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Research article

Effects of Dietary Protein Levels on the Stage Development and Production Performance of Nile Tilapia

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Abstract: Lack of understanding in selecting age-appropriate protein levels in the tilapia diet would result in low growth and productivity. Therefore, the effect of dietary protein levels on tilapia development and the productive potential of fry raised in concrete ponds according to the CRD experimental protocol was investigated using three treatments with four replications. Fifteen breeder fish/ponds with a male-to-female ratio of 1:2 were fed with 15.5, 25, and 30% protein for 20 weeks. The water quality was monitored and the fish's mouth was tapped every 2 weeks. The results showed that feeding of 25% protein affected egg development at all stages (Zygote, Cleavage, Gastrula, Segmentation, Pharyngula, Hatching, Early larva, and Late larva) as well as production cost better than other feeds ($p<0.05$). Breeders fed 30% protein had a higher average weight, specific growth rate, condition factor, and feed efficiency but the lowest feed intake, feed rate, and feed conversion rate ($p<0.05$). A 25% protein diet provides adequate nutrition for hatching and fries production while keeping the pond water safe for their lives.

Keywords: Nile tilapia; Dietary protein; Fish growth; Fish egg; Water quality

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1. Introduction

Nile tilapia (*Oreochromis niloticus*) is one of the fish farmed on every continent in the world, including 135 countries [1]. This fish farming tends to increase and is the world's second-largest fish farming, inferior to the carp group. Thailand is the world's fifth-largest producer of tilapia [2] and tilapia is the first rank fish farming industry in Thailand. Tilapia produces up to 217.9 tons, accounting for 52.7% of all freshwater aquaculture [3]. Tilapia fry can be made in earthen ponds, net pens, and concrete ponds. The production of tilapia fry in concrete ponds is widely popular in Thailand. The pond size will be in the range of 2-10 meters in length, 2-4 meters in width, and 0.8-1 meters in depth, with breeders in the ratio of 1:3 (3-4 fishes per square meter) [4]. Producing fry in concrete ponds results in more accessible fry collection than in earthen ponds and net pens. Still, its production cost is higher, which is a significant weakness in the production system, especially the cost of food, which accounts for more than 50% of the total cost. Besides, it is necessary to manage the water system in the fishpond for suitable quantity and quality or to improve quality for tilapia farming, such as pH 6.5-8.0 and dissolved oxygen concentration (DO) of not less than 4 mg/L [5].

Fish feeds protein mainly significantly impacts the growth, survival rate, cost-benefit analysis, yield, and quality of farmed fish. A feed with appropriate protein levels for the development of fish of various ages and species is critical, whereas a meal with lower protein levels than the fish requires results in low growth rates and yields. [6]. The protein requirement from nutrients for tilapia is approximately 1 g/kg of fish per day, and breeders require protein in the range of 25-50% [6], fat in the range of 5-9% [8], and digestible energy in the range of 8.2-9.4 kcal/g protein [9]. Most aquaculture farms in Thailand use commercially available ready-made pellets with varying protein levels, including some using low-quality raw materials to reduce production costs. When the farmers use them to raise fish, they will impact the fish's development rate, low survival rate, and loss—as such, selecting the proper feed for tilapia production in concrete ponds is critical.

This study aims to determine the production potential of fry in small concrete ponds fed with various protein levels. The types of feeds with different protein levels have been selected from the local commercially available to assist the farmer in selecting the best feeds for fish farming.

2. Materials and Methods

2.1 Fish sample preparation

The male and female breeders of 180 and 120 tilapia (*O. niloticus*) were taken from the Nong Khai Inland Fisheries Research and Development Center in Nong Khai Province, Thailand. They were raised in six concrete ponds of two square meters in size, separated by sex, and filled with about 1 cubic meter of freshwater so that the fish could adapt to the experimental conditions. The fish were fed twice a day (9 a.m. and 3 p.m.) at a satiation level with a 25% protein diet (Charoen Pokphand Foods Public Co., LTD, Samut Sakhon, Thailand) for one week at the Faculty of Interdisciplinary Studies, Khon Kaen University Nong Khai Campus.

