system in India. Though different grasshopper meals have been tried for inclusion in fish diets but there is no data available on the utilisation of grasshopper meal in the diet of cultivable carps. Keeping this information in mind, the present study has been undertaken for nutritional evaluation of a grasshopper species, *O. hyla hyla* as a partial substitute of fishmeal in the compound diets of an Indian major carp, *L. rohita.*

Materials and methods

Procurement of grasshopper biomass

The multivoltine grasshopper species, *O. hyla hyla*, was reared in the insectariums of the Department of Zoology following the method as suggested by Haldar, Das, and Gupta (1999). Five thousand adult insects (average wet weight of each individual 0.305 ± 0.019 g) were procured for *Oxya* meal (OM) preparation. Prior to procurement, insects were kept deprived of food for 24 h to make their gut empty. Wings and appendages were removed to get rid of excess chitin, and the insects were dried under a hot air oven at 40 °C until weight became constant. The dried insects were milled and used for fish feed formulation.

Estimation of proximate compositions of ingredients and feeds

Fishmeal (FM) (Bombay duck, *Harpadon nehereus*), OM, soybean meal (SBM), mustard oil cake (MOC), rice bran (RB) and wheat flour (WF) were taken as major feed ingredients. Proximate compositions of the dried ingredients were estimated following the methods of AOAC (2005). Nitrogen content was estimated using Micro-Kjeldahl digestion and distillation method, and crude protein content was calculated by multiplying the value of nitrogen content with the factor 6.25. The crude lipid was estimated by ether extraction method using Soxhlet apparatus. After lipid extraction, sample was digested with 1.25% H_2SO_4 and 1.25% NaOH and subjected to 550 ± 50 °C in muffle furnace till the sample was burnt to ashes and crude fibre was determined as loss on ignition of the dried lipid-free residue. Ash content was determined by subjecting the samples in muffle furnace at 550 ± 50 °C for about 6 h. Carbohydrate content was calculated by difference method (100—sum of protein, fat and ash). Nitrogen-free extract (NFE) was calculated by subtracting the sum of crude protein, fat, ash and fibre from 100.

Diet formulation

Seven iso-nitrogenous, experimental diets (D1 to D7) containing about 33.62 ± 1.76% crude protein were prepared taking 30% of the ingredients from animal sources. Of these 30% ingredients, FM to OM ratio from D1 to D7 were 30:0, 25:5, 20:10, 15:15, 10:20, 5:25 and 0:30. Therefore, FM was substituted by OM in D1 to D7 at a rate of 0%, 17%, 33%, 50%, 67%, 83% and 100% respectively. Diet 1 (D1) was without OM and was considered as reference diet (RD). The amount of SM and MOC were provided with 22% and 10% respectively in all diets. Desired crude protein level was adjusted by balancing the proportion of RB and WF following Pearson's square model (De Silva & Anderson, 1998). Carboxy methyl cellulose (CMC) (3%) was added as a binder, and chromic oxide (1%) was used as non-digestible marker to estimate apparent digestibility. Vitamin premix and minerals were added (Table 2). All the ingredients were dried, milled and mixed well by adding water, after which they were passed through a hand pelletizer of 0.5 mm pellet diameter. The pellets were dried in hot air oven for 24 h, crumbled and stored in air tight containers. Proximate compositions of the diets were estimated following the methods of AOAC (2005). Metabolisable energy (ME) of the diets was calculated considering caloric values of protein 4.5 Kcal g^{-1}, lipid 8.51 Kcal g^{-1} and carbohydrate 3.49 Kcal g^{-1} according to Jauncey (1982).

