



# Effects of Different Fish Oil Levels on the Ovarian Development and Breeding Performance of Asian Catfish [*Pangasianodon hypophthalmus* (Sauvage, 1878)]

Suneetha Nuthakki <sup>a\*</sup> and Sumanth Kumar Kunda <sup>a</sup>

<sup>a</sup> Department of Zoology & Aquaculture, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur District, Andhra Pradesh-522510, India.

## Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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## ABSTRACT

This study was conducted to evaluate the effects of different levels of dietary fish oil on the ovarian development and breeding performance of *Pangasianodon hypophthalmus*. To observe the effects of different levels of dietary fish oil diets on the ovarian development and breeding performances of *Pangasianodon hypophthalmus* an experiment was conducted. Isonitrogenous diets namely Diet-1, 2, 3 and Diet-4 contained 5%, 10%, 15% and 20% of fish oil levels tested on broodstock of *P. hypophthalmus* along with control. After a three-month rearing period, the fish were subjected to

\*Corresponding author: Email: [suneethan3@gmail.com](mailto:suneethan3@gmail.com);

breeding induction to assess their reproductive performance. The largest ova diameter (1.20 mm) was observed in fish fed Diet-3, while the smallest was recorded in the control group. Fecundity was also highest in fish fed Diet-3 (15% lipid content) and lowest in the control group. Fertilization and hatching rates were significantly higher in the Diet-3 group, at  $90.5 \pm 3.01\%$  and  $62.0 \pm 1.57\%$ , respectively, though no significant differences were noted between Diet-2 and Diet-4. Larval survival was greatest in fish fed Diet-3. This diet, containing the recommended lipid level of 15%, resulted in significantly ( $p < 0.05$ ) higher ovary weight, absolute fecundity, fry production, and fry survival. The findings of this study demonstrate that diets with higher lipid levels enhance reproductive performance, particularly in terms of fecundity and larval survival.

**Keywords:** *Pangasius*; lipids; ovarian development; breeding performance; larval survival.

## 1. INTRODUCTION

“Air-breathing catfishes such as Indian magur (*Clarias batrachus*) and striped catfish (*Pangasianodon hypophthalmus*) are highly popular varieties and have great commercial importance in the country. *Pangasianodon hypophthalmus* is one of the candidate species for aquaculture because of its omnivorous feeding habits, rapid growth, disease resistance, and ease in the acceptance of artificial feeds” (Seshagiri et al., 2021). It was introduced into India during 1997 accidentally via Bangladesh and there is a continuous eagerness among the fish farmers in the Andhra Pradesh for its culture and propagation. *P. hypophthalmus* can be cultured as monoculture or polyculture. *Pangasius* can grow to 1.5 to 3.0 kg in one year, and at an annual yield of 10 to 15 t/ha in pond culture. *Pangasius* culture was estimated about 40,000 hectares in the country with the current production levels of 500,000 to 600,000 metric tons per year (NFDB, 2008). Nowadays the expansion of culture of this species faces a major problem due to lack of a good quality of seed because of lack of knowledge about the broodstock nutrition of *P. hypophthalmus*. The feed which is formulated and broadcasted in its farming is based on the nutritional requirement of channel catfish, *Ictalurus punctatus* (Bell & Sargent, 2003; Shim & Chua, 1986; Watanabe & Vassallo-Agius, 2003).

“Nutrition is known to have a profound effect upon breeding performance of fish such as gonadal development, fecundity, egg and larval quality of fish” (Torsabo et al., 2022). “For the initiation of study on the nutrition of broodstock, it is necessary to determine whether spawning and egg quality are influenced by nutritional quality of broodstock diets or not. Reproductive performance of the *Pangasius* is an important factor for the successful mass production of offsprings. Development of broodstock diet and

different feeding regimes improve egg quality and seed production” (Torsabo et al., 2024). Some of the basic nutrients affect the gonadal development of the fishes, also increase the fecundity by the vitellogenesis process (Izquierdo et al., 2001). The proper feed with balanced nutrients raises the aquaculture production economically both qualitative and quantitatively (Schneider et al., 2005).