2.2 Experimental design

The study used a Completely Randomized Design (CRD) with four replications. Three commercially available floating pellet diets fed the fish: a 15.5% protein diet (herbivorous fish feeds, T1), a 25% protein diet (large catfish feed, T2 control) [4], and a 30% protein diet (small catfish feed, T3), with pellet sizes of approximately 5, 5, and 3 mm, respectively. The male and female breeders from each protein level were randomly weighed and placed in concrete ponds 1.8 x 1.8 square meters in size filled with water at a depth of 0.55 meters at a sex ratio of 1:2 (male:female) or 15 fish/pond with a mean weight of 107.51 ± 2.25 g and 92.79 ± 2.12 g of male and female fish, respectively. They were fed saturated diets (1-2% of their body weight) twice daily (9 a.m. and 3 p.m.) for 20 weeks. Also, the water was changed to 100% of its total volume every two weeks. Throughout the experiment, there were no substitutions of breeders, and their mouths were tapped every two weeks using Sousa's method. [10]

2.3 Data collection

Water quality was measured weekly, including water temperature (T) and dissolved oxygen concentration (DO), by using YSI (model 52, Yellow Spring Instrument Co., Yellow Springs, OH, USA). The pH of the water was measured with a pH meter (Digital Mini-pH Meter, 0-14PH, IQ Scientific, Chemo-science (Thailand) Co., Ltd, Thailand), and total ammonia in nitrogen form (NH_3-N) was determined by using the Phenate-hypochlorite method [11]. Biomarkers were analyzed every two weeks, including total bacteria and coliforms of *Escherichia coli* and *Streptococcus* sp., using colony count techniques. [12]

Fish growth was measured by weighing the fish on a 2 decimal scale, and the total length was measured at the end of the experiment with a vernier (no feeding for one day before weighing). Fish weight gain, remaining fish, and the weight of feed eaten by the fish were all recorded to calculate weight gain, specific growth rate, condition factor (K) = (100 x bodyweight) ÷ (total length)^3, feed conversion rate, and feed efficiency. Eggs were collected every two weeks and separated by egg stage, according to [13] before being calculated: spawning rate = 100 x (number of tapped mouth female breeder ÷ total female breeder released), fecundity = number of eggs ÷ female breeder weight (g), egg output = (number of eggs ÷ area (sq.m.)) ÷ day, female productivity = (number of eggs ÷ total weight of initial female breeder (kg)) ÷ day and egg production according to Surajit method [14]. The costs and benefits of the products were determined according to Shaha's method [15].

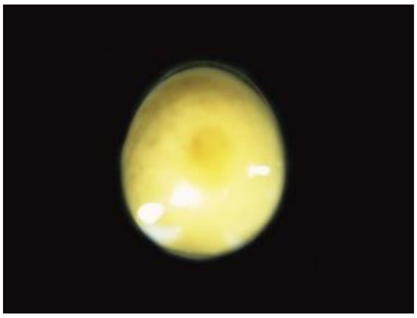
2.4 Data analysis

Observed data were analyzed for variance (ANOVA) according to the CRD experimental scheme. The mean difference ± standard error (SE) was compared using Duncan's Multiple Range Test at 95% confidence levels by the SPSS package.