Experimental design

The feeding trials of the experimental diets were conducted on *L. rohita* fingerlings in circular tanks of (26 L) filled with tap water. Fingerlings were procured from local fish farm at Santiniketan, West Bengal, and acclimatised to aquarium conditions and supplementary feeding for 20 days. After acclimation fingerlings were weighed to record initial weight. Fingerlings (average initial weight 1.50 ± 0.19 g) were randomly distributed to the circular tanks at 20 fish in each tank. Experiments were conducted in triplicate for each dietary treatment and continued for 100 days. Fish were fed with known amount of feed at 3% body weight once daily at 10.00 am. The amount of feed was adjusted with increase of body weight in every 15-day interval. Fish were allowed to feed for 4 h, and uneaten feeds were collected, oven dried, weighed and subjected to further analysis. Experimental tanks were kept under continuous aeration to avoid oxygen deficiency. About 50% water of the tank was changed in every alternate day and was scrubbed once a week to prevent algal growth. Water quality was checked throughout the experiment period. Faecal matters were collected before providing feeds by careful siphoning method (Spyridakis, Metailler, Gabaudan, & Riaza, 1989) and were oven dried at 60 °C and used for analysis.

Water parameter analysis

Water temperature, pH, dissolved oxygen (DO) and hardness of experimental tanks were estimated in every fortnight following the methods of APHA (1990). Water temperature was in the range of 25.4 to 30.5 °C, pH 7.14 to 7.61, DO 4.14 to 7.82 mg L^{-1} and hardness was 172.4 to 189.6 mg L^{-1}.

Estimation of growth performance and survival rate

Growth performances of the fingerlings were recorded as percentage of wet weight and length gain, percent of specific growth rate (%SGR), percent of average daily weight gain (%ADG) and condition factor (K). Survival rate was estimated as percent of surviving fish. The parameters were measured following the methods of De Silva and Anderson (1998) and Moyle and Cech Jr (1996) and were calculated as follows:

$$\text{Weight gain }(\%) = 100 \times \frac{(\text{Wt}_2\text{-Wt}_1)}{\text{Wt}_1}$$

$$\text{Condition factors (K)} = \frac{\text{weight (g)}}{\text{length (cm)}^3} \times 100$$

$$\text{Specific growth rate }(\text{SGR}\%\text{day}^{-1}) = 100 \times \frac{(\text{InWt}_2 - \text{InWt}_1)}{(t_2 - t_1)}$$

$$\text{Average daily weight gain }(\%\text{ADG}) = 100 \times \frac{(\text{Wt}_2 - \text{Wt}_1)}{\text{Wt}_1 \times (t_2 - t_1)}$$

where Wt_2 = final wet weight, Wt_1 = initial wet weight and $t_2 - t_1$ = period of experiment.

$$\text{Survival rate }(\%) = 100 \times \frac{\text{total number of surviving fish}}{\text{total number of fish stocked}}$$

Estimation of feed utilisation indices

Feed utilisation indices such as feed conversion ratio (FCR) and protein efficiency ratio (PER) were estimated following the methods of De Silva and Anderson (1998). Protein retention value (PRV) and lipid retention value (LRV) were estimated following the method of Xie et al. (2011). Nitrogen metabolism (Nm) was calculated according to Jamabo and Alfred-Ockiya (2008).

Apparent protein digestibility (APD) was estimated by estimating chromic oxide in the diets and faeces following the spectrophometric method (Divakaran, Leonard, & Ian, 2002). Protein levels were estimated in the diets and faecal samples following AOAC (2005) methods. Apparent protein digestibility (APD) value was then calculated by using the formulae (De Silva & Anderson, 1998) as follows:

$$\text{Feed conversion ratio (FCR)} = \frac{\text{dry weight of feed consumed (g)}}{\text{wet weight gain (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{wet weight gain (g)}}{\text{crude protein fed (g)}}$$

$$\text{Protein retention value (PRV)} = 100 \times \frac{\text{fish protein gain (g)}}{\text{protein intake (g)}}$$

$$\text{Lipid retention value (LRV)} = 100 \times \frac{\text{fish lipid gain (g)}}{\text{lipid intake (g)}}$$

$$\text{Nitrogen metabolism (Nm)} = \frac{0.54 \times (\text{Wt}_2 - \text{Wt}_1) \times t}{2}$$

[where Wt_1 = initial weight, Wt_2 = final weight, and t = number of days]

$$\text{Apparent protein digestibility (APD)} = 100 - \left(\frac{\%\text{chromium in food}}{\%\text{chromium in faeces}} \times \frac{\%\text{Protein in faeces}}{\%\text{Protein in food}}\right)$$

