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Posted Date: 19 June 2024

doi: 10.20944/preprints202406.1037.v1

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Vitamin Solutions Effects on Reproduction of Broodstock, Growth Performance and Survival Rate of *Pangasius* Catfish Fingerling

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Abstract: A feeding study was conducted to evaluate the different diets supplemented with vitamin solution effect on broodstock reproduction, growth performances, and the survival rates of larvae and fry of *Pangasius* catfish during over-spawning season. The experiment of broodstock cultivation was set up as a diet supplement design with six different diets consisting of vitamin solution, each diet fed to triplicate groups of fish, the stocking density of 10 broodstock fish (07 females and 03 males) per hapa net. Fish larvae and fry were reared in the hatchery for 45 days after hatching to evaluate growth performances and survival rates of fish fingerling. The growth and reproductive performances of breeders fed with different test diets showed that there were differences among the six tested diets ($P < 0.05$). The highest final body weight (FBW), weight gain (WG), daily weight gain (DWG), specific growth rate (SGR) of broodstock and survival rate of *Pangasius* fingerlings were found in Treatment 5, which contained 0.6% H-OVN for *Pangasius* Broodstock mixed with 12.6% algal oil and Treatment 3, which contained 0.6% vitamin premix H-OVN for *Pangasius* Broodstock compared to other Treatments. With these test diets, the Gonadosomatic index (GSI), relative fecundity index (RFI), fertilized egg, hatching rates of eggs, and survival rate of fingerling ranged between a 6.7–12.8%, 104,267–199,512 eggs/kg, 62.2–93.6%, 43.5–76.2%, and 45.3–66.3%, respectively ($P < 0.05$). The findings of this study showed that the diet containing 35% CP contents supplemented with 0.6% vitamin premix H-OVN mixed with algal oils showed the highest results in terms of growth, reproductive performance indices, and survival rates of *Pangasius* catfish fingerling.

Keywords: *Pangasius* catfish; broodstock nutrition; reproduction; growth performances; fingerling; survival rates

1. Introduction

Pangasius catfish is an important commercial aquaculture species with a high economic value for fish farming with an annual production of approximately reached 1.52 million tonnes [1]. Recently, it is reported that the low survival rate and unstable supply of fish fingerlings throughout the year could be one of the major hindrances and bottlenecks for the continued growth of the *Pangasius* aquaculture industry sector in the Mekong Delta, Vietnam [2–4]. *Pangasius* broodstocks spawn all year round, but it has low and variable survival rates for fish fry and fingerling, rarely reaching over 20–25% during the main spawning season and only 12–15 % end-breeding season [2,4–7]. In the Mekong Delta, Vietnam, most *Pangasius* catfish hatchery operations and nursery farming typically feed their broodstock fish with the same commercial diet as used for grow out of the produced

 doi:10.20944/preprints202406.1037.v1

commercial fish. This diet, containing 28–30% available crude protein (CP) and 3.5–5% lipid contents, may not contain the appropriate feed nutrient levels to optimize spawning success of brooders and to improve the quality of fish fry [4,5,8,9]. Many scientific researchers stated that low quality broodstock nutrition, feeding, and feed management is one of the major challenges for fish producers, alongside decreased genetic integrity, inbreeding of fish, and seed degeneration [3,4,7,10–12]. However, many research scholars emphasize that better quality broodstock diets, formulated to increase successful reproduction and better fish management practices, would allow producers not only to reduce the number of broodstock needed to meet eggs and fry production goals but also produce better quality fry and meet fry demands of fish farmers' year round [13–16].

Many studies are being carried out around the world to investigating the human health benefits of fungi and algae [17–19]. Several species of algae and fungi including lichenized fungi (lichens), have the ability to biosynthesize biologically active compounds and are potential sources of natural antibiotics and antioxidants that could be used as supplementary medicine and food sources for human and aquatic animals [58]. The main nutrients found in mushroom-fruiting bodies are proteins, carbohydrates, fats, including essential fatty acids (EFA), fibre, and vitamins and minerals. Algae biomass and lichens are a renewable source of many valuable active substances that have a wide range of applications in many industries, such as food, chemical, agriculture (including animal and aquatic feeds), pharmaceuticals, cosmetics, and medicines [17,18,20]. Microalgea provides essential amino acids, valuable triglycerides such as lipids, vitamins, and pigments, making them suitable as nutritional supplements in animal feed and aquafeed formulations [17,18,21,22]. It is also reported that microalgea supplements in diets improved the fatty acid profile of farmed fish and shrimp by improving the ω-3/ω-6 ratio, increasing polyunsaturated fatty acid (PUFA) content, and enriching long chain PUFAs [21,23]. Studies on application of microalgae species rich in EPA or DHA in aquaculture include Pacific white shrimp, Giant tiger prawn, Giant freshwater prawn [21,24,25], Gibel carp [23], Tilapia [22,25], European seabass [26], and Common carp [25]. Several studies reported that microalgae oils have the potential to replace fish meal and fish oils in aquaculture and ensure sustainability standards. It can be used directly as supplement sources in animal feed and aquafeed feed formulations to improve reproduction, produce good quality of fish eggs, fish fry and yields [18,22,27].

In recent years, dietary protein, lipid (fats, fatty acids), vitamin and energy requirements of many commercial catfish species (included Channel catfish, Black catfish, Bagrid catfish, African catfish have been widely examined [28–39], while studies focusing specifically on broodstock nutrition of *Pangasius* catfish species are still limited. This absence of research may be because it is generally considered to be of high cost as it requires a long period of feeding broodstock fish before any effects can be seen on fish fecundity, egg quality, hatching success [13–15]. The quality of feed ingredients, feed quality and feed utilization by broodstock fish species is a key factor to improve the reproductive performance, egg and sperm quality and hatchability and enhanced survival rate of fish species [13–15]. The quality of feed ingredients, feed quality and feed utilization by broodstock fish species is a key factor to improve the reproductive performance, egg and sperm quality, hatchability and enhanced survival rate of fish species [13–15]. Up to now little research is available on broodstock nutrition and the potential effects on reproductive performance, fish fry survival rate fry, and fingerling and seed quality from adding vitamins, fatty acids from algae oil and fungi oil to the food of *Pangasius* catfish broodstock. A study on striped catfish in Vietnam recommended that a standard for the conditioning feeds of *Pangasius* catfish broodstock needs to be developed [40]. Therefore, this feeding trial was conducted to evaluate the effects of different diets supplemented with vitamin solution effect on growth performances, broodstock reproduction, hatchability, and the survival rates of fry and fingerling of *Pangasius* catfish. The hypothesis tested in this study was that a dietary of approximate 35% CP contents supplemented with different vitamin solution, and plant oils (algal oil, and fungal oil) can improve the reproductive performances, eggs quality of broodstock, and enhance the survival rates of fry and fingerlings of *Pangasius* catfish. The findings of this research will provide valuable information for *Pangasius* catfish farmers and the fish production industries in the Mekong Delta, Vietnam.

