

Eri silkworm pupae as fish meal replacement in common carp (*Cyprinus carpio* L.) feed in indoor aquaria

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ABSTRACT: Eri silkworm (*Samia ricini* D.) pupae based fish feed, for common carp (*Cyprinus carpio* L.) advanced fry, in indoor aquaria was evaluated with eight different diets. Eri silkworm pupae meal (ESWPM) based fish feeds influenced the growth of common carp significantly. Fortnightly growth measurements of the carp in eight treatments showed maximum in ESWPM alone and in ESWPM 100 per cent treatments. The proximate nutrient and amino acid composition varied in the fish feed diets. ESWPM based fish feed recorded significant impact on the carp with reference to its weight gain, specific growth rate, feed conversion ratio and relative growth rate. The technology got patent.

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KEYWORDS: By-product utilization, *Samia ricini*, pupae, *Cyprinus carpio*, feed

Fish meal has long been a significant source of protein in the fish feed industry. Furthermore, wild fish catches have been progressively dropping due to aquaculture's heavy reliance on them as a feed source for farmed fish (FAO, 2014). Aquaculture feed costs make up between 40 and 70 percent of the price of the fish produced (Wilson, 2002; Rana *et al.*, 2009). Due to the shortage of fish meal, other protein sources with comparable nutritional characteristics have been thoroughly researched (Daniel, 2018). Studies on insect protein as a partial or complete substitute for the fish meal are attempted (Van Huis, 2013). Antifungal and antibacterial peptides in insect meal may also help the meal's shelf life.

Some conflicting opinions also arise about chitin-

containing insect-based fish feed, which reduces nutrient absorption and digestibility and interferes with the fish's normal physiological functions. However, the three enzymes required for chitin digestion: chitinase, chitobiose, and lysozyme (Lindsay *et al.*, 1984; Fines and Holt, 2010), are present in both carnivorous and omnivorous fish species. Further, Aquatic organisms' immune systems are modulated by chitin, its derivatives, and active substances found in insect exoskeletons, such as antimicrobial peptides (AMPs). Chitin intake in moderate proportion can enhance fish gut health, immunity, and resistance to infectious diseases (Hoffman *et al.*, 1997; Kim and Rajapakse, 2005; Lin *et al.*, 2012; Harikrishnan *et al.*, 2012). Chitin is responsible for some of the immunostimulatory

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effects of insect meal (Su *et al.*, 2017; Li *et al.*, 2017; Gasco *et al.*, 2018; Henry *et al.*, 2018).

According to Chakrabarthy *et al.* (1973), silkworm pupae are a better meal for Indian major carps than mustard oil cake and rice bran. Among silkworms, the two popular types are mulberry silk worm (MSW), feeding on mulberry, and eri silk worm (ESW), feeding on castor. Using the under-utilized by-products, MSW and ESW pupae, containing nearly 54 and 62 per cent protein, respectively, as an alternative protein source provides ample scope (Zegeye, 2020; Altomere *et al.*, 2020). Moreover, the enormous potential for using ESW pupae as a feed resource is made possible by their composition, which includes 26.21 per cent lipid, 8 per cent moisture content, 56.83 per cent Poly Unsaturated Fatty Acids (PUFA), high methionine and lysine content, low omega-6/omega-3 fatty acid, and sodium: potassium ratio (Mrinal Ray and Gangopadhyay, 2021).

One of the most popular and commercially significant freshwater fish is the common carp, *Cyprinus carpio*, which accounts for 11 per cent of global freshwater aquaculture production (FAO, 2014). Global production of common carp accounts 3.4 per cent of the world's fish production. During its transitions from the advanced fry to the fingerlings stage, insects become one of its main sources of nutrition (Aquaculture feed and fertilizer resource information system, FAO, 2014). The current study aimed to determine ESW pupae's suitability as a protein supplement replacing fish meal in common carp feed.

MATERIALS AND METHODS

The ESW culture was maintained in the Department of Entomology, Faculty of Agriculture, Annamalai University, Tamil Nadu, India. On the fifth day of spinning, cocoons were cut open, pupae taken out, dried in a hot air oven (Technico, TLPL 131) at 60°C for 12 hours, and made into powder. Various ratios of feed ingredients, *viz.*, ESW pupae powder, fish meal, groundnut cake, rice bran, fish oil, tapioca flour, and vitamin and mineral premix (Table 1), were ground and sieved through a 250-micron

mesh. The ingredients were combined with enough water, mixed into a soft dough, cooked under pressure for 15 minutes, cooled, and then pushed through a manual noodle's maker (with a 1mm diameter sieve plate) to obtain noodle-like threads. They were then baked in a hot air oven at 60°C for six hours to lower the moisture content to 8 per cent, broken and sieved to obtain the required pellet size (0.5 - 1.5mm), depending on the age of the fish. The prepared isocaloric and isonitrogenous ESW pupae-based fish feeds were packed in airtight plastic containers separately and stored at room temperature.