Estimation of body indices, carcass composition

Hepatosomatic index (HSI), relative length of gut (RLG) and digesto-somatic index (DSI) were

Table 1 Proximate composition of feed ingredients (% of dry matter)

Components	OM	FM	SBM	MOC	RB	WF
Crude protein	64.67 ± 1.61	55.30 ± 2.50	37.64 ± 2.48	35.60 ± 1.43	13.44 ± 0.65	10.75 ± 0.23
Crude lipids	2.58 ± 0.09	10.32 ± 1.08	5.36 ± 0.05	8.91 ± 0.87	5.04 ± 0.11	0.92 ± 0.04
Crude fibre	9.23 ± 0.70	4.10 ± 0.40	7.56 ± 0.13	5.35 ± 0.10	22.65 ± 0.40	0.81 ± 0.06
Ash	4.59 ± 0.18	15.59 ± 0.34	5.59 ± 0.53	8.36 ± 1.30	21.57 ± 0.42	0.55 ± 0.03
NFE	18.94 ± 1.76	14.61 ± 2.07	45.88 ± 4.65	40.97 ± 1.48	37.14 ± 1.06	86.89 ± 0.16

Means with ±SD are values of triplicate groups. *OM Oxya* meal, *FM* fishmeal, *SBM* soybean meal, *MOC* mustard oil cake, *RB* rice bran, *WF* wheat flour, *NFE* nitrogen-free extract

examined, following the methods of Xie et al. (2011). Carcass compositions (moisture, protein, lipid and ash content as % of wet weight of tissue) of the fish were estimated following the methods of AOAC (2005).

$$\text{Hepatosomatic index (HSI)} = 100 \times \frac{\text{liver weight (g)}}{\text{weight of the fish (g)}}$$

$$\text{Relative length of Gut (RLG)} = \frac{\text{Gut length (cm)}}{\text{Total length of the fish (cm)}}$$

$$\text{Digesto–somatic index (DSI)} = 100 \times \frac{\text{wet weight of digestive tract (g)}}{\text{wet weight of the fish (g)}}$$

Statistical analysis

Values were presented as means ± standard deviation (SD). A one-way analysis of variance (ANOVA) was used to compare the values obtained for the different parameters using Microsoft excel 2007 software. Duncan's multiple range tests (DMRT) were used to determine whether mean values differed significantly or not. Significance was accepted at probabilities of 0.05 or less.

Results

Proximate composition of ingredients and feeds

Proximate compositions of the feed ingredients are represented in Table 1. Results revealed that crude protein content of OM (64.67 ± 1.61%) was higher than FM (55.30 ± 2.50%). SBM and MOC contain about 37.64% and 35.6% crude protein on dry matter basis. The proximate

Table 2 Ingredients and proximate composition of the formulated diets (%)

Ingredient (%)	RD	TD					
	D1	D2	D3	D4	D5	D6	D7
Oxya meal	0	5	10	15	20	25	30
Fishmeal	30	25	20	15	10	5	0
Soybean meal	10	10	10	10	10	10	10
Mustard oil cake	22	22	22	22	22	22	22
Rice bran	25	30	12	4	10	12	13
Wheat flour	8	3	21	29	23	21	20
CMC	3	3	3	3	3	3	3
Vitamin and minerals	1	1	1	1	1	1	1
Chromic oxide	1	1	1	1	1	1	1
Proximate composition (% of dry matter)							
Crude protein	33.15 ± 1.69	33. 81 ± 1.70	33.08 ± 1.72	33.55 ± 1.86	33.33 ± 1.97	34.16 ± 1.87	34.23 ± 1.70
Crude lipid	6.84 ± 0.21	6.76 ± 0.44	5.78 ± 0.48	5.40 ± 0.43	5.05 ± 0.86	4.64 ± 0.50	4.24 ± 0.32
Carbohydrate	48.05 ± 1.80	46.47 ± 1.82	51.97 ± 1.79	52.28 ± 1.94	52.99 ± 1.99	53.93 ± 2.08	54.72 ± 1.70
Ash	11.97 ± 0.45	12.95 ± 0.35	9.17 ± 0.32	8.78 ± 0.43	8.62 ± 0.47	7.26 ± 0.39	6.81 ± 0.39
Crude fibre	8.62 ± 0.33	10.05 ± 0.24	7.17 ± 0.50	7.35 ± 0.48	7.82 ± 0.64	7.67 ± 0.44	7.92 ± 0.76
Nitrogen-free extract	39.43 ± 1.92	36.42 ± 1.78	44.80 ± 1.87	44.93 ± 1.80	45.17 ± 1.99	46.27 ± 2.18	46.81 ± 1.89
ME (Kcal 100 g^{-1})	344.96 ± 2.32	336.81 ± 3.36	354.42 ± 3.92	353.68 ± 3.26	350.68 ± 3.36	354.71 ± 3.82	353.46 ± 2.60