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2. Materials and Methods

2.1. Study Site and Research Layout

The experiments were carried out at the Pangasius catfish broodstock farm and hatchery in My Thoi wards, Long Xuyen city, An Giang province, Vietnam. Two experiments were carried out, one outdoors and one indoors. In the outdoor experiment, 180 *Pangasius* catfish brooders were fed six test diets supplemented with differing amounts of vitamin premixes, algal oil, and fungal oil in a series of 18 hapa nets in an earthen pond of 1,500 m^2, with three replicates for each diet. The experiment was done during October–February, which is over spawning season. The experiments of breeding, larvae rearing and fingerling rearing were conducted in an indoor hatchery. These experiments aimed to evaluate the quality of eggs, egg hatchability, growth performances, fingerling production, and the survival rates of fingerlings of *Pangasius* catfish. The research layout of this study is presented in Figure 1

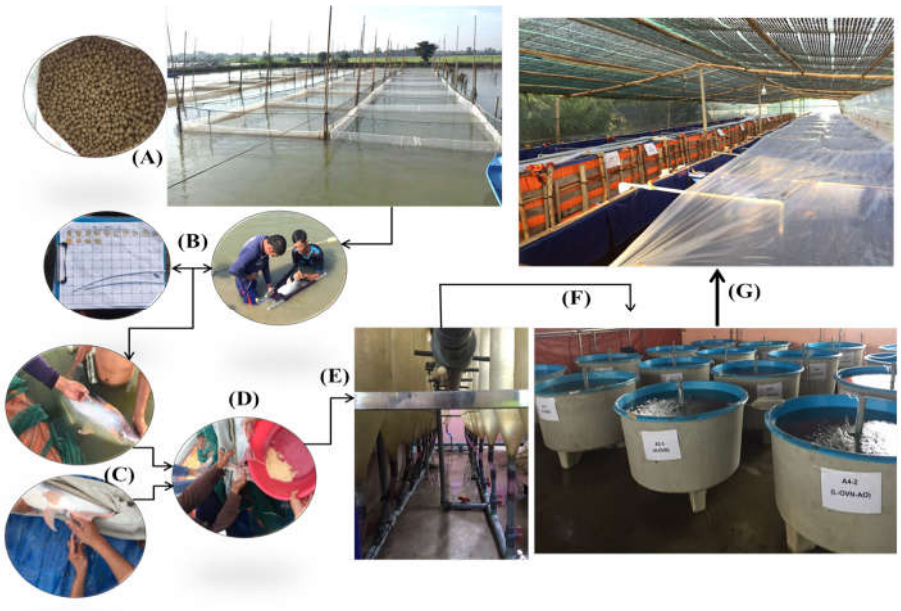

Figure 1. The layout of experimental broodstock rearing, reproductive performance, and larvae rearing of *Pangasius catfish* in offsprawning season as following steps: **(A)**: Experiment of broodstock fish rearing and feeding; **(B)**: Broodstock selection for induced prawning; **(C)**: Broodstock injection and sperm quality checking; **(D)**: Egg stripping and fertilizing; **(E)**: Fertilized egg incubating in the hatching incubator system; **(F)**: Larvae after hatching for two days nursing; **(G)**: Experiment of larvae and fingerlings reared for 45 days after hatching.

2.2. Pond Preparation and Management of the Broodstock

The earthen pond used for the broodstock experiment was prepared by pumping out the water and treating it with 150 kg lime (CaCO3). After this the pond was filled with new freshwater from a reservoir pond. A system of 18 hapa-nets with 4.0 mm mesh size were used to test six treatments in triplicates. Each hapa-net was suspended and tied to four Melaleuca poles. The sides and bottoms of each hapa-net were scrubbed and cleaned every two months, and at least 20–30% of the water was exchanged monthly during cultivation of the broodstock fish.

2.3. Selection Criteria of Broodstock

The broodstock were obtained from a broodstock fish pond at the Pangasius catfish farm of NAVICO. A total of 180 brooders fish at the pre-maturation stages, which were at 3–3.5 years old, were selected from the broodstock fish pond and transferred to 18 hapa-net system (6 treatments in triplicates), where each hapa-net was 3.5 m x 3.5 m x 3.0 m (length x width x depth) (Figure 1). At the beginning of the experiments the body weight, length and belly width of the broodstock fishes were measured. The female breeders were checked and selected based on having a large soft belly, healthy external appearance, good agility condition, and uniform eggs size. The male breeders were selected

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based on the thickness of the milt/semen, obtained by hand stripping, healthy external appearance, good agility condition, and large size [41].

2.4. Experimental Diet Preparation and Feeding Practice

The experimental diets were formulated to meet the nutrient requirements for striped catfish broodstock, with approximately 35% CP supplemented with vitamin premix, algal and fungal oils (Table 1). The experimental feed ingredient sources were: soybean meal (45% CP) and Kien Giang fish meal (55% CP), poultry by-product meal (65% CP), and soybean oil, which were purchased from the local markets in An Giang and Dong Thap provinces. Fish oil (Tuna oil), choline chloride (50% choline), mineral premix for fish, vitamin premix algal oil and fungal oil were provided by DSM SINGAPORE INDUSTRIAL PTE. LTD.

Table 1. Formulation and chemical composition of test diets (g/kg DM) used for broodstock of *Pangasius* catfish species.

Raw materials	Experimental diets					
	T1	T2	T3	T4	T5	T6
Fish meal (578 g/kg CP)	75.0	75.0	75.0	75.0	75.0	75.0
Poultry by-product meal (648 g/kg CP)	184.1	184.1	184.1	184.1	184.1	184.1
Wheat flour (158 g/kg CP)	288.1	288.1	288.1	289.2	289.2	288.0
Soybean meal (490 g/kg CP)	360.0	360.0	360.0	360.0	360.0	360.0
Soybean oil	38.9	38.9	38.9	47.6	47.6	47.2
Fish oil	40.0	40.0	40.0	17.6	17.6	17.6
Choline chloride	5.0	5.0	5.0	5.0	5.0	5.0
Mineral premix[a]	3.0	3.0	3.0	3.0	3.0	3.0
Vitamin premix (Rovimix 2020)[b]	6.0	–	–	–	–	–
Vitamin premix (L-OVN)[c]	–	6.0	–	6.0	–	–
Vitamin premix (H-OVN)[d]	–	–	6.0	–	6.0	6.0
Algal oil[e]	–	–	–	12.6	12.6	12.6
Fungal oil	–	–	–	–	–	1.5
Actual chemical composition (g kg^{-1} DM)						
Dry matter	901.0	904.1	885.2	897.0	895.3	930.2
Crude protein	350.1	350.5	350.0	351.5	358.5	351.5
Crude fat	65.2	66.8	65.2	68.4	73.8	69.8
Crude fibre	28.9	34.4	48.8	46.4	40.9	44.5
Ash	103	110	109	110	110	118