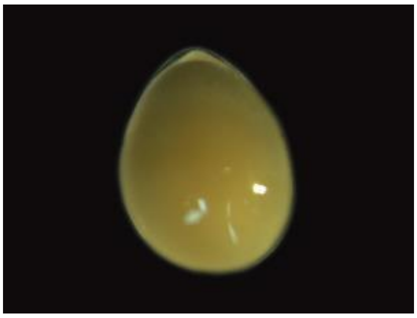
3. Results and Discussion

3.1. Results

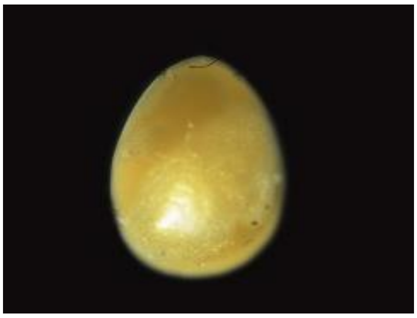
The results of mouth-tapping egg development in female breeders revealed that egg development of fish fed 15.5% protein had the highest cleavage and segmentation stages at 6.77 percent, followed by zygote stage (2.26%) and pharyngula, hatching, and only 1.50% of late larva stage. Still, no egg development was found in the gastrula and early larva stages. Egg development in fish fed 25% protein was discovered at various stages, in descending order: Late larva (10.53%) > segmentation (9.02%) > hatching (8.27%) > cleavage stage (7.51%) > zygote stage (5.26%) > early larva stage (4.51%) > pharyngula stage (3.01%), but no eggs were developed during the gastrula stage. Egg development was observed in descending order in fish fed 30% protein: As shown in Figure 1 and Table 1, cleavage (8.27%) > segmentation (6.77%) > zygote, pharyngula, and late larva (3.76%) > early larva (3.01%) > hatching (1.50%) > gastrula (0.75%).



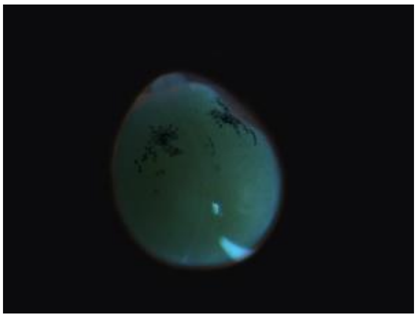
a. Zygote period



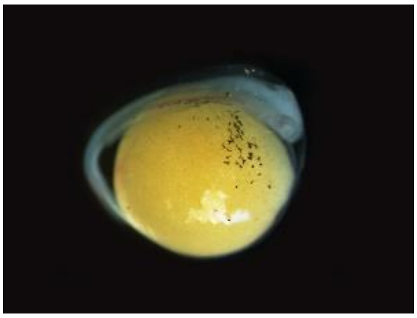
b. Cleavage period



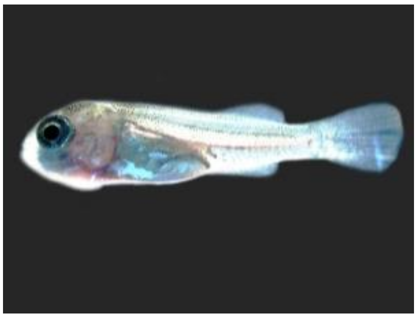
c. Gastrula period



d. Segmentation period




e. Pharyngula period f. Hatching period

g. Early larva period h. Late larva period

Figure 1. The developmental egg stages observed on Nile tilapia

Table 1. Percentages in developmental stages of Nile tilapia eggs as influenced by various percentages of feed protein diets (n=120)

Periods	**Diet (% protein)**		
	15.5	**25.0**	**30.0**
Zygote	2.26	5.26	3.76
Cleavage	6.77	7.51	8.27
Gastrula	None	None	0.75
Segmentation	6.77	9.02	6.77
Pharyngula	1.50	3.01	3.76
Hatching	1.50	8.27	1.50
Early larva	N/A	4.51	3.01
Late larva	1.50	10.53	3.76

The spawn rate per cycle of the female breeder ranged from 1.29 to 1.67% when the fish were fed 30, 25, and 15.5% protein, respectively, which was not statistically different (Table 2). The female breeder fed 30 percent protein had the highest fecundity at 5.20 eggs/g, which was significantly different. The total egg production, egg output, and female productivity of tilapia were 820.59 eggs, 1.81 eggs/m^2/day, and 4.14 eggs/kg total initial female/day, respectively when fed 30% protein. The results were not significantly different from those fed 25% protein ($p < 0.05$).

Table 2. Mean values of spawning rate, fecundity, egg production, egg output, and female productivity of Nile tilapia, measured at two weeks intervals (n=120)

Diet (% protein)	Spawning rate (%)	Fecundity (eggs/g female spawned)	Egg production (eggs)	Egg output (eggs/m²/day)	Female productivity (eggs/kg total initial female/day)
15.5	1.67 ± 0.30	2.03 ± 0.23^{c}	411.92 ± 111.60^{b}	0.91 ± 0.25^{b}	2.06 ± 0.50^{b}
25.0	1.60 ± 0.13	3.89 ± 0.25^{b}	815.17 ± 90.68^{a}	1.80 ± 0.20^{a}	4.07 ± 0.46^{a}
30.0	1.29 ± 0.11	5.20 ± 0.33^{a}	820.59 ± 92.92^{a}	1.81 ± 0.20^{a}	4.14 ± 0.49^{a}
F-test	ns	**	*	*	*
C.V. (%)	5.87	3.84	6.43	6.13	7.69

Letter (s) in each column indicated statistically significant presented as standard errors of means (ns=p > 0.05; *=p < 0.05; **=p < 0.01). C.V. = coefficient of variation.

