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NUTRITIONAL EVALUATION OF SHORT-HORNED GRASSHOPPER (*Oxya hyla hyla* Serville) AS A SUBSTITUTE FOR SOYBEAN MEAL IN COMPOUND DIETS OF ROHU (*Labeo rohita* Hamilton)

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ABSTRACT

The growth effects of partial substitution of soybean meal for grasshopper meal (Oxya hyla hyla) in compound diets of Rohu fish (Labeo rohita) were evaluated for 75 days at the aquaculture research center in IAAS, Paklihawa Campus with 16 meshed cages of 1 m³size, stocked with 12 fingerlings³. Completely Randomized Design was used with 4 treatments and 4 replications. Treatments included the incremental substitution of Soybean meal (SM) for Grasshopper meal (GM) as 0% GM, 10% GM, 20% GM and 30% GM. Feeding rates were adjusted every month based on observed body weight. Fish parameters were observed at 16, 27, 45, 60 and 75 days after stocking, whereas, the physical water parameters were observed daily. During the study period, the recorded mean water temperature, pH, dissolved oxygen and survivorship were 19°C, 8.2, 6.4 mg/L and 64.6% respectively. Average growth rate, relative growth rate, specific growth rate, daily weight gain and protein efficiency ratio showed no significant difference (p>0.05) among the treatments. The Feed Conversion Ratio of 20% GM and 30% GM were statistically similar and superior to 0% GM and 10% GM as well. To sum up, we did not have enough evidence to prove the superiority of grasshopper meal incorporated diet on rohu, perhaps because of a lower feed efficiency.

Keywords: Cage fish culture, Compound diets, Insect-protein substitution, Short-horned grasshopper

1. INTRODUCTION

Feed is considered the most important input in fish farming because it occupies about 50% of the whole farming cost (Craig et al., 2017). The fish feed has different ingredients originating from plant and animal sources. Plant sources are limited in the supply of nutrients like Vitamin B12 (Murphy & Allen, 2003; Rizzo et al., 2016; Watanabe et al., 2014). Furthermore, plant-based ingredients may contain anti-nutrients like phytic acid (Beckhout & Depaepe, 1994), glucosinolates (Liener, 1980), phytosterols (Ostlund et al., 2003), quinolizidine alkaloids like lupinin (Wink et al., 1998), various oligosaccharides (Wiggins, 1984), and protein inhibitors like trypsin. chymotrypsin, elastases and carboxypeptidases (Liner, 1980) have suppressive effects on the growth of feeding animal. Feed needs to be abundant in micronutrients to fulfil daily nutritional requirements. A high protein content is critical for fish growth and development. The amount of crude protein required in fish feed depends upon different aspects like feeding habits, temperature, water quality, genetic makeup and the growth stage of the fish (Craig et al., 2017). In a study with Rohu (*L. rohita*), 30% crude protein was found to be sufficient to obtain optimum growth (Singh et al., 2006).

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Insects are high in crude protein, fat, minerals, vitamins, and fibres (Mintah et al., 2020). An average insect has 29.6% dry chitosan, which has cholesterol-reducing properties (Caparros Megido et al., 2014) along with antiviral, anticancer, antifungal, antimicrobial, and bacteriostatic effects (Piccolo et al., 2017), in its body (Tauber, 1898). Globally, honeybee (*Apis mellifera*), silkworm (*Bombyx mori*), African palm weevil (*Rhynchoporus phoenicis*), yellow mealworm (*Tenebrio molitor*), mopane caterpillar (*Imbrasia belina*) and crickets (*Acheta domesticus*) are among the most commonly edible insects (Tang et al., 2019). About 1.9 billion people around the world have insects regularly in their diets (van Huis et al., 2015). The high protein content of insects assures a nutritional supplement. Insects are good sources of essential amino acids (Zielińska et al., 2015).