Note: [a]Mineral premix contained (g kg^{-1}): Copper sulphate pentahydrate (13.33), Iron sulphate monohydrate (64.52), Manganese oxide (22.22), Zine oxide (62.50), Combalt (0.66), Idoine (33.33), Selenium (2.96), Filler (800.48). [b]Composition of vitamin Rovimix C-2020 contained (g kg^{-1}): vitamin A (0.42), vitamin E (20.0), vitamin C (35.71), vitamin K (1.94), vitamin B1 (0.45), vitamin B2 (1.25), vitamin B6 (1.02), vitamin B12 (0.25), niacin (4.0), pantothenic acid (3.33), folic acid (0.46), biotin (0.83), choline (166.67), anticaking (10.0), BHT (0.2), alpha cellulose (753.46). [c]Vitamin premix L-OVN contained (g kg^{-1}): Vitamin A (1.33), Vitamin E (83.33), Vitamin C (238.09),

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Vitamin K (1.94), Vitamin B1 (1.81), Vitamin B2 (3.13), Vitamin B6 (3.05), Vitamin B12 (0.40), Niacin (13.33), Pantothenic acid (7.41), Folic acid (0.83), Biotin (0.83), Choline (166.67), Anticaking (10.0), BHT (0.2), Alpha cellulose (467.64). [d]Vitamin premix H-OVN contained (g kg^{-1}): Vitamin A (1.83), Vitamin E (166.67), Vitamin C (476.19), Vitamin K (1.94), Vitamin B1 (3.62), Vitamin B2 (3.13), Vitamin B6 (5.08), Vitamin B12 (0.40), Niacin (13.33), Pantothenic acid (7.41), Folic acid (2.08), Biotin (0.83), Choline (166.67), Anticaking (10.0), BHT (0.2), Alpha cellulose (140.62). [e]Algal oil contained EPA + DHA: EPA-content (110 mg g^{-1}), DHA + EPA content (505 mg g^{-1}), TotOx (2*Peroxide value + anisidine value: (6.0)), Free fatty acid (3.6%), Moisture (0.33%). All products of vitamin solution for *Pangasius* Broodstock, algal oil + fungal oil were provided by DSM SINGAPORE INDUSTRIAL PTE. LTD, (Company Registration No. 199100649D), a company incorporated in Singapore and having its registered office at 30 Pasir Panjang Road, #13-31, Mapletree Business City, Singapore 117440 trading as DSM NUTRITIONAL PRODUCTS ASIA PACIFIC (Business Registration No 53192777).

The detailed composition of vitamin premix, mineral premix and algal oil compounds are presented in Table 1. Two tonnes of floating pelleted feeds with 5.0 mm diameters of the six test diets of the experiment were produced at the Aquafeed production of Dong A plant, Cao Lanh city, Dong Thap province, Vietnam. The broodstock fishes were reared and fed with the experimental diets for 65 days. Broodstock fishes of each treatment were fed by hand to apparent satiety, at a rate of about 3–5% of body weight, twice a day at 8:00–9:00AM and 4:00–5:00 PM. The chemical composition of the test ingredients and diets are shown in Table 2 and Table 3.

Table 2. Proximate chemical composition and amino acid contents (g/kg DM) of feed ingredients.

	Feed Ingredients			
	Soybean meal	**Wheat flour**	**Fish meal**	**Poultry byproduct meal**
DM	895	888	911	953
CP	490	158	578	648
Lipid	12.0	13.0	70.0	72.0
Ash	58.0	15.0	185	259
Crude fibre	26.0	4.0	4.0	26.0
Essential amino acids				
Histidine	13.5	3.0	8.2	6.9
Isoleucine	22.8	4.8	19.3	11.3
Leucine	38.3	9.0	37.4	23.5
Lysine	30.8	2.9	25.1	19.9
Methionine	2.4	1.6	8.3	4.9
Phenylalanine	24.8	5.9	0.2	13.0
Valine	24.0	6.2	27.1	17.2
Threonine	19.2	3.7	19.5	12.0
Total	**175.8**	**37.1**	**145.1**	**108.7**

Table 3. Amino acid and fatty acid profiles (g/kg DM) in test diets used for broodstock of Pangasius catfish.

Essential amino acids	**Experimental Diets**					
	T1	**T2**	**T3**	**T4**	**T5**	**T6**

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Histidine	6.4	6.4	6.4	6.4	6.4	6.4
Isoleucine	12.2	12.2	12.2	12.2	12.2	12.2
Leucine	23.8	23.8	23.8	23.8	23.8	23.8
Lysine	17.2	17.2	17.2	17.3	17.3	17.3
Methionine	4.6	4.6	4.6	4.6	4.6	4.6
Phenylalanine	7.7	7.7	7.7	7.7	7.8	7.8
Valine	16.9	16.9	16.9	17.0	17.0	17.0
Threonine	12.1	12.1	12.1	12.1	12.1	12.1
Total	**101.0**	**101.0**	**101.0**	**101.1**	**101.3**	**101.3**

Note: T1 (Treatment 1): Rovimix 2020, 0.6% (C-2020); T2 (Treatment 2): L-OVN for *Pangasius* Broodstock 0.6% (L-OVN); T3 (Treatment 3): H-OVN for *Pangasius* Broodstock 0.6% (H-OVN); T4 (Treatment 4): L-OVN for *Pangasius* Broodstock 0.6% + 12.6% algal oil; T5 (Treatment 5): H-OVN for *Pangasius* Broodstock 0.6% + 12.6% algal oil (H-OVN-AO); T6 (Treatment 6): H-OVN for *Pangasius* Broodstock 0.6% + 12.6% algal oil + 1.5% fungal oil (H-OVN-AO/FVO).

2.5. *Broodstock Experimental Design*

The experiment was set up as a factorial design with six different diets fed in triplicate groups of *Pangasius* catfish broodstock cultured in a hapa net system in an earthen pond (1,500 m^2) with a depth of about 2.5 m. Ten fishes with a mate ratio of 3 males : 7 females were reared in each net for about two months to acclimatize them to the conditions in the hapa net. Before the experiment began all the fish were fed daily on the same commercial diet, containing 24% CP. Feeding was carried by hand, from a small boat, between 4 and 5pm each day, at a rate of 3-5% of body weight, until fish reached apparent satiety. The feed was distributed to each treatment using a small boat.

2.6. *Induced Spawning and Larvae Rearing Practices*

2.6.1. Mature Broodstock Fish Selection for Induced Spawning

After 65 days of intensive feeding of the broodstock fish with the experimental diets, the egg quality and development status of individual female fish were checked with the help of a catheter. Mature females were identified by their big, round and soft bellies, along with reddish, swollen ventral genital pores. Male broodstock fish were identified by observation of their genital papilla, which oozes milt/semen when they were ready to breed, the presence of a slight stripe on the abdomen, and the quantity and quality of their sperm/milt were checked by stripping.