Means with ±SD are values of triplicate groups. *RD* reference diet; *TD* test diet; *CMC* carboxy methyl cellulose, vitamin and mineral mixture (Supradyn, Abbott Healthcare Pvt. Ltd., Mumbai, India); *ME* metabolised energy (Kcal 100 g^{-1})

Table 3 Growth parameters, condition factor and survival rate of *L. rohita* fingerlings after 100 days feeding

	RD	TD					
	D1	D2	D3	D4	D5	D6	D7
Length gain (%)	33.71 ± 1.62[a]	32.40 ± 5.56[a]	31.61 ± 3.01[a]	31.94 ± 3.53[a]	27.34 ± 2.65[b]	29.82 ± 2.61[b]	28.26 ± 1.91[b]
Weight gain (%)	155.85 ± 29.61[a]	158.05 ± 27.07[a]	164.72 ± 20.48[a]	177.03 ± 24.55[a]	144.90 ± 30.33[b]	146.11 ± 31.04[b]	140.79 ± 34.13[b]
K	1.79 ± 0.15	1.80 ± 0.08	1.78 ± 0.11	1.77 ± 0.14	1.72 ± 0.15	1.76 ± 0.19	1.77 ± 0.11
SGR (%)	0.41 ± 0.06[a]	0.42 ± 0.06[a]	0.43 ± 0.04[a]	0.44 ± 0.04[a]	0.37 ± 0.05[b]	0.39 ± 0.07[b]	0.36 ± 0.06[b]
ADG (%)	1.63 ± 0.41[a]	1.65 ± 0.38[a]	1.69 ± 0.23[a]	1.79 ± 0.25[a]	1.40 ± 0.29[b]	1.50 ± 0.41[b]	1.62 ± 0.38[b]
Survival rate	81.67 ± 5.77[b]	88.33 ± 2.89[a]	88.33 ± 2.89[a]	88.67 ± 2.89[a]	88.33 ± 2.89[a]	88.33 ± 2.89[a]	80.00 ± 5.00[b]

Means with ±SD are values of triplicate groups. Figures in the same row with same superscripts are not significantly different (one-way ANOVA followed by DMRT, $p > 0.05$). *RD* reference diet, *TD* test diet, *K* condition factor, *SGR* specific growth rate, *ADG* average daily weight gain

compositions of the prepared diets are presented in Table 2 which shows that diets are iso-nitrogenous containing about 33% crude protein.

Growth performance and survival rate of the fish

During feeding experiments, it has been noticed that OM-incorporated experimental diets were acceptable and palatable to the fish and fish grew significantly ($p < 0.05$) from their initial wet weight and length after 100 days feeding. The results showed that feeding on OM-incorporated diets caused no significant differences in condition factor, growth performances and percent of survival among the fish of different groups (Table 3). SGR (%day^{-1}) and ADG (%) of the fish fed with experimental diets and reference diet (D1) were similar. Wet weight and length gain the fish fed with D3 and D4 were better than the fish fed on reference (D1) diet but the fish fed on D6 and D7 diets containing higher level of OM showed inferior results.