Lipids are identified as major dietary factors in the broodstock diet that reveals successful reproduction and survival of larvae. Lipids in particular highly unsaturated fatty acids (HUFA), play as key nutrients affecting breeding performance in finfish and shellfishes (Torsabo et al., 2022). Also, HUFA play an important role in regulating hormonal levels, which control different stages of reproduction, such as steroidogenesis and ovulation. Requirements of HUFA are different from fish species to species, environment and season (Izquierdo et al., 2001). Under field conditions, fish growers made the assumption that the fish could use feed to develop and reproduce. In hatchery systems, broodstocks are fed balanced diets with varying amounts of dietary nutrients. Nonetheless, several studies have shown that omega-3 and omega-6 fatty acids are essential for fish reproduction.

Several researchers have highlighted the importance of dietary nutrients on breeding performance of brood fish both quantitative and qualitatively (Kithsiri, 2008). Among the macro nutrients lipids and fatty acids play vital role on somatic growth and breeding performance of fish. However, as per the literature available there is no report on breeding performance of *pangasius*, their nutrient utilisation and metabolic activity of juveniles with respect to different lipid diets. Hence, the present study was undertaken to investigate the effect of fish oil diets on the breeding performance and gonadal development of *pangasius*.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Site and Experimental Animals

Experiments were conducted during the years 2021-2023 at MNR fish farm and pangasius hatchery at Akiveedu, Andhra Pradesh. The *P.hypophthalmus* brooders purchased from the farmers and reared in cultured tanks for breeding purpose. The broodstock mean initial weight of male was 2.5 kg and females were used with 5.0 kgs. Feeding of fishes was done using 28% crude protein.

### 2.2 Experimental Design

The experiment on the breeding performance of *Pangasius* utilized four treatment groups, each with replicates, for a total of eight circular tanks, each with a capacity of 1,000 liters, along with a control group. Male and female fish were stocked in a 2:1 ratio. Prior to stocking, the tanks were cleaned with potassium permanganate to ensure hygiene. The experimental trials were conducted using a Completely Randomized Design (CRD).

### 2.3 Experimental Diets

Four practical diets were prepared using fish oil with different levels namely Diet-1 (5% Fish oil), Diet-2 (10% Fish oil), Diet-3 (15% Fish oil), Diet-4 (20% Fish oil) and control (without oil). All the feed were prepared with uniform protein and energy levels. Composition of ingredients used for preparation of diets is represented in Table 1. Feed ingredients including fish meal, soya bean meal, ground nut oil cake, rice bran, corn flour and wheat flour were procured from the market. Each ingredient was dried and pulverized before preparation of feed. For every diet, the components were combined and weighed separately. After adding water to make the dough, the mixture was baked for fifteen minutes. Following that, a vitamin-mineral mixture and oil containing 0.5% BHT were added. A 3 mm diameter pelletizer was used to create the pellets. The pellets were stored at 60°C for three to four hours after being oven dried. The pellets were labeled appropriately and placed in an airtight container after drying. Following feed formulation, the proximate composition of the formed feeds was ascertained using the standard procedures provided by the Association of Official Analytical Chemists (AOAC, 1995). Table 1 displays the proximate content of the various feeds.

### 2.4 Feeding and Sampling of the Experimental Fish

The broodfish were fed two times a day at 9am and 6pm. In first few days, feeds were applied at the rate of 3% of the body weight. Sampling of fish was done fortnightly. During sampling all fish from each tank were caught with the help of hand net after lowering the water level. Then the weight of each fish was taken with a digital weighing balance (Digitech, 0.1 -10kg). At the time of each sampling all the tanks were washed with potassium permanganate and the tanks were refilled with fresh water.