Mature male and female broodstock fish in good condition from each treatment were selected, marked, and quarantined for 1–2 days in separate rectangle tarpaulin tanks with 10 m^3 of water volume for female breeders and 5 m^3 of water volume for male breeders for their acclimation to the water environmental condition in the hatchery before the induced breeding procedure commenced

2.6.2. Induced Stripping Practices of Broodstock

The mature broodstocks were induced to spawn in hatcheries using human chorionic gonadotropin (hCG) injection. The female fish were given 4 injections, each injection with a different dose of hCG: 200, 300, 700 and 2,700 UI/kg, while male fish were injected only once, at the same time as the final injection for the females. The detailed protocol of hCG doses, time for fish injection, and injection dose calculation are given in the supplemental information. Females ovulated from 10–12 hours after the last injection, with a water temperature of 27–28°C. Eight hours after injecting the last dose of hCG, female breeders were checked for eggs by slight stripping on the belly to ensure that the ripe eggs were at stage IV condition. Eggs and milt/semen of fish breeders from each treatment were then drily striped and slowly poured into each other in small plastic tubs, each stripping into a separate tub. All eggs and body weight of each female from different treatments were weighed for their gonadosomatic index determination. Milt/semen and eggs of different treatment were stirred

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around two minutes by using the chicken feathers and then washed 2–3 times with clean water (Figure 1). A tannin solution with 5% concentration was added to the tub to remove adhesiveness (stickiness). The mixture was slowly poured on the mixed eggs in a plastic tub of each treatment and stirred for 1–2 minutes, and then thoroughly rinsed with clean water 2–3 times. One gram of eggs of each treatment was sampled and measured in triplicate to evaluate the egg fecundity, eggs number, and eggs size, using a microscope (Carl Zeiss Microscopy, Germany) at 4x and 10x magnifications. The wet weight of eggs was determined using an electronic balance (Mettler Toledo, Swiss).

2.6.3. Eggs Incubation and Larvae Fish Nursing Practices

An incubator system was set up in a closed re-circulation system in a series of 18 hatching jars (Weiss-shaped incubators) for six treatments in triplicates with a water volume of about 18 L for each hatching jar (Fig. 1). The water supply for the hatching jars of the incubator system was taken from a reservoir tank, treated with a biological filter and passed through an ozone generator before being supplied to each hatching jar. The water flow through the incubator system had been adjusted to moderate flow so the eggs were stirred and not allowed to settle at the bottom of the jars. Fertilized eggs were stocked at an average density of 44,340 (15,800–88,890) eggs per hatching jar. The fertilized eggs of each treatment were incubated in the hatching jar system for 35–36 hours at a water temperature of 25–27^{0}C during December.

After hatching, all larvae fishes were moved and delivered into 18 different nursing tanks (six treatments in triplicates) with 0.5 m^3 of water in each tank and reared for 48 hours. Aeration with two air-stone diffusers was provided to each tank via a moderate-pressure electrical blower. The total length and height of larvae after hatching were measured under a microscope (Carl Zeiss Microscopy, Germany), and the wet weight of hatchlings was measured using an electronic balance (Mettler Toledo, Swiss).

2.7. *The Experiments of Larvae to Fingerling Rearing*

The larvae remained in the nursing tanks for the first 48 hours, until the yolk sacs had been absorbed, and then transferred to larger indoor tanks for the next 15 days, during December 2020 to January 2021. There were 24 tarpaulin tanks, placed indoors, each tank with a water volume of 1 m3 (1 m x 1 m x 1 m for each tank) and a stocking density of 15,000 larvae/tank. They were supplied with water which had been treated with a biological filter. The experiments were covered by a blue tarpaulin and a green net for sun and rain shade to control the temperature during the winter season (Figure 1). The rearing tarpaulin tanks of the experiment were also covered with a long white tarred canvas to maintain the water temperature at nighttime (Fig. 1, G). Two days after hatching and until the 5th-day, the larvae fishes was fed with Artemia (Vinh Chau Artemia products) at a rate of Artemia to larvae of 5 : 1. Between 6–15 days after hatching the larvae fishes were also fed a mixture made up of 50% brine shrimp + 50% of UV-milk powder feed with 42% CP content (Commercial feed powder (Name: Tomboy TB0). The larvae were fed daily 4 times to apparent satiety at 8.00 AM, 11:00 AM, 2:00 PM, and 5:00 PM. At the beginning of the experiment, a sample of 30 larval fish from the nursing tank from each treatment was weighed using a digital scale and measured using a microscope (Mettler Toledo, Swiss) for the evaluation of growth performances indices. All larvae fish from each experimental treatment were harvested, counted and weighted at the end of the experiments to estimate production and final survival rates of the fish.

The second experiment of fry to fingerling rearing was conducted after finishing and harvesting the fish larvae from first experiment. 2,000 fry per tarpaulin tank were reared for 45-day using the same facility, equipment and diet treatments (6 treatments in 4 replicates) as the first experiment of larvae rearing (Fig.1). Fish were fed 2 times per day with the experimental milled diets to apparent satiety at 8.00 AM and 5:00 PM. At the beginning and in the end of the experiment, a sample of 30 fish in each nursing tank from each treatment were weighed and measured in the same way as in the first experiment. Fish of each treatment during the experiment were collected every fortnight to measure weight and length gains for the evaluation of growth performances indices. All fry of each experimental treatment were harvested, counted and weighted at the end of the experiments to estimate fingerling production and final survival rates of fingerlings.

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2.8. *Water Quality Monitoring*

Temperature (T^{o}C), Dissolved oxygen (DO mg/L), and pH in the earthen pond of the broodstock cultivation and the rearing tank system for fish larvae were recorded daily with a DO meter. Water samples for nitrogen (NO_2^- mg/L) and ammoniac (NH_3^+) analyses were collected twice a month and kept cool in the refrigerator until they were analysed using the Hach Lange cuvette test method (DR2800 visual spectrophotometer, Hach Lange Gmbh, Germany).

2.9. *Chemical Analysis*

Samples of experimental test ingredients and diets were analyzed by using standard methods [42]. Dry matter was determined by drying in an oven at 105^{0}C for 24 hours. Nitrogen (N) was determined by the Kjeldahl method and crude protein (CP) was calculated as N x 6.25. Crude fat (EE) content was analyzed using the Soxhlet method after acid hydrolysis of the sample. Ash content was determined by incineration in a muffle furnace at 550^{0}C for 12h. Amino acid profiles of ingredients and diets (Table 2 and Table 3) were analysed by high-performance liquid chromatography according to [43]. The fatty acid composition of the diets (Table 3) was determined using the total lipid extracts of the diets that were transesterfied with boron triflouride. Laboratory analysis of feed ingredients and diets was conducted at the Advanced Laboratory, Department of Science, Can Tho University, Vietnam.