Cyprinus carpio advanced fries with a mean weight range from 0.300 – 0.450g were purchased from a commercial fish farm (M/s Shakthi fish farm). They were acclimatized for about two weeks with a continuous oxygen supply using an aquarium air pump (Sebo, AP 500) and commercial fish feed (Vrudhi Plus, Godrej Agrovvet Limited, Mumbai). Then they were randomly stocked into an experimental glass tank (60cm x 45cm x 45cm). Feed was given daily once with a ratio of five per cent fish body weight. The faecal matter was siphoned out and a 75 per cent water change was done at weekly intervals. The experimental design was planned as a Completely Randomised Design (CRD) consisting of eight treatments, three replications, and ten fish per replication. Growth indices like Weight Gain (g), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Relative Growth Rate (RGR), Survival Rate (%) and Fish In: Fish out (FIFO) (%) were calculated for ten weeks. Experimental feeds' proximate nutrient composition and essential amino acid composition analysis using three samples from each treatment were carried out following Sadasivam and Manickam (2005) and Bandlamori *et al.* (2012) protocol, respectively. The data were subjected to one-way ANOVA and analysed using SPSS Statistical package version 16.

Formulas for growth indices:

Weight Gain (g) = Final wet weight – Initial wet weight

Table 1. Composition of experimental diets (per 100 g) in the treatments

Feed Composition	ESWPM 20%	ESWPM 40%	ESWPM 60%	ESWPM 80%	ESWPM 100%	ESWPM alone	Control	Positive* Control
Fish meal (g)	24	18	12	6	0	-	30	-
Eri Silkworm pupae powder (g)	6	12	18	24	30	62	0	-
Groundnut cake powder (g)	20	20	20	20	20	-	20	-
Rice husk (g)	37	37	37	37	37	-	37	-
Fish oil (ml)	2	2	2	2	2	-	2	-
Tapioca flour (g)	10	10	10	10	10	-	10	-
Vitamin and Mineral premix (g)	1	1	1	1	1	-	1	-
Bentonite	-	-	-	-	-	3	-	-
Cellulose	-	-	-	-	-	35	-	-

ESWPM - Erisilk worm pupae meal; *Commercial fish feed used as such

$$\text{Specific Growth Rate (SGR)} = \frac{(\ln(W_t) - \ln(W_0))}{t(d)} \times 100$$

W₀[g]= the weight in grams at the beginning of the period

W_t [g]= the weight in grams at the end of the period

t[d]= period, expressed in number of days

Ln = natural logarithm

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Feed Fed}}{\text{Wet weight gain}}$$

$$\text{Relative Growth Rate (RGR) (\%)} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Initial Weight}}$$

$$\text{Survival (\%)} = \frac{\text{Number of live fishes at end of the experiment}}{\text{Total numbers of fishes Stocked}} \times 100$$

$$\text{Fish In: Fish Out (FIFO) (\%)} = \text{FCR} \times (\% \text{ Fish meal} + \% \text{ Fish oil in feed})$$

RESULTS AND DISCUSSION

Impact of eri silkworm pupae-based fish feed on common carp's growth performance

It was found that the ESW pupae-based fish feed significantly influenced common carp's growth indices. This performance of ESW pupae meal (ESWPM) at 20 and 40 per cent, recorded poor fortnight weight gains (Table 3). Such poor performance resulted due to the poor protein content of the feed fed (as both treatments contain more proportion of fish meal over ESWPM than other treatments). Nandeesh *et al.* (1990) reported such a reduced growth in common carp when fed with a diet containing 10 and 20 per cent non-defatted pupae meal. In contradiction to poor performance of ESWPM treatment, Jayaram and Shetty (1980a) reported better growth in 30 per cent defatted pupae meal fed catla and common carp.