The use of insects as protein sources for feed formulation is not something new though. Insect-based feed is suitable for poultry (Pieterse et al., 2019; Veldkamp et al., 2012), fish (Lock et al., 2016), and pigs (Sogari et al., 2019; Spranghers et al., 2018). The use of mixed protein produces better growth than the use of a single type of animal/plant protein (Attalla & Mikhail, 2008). Asia is among the more tolerant places for insect-based feed. China, more importantly, considers the use of insects in feed formulation unless the producers do not break the government rules concerning feed and its additives (Lähteenmäki-Uutela et al., 2018). In North Korea, insect-based feed is prohibited (Jo & Lee, 2016). However, insects are common food and feed ingredients in South Korea (Han et al., 2017). Annex II of the Regulation (European Union) 2017/893 permits the use of certain insects like house cricket (*A. domesticus*), banded cricket (*Gryllodes sigillatus*), field cricket (*Gryllus assimilis*), yellow mealworm (*T. molitor*), lesser mealworm (*Alphitobius diaperinus*), black soldier fly (*Hermetia illucens*), and the common house fly (*Musca domestica*). In the US, the black soldier fly (*H. illucens*) is the only insect permitted to replace fish meal (Lähteenmäki-Uutela et al., 2018). Canada permits the use of the black soldier fly in all poultry and aquaculture after 2018 (Lähteenmäki-Uutela et al., 2018).

Out of all the insects, grasshoppers (Orthoptera) are among the largest diverse groups in the animal kingdom (Paulraj et al., 2009). They are oligophagous feeders with definite host preferences (Mulkern, 1967). *Oxya hyla hyla* (Orthoptera: Acrididae) is a multivoltine polyphagous pest of Poaceae (Das et al., 2012). These grasshoppers are green in colour. Brown bands run laterally from each eye up to the episternum with relatively short filiform Antennae. *O. hyla hyla* contains 64% protein constituting all essential amino acids (30% of DM, Glutamate, and Glutamine; 6% of DM, Serine), 28% carbohydrate, and only 2.58% fat (Ghosh and Mandal, 2019). It is fair to assume that the nutritional findings from close relatives of *O. hyla hyla* are similar to its very nutritional characteristics, if not the same. For instance, *O. fuscovittata* is confirmed to have 25% to 50% fish meal replacement potential in the case of black molly fish, *Poecilia sphenops* (Ganguly et al., 2014). A study suggests that 50% replacement of fish meal in the Rohu diet doesn't change its flesh quality, growth performance and feed utilization indicators (Ghosh & Mandal, 2019b).

The fish component in this study is Rohu (*L. rohita*) (Cypriniformes: Cyprinidae), which is a commonly distributed fish of Nepal (Neupane & Rajbanshi, 2022). It has a wide range of feeding niches from the bottom to the column (Rahman et al., 2008) and is fit for intensive farming (Jhingran & Pullin, 1985) making it suitable for cage culture (Kakati et al., 2018; Balkhande et al., 2019). Various animal proteins have been incorporated into the diets of Rohu to produce good growth performances (Ghosh & Mandal, 2019a; Sampathkumar & Raja, 2019). Reporting fish growth rates is challenging since the growth pattern varies with growth conditions and fish maturity. There are certain growth parameters such as absolute growth rate (AGR), specific growth rate (SGR) and relative growth rate (RGR) used to report fish growths (Hopkins, 1992). This study aims to understand the bio-efficacy of rice grasshopper incorporated feed in the growth and development of Rohu.

2. METHODOLOGY

2.1 EXPERIMENTAL SETUP

The study was conducted inside semi-submerged nylon cages in an earthen aquaculture pond at the Aquaculture Research center at the Institute of Agriculture and Animal Science, Paklihawa campus, Rupandehi. The cages had a 15 cm slit opening on the upper face. This slit was used for feeding, fish sampling and other maintenance activities. Each cage was installed half a meter apart from consecutive cages. The first cage was about a meter away from the pond's edge. The cages were fixed using sturdy ropes and a load was tied to the lower face of each cage. The experiment included four treatments (T0, T1, T2 and T3) and four replications. A completely randomized design was used with the treatments are mentioned in Table 1.