2.10. *Calculation*

The following calculations were made:

Fertilization (%) = [Number of fertilized eggs / total number of eggs in the batch] × 100.

Hatching rate (%) = [Number of hatched eggs / total number of eggs in the batch] × 100.

Ripe eggs (%) = (Number of eggs with yolk position near one edge of the egg / total number of eggs counted) × 100.

Gonadosomatic index (GSI%) = (Gonad weight / total body weight) × 100.

Relative fecundity index (RFI) = (Total number of eggs in female ovary/Total weight of female).

Weight gain = Final body weight – Initial body weight.

Length gain = Final body length – Initial body length

Daily weight gain (DWG) = (W_f – W_i) / T), where W_f and W_i refer to the mean final weight and the mean initial weight, respectively, and T is the feeding trial period in days.

Specific growth rate (SGR%) = [(ln W_f – ln W_i) / T] × 100.

Food conversion ratio (FCR) = [total feed intake (g)/total wet weight gain (g)].

Survival rate [(SR%) = (TF_f / TF_i) × 100], where the TF_f is the total number of fish at the finish (harvest) and TF_i is the total number of fish at the start.

2.11. Statistical Analysis

All data on induced spawning, egg fecundity, early lifestage development, hatching rate, growth performances of broodstock and larvae fish, survival rate of fry and fingerling, and water quality parameters were statistically analyzed by General Linear Model (ANOVA), using Pairwise Comparison and Tukey method for treatment comparisons ($P \leq 0.05$ level of significance), MINITAB Statistic program (version 16).

3. Results

3.1. Chemical Composition and Essential Amino Acid Content of Feed Ingredients and Diets

The result of the nutrient contents and amino acid profiles of feed ingredients showed that it was highest in poultry by-product meal, followed by fish meal, soybean meal, and wheat flour (Table 2).

The chemical analysis composition of the six diets used for *Pangasius* catfish broodstock cultivation is presented in Table 1 and 3. The crude protein (CP) contents of the experimental diets were approximately 350 g/kg DM. The highest crude fat contents were found in the diets of Treatment 5, Treatment 4 and Treatment 6, which were supplemented with algal and fungal oils compared to other test diets. The contents of crude fibre and ash ranged between 28.9–48.8 and 103–118 g/kg DM,

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respectively. Amino acid and fatty acid profiles in six test diets fed to the *Pangasius* catfish broodstock were quite similar among the experimental diets (Table 3).

3.2. Growth Performance Indices of Broodstock

The broodstock fish from each treatment was measured to obtain the final fish body indices at the end of experiment. The growth performance indices of the experimental fishes are shown in Table 4. The results showed that average final body weight (BW) and weight gain (WG) of female broodstock fish at the end of the experiments were 6.11±0.0.46 (5.57–6.95) kg/fish and 1.24±0.48 (0.63–1.95) kg/fish (wet weight basis), respectively with slight differences between treatments ($P < 0.05$). There were significant differences ($P < 0.05$) in daily weight gain (DWG), and specific growth rate (SGR) between treatments of the broodstock fish (Table 4). The highest WG, DWG and SGR were found in Treatments 5 (diets supplemented with 0.6% vitamin premix H-OVN and 1.26% algal oil), followed by Treatment 1 (diets supplemented with 0.6% vitamin premix C-2020), and the lowest found in Treatment 4 (diets supplemented with 0.6% vitamin premix H-OVN) (Table 4).

Table 4. Growth and reproductive performances indices of broodstock of *Pangasius* catfish fed with test diets for 65 days.

Indices		Experimental Treatments						*P-value*
		T1	T2	T3	T4	T5	T6	
Growth performance indices of brooder fish								
Initial body weight (kg)		4.4±0.7	5.0±0.9	5.2±0.9	4.9±0.8	5.0±0.1	4.7±0.1	0.841
Final body weight (kg)		6.2±0.9^{b}	6.1±0.7^{b}	6.0±0.01^{b}	5.6±0.6^{c}	7.0±1.1^{a}	6.0±0.2	0.024
Weight gain (kg)		1.8±0.2^{ab}	1.0±0.3^{b}	0.9±0.9^{bc}	0.6±0.3^{c}	2.0±0.9^{a}	1.3±0.2^{ab}	0.027
Daily weight gain (kg)		0.04±0.01^{ab}	0.02±0.01^{b}	0.02±0.02^{bc}	0.02±0.01^{c}	0.10±0.02^{a}	0.02±0.01^{ab}	0.027
Specific growth rate (SGR%)		0.5±0.4^{ab}	0.3±0.2^{b}	0.3±0.2^{bc}	0.2±0.1^{c}	0.6±0.2^{a}	0.4±0.01^{ab}	0.027
Food conversion ratio (FCR)		1.8±0.3	1.8±0.4	1.7±0.9	1.8±0.2	1.6±0.1	1.7±0.3	0.100
Reproductive performance indices								
Egg size (µm)	Before injecting hCG	1.0±0.1	0.9±0.01	0.9±0.1	1.0±0.01	1.0±0.1	0.9±0.1	0.07
	After injecti	1.1±0.03^{ab}	1.1±0.1^{b}	1.0±0.3^{c}	1.0±0.5^{bc}	1.1±0.3^{a}	1.0±0.1^{ab}	0.001

 doi:10.20944/preprints202406.1037.v1

	ng hCG						
Gonad weight (g/fish)	750.1±54.8[a]	366.7±100.1[b]	400.0±109.5[ab]	466.7±264.6[ab]	754.02±248.1[a]	500.1±100.6[ab]	0.004
Gonad somatic index (GSI%)	12.2±0.98[a]	6.0±1.7[b]	6.7 ± 1.8[ab]	8.4±4.8[ab]	12.8±0.8[a]	8.4±0.1[ab]	0.027
Relative fecundity index (egg/kg)	152,158±7,467[a]	90,014±2,467[b]	104,267±7,381[ab]	133,642±1,503[a]	199,512±7,467[a]	119,748±1,166[ab]	0.022
Total number of eggs in female ovary (egg)	1,227,000±107.4[a]	546,383±303.7[b]	625,600±452.3[ab]	744,387±119.9[ab]	1,057,500±232.1[a]	712,500±106.9[ab]	0.047

Note: The values represent the mean ± SD (Standard Deviation). Means within rows with different superscript letters are significantly different (P < 0.05).3.4. Growth performance indices of larvae fish reared at 15 days after hatching.