### 2.5 Parameters Studied for Breeding Performance

Standard breeding protocol was followed for pangasius (Chaturvedi et al., 2015.) Reproductive performance was determined in terms of ovary weight, ovary volume, ova diameter, absolute fecundity and number of fry produced throughout the breeding period. Five fishes of each treatment were used to determine the reproductive performance. Dissections were carried out under a binocular stereoscopic microscope (Unilab GE-48). After taking all ovary measurements, one portion of ovary were then preserved in 4% formalin for the measurement of egg diameter.

### 2.6 Fecundity

“For the estimation of fecundity, the ovaries of females were weighed; three sub-samples were taken from the anterior, middle and posterior sections of each ovary and weighed. Then the total number of eggs in each ovary sub sample was proportionally estimated to calculate absolute fecundity using the following equation” (Yelden and Avsar, 2000).

$$AF = nG/g$$

Whereas, AF = Absolute fecundity  
n = Number of eggs in sub samples  
G = Ovary weight (g)

### 2.7 Spawning Fecundity

Total number of eggs laid by fish after spawning. The eggs of *P.hypophthalmus* were collected in a plastic jar. The eggs were measured in beaker of known volume. Total number of eggs laid (approx.) calculated by following formula:

Total no. of eggs laid = Average no. of eggs in each sample beaker x no. of beakers of eggs.

**Table 1. Ingredient composition of experimental diets**

Ingredient composition	Dietary lipid levels				
	Control	Diet-1	Diet-2	Diet-3	Diet-4
Fish meal	21.21	21.21	21.21	21.21	21.21
Soya bean meal	22.20	22.20	22.20	22.20	22.20
GNOC	25.09	25.09	25.09	25.09	25.09
Rice bran	12	11	9	7	3
Corn flour	8	6	4.5	3	2.5
Wheat flour	8	6	4.5	3	2.5
Fish oil	-	5	10	15	20
Vitamin-mineral mix <sup>a</sup>	2	2	2	2	2
BHT	0.5	0.5	0.5	0.5	0.5
<b>Proximate composition</b>					
Dry matter	93.76	94.37	94.71	95.27	95.02
Crude protein	28.82	28.89	28.08	28.16	28.94
Ether extract	1.14	5.95	10.53	16.05	19.88
Ash content	8.98	8.17	7.01	6.94	7.9
Carbohydrate	54.06	50.99	47.38	41.85	37.28
Energy	374.7	397.7	424.6	452.4	467.8

Calculated digestible energy, DE (Kcal/100g) = (CP% x 4) + (EE% x 9) + (TC% x 4); DE = Digestible Energy, CP = Crude Protein, EE = Ether Extract; Carbohydrates (calculated by difference).

<sup>a</sup>Vitamin-mineral mix (Emix™ plus) (quantity/ 2.5 kg): Vitamin A-55,00,000 IU; Vitamin D3-11,00,000 IU; Vitamin B2-2000 mg; Vitamin E-750 mg; Vitamin K-1000 mg; Vitamin B6-1000 mg; Vitamin B12-6 mg; Calcium panthothenate-2500 mg; Niacinamide-10 gm; Cholinechloride-150 gm; Mn-27,000 mg; Iodine-1000 mg; Fe-7500 mg; Cu-2000; Zn-5000 mg; Co-450mg; Ca-500 g; P-300 g; Se-50 ppm; L-Lysine-10 g; DL-methionine-10 g; Satawari-2500 mg; Carrier-q.s; Lactobacillus-120 million units and yeast culture-3000 million units

## 2.8 Fertilization Rate

The fertilization rate was calculated through random sampling by examining 2-3 samples from each breeding tank by using following formula:

Fertilization rate (%) = (Average no. of fertilized eggs in a sample) / (Average no. of eggs in a sample) x100

## 2.9 Hatching Percentage

Percentage of hatching was calculated by following formula:

Hatching % = (Total no. of spawn) / (Total no of fertilized eggs) x 100

## 2.10 Total Larval Production

Total number of larvae were harvested after three days of yolk sac absorption and estimated their survival percentage.