Table 1. Specification of different treatments

Treatments	Details
T0	0% soybean meal replaced with grasshopper meal
T1	10% soybean meal replaced with grasshopper meal
T2	20% soybean meal replaced with grasshopper meal
T3	30% soybean meal replaced with grasshopper meal

2.2 COLLECTION OF RICE GRASSHOPPERS (*Oxya hyla hyla*)

The grasshoppers were collected from the rice fields in Rupandehi and Parasi by handpicking as well as sweeping. Both nymphs and adult grasshoppers were collected without sexual sorting. The insects were abundant in green and damp rice fields. Grasshoppers were found to be relatively docile right after dusk.

2.3 INSECT PROCESSING

The collected insects were transferred into airtight containers to kill the insects by suffocation. Dead insects were sun-dried for 5 days. The dried insects were pulverized and passed through a wire mesh (2mm size).

2.4 PROXIMATE ANALYSIS

Proximate analysis displayed a high crude protein (CP) content of 63.21% in grasshopper meal, GM (Table 2). The CP in GM was second to fish meal. The fibre content in GM (fibre % = 7.5%) was highest among other protein sources, fish meal and soybean meal (Table 2). Moisture content in GM was as low as 5.7% (Table 2).

Table 2. Proximate analysis of feed ingredients

Ingredients	Crude protein%	Crude fiber%	Moisture%	Crude fat%
Grasshopper meal (GM)	63.2	7.5	5.7	2.9
Fish meal (FM)	67.5	6.3	4.5	NA
Soybean meal (SM)	35.5	6.7	2.0	14.7
Rice bran (RB)	13.5	8.4	9.9	12.9
Mustard oil cake (MOC)	40.6	NA	NA	NA

2.5 FEED PREPARATION

Different diets were formed specific to the treatment details (Table 3). After formulating the feed, 2g of commercially available vitamin premix (Table 4) and 2g of table salt were added for every 100g of formulated feed. Feed pellets were produced in a manually operated pellet machine (pellet size 3mm). The pellets were sun-dried for 4 days until crumbly in texture. The dry pellets were packed in labelled packs specific to each cage. The poly bags were then stored under dry conditions to avoid infection.

Table 3. Composition of different lab-formulated diets (in every 100g)

Ingredients	Amount in grams			
	T0	T1	T2	T3
Rice bran	39.5	42.5	45.5	48.8
Mustard Oil Cake	18.5	15.5	12.5	9.3
Soybean Meal	30	27	24	21
Fish Meal	12	12	12	12
Grasshopper Meal	0	3	6	9
Estimated CP%	31.6	31.6	31.6	31.5

Table 4. Composition of the commercial vitamin premix (in 1kg) used in feed formulation

S. N	Constituent	Content in 1kg of premix
1	Vitamin A	7,00,000 I. U
2	Vitamin D3	70,000 I. U
3	Vitamin E	250 mg
4	Cobalt	150 mg
5	Copper	1200 mg
6	Iodine	325 mg
7	Iron	1500 mg
8	Magnesium	6000 mg
9	Potassium	100 mg
10	Sodium	5.9 mg

S. N	Constituent	Content in 1kg of premix
11	Manganese	1500 mg
12	Sulphur	0.72%
13	Zinc	9600 mg
14	DL-Methionine	1000 mg
15	Calcium	25.5%
16	Phosphorus	12.75%

Note: The composition mentioned above are all factory-labelled figures.

2.6 FISH STOCKING AND ACCLIMATIZATION

Each cage was stocked with 2-month-old Rohu fingerlings (Average size 5.6g) at the rate of 12 fingerlings per m^3. Each group was weighed individually for the estimation of the initial stocking weight in each cage. Acclimatization was done for five days using commercial feed purchased from the source hatchery (Table 5).

Table 5. Composition of commercial feed (size 2mm) used for acclimatization

S. N	Feed elements	Max. Content (%)
1	Crude protein	32-34
2	Crude fat	5
3	Crude fiber	5.5
4	Moisture	11

Note: The composition mentioned above are all factory-labelled figures.