Feed quality and feed utilisation

Data obtained regarding feed quality and feed utilisation assessment were presented in Table 4. The results (Table 4) showed that FCR of the experimental diets were not affected rather improved in D3 and D4 diets. Similarly, PER was improved in D3 and D4 diets. However, feeds (D5, D6 and D7) which were prepared with higher proportion of OM showed significantly ($p < 0.05$) reduction in PER and Nm. Protein and lipid retention values in the fish fed on test diets did not differ significantly ($p < 0.05$) from the control. APD of the feeds D2, D3 and D4 was similar to reference diet (D1) but APD reduced significantly in D5 to D7.

HIS, RLG, DSI and carcass composition

Feeding experiments showed that feeding on OM-incorporated diets had no negative impact on HSI, RLG and DSI (Table 5). The proximate compositions of carcass on wet matter basis of the fish fed on experimental diets revealed that carcass protein and lipid contents were significantly increased in the fish fed on D2, D3 and D4 diets than D1 fed fish. However, in fish fed with D5 to D7 diets carcass lipid content was decreased (Table 6).

Discussion

Protein is an essential and most important component of a fish feed, and it should be at optimum level to obtain the best growth performance of fish. The conventional protein source for aqua-feed industry is fishmeal. Due to resource limitation, fishmeal production is inadequate with respect to its demand and expensive. Thus, cheaper and efficient substitutes of fishmeal are necessary. Soybean meal, rapeseed meal and oil cakes are

Table 4 Feed utilisation parameters of *L. rohita* fingerlings after 100 days of feeding

	RD	TD					
	D1	D2	D3	D4	D5	D6	D7
FCR	1.89 ± 0.38[a]	1.99 ± 0.48[a]	1.82 ± 0.22[b]	1.72 ± 0.20[b]	2.19 ± 0.40[a]	2.05 ± 0.39[a]	1.95 ± 0.41[a]
PER	1.59 ± 0.08[b]	1.62 ± 0.09[a]	1.67 ± 0.10[a]	1.74 ± 0.12[a]	1.36 ± 0.07[c]	1.40 ± 0.06[c]	1.54 ± 0.17[b]
PRV	1.45 ± 0.17	1.43 ± 0.14	1.46 ± 0.24	1.48 ± 0.23	1.46 ± 0.31	1.44 ± 0.29	1.46 ± 0.37
LRV	2.68 ± 0.27	2.63 ± 0.38	2.53 ± 0.39	2.73 ± 0.30	2.50 ± 0.39	2.72 ± 0.44	2.61 ± 0.56
ARV	1.09 ± 0.16	1.13 ± 0.20	1.09 ± 0.30	1.15 ± 0.20	1.27 ± 0.22	1.27 ± 0.23	1.20 ± 0.17
Nm	64.04 ± 6.61[a]	58.81 ± 6.88[b]	65.80 ± 3.20[a]	67.39 ± 2.54[a]	56.97 ± 1.27[b]	59.51 ± 8.21[b]	60.86 ± 4.23[b]
APD	80.28 ± 1.23[a]	78.11 ± 2.28[b]	80.37 ± 1.61[a]	81.66 ± 1.65[a]	78.55 ± 2.62[b]	77.97 ± 2.57[b]	79.12 ± 2.56[b]

Means with ±SD are values of triplicate groups. Figures in the same row with different superscripts are significantly different (one-way ANOVA followed by DMRT, $p < 0.05$). *RD* reference diet, *TD* test diet, *FCR* feed conversion ratio, *PER* protein efficiency ratio, *PRV* protein retention value, *LRV* lipid retention value, *ARV* ash retention value, *Nm* nitrogen metabolism, *APD* apparent protein digestibility

Table 5 Effect on HSI, RLG and DSI of *L. rohita* fingerling after 100 days of feeding