## 2.11 Physico-chemical Parameters of Water

The samples were analyzed for 8 physicochemical parameters following the APHA (1998) standard methods. Parameters studied are water temperature, pH, total alkalinity, dissolved oxygen, total hardness, nitrite, nitrate and ammonia.

## 2.12 Statistical Analysis

Mean values of all parameters were subjected to one way ANOVA to study the treatment effect and Duncan's Multiple Range Test (DMRT) was performed to determine the significant differences between the mean values. Comparisons were made at the 5% probability level.

## 3. RESULTS AND DISCUSSION

### 3.1 Effects of Dietary Lipids on Ovarian Development and Fecundity

#### 3.1.1 Ovarian development

The ovarian length, ovarian weight and ovum diameter are presented in Table 2. The fish fed Diet-3 and Diet-4 diets had higher ovary length and ovary weight respectively and were observed significantly (P<0.05) different than the fish fed other groups. Those values were found to be lowest in the fish fed control diet. The ova diameter was observed highest in fish fed Diet-3 (1.20mm) and lowest was observed in control diet. A significant (p<0.05) difference was observed in ovum diameters of fish fed different dietary fish oil levels. It was reported that higher

ovary weight was observed when fish were fed higher lipid levels (Xiong, 2022). The fecundity, total number of eggs produced by a fish in single spawn, has been used to determine reproductive performance of fish.

In case of fecundity, it was found that number of ova/g body weight of fish was the highest in Diet-1 and the lowest in control. The fecundity was estimated as ova per gram ovary, ova per gram body weight, absolute fecundity and relative fecundity are presented in Table 3. Absolute fecundity of pangasius was varied from 3.5-4.0 lakhs and was significantly ( $p<0.05$ ) different among the dietary treatments. The average number of ova per gram ovary and ova per gram body weight are shown in Fig. 1. Statistical analysis by ANOVA showed that there was significant difference among the four different treatments. The results were supported by Duray et al., (1994) who reported that rabbit fish (*Siganus guttatus*) brood stock fed with dietary lipid level 18% yielded a higher relative fecundity (eggs/g body weight) compared to 12% lipid diet. Therefore, it seems that dietary lipid requirement

is species specific as far as its requirement is concerned in gonadal development and breeding performance of fish.

### 3.2 Effects of Fish Oil Diets on Breeding Performance

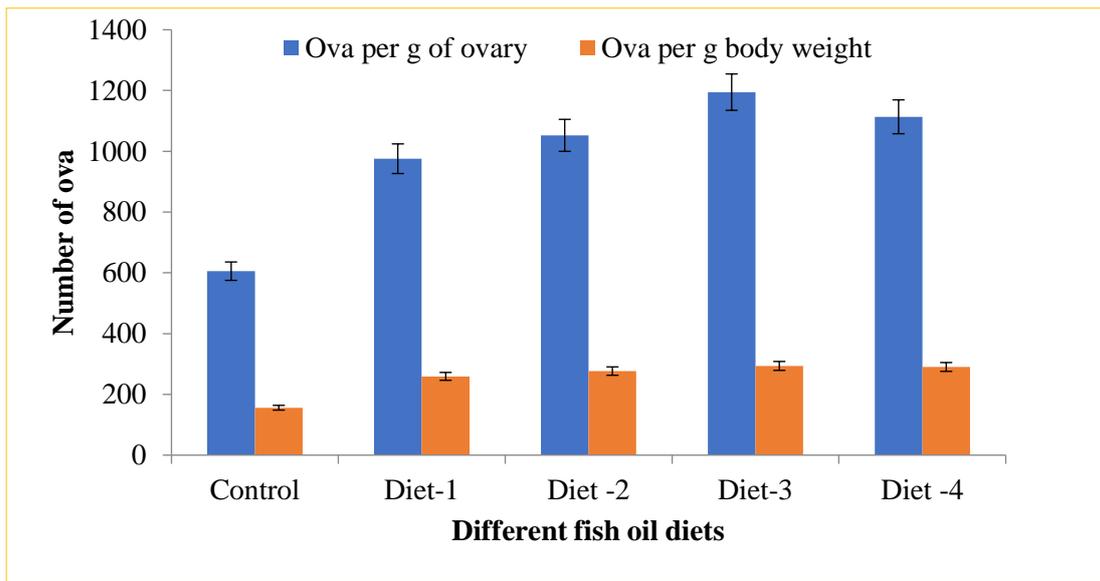
#### 3.2.1 Fertilization rate and hatching rate