During the first fortnight measurement, the maximum growth was recorded in ESWPM 100 per cent (5.1g) and ESWPM alone (5g) both treatments recorded on par with each other. Positive

Table 2. Proximate nutrient and essential amino acid composition of experimental feeds in the Treatments

Composition	ESWPM (20%)	ESWPM (40%)	ESWPM (60%)	ESWPM (80%)	ESWPM (100%)	ESWPM alone	Control	Positive Control (Commercial fish feed)
Crude protein (%)	38.1	38.3	38.0	38.4	38.5	38.5	38.4	38
Crude lipid (%)	8.3	8.2	8.1	8.0	8.6	9.4	8.2	8.5
Crude fibre (%)	1.1	1.3	1.1	1.2	1.1	1.2	1.2	0.8
Metabolizable energy (MJkg ⁻¹)	17.5	17.2	17.4	17.3	17.6	18	17.3	17.0
Iron (mg 100 g ⁻¹)	13.1	13.0	13.3	13.5	13.8	13.5	13.7	12.9
Zinc (mg 100 g ⁻¹)	20.5	21.0	21.8	20.9	21.3	21.9	21.2	20.6
Calcium (mg 100 g ⁻¹)	1.4	1.5	1.5	1.9	2.0	2.1	1.4	1.3
Phosphorus (mg 100g ⁻¹)	1.1	1.2	1.3	1.2	1.4	1.5	1.4	1.5
Methionine (g 100 g ⁻¹)	1.1	1.1	1.4	1.3	1.5	1.6	1.5	1.4
Cystine (g 100 g ⁻¹)	1.3	1.3	1.2	1.4	1.5	1.6	1.9	1.6
Lysine (g 100 g ⁻¹)	2.7	2.5	3.3	3.0	3.3	3.7	4.0	3.2

control and ESWPM 80 per cent recorded next best weight gains. However, the trend was little bit changed in the second fortnight, ESWPM alone recorded higher value (12.6 g) followed by ESWPM 100 per cent (12.1 g). In third fortnight measurement, ESWPM 100 per cent and ESWPM alone both treatments were on par with each other. It was followed by positive control and ESWPM 80 per cent recorded on par with each other. Control treatment and ESWPM 60 per cent also were on par with each other. In fourth fortnight interval, ESWPM 80 per cent recorded weight a head than positive control. In last fortnight measurement (fifth fortnight interval), trend recorded in third fortnight interval evidenced. Irrespective of all the fortnight interval measurements ESWPM 40 and 20 per cent records the poor growth performance (Table 3). Weight gain (%) in experimental feed-fed fish over positive control represent the significant performance of ESWPM alone (23.57 % increase over positive control) and ESWPM 100 per cent (20.29 % increase over positive control) among other treatments.

Rangacharyulu *et al.* (2003) reported a 13 per cent higher weight gain when fish meal was entirely replaced with silkworm pupae silage in the Indian major carp's diet. The reason behind the enhanced performance is due to the presence of hydrolysed protein in ensiled silkworm pupae as compared to the complex proteins in fish meal (Manikandavelu *et al.*, 1992; Anon, 1999). Wan *et al.* (2017) reported that silkworm pupae meal is an attractive and sustainable functional feed component in carp diet with enhanced growth performance. Better protein and fat digestibility of carp diet containing silkworm pupae meal than the fish meal was reported by Nandeeshu *et al.* (1990) were in line with the present study findings. However, Nandeeshu *et al.* (1989 a, b) and Ji *et al.* (2015) reported least growth performance, impaired antioxidant enzyme status, decreased digestive function, and unfavourable changes in hepatic and intestinal morphology in fish fed with defatted silkworm pupae entirely or in combination with fish meal at different proportions. Further, Nandeeshu *et al.* (2000) found 50 per cent silkworm pupae

Table 3. Weekly growth performance (weight gain) of fish fed on experimental feeds (g) at fortnightly intervals

Treatments	Initial	1 st	2 nd	3 rd	4 th	5 th
ESWPM 20 %	0.358 (0.598) ^b	2.4 (1.549) ^d	4.8 (2.191) ^f	9 (3.000) ^e	14.4 (3.860) ^e	19.8 (4.505) ^e
ESWPM 40 %	0.333 (0.576) ^b	2.4 (1.549) ^d	5.7 (2.387) ^e	10.2 (3.193) ^d	15.2 (3.964) ^e	21.3 (4.669) ^d
ESWPM 60 %	0.337 (0.580) ^b	3.5 (1.871) ^c	8.8 (2.966) ^d	14.3 (3.781) ^c	18.7 (4.382) ^d	28.2 (5.357) ^c
ESWPM 80 %	0.333 (0.576) ^b	3.9 (1.975) ^b	9.8 (3.130) ^c	16.2 (4.025) ^b	22.5 (4.796) ^b	31.1 (5.621) ^b
ESWPM 100 %	0.365 (0.604) ^a	5.1 (2.258) ^a	12.1 (3.480) ^b	19.9 (4.462) ^a	27.2 (5.263) ^a	36.2 (6.060) ^a
ESWPM alone	0.424 (0.652) ^a	5 (2.236) ^a	12.6 (3.559) ^a	20.5 (4.528) ^a	28 (5.338) ^a	37.1 (6.132) ^a
Control	0.404 (0.635) ^a	3.5 (1.871) ^c	8.6 (2.932) ^d	14.2 (3.768) ^c	18.6 (4.370) ^d	27.9 (5.329) ^c
Positive Control (Commercial feed)	0.414 (0.643) ^a	3.8 (1.949) ^b	9.5 (3.082) ^c	15.6 (3.949) ^b	21.3 (4.669) ^c	30.2 (5.543) ^b
SEd	0.008	0.023	0.036	0.047	0.054	0.065
C.D(0.05)	0.016	0.046	0.072	0.094	0.109	0.130