2.7 FEEDING RATE

The daily ration was fed at 9 AM every day. Fish were fed manually by 8% of their body weight (Ahmed & Abid, 2009) and the rates were adjusted every two weeks with changes in body weight observations.

2.8 DATA COLLECTION

Fish sampling was done randomly from each cage every fortnight starting 16 days after stocking (DAS). Sampling was done at the rate of 30% of the stocking population. Data observation was done at five time points throughout the study period at 16 DAS, 27 DAS, 45 DAS, 60 DAS and 75 DAS. Water quality parameters were observed every day at 3-time points (8 AM, 12 PM and 4 PM).

2.9 GROWTH PARAMETERS

Various growth parameters were calculated using the data obtained from various observations. All the parameters enumerated after the research activity have been mentioned below:

a. Absolute Growth Rate, AGR (g) = Final Mean Weight(g) – Initial Mean Weight(g) (Hopkins, 1992)

b. Relative Growth Rate, RGR (%) = (mean weight gain / initial mean weight) * 100 (Hopkins, 1992)

c. Specific Growth Rate, SGR (%) (Hopkins, 1992)

d. Feed Conversion Ratio, FCR (Ponzoni et al., 2013)

e. Daily Weight Gain, DWG (g) (Prein et al., 1993)

f. Protein Efficiency Ratio, PER (Zeitoun et al., 1976)

3. RESULTS

3.1 WATER QUALITY PARAMETERS AND FISH SURVIVORSHIP

The average weekly water temperature ranged from 16.4^{o}C to 23.1^{o}C (Figure 1). The temperature readings decreased gradually from the first week of the study to the sixth week. The seventh week saw a slight dip in average temperature. There was an overall decreasing trend of temperature throughout the study period. The dissolved oxygen (DO) was observed between 5.2 mg/L to 7.5 mg/L (Figure 2). The DO level was on the lower end of the range in the beginning which kept an increasing trend throughout the study period. The pH of the pond water ranged between 7.3 and 8.7 (Figure 3). The Secchi disc reading was between 14.6 cm to 19.6 cm. The overall mean fish survivorship observed was 64.58%. The mean survivorship for T0, T1, T2 and T3 were 50%, 70.8%, 66.7% and 70.9% respectively.