Average fertilization rates were recorded as  $70.5\pm 2.55$ ,  $74.95\pm 1.53$ ,  $88.53\pm 3.45$ ,  $90.5\pm 3.01$  and  $85.1\pm 1.51$  in control, Diet-1, Diet-2, Diet-3 and Diet-4, respectively (Table 2). The highest fertilization rate was observed in Diet-3 and lowest fertilization rate was observed in control. Statistical analysis by ANOVA showed that there was a significant ( $P<0.01$ ) difference among different treatments. Diet-3 was significantly ( $P<0.05$ ) different from Diet-1, Diet-2 and Diet-4, but there was no significant difference between Diet-2 and Diet-4 (Fig. 2). During the experiment, the average hatching rates were found to be  $45.5\pm 3.50$ ,  $52.5\pm 1.5$ ,  $60\pm 1.51$ ,  $62\pm 1.57$  and  $60.33\pm 1.42$ , respectively in control, Diet-1, Diet-2, Diet-3 and Diet-4. The highest hatching

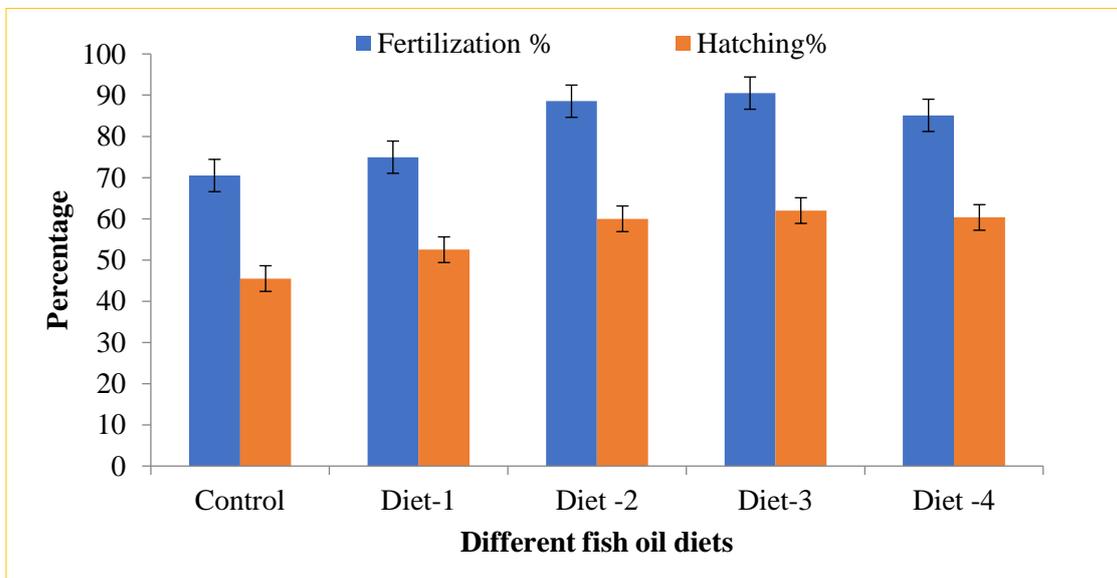
**Table 2. Breeding performance of pangasius fed different levels of fish oil diets**