	RD	TD					
	D1	D2	D3	D4	D5	D6	D7
HSI	0.71 ± 0.06	0.71 ± 0.11	0.70 ± 0.10	0.71 ± 0.07	0.72 ± 0.10	0.72 ± 0.11	0.70 ± 0.09
RLG	3.43 ± 0.07	3.43 ± 0.10	3.44 ± 0.06	3.43 ± 0.09	3.42 ± 0.10	3.45 ± 0.06	3.43 ± 0.08
DSI	4.25 ± 0.08	4.28 ± 0.13	4.28 ± 0.11	4.26 ± 0.08	4.30 ± 0.06	4.29 ± 0.13	4.26 ± 0.09

Means with ±SD are values of triplicates groups. Figures in the same row with same superscripts are not significantly different (one-way ANOVA, $p > 0.05$). *RD* reference diet, *TD* test diet, *HSI* hepatosomatic index, *RLG* relative length of gut, *DSI* digest-somatic index

efficiently used in fish feed formulation (Dossou et al., 2018; Webster et al., 1992, 1995). However, animal-derived protein sources are found to be better than plant sources because of higher amino acid compatibility and less content of anti-nutritional factors. The present study demonstrated the feasibility of the use of a grasshopper *O. hyla hyla* meal as an alternate protein source in feed formulation for an Indian major carp, *L. rohita*. The dietary protein requirement for *L. rohita* has been suggested to be 30–35% to obtain high feed efficiency and growth performance (Chakrabarty, Chowdhry, & Chakrabarty, 1999; Rangacharyulu et al., 2000). Hence, 33% dietary protein was considered for the preparation of experimental diets. The major ingredients from plant protein sources like SBM and MOC were taken in a fixed proportion for all diets and keeping in mind that if quality of feeds differed that would be due to substitution of FM by OM. The results after 100 days feeding experiments clearly revealed that acceptability of the feeds was not affected due to OM incorporation rather feeding on these diet fish grew significantly ($p < 0.05$) from their initial wet weight and length. The results showed that diets (D2, D3 and D4) with up to 50% replacement of FM by OM did not affect food consumption, feed utilisation and growth performances of the fish.

Condition factor indicates health status of the fish, and it is used to determine the feeding activity (Lizama & Ambrosio, 2002). In the present study, condition factor of the fish of different feeding groups showed no significant variations. This indicated that OM-incorporated diets had no adverse effect in maintaining good health status of the fish. The growth performances viz., wet weight gain (%) and length gain (%), SGR (% day^{-1}) and ADG (%) of the fingerlings fed on D2, D3 and D4 diets were almost similar to the D1 fed fish. Another work with insect-containing diets (Begum, Chakraborty, Zaher, Abdul, & Gupta, 1994) showed that feeding with the diets containing 50% of its protein contributed by silkworm pupae resulted significantly better SGR, FCR and PER for *L. rohita*. FCR and PER indicate nutritional value of feeds available for the growth of fish (De Silva & Anderson, 1998). The result of the present study showed that FCR and PER values of the diets, D3 and D4, were similar to the reference diet (D1) but the results were unsatisfactory when more than 50% FM was replaced by OM. This may be due to improper balance of required amino acids. Thus, up to 50% replacement of FM by OM could be successfully implemented.

Nutritional value of a feedstuff depends on its digestibility. According to Salim, Aziz, Sultan, and Mustafa (2004), digestible ingredients maximise nutrient availability and growth of fish and check the waste production. Digestibility of FM in carps varies from 80–85% depending on the origin and processing of fishmeal (Ogino & Chen, 1973). Bairagi, Ghosh, Sen, and Ray (2002) observed 89% apparent protein digestibility (APD) of a 40% FM containing diet fed to *L. rohita* fingerlings. APD of the present formulated feeds fed to *L. rohita* fingerlings were found to be from 77.9 to 81.6% having no significant differences. Similar results were obtained by Mukhopadhyay and Ray (1999) where they observed that APD of copra meal substituted diets of *L. rohita* were from 81.1 to 88.9%. Nitrogen metabolism of *L. rohita* fed on the diets D3 and D4 did not differ significantly to that D1 diet. Moreover, OM-incorporated

Table 6 Carcass composition (% of wet weight) of *L. rohita* fingerlings after 100 days feeding