Tilapia breeders fed different diets for 20 weeks revealed that the water temperature ranged from 27.22 to 27.66 °C, which was not statistically different (Table 3). The dissolved oxygen concentration, pH, and total bacterial count ranged from 5.20 to 5.29 mg/L, 8.01 to 8.04, and 201.33 to 214.80 CFU/mL, respectively, which were not statistically different. However, the highest amount of ammonia was 0.104 mg/L, and the lowest was 0.044 mg/L when the fish were fed 30 and 15.5% protein, respectively, which differed significantly. *Escherichia coli* and *Streptococcus* sp. were not found in water throughout the culture.

Table 3. Mean water temperature values, dissolved oxygen, pH, and the total amount of ammonia. These were measured at week intervals, while total bacteria and coliforms of *Escherichia coli and Streptococcus* sp. were measured at two weeks interval

	Diet (% protein)			F-test	C.V. (%)
	15.5	25.0	30.0		
Water temperature (°C)	27.66 ± 0.18	27.35 ± 0.14	27.22 ± 0.10	ns	4.11
Dissolved oxygen (mg/L)	5.29 ± 0.05	5.23 ± 0.07	5.20 ± 0.02	ns	9.45
pH	8.01 ± 0.02	8.04 ± 0.01	8.04 ± 0.01	ns	1.50
Total ammonia (mg/L)	0.044 ± 0.002^{c}	0.088 ± 0.002^{b}	0.104 ± 0.003^{a}	**	5.62
Total bacteria (CFU/mL)	201.33 ± 44.82	207.73 ± 33.12	214.80 ± 29.68	ns	5.93
Coliforms (cfu/mL)					
Escherichia coli	None	None	None	-	-
Streptococcus sp.	None	None	None	-	-

Letter(s) in each row indicated statistically significant effect due to treatments presented as standard errors of means (ns=p > 0.05; **=p < 0.01). C.V. = coefficient of variation.

The initial mean weight of fish was in the range of 95.26-100.92 g, which was not statistically different. At the end of the experiment, tilapia breeders had a 100% survival rate across all treatments, with the highest mean values of final weight, weight gain, specific growth rate, and condition factor (*K*) of 256.93 g, 161.68 g, 0.71%/day, and 2.20, respectively, when fish were fed 30% protein. When the data were statistically analyzed, it was discovered that the mean final weight, mean weight gain, specific growth rate, and condition factor (*K*) were all significantly different (Table 4).

Table 4. Mean values of final weight, weight gain, specific growth rate, and condition factor *(K)* of Nile tilapia as influenced by various percentages of protein diets (n=120)

Diet (% protein)	Mean initial weight (g)	Mean final weight (g)	Weight gain (g)	Specific growth rate (%/day)	Condition factor (K)
15.5	100.92 ± 6.42	213.86 ± 8.43^{b}	112.94 ± 4.07^{c}	0.54 ± 0.03^{c}	1.89 ± 0.01^{b}
25.0	96.90 ± 3.95	233.98 ± 2.98^{b}	137.08 ± 1.92^{b}	0.63 ± 0.02^{b}	1.97 ± 0.01^{b}
30.0	95.26 ± 5.35	256.93 ± 6.70^{a}	161.68 ± 2.51^{a}	0.71 ± 0.02^{a}	2.20 ± 0.09^{a}
F-test	ns	**	**	**	**
C.V. (%)	2.94	5.49	4.34	7.46	5.02

Letter *(s)* in each column indicated statistically significant presented as standard errors of means *(*ns=p > 0.05; **=p < 0.01*)*. C.V. = coefficient of variation.

Mean values of total feed intake and feed intake rates were the lowest at 4,482.66 g and 2.13 g/fish/day, respectively, when fed 30% protein. According to the statistical analysis, the mean values of