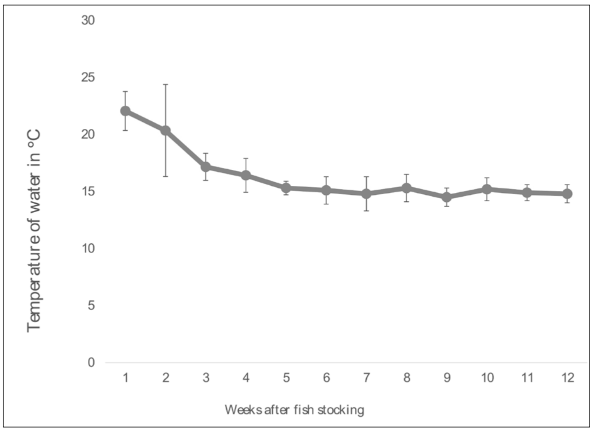

Figure 1. Weekly temperature (°C) of pond water after fingerling stocking

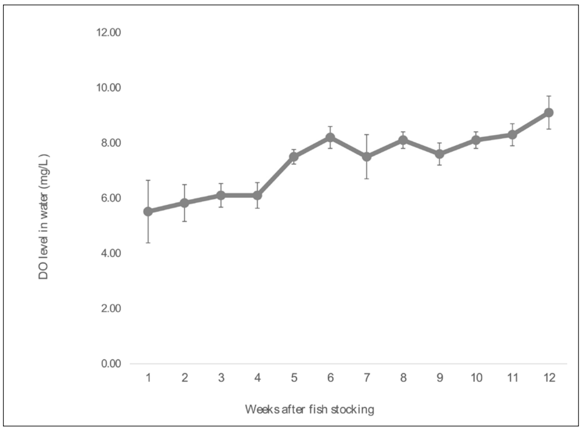

Figure 2. Weekly Dissolved Oxygen (DO) of pond water after fingerling stocking

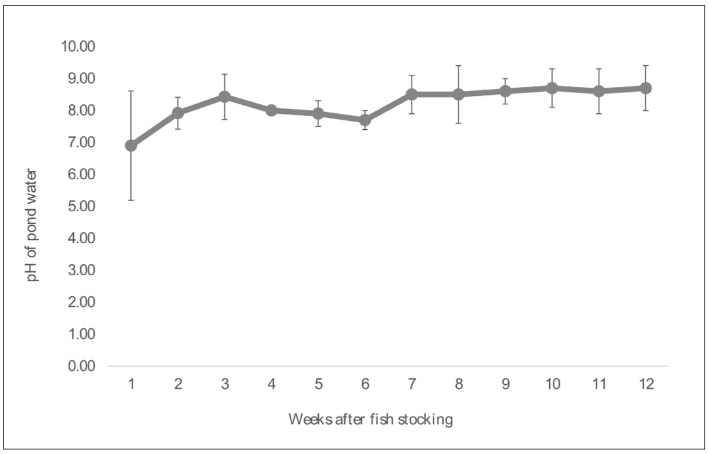

Figure 3. Weekly pH of pond water after fingerling stocking

3.2 FISH GROWTH PARAMETERS

There was no significant difference (p>0.05) between the stocking weights of different cages (Table 2). Similarly, there was no significant difference (p>0.05) between the harvest weights of different cages (Table 6). AGR, SGR and RGR, in all observations, were statistically non-significant at a 95% confidence interval (Table 7, Table 8 and Table 9).

Table 6. Stocking weight and harvest weight of fish in different cages

Treatments	Stocking weight (g)	Harvest Weight (g)
T0	71.4±5.8	123.3±12.7
T1	74.7±2.7	140.9±1
T2	60.2±3.1	148.9±18.8
T3	61.4±4.8	143.7±9.1
LSD	13.2	41.9
F-test result	NS	NS

Note: T0= 0% Grasshopper meal substitution; T1= 10% Grasshopper meal substitution; T2= 20% Grasshopper meal substitution; T3= 30% Grasshopper meal substitution.

Table 7. Observations on absolute growth rates between different time points

Treatments	AGR (g.) in given DAS					
	0-75 DAS	0-16 DAS	16-27 DAS	27-45 DAS	45-60 DAS	60-75 DAS
T0	4.3±0.8	0.8±0.5	1.0±0.6	1.0±0.3	0.6±0.2	1.0±0.2
T1	5.5±0.9	1.9±1.0	0.8±0.4	1.3±0.3	0.4±0.1	1.1±0.3
T2	7.4±1.6	1.3±0.7	2.9±1.9	1.3±0.4	1.0±0.2	0.9±0.1
T3	6.9±0.6	1.4±0.6	1.6±0.7	1.8±0.4	1.2±0.3	1.0±0.4
LSD	3.2	2.3	3.3	1.0	0.7	0.8
F-test result	NS	NS	NS	NS	NS	NS

Note: T0= 0% Grasshopper meal substitution; T1= 10% Grasshopper meal substitution; T2= 20% Grasshopper meal substitution; T3= 30% Grasshopper meal substitution.