	RD	TD					
	D1	D2	D3	D4	D5	D6	D7
Moisture	78.32 ± 1.19	78.74 ± 1.11	78.71 ± 1.15	78.98 ± 1.27	78.74 ± 1.48	78.22 ± 1.16	78.10 ± 0.98
Protein	15.94 ± 0.47[b]	16.06 ± 0.59[a]	16.31 ± 0.34[a]	16.41 ± 0.34[a]	16.32 ± 0.24[a]	16.13 ± 0.48[a]	16.04 ± 0.56[a]
Lipid	4.92 ± 0.15[a]	4.72 ± 0.23[a]	4.72 ± 0.17[a]	4.75 ± 0.25[a]	4.62 ± 0.28[b]	4.67 ± 0.29[b]	4.65 ± 0.34[b]
Ash	3.24 ± 0.37[b]	3.57 ± 0.23[a]	3.50 ± 0.30[a]	3.56 ± 0.21[a]	3.62 ± 0.31[a]	3.68 ± 0.35[a]	3.32 ± 0.30[a]

Means with ±SD are values of triplicate groups. Figures in the same row with same superscripts are not significantly different (one-way ANOVA, $p > 0.05$). *RD* reference diet, *TD* test diet diets had no negative effect on the protein, lipid and ash retention.

HSI increases due to conversion of excess glucose into glycogen and its deposition or decreases due to depletion of glycogen storage in case nutrient deficiency (Debnath et al., 2007). In this study, HSI of the fish fed on reference diet and test diets neither increased nor decreased and that indicated no nutrient deficiency was occurred by feeding on test diets. Moreover, test diets caused no adverse effects on RLG and DSI of the fish. The carcass composition often determines the flesh quality of fish and it depends on the feeding status. Feed quality and feeding rate affect the body composition of fish (Adebayo, Balogun, & Fagbenro, 2000; Hassan & Jafri, 1994; Khan, Jafri, & Chadha, 2004; Lovell, 1992; Panda, Mishra, & Samantaray, 1999). Hence, carcass composition and organoleptic properties of the fish flesh are taken into account during quality feed formulation. The present study revealed that protein and lipid content in the flesh were improved in D2, D3 and D4 diet fed fish than the fish fed on reference diet.

Conclusion

The results of this study revealed that up to 50% replacement of FM by OM meal in the diets of *L. rohita* fingerlings did not affect the growth performances, feed utilisation parameters and flesh quality of the fish. Hence, the short horn grasshopper *O. hyla hyla* may be a new protein-rich ingredient in aqua feed formulation and may be used as partial replacer of fishmeal in the compounded diets of fish.

Abbreviations

%ADG: Percent of average daily growth; %SGR: Percent of specific growth rate; ANOVA: Analysis of variance; APD: Apparent protein digestibility; CMC: Carboxy methyl cellulose; CO: Chromic oxide; CP: Crude protein; D: Diet; DO: Dissolved oxygen; DSI: Digesto-somatic index; FCR: Feed conversion ratio; FM: Fishmeal; H_2SO_4: Sulphuric acid; HIS: Hepatosomatic index; K: Condition factor; LVR: Lipid retention value; ME: Metabolisable energy; MOC: Mustard oil cake; NaOH: Sodium hydroxide; NFE: Nitrogen-free extract; Nm: Nitrogen metabolism; OM: *Oxya* meal; PER: Protein efficiency ratio; PRV: Protein retention value; RB: Rice bran; RD: Reference diet; RLG: Relative length of gut; SBM: Soybean meal; SD: Standard deviation; TD: Test diet; WF: Wheat flour

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Availability of data and materials

Published data of this article may be used for academic and non-commercial research purposes.

Authors' contributions

SG designed and carried out the experiments, collected samples, involved in analysis and interpretation of data and manuscript writing. DKM suggested the concept of the work, designed the study and involved in interpretation of data and manuscript writing. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

Rearing and handling of grasshoppers and cultivable fish used in this study have been conducted in compliance to the guideline of the 'Institutional Animal Ethics Committee' of Visva-Bharati University, India.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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