Parameters	Control	Diet-1	Diet -2	Diet-3	Diet -4
Fish weight (g)	$3205.50\pm 4.50^a$	$3017.55\pm 7.57^{ab}$	$3140.5\pm 4.22^{abc}$	$3676.5\pm 6.51^c$	$3258.5\pm 3.50^{bc}$
Ovary length	$7.01\pm 0.34^a$	$7.69\pm 0.57^a$	$8.59\pm 1.04^a$	$10.37\pm 1.33^a$	$9.92\pm 0.69^a$
Ovary weight	$20.90\pm 0.34^a$	$23.31\pm 1.44^{ab}$	$37.52\pm 2.96^{bcd}$	$51.05\pm 3.01^{cd}$	$41.54\pm 2.24^d$
Ova per g of ovary	$605.50\pm 50.55^a$	$975.50\pm 75.45^{bcd}$	$1052.50\pm 72.50^{bcd}$	$1194.64\pm 29.33^d$	$1113.50\pm 63.44^{cd}$
Ova per g body weight	$156.02\pm 2.61^a$	$259.17\pm 2.01^{abcd}$	$276.73\pm 11.87^{bcd}$	$293.58\pm 6.52^{ab}$	$290.12\pm 13.65^d$
Ova diameter	$0.94\pm 0.02^c$	$1.17\pm 0.05^a$	$1.17\pm 0.09^a$	$1.20\pm 0.12^b$	$1.18\pm 0.02^a$
Absolute fecundity (lakhs)	$3.5\pm 0.88^a$	$3.6\pm 0.75^{abc}$	$3.8\pm 0.56^{bcd}$	$4.0\pm 0.43^d$	$3.6\pm 0.6^d$
Relative fecundity (lakhs)	$1.14\pm 0.35^a$	$1.35\pm 0.45^{abc}$	$1.44\pm 0.75^{bcd}$	$2.1\pm 0.5^{cd}$	$2.05\pm 0.58^d$
Size of fertilized eggs (mm)	$2.63\pm 0.38^a$	$2.29\pm 0.51^a$	$2.41\pm 0.14^a$	$2.06\pm 0.16^a$	$2.58\pm 0.38^a$
Fertilization%	$70.5\pm 2.55^a$	$74.95\pm 1.53^{abc}$	$88.53\pm 3.45^d$	$90.5\pm 3.01^d$	$85.1\pm 1.51^{cd}$
Hatching%	$45.5\pm 3.50^a$	$52.5\pm 1.5^b$	$60\pm 1.51^{bcd}$	$62\pm 1.57^d$	$60.33\pm 1.42^{cd}$
No. of larvae (lakhs)	$1.2\pm 0.13^a$	$1.39\pm 0.30^b$	$1.5\pm 0.11^{bc}$	$1.6\pm 0.09^c$	$1.566\pm 0.15^c$
Survival rate	$28\pm 1.41^a$	$32.5\pm 1.70^{abc}$	$35.5\pm 2.12^{bcd}$	$40.5\pm 0.7^g$	$38.0\pm 1.24^{ef}$

Data expressed as Mean  $\pm$  SE (n=3). Mean values in the same row with different superscripts differ significantly ( $P<0.05$ )



**Fig. 1. The average number of ova per gram ovary and ova per gram body weight of pangasius fed different experimental diets**



**Fig. 2. The relative percentage of fertilization rate and hatching percentage of pangasius fed different experimental diets**

rate was found in Diet-3 and lowest in control. The ANOVA test showed that there was a significant difference ( $P < 0.01$ ) between different responding treatments. DMRT showed that Diet-4, Diet-3 and Diet-2 were significantly different ( $P < 0.05$ ) from Diet-1 and Control, but there was no significant difference between Diet-2, Diet-3 and Diet-4.