Table 8. Observations on relative growth rates between different time points

Treatments	RGR (%) in given days after stocking (DAS)					
	0-75 DAS	0-16 DAS	16-27 DAS	27-45 DAS	45-60 DAS	60-75 DAS
T0	73.4±15.2	18.7±4.9	17.5±6.7	12.1±2.6	7.2±2.6	10.5±3.0
T1	88.2±2.1	29.6±15.0	12.3±7.0	15.2±4.0	4.3±1.2	10.0±2.6
T2	149.1±33.9	53.3±23.1	58.3±40.0	13.9±1.5	10.7±2.5	8.1±1.3
T3	136.2±15.2	57.4±21.3	22.7±10.2	24.1±7.3	12.8±5.0	10.3±4.2
LSD	64.6	54.2	65.4	13.6	9.7	9.1
F-test result	NS	NS	NS	NS	NS	NS

Note: T0= 0% Grasshopper meal substitution; T1= 10% Grasshopper meal substitution; T2= 20% Grasshopper meal substitution; T3= 30% Grasshopper meal substitution.

Table 9. Observations on specific growth rates between different time points

Treatments	SGR (%/day) in given Days After Stocking (DAS)					
	0-75 DAS	**0-16 DAS**	**16-27 DAS**	**27-45 DAS**	**45-60 DAS**	**60-75 DAS**
T0	0.3±0.1[b]	0.5±0.1	0.5±0.1	0.2±0.1	0.2±0.1	0.3±0.1
T1	0.4±0.0[ab]	0.7±0.3	0.4±0.2	0.3±0.1	0.1±0.0	0.3±0.1
T2	0.5±0.1[a]	0.6±0.3	1.5±0.9	0.3±0.0	0.3±0.1	0.2±0.0
T3	0.5±0.0[a]	0.7±0.3	0.8±0.3	0.5±0.1	0.3±0.1	0.3±0.1
LSD	0.2	0.8	1.5	0.3	0.2	0.2
F-test result	S	NS	NS	NS	NS	NS

Note: Post-hoc test by using DMRT; T0= 0% Grasshopper meal substitution; T1= 10% Grasshopper meal substitution; T2= 20% Grasshopper meal substitution; T3= 30% Grasshopper meal substitution.

3.3 FEED EFFICIENCY PARAMETERS

The PER and DWG means were significantly different to each other at a 95 % confidence interval (Table 10). PER was the highest in T2 (0.77±0.16) and the lowest in T0 (0.42±0.08). Means of FCR were found to be significantly different after one-way ANOVA at a 5% level of significance (Table 10). Both T2 (4.64±0.85) and T3 (4.63±0.36) were statistically similar to each other and had the lowest ratios among all the treatments. T1 (6.88±0.94) was similar to all other treatments.

Table 10. Feed efficiency indicators based on mean weight gain and feed consumed within 75 days after stocking

Treatments	PER	FCR	DWG (g)
T0	0.4±0.1	8.2±1.1[a]	0.1±0.0
T1	0.5±0.1	6.9±0.9[ab]	0.1±0.0
T2	0.8±0.2	4.6±0.9[b]	0.1±0.0
T3	0.7±0.1	4.6±0.4[b]	0.1±0.0
LSD	0.3	2.7	0.0
F-test result	NS	S	NS

Note: T0= 0% Grasshopper meal substitution; T1= 10% Grasshopper meal substitution; T2= 20% Grasshopper meal substitution; T3= 30% Grasshopper meal substitution.

4. DISCUSSION

Fish growth rates are directly proportional to metabolism (Wood & McDonald, 1997) and fish activity (Bartolini et al., 2015). Similarly, temperature plays a significant factor in fish development (Britz et al., 1997; Houlihan et al., 1993). The best temperature range for rearing *L. rohita* is 24-26°C (Kausar & Salim, 2006). However, the temperature range in this research is lower than the optimum temperature. This is one of the reasons that the present study observed low weight gain in the fish. In another study, it was possible to obtain a higher weight gain in fish reared in a polyhouse system with an inner temperature of 19°C when it was 14.8°C outdoors (Khan et al., 2004). A pH of 6.5 to 7.5 is the most favourable range and a DO level of above 5 mg/L is favourable for a productive fishpond (Wagle et al., 2018). The weekly observed DO (mean = 6.4 mg/L) levels were favourable for the pond culture of fish. However, the weekly pond pH (mean = 8.1) was slightly higher than the optimum level. The earthen pond produced higher turbidity while performing research activities like feeding, sampling and cage/pond maintenance. This turbidity has a negative influence on fish reared in ponds and cages (Lall & Tibbetts, 2009).