Mean of three replications; Values within parentheses are square root transformed

inclusion diet were optimal to maintain growth performance and meat quality in common carp, contradicting the present finding of optimal growth performance in inclusion diets containing ESWPM above 60 per cent. As the fish meal incorporated might be contaminated, the resulting protein quality might also be inferior leading to conflicting results. Moreover, eri silkworm pupae's nutritive profile is completely different from mulberry silkworm pupae and fishmeal.

Poor weight gain recorded in fish fed with low proportion of ESW pupae powder might be due to amino acid profile imbalance. This was corroborated by the Kaushik and Seiliez (2010) and Wan *et al.* (2017). This was evidenced by the differences in amino acid profile of different feeds. The proximate nutrient and amino acid composition different

treatment fish feeds indicated variation (Table 2).

Similarly, Hora and Pillay (1962) found higher silkworm pupae incorporation led to offensive odour. Such odour related issues were observed in the present study only in the treatment ESWPM alone. Remaining ESW pupae-based fish feeds was in tune with the findings of Jayaram and Shetty (1980b) who reported no adverse flavour.

There was significant impact of eri silkworm pupae-based fish feed on common carp's growth indices (Table 4). Among the experimental feeds, fish fed with ESWPM alone recorded the highest weight gain of 36.67g followed by fish fed with ESWPM 100 per cent (35.86 g) and positive control (29.81g). Treatment ESWPM 60, 40 and 20 percent recorded least weight gains. Wan *et al.* (2017) studied the

Table 4. Growth indices of different experimental feeds fed common carp

Treatments	Weight gain	Specific Growth Rate (SGR)	Feed Conversion Ratio (FCR)	Relative Growth Rate (RGR)	Survival Rate (%)	Fish In: Fish Out (FI:FO) (%)
ESWPM 20 %	19.44	2.79	2.5	54.3	100	20
ESWPM 40 %	20.96	2.79	2.3	62.94	100	32.2
ESWPM 60 %	27.86	3.21	1.7	82.67	100	34
ESWPM 80 %	30.76	3.34	1.6	86.48	100	41.6
ESWPM 100 %	35.86	3.68	1.3	92.37	100	-
ESWPM alone	36.67	3.93	1.3	98.24	100	-
Control	27.49	3.46	1.8	68.04	100	57.6
Positive Control (Commercial feed)	29.81	3.61	1.6	72	100	-

effect of marine (Ragworm) and terrestrial invertebrate meals (Silkworm Pupae) in the diet of *C. carpio* and reported an increased weight gain of 12 per cent in diets containing fish meal + silkworm pupae when compared with the reference control diet.

Maximum percent increase in fish weight per day indicated by the parameter, Specific Growth Rate (SGR) was recorded in ESWPM alone. On an average, it recorded 3.93 per cent daily weight gain. The ESWPM 100 per cent and positive control recorded 3.68 and 3.61 per cent daily weight gains, respectively (Table 4).

Least Feed Conversion Ratio (FCR) of 1.3 was obtained from ESWPM alone and ESWPM 100 per cent followed by ESWPM 80 per cent and positive control records 1.6. Poor FCR noticed in ESWPM 40 and 20 per cent, hence both this feed composition is not recommended for commercial pisciculture (Table 4).

Relative Growth Rate (RGR) was higher in ESWPM alone (98.24) followed by ESWPM 100 per cent (92.37). This denotes the significant better performance than other treatments. Least RGR

values 62.94 and 54.3 were in ESWPM 40 and 20 per cent respectively. No negative impact on survival rate (%) was recorded in all the experimental diets fed fishes. Trend in FIFO was exact *i.e.*, the feed contains higher proportion of fish meal records higher values correctly.

From the present research, it was found that treatments, ESWPM alone and ESWPM replacing 100 per cent fish meal performed better than other experimental feeds. The technology got the patent from the Controller General of Patents, Designs & Trade marks, Chennai [No.136821 dt 18-12-2023]. However, the turbidity and the bad odour caused by ESWPM within few days after application need to be addressed.

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