Studies have shown that the dietary lipid levels play a major role in reproductive performance of

fish (De Silva and Radampola, 1990; Chong et al., 2004). The reproductive performance in terms of gonadal development, fecundity, egg quality and number of larvae production obtained from the females fed with Diet-3 (15% lipid) was significantly higher compared to those fed with control and other experimental diets (Table 3). Results of the study indicate that the higher reproductive performance of fish fed with higher concentration of the lipids in the formulated diets would indicate that the nutritional quality of the

**Table 3. Physico-chemical parameters of water in rearing tanks during the study period**

Parameters	Control	Diet-1	Diet -2	Diet-3	Diet -4
Temperature (°C)	24.2-26.7	23.8-27.2	25.3-26.5	23.5-26.8	23.4-26.9
pH	7.8-8.1	7.8-8.1	7.5-8.4	7.5-7.9	7.8-8.4
DO (mgL <sup>-1</sup> )	6.9-7.1	6.9-7.1	5.7-7.2	5.6-6.8	5.3-6.5
Alkalinity	125-150	105-155	120-170	120-165	115-155
Hardness (mgL <sup>-1</sup> )	221-244	220-241	228-238	241-245	238-247
Ammonia-N (mgL <sup>-1</sup> )	0.16-0.21	0.17-0.21	0.22-0.27	0.14-0.19	0.18-0.25
Nitrite-N (mgL <sup>-1</sup> )	0.001-0.002	0.001-0.002	0.001-0.002	0.003-0.005	0.002-0.003
Nitrate-N (mgL <sup>-1</sup> )	0.02-0.04	0.03-0.04	0.02-0.04	0.04-0.06	0.05-0.07

broodstock diet influences reproduction. Similar findings were reported in seabass (Bentley et al., 2009) and spotted babylon (Sangsawangchote et al., 2010).

“Fertilization success is another measure of egg quality which was also highly variable (70.5-90.5%) in the study and it is consistent with the findings of earlier studies” (Nandi et al., 2001). Watanabe et al., (2003) reported “a high variability (10-100%) in overall fertilization rate when fish fed with 9 to 15% lipid”. Similarly, hatching success observed in this study range of 45.5 to 62.45% was found higher in Diet-3 diet and lowest in control during spawning. In contrast, Navas et al., (2001) also found that *D. labrax* brood stock fed with a natural diet produced eggs with a higher hatching rate than fish fed with artificial diets containing 10 to 17% lipids. The lack of lipid level in the diet might be reported lower hatching success in control diet.

### 3.3 Physico-chemical Parameters of Water in Rearing Tanks During the Study Period

Water temperature, dissolved oxygen and pH during the brood rearing period in the tanks were found to be in the desirable range according to Boyd (1979), Jhingran and Pullin (1985) and Rahman et al. (1982). There was no indication of the adverse effect of water quality parameters on the survival of pangasius broodstock and larval survival (Table 3). The goal of the present work was to find out if there is any positive impact of different levels of fish oil diet on breeding performances of the female of pangasius. The results presented here indicated that there was a positive correlation between dietary lipid levels and breeding performance.

Based on the results, it can be concluded that fish oil diets positively influence the breeding

performance of female *Pangasius* broodfish. The best fertilization rate and hatching rate of the eggs were obtained with fish fed Diet-1 which contained 15% level of fish oil (James et al., 1993).

## 4. CONCLUSION

It is evident from the present results that the fertilization and hatching rates were significantly higher in the Diet-3 group, at 90.5±3.01% and 62.0±1.57%, respectively, though no significant differences were noted between Diet-2 and Diet-4. Larval survival was greatest in fish fed Diet-3. The findings of this study demonstrate that diets with higher lipid levels enhance reproductive performance, particularly in terms of fecundity and larval survival.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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