The mean AWG and RWG obtained at the end of the study are much higher than the results obtained in the study conducted at Tarahara, Nepal (Wagle et al., 2018). However, both AWG and RWG had no significant difference between the treatments. Furthermore, SGR was significantly different between treatments at 0-75 DAS. The Specific Growth Rates obtained from our study were inferior to the results from Wagle et al. (2018) at a comparable duration. The highest DWG was observed in 20% GM and 30% GM which was 0.1±0.0 g in both diets. However, the daily gains showed no significant difference from other treatments.

An exponential model is common in analyzing fingerling growths since it can produce reliable results with only mean initial and final weights (Gamito, 1998). The exponential model fits in growth analysis for a short growth period (Cuenco et al., 1985; Vinberg & Duncan, 1971; Weatherley, 1987). However, it is preferred in fish growth analysis more than other models for its easiness (Barnabé, 1994; Porter & Gordin, 1986). SGR is the most suitable parameter to measure growth in fingerlings (Hopkins, 1992). These rates assume an exponential model for growth. However, even this measure was observed to be non-significant at a 95% confidence interval between all five observation points except for SGR at 0-75 DAS.

Both the stocking and harvest weights were statistically non-significant . Stocking weights, not being different between cages, is a necessary requirement to prove no difference between the stocked fishes. This weeds out any possibility of variation just because of differences in average fish sizes. However, the non-significant differences (p>0.05) between harvest weights imply that there is no effect of partial substitution of grasshopper meal for soybean meal on the growth and development of Rohu. Ingredient substitution rates could be increased in further studies to explore if such non-significant results are just because of insufficient substitution of grasshopper meal.

Protein efficiency ratios were statistically similar to each other. However, the PER was found to be the highest in 20% GM. Feed conversion ratios were significantly different at a 95% confidence interval. Diet with 20% GM and 30% GM had the lowest feed conversion ratios. FCR decreased significantly with the increasing proportion of grasshopper meal.

A similar study was done by replacing soybean with cottonseed meal in grass carp fingerlings (Zheng et al., 2012). Soybean meal was replaced because it contains different antinutritional compounds that hurt fish intestinal mucosa (Francis et al., 2001). The antinutrients display severe effects on the mucosal layer if the proportion of soybean is greater than 50% in the feed formulation (Burrells et al., 1999). The findings on FCR for this study were contradictory to our findings. FCR for grass carp feed increased with increasing rate of cottonseed meal. However, our findings suggest that FCR decreases with an increased rate of grasshopper meal.

When the figures for PER and FCR are compared between the two studies, the average means are much higher in our study. Our FCR ranges between 4.6±0.9 to 8.2±0.9 whereas, the study by Zheng et al. (2012) shows an FCR ranging between 1.4±0.1 to 1.7±0.1. Similarly, our PER ranged between 0.4±0.1 and 0.8±0.1 in contrast to the findings from Zheng et al. (2012) ranging between 1.7±0.1 and 2±0.1. There is this huge gap in FCR/PER between our results and Zheng et al. (2012) because we were unable to extract the feed losses.

5. CONCLUSION

In conclusion, *O. hyla hyla* is as rich as fish meal in terms of being a protein source. It was hypothesized that a partial substitution of soybean meal with grasshopper meal would produce better results on the growth and development of Rohu. However, the study lacked the evidence to prove the hypothesis. We believe that using separate aquariums would have made it possible to extract feed deposits. It would have given us a better estimate of FCR and PER. Another reason for the non-significant effects of insect meal substitution could be the use of smaller substitution rates. As a result, we recommend the use of a higher substitution rate in further studies. As *L. rohita* is a tropical fish, we recommend the use of polyhouse structure around the rearing pond to increase temperatures during the winters for a higher weight gain.

DECLARATION

The authors declare no conflict of interest.

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