3.3. Reproductive Breeding, Hatching, and Early Life-Stage Development

The results showed that the average diameter of the egg before and after fertilization ranged from 925–985 µm and 1,023.3–1,133.3 µm, respectively (P = 0.001–0.007). The egg sizes after fertilization were 1.1–1.2 times larger than the sizes before fertilization (Table 4). The total number of eggs in the female ovary (egg) ranged from 546,383–1,227,000 and was significantly different between treatments (P < 0.05). The average gonad weight of females was highest in Treatment 5 (754 g/fish) and Treatment 1 (750 g/fish), followed by the Treatment 6 (500 g/fish), Treatment 4 (466.7 g/fish), Treatment 3 (400 g/fish), and Treatment 2 (366.7 g/fish) (P < 0.05). Gonadosomatic index (GSI%) values were found highest and lowest in Treatment 5 and Treatment 3 (6.0%), respectively (P < 0.05) (Table 4). The relative fecundity index (RFI) ranged between 90,014–199,512 egg/kg and were significantly different between each treatment (P < 0.05). Fig. 2 showed that egg fertilization and hatching ratios varied between treatments with a range of 62.2–85.9% and 43.5–87.3%, respectively (P = 0.001–0.027). The highest proportion of egg fertilization was recorded for Treatment T5 (85.9%) followed by Treatment 6 (85.1%) and Treatment 1 (82.4%), and the highest hatching rate was in Treatment 1 (87.3%) followed in descending order by Treatment 5 (76.2%), Treatment 2 (73.7%), Treatment 6 (70.3%), Treatment 4 (54.6%), and Treatment 3 (43.5%) (Fig. 2).

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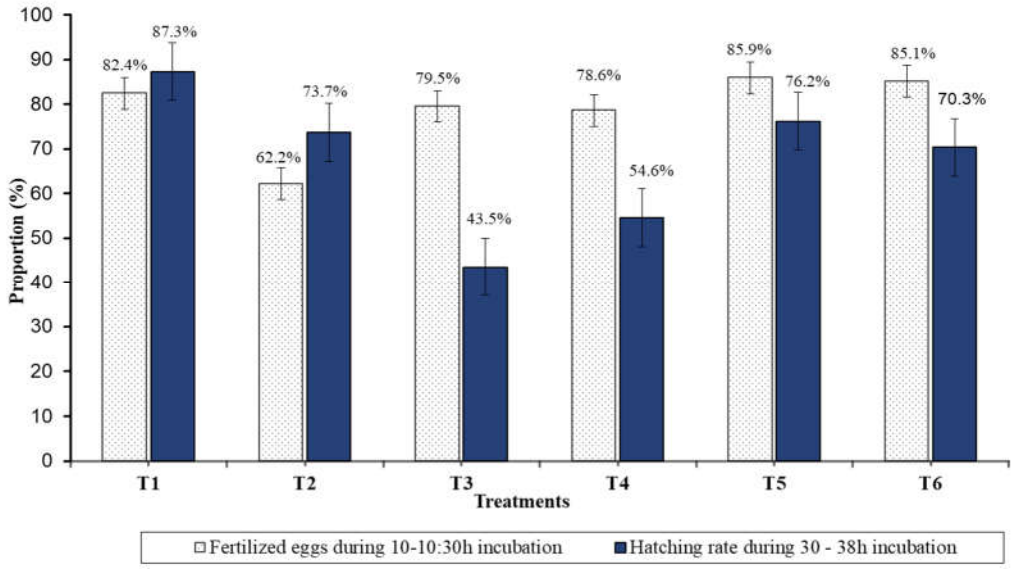

Figure 2. The proportion (%) of fertilized eggs and hatching rates of *Pangasius* catfish.

There were significant differences ($P = 0.001–0.033$) between treatments in final body weight gain (BWG), final length, daily weight gain (DWG), and specific growth rate (SGR) of Pangasius catfish larvae at 15 days after hatching (Table 5). The highest and lowest final BW, final fish length, WG, and DWG were recorded in Treatment T3 (diets containing 0.6% vitamin premix H-OVN) and Treatment 2 (diets containing 0.6% vitamin premix L-OVN), respectively. The specific growth rates (SGR%) were highest in Treatment 2 (10.1%) and Treatment 4 (9.2%), followed by Treatment 6 (8.6%), Treatment 1 (8.5%), T3 (8.2%), and Treatment 5 (6.1%) ($P < 0.05$). The data in Table 5 and Fig. 3 show that the total number of fish larvae and final survival rate of larvae 15 days after rearing ranged between 4,015–8,402 fish and 23.4–56.0%, respectively ($P < 0.05$). The highest number and highest survival rates of fish were found in Treatment 5 (56.0%) in the diets containing 0.6% vitamin premix H-OVN, followed in descending order by Treatment 3 (40.4%), Treatment 1 (36.0%), Treatment 6 (32.7%), and the lowest of final survival rates of fish were found in Treatment 2 (29.4%) and Treatment 4 (22.7%) in the diet which contained 0.6% vitamin premix L-OVN-AO) + 1.26% algal oil ($P < 0.05$).

Table 5. Growth performance indices and fingerling production of *Pangasius* catfish fingerlings reared at 30 days after hatching, and 45 days after hatching.

Indices	Experimental Treatments					
	T1	**T2**	**T3**	**T4**	**T5**	**T6**
Body indices of fingerling reared at 15 days **(n = 15,000 larvae/tank)**						
BWG (mg)	12.1±0.1 ^{c}	11.11±0.01^{d}	15.6±0.1^{a}	14.4±0.2^{b}	14.3±0.2^{b}	12.9±0.2^{c}
Length (mm)	14.9±0.4^{bc}	14.82 ± 0.4^{c}	15.4±0.5^{a}	15.2±0.4^{ab}	15.1±0.4^{bc}	15.0±0.4^{bc}
DWG (mg)	0.6±0.01^{d}	0.60±0.01^{e}	0.8±0.01^{a}	0.8±0.01^{b}	0.8±0.01^{b}	0.6±0.01^{cd}
SGR%	8.5±0.6^{c}	10.05±0.7^{a}	8.2±0.4^{c}	9.2±0.9^{b}	9.1±0.6^{b}	8.6±1.0^{c}
Body indices of fingerling reared at 30 days **(n = 2,000 fry/tank)**						
BWG (mg)	63.0±35.2^{b}	147.0±24.5^{a}	41.0±9.4^{d}	23.0±15.2^{e}	47.0±11.3^{c}	39.0±18.7^{d}

 doi:10.20944/preprints202406.1037.v1

Length (mm)	25.0±2.2[a]	22.0±2.0[ab]	18.3±0.5[b]	23.6±0.6[a]	18.0±0.4[b]	24.0±1.4[a]
DWG (mg/d)	4.9±0.3[b]	11.3±1.5[a]	3.2±0.8[d]	1.7±0.9[e]	3.6±1.5[c]	3.0±19[d]
SGR (%)	6.9±1.2[c]	10.6±2.2[a]	4.4±1.6[e]	2.6±1.7[f]	6.4±3.0[d]	4.7±2.1[e]
Body indices of fingerling reared at 45 days **(n = 2,000 fry/tank)**						
BWG (mg)	947.0±64.0[a]	313.0±58.7[d]	708.0±53.2[b]	431.0±66.4[c]	304.0±51.2[d]	499.0±98.8[c]
Length (mm)	60.1±1.7[a]	41.5±1.2[b]	41.5±0.9[b]	56.5±1.0[a]	37.0±0.7[b]	57.5±1.3[a]
DWG (mg/d)	79.0±1.5[a]	26.0±2.3[d]	59.0±3.3[b]	36.0±2.9[c]	25.0±3.2[d]	42.0±5.4[c]
SGR (%)	6.8±0.7[b]	2.7±0.6[d]	9.0±1.5[a]	4.0±2.2[c]	4.12±2.2[c]	4.0±1.8[c]
Total number of survival larvae (fish)	466.7±12.7[b]	439.3±94.7[b]	1326.3±560.5[a]	413.3±242.7[b]	781.3±173.2[b]	603.7±251.7[b]

3.4. *Growth Performance and Survival Rate of Fingerling Reared at 30 and 45 Days after Hatching*

The body weight gain (BWG), final length, daily weight gain (DWG), and specific growth rate (SGR%) of fish fry 30 and 45 days after hatching are presented in Table 6. The results of growth performance indices showed that there were significant differences among the test diets ($P < 0.05$). The fish body indices 45 days after hatching were 2.1–18.7 times higher than the body indices 30-days after hatching (Table 5). The total number and survival rate of fingerlings 45-days after hatching ranged between 413.3–1,326.3 fingerlings and 22.0–66.3% ($P < 0.05$). The highest final survival rate of fingerling were recorded in Treatment 3 (66.3%), Treatment 5 (40.4%), followed by Treatment 6 (36.3%), and Treatment 1 (23.3%), while the lowest survival rates were found in Treatment 4 (22.7%), and Treatment 2 (22.0%).

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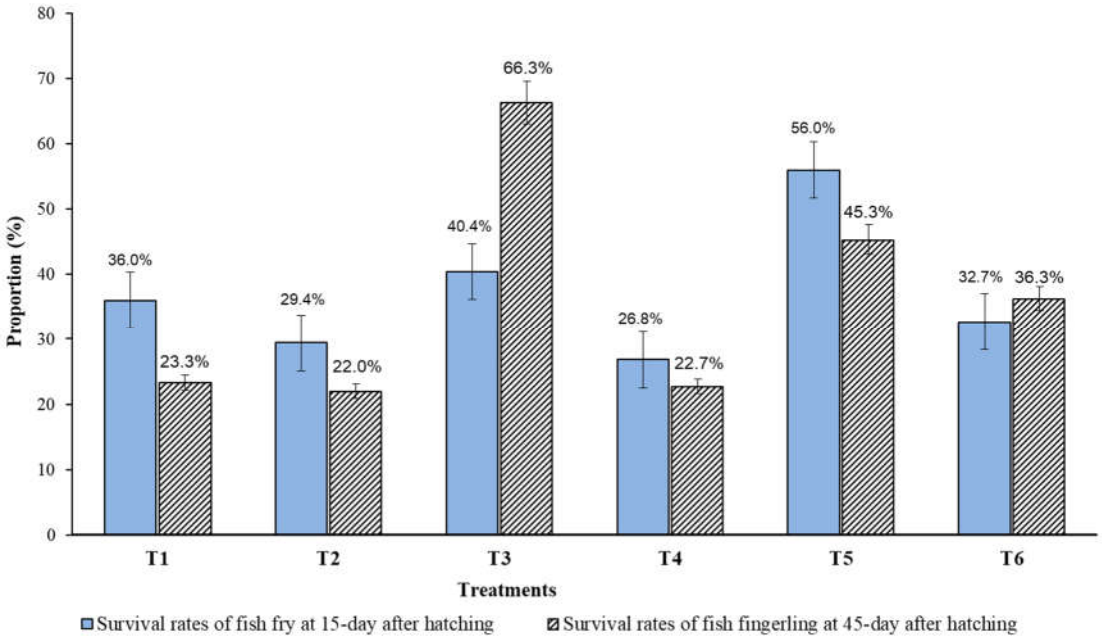

Figure 3. The survival rate of fish fry at 15 days and fish fingerling at 45 days after hatching of *Pangasius* catfish.

3.5. *Water Quality Monitoring*

Average temperature, pH, DO, NH_3/NH_4^+ and NO_2^- of the water in the earthen pond where the broodstock were reared ranged between 24.8–29.8^{0}C, 7.30–8.05, 1.7–3.1 mg/L, 0.11–0.15 mg/L and 0.35–0.58 mg/L, respectively. The water quality parameters of the fish larval and fingerling rearing tanks - ranged between 25–27°C,7.9± 0.1 (7.8–8.1) pH, 1.9±1.1 (0.8–3.2) NO_2^- (mg/L), and 0.05 ± 0.03 (0.02–0.10) NH_3/NH_4^+ (mg/L).

4. Discussion

A diet rich in functional ingredients such as n-3 LC-PUFA fatty acids, essential amino acids, vitamin antioxidants, and prebiotic compounds has been shown to improve the broodstock, survival rate of larvae, yield, and farmed fish quality [19,44–46]. Colombo [44] reported that maternal nutrition directly influence on the quality of the larvae and fingerlings. Lipids (fats and fatty acids) from fish oils, vegetable oils, microalgae and algal oils are an essential macronutrient for growth performances of fish and they provide at least three key essential fatty acids (EFA), which contain n-3 LC-PUFA, specifically DHA (22 : 6n-3) and EPA (20 : 5n-3). These substances are important for the metabolism of terrestrial animals and fish and contribute to their growth and physiological functions [17,18,20,47,48]. Several researchers have shown that diets containing highly unsaturated fatty acids (HUFA), such as n-3 and n-6 HUFA influence gonadal development, eggs quality, fecundity, hatching and larvae survival rates [15,20,49–51]. It is [50,52] reported that the dietary manipulation of n-3 and n-6 highly unsaturated fatty acid could improve levels and ratios of AA, EPA, and DHA , which were transferred to the resulting eggs with improvements in early survival and hatching success for European sea bass (*Dicentrarchus labra*) and Channel catfish (*Ictalurus punctuates*).

In recent years, the microalgal biomass market produces about 5,000 tonnes of dry matter per year and generates a turnover of approximately US$ 136.25 million per year [53,54]. Ślusarczyk, Adamska [17] reported that both fungi and algae are a potential source of natural antibiotics and antioxidants that would be safe to use and have no side effects. It is indicated that fungi and algae have the potential to replace fish meal and fish oil in aquaculture and ensure sustainability standards. Algae provide essential amino acids, valuable triglycerides such as lipids, vitamins, and pigments, making them suitable as nutritional supplements in livestock feed and aquafeed formulations [17–19]. Several previous studies have reported that among the lipids in algae there are essential

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unsaturated fatty acids (EFAs) including arachidonic acid, eicosapentaenoic acid and the rare γ-linolenic acid (GLA) [17,48,54].

Vitamins are organic compounds essential for supporting the normal growth and health of fish. They represent a significant cost in food fish and aquafeed production [55]. Since fish often cannot synthesize vitamins and essential amino acids, they must be supplied in their diets [15,16,55]. Vitamin premixes commonly used in food fish production diets are often considered adequate for farmed fish [55]. Consequently, the relative importance of fatty acid and vitamin premix contents for reproductive performance and fish fry production can be qualitatively assessed by testing them as supplements in broodstock fish diets. The chemical composition, the content of essential amino acids and fatty acids of the experimental diets used in this experiment were comparable with the values reported for broodstock diets of European Sea Bass [50], Gilthead sea bream [16] and Channel catfish [56]. Also the content of crude protein, crude fat and the composition of the vitamin premix compounds of the test diets in this study were in good agreement with feed given to adult channel catfish, tilapia, African catfish and striped catfish [28,39,57].

Our results show that there were significant differences ($P < 0.05$) among the test diets in growth performance indices (final body weight, total weight gain, daily weight gain, specific growth rate) and reproduction indices (gonad somatic index, relative fecundity index, total number of eggs in the ovary, percentage of fertilized eggs, and hatching ratios) of the broodstock (Table 4 and Figure 2). The values for the diets in Treatment 5 were the highest, followed in descending order by Treatment 1, Treatment 6, Treatment 2, Treatment 3, and Treatment 4. This indicates that the Pangasius catfish broodstock received the diet nutrients well, without compromising growth and reproductive performance indices. [15] and [58] reported that gonadal development, fecundity and egg fertilization, egg size, and total egg volume all increased in some fish species when certain dietary proteins and essential nutrients, such as essential amino acids, vitamins, and fatty acids, were available in the feed..

The broodstock SGR in the present study was similar to values observed in a study on striped catfish breeders after six months feeding with a trial feed containing a 35% crude protein and a supplementary vitamin premix [59,60]. The GSI (%) and RFI (egg/kg) values in the present study were much higher than GIS (4.73–9.21%) and RFI (65,000–168,900 eggs/kg) previously reported for Pangasius catfish broodstock fed different dietary protein (25–45% CP) [59,61]. In general, the relative fecundity values (egg/kg) in this study were comparable to values (117,000–153,000 eggs/kg) of striped catfish broodstock spawners obtained by [4,59,62]. However, it was much greater than value found for Basa (*Pangasius bocourti*) and *Pangasius* catfish broodstock reported by Cacot, Legendre [63], Cacot [64]. The fertilized egg incubation period in this experiment lasted for 33–36 hours to complete the hatching process and is similar to the hatching periods reported for Asian Pangasius catfish [65]. The average hatching rate of eggs in the present study during the off? breeding season was approximate 78.5% ($P < 0.05$), which was in the range of the 70–80% hatching rates reported for *Pangasius* sutchi during peak season in Bangladesh [66] and Vietnam [4,5], Malaysia [67] and Nepal [62]. However, the hatching rate of the present study was much higher than the average values of 55–65% reported for Tra catfish (*Pangasius hypophthalmus*) and Basa catfish (*Pangasius* bocourti) in Vietnam [63,65,68] and 30% reported for yellowtail catfish (*Pangasius pangasius*) in Bangladesh [68].

The survival rates of fingerlings reared for 45 days after hatching ranged between 22.0% and 66.3%. The highest final survival rate was recorded in Treatment 3 (66.3%), followed by Treatment 5 (45.3%), Treatment 6 (36.3%), Treatment 1 (23.3%), Treatment 4 (22.7%), and Treatment 2 (22.0%) ($P<0.05$). These results suggest that diets for Pangasius broodstock supplemented with 0.6% H-OVN, as well as those supplemented with 0.6% H-OVN mixed with 12.6% algal oil, can improve growth performance, reproduction of the broodstock, and survival rates of fingerlings. This improvement is likely due to the adequate provision of lipids, essential amino acids, and vitamins, which regulate metabolism and intestinal flora. The final survival rates (22.0–66.3%) of fingerlings in the present study during the over spawning season were 1.4 to 2.0 times higher than the survival rates (20–25%) during the main spawning season and (12–15%) during the over spawning season reported for Pangasius catfish by fish farmers in the Mekong Delta [2,4–7].

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5. Conclusions

This study demonstrated that the broodstock of *Pangasius* catfish effectively absorbed the nutrients from the test diets, resulting in increased growth and improved reproductive performance indices. Our research found that diets containing 35% crude protein (CP), supplemented with 0.6% vitamin premix H-OVN (Treatment 3), and diets containing 35% CP, supplemented with 0.6% vitamin premix H-OVN and 1.26% algal oil (Treatment 5), produced the best results. These diets led to optimal growth, enhanced reproductive performance, and the highest final survival rates of fry and fingerlings. The findings of this research provide valuable information for *Pangasius* catfish farmers and the fish production industries in the Mekong Delta, Vietnam. Further studies on these diets for *Pangasius* catfish broodstock, along with a cost-benefit analysis under commercial farm conditions, are recommended to confirm the advantages demonstrated by this study.

Author Contributions: Conceptualization, design of the experiment and acquisition of funding: Chau Thi Da. Conduct of experiments and data collection: Chau Thi Da and Bui Thi Kim Xuyen. Formal analysis and writing—original draft preparation: Chau Thi Da and Bui Thi Kim Xuyen. Writing—review and editing: Chau Thi Da, Charles Howie, Hakan Berg, Bui Thi Kim Xuyen, Thi Kieu Oanh Nguyen, Pham Thi Thu Ha, Thai Minh Quan, Van Tai Tang and Minh Tan Pham. All authors have read and agreed to the published version of the manuscript.

Funding: Department of Physical Geography, Stockholm University, Sweden

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available from the corresponding author on request.

Acknowledgments: The authors wish to thank DSM SINGAPORE INDUSTRIAL PTE. LTD, a company incorporated in Singapore for funding this research for a researcher team of Ton Duc Thang University, Vietnam to carry out this research. The authors wish to thank NAVICO Company, An Giang province for providing striped catfish broodstock and necessary facilities to conduct experiments and technician staff to support and assist the implementation of these experiments. On behalf of authors, I would like to thank Professor Torbjörn Lundh, Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Sweden for his valuable comments and advice on the feed nutrition for this research. Finally, we would also would like to thank the laboratory staff of the Advanced Laboratory, Department of Science, Can Tho University, Vietnam, and Mr. Tran Quang Dien and Mr. Qui, students of the Aquaculture Department, Faculty of Agriculture and Natural Resources, An Giang University, Dong Nai Technology University Vietnam, for their support and assistance during this study. Finally, the authors also thank professor Hakan Berg (Stockholm University, Sweden) and Dr. Charles Howie Former adviser in education to Faculty of Agriculture, An Giang University, and Agricultural Consultant for language revision of this paper.

Conflicts of Interest: The authors declare no conflict of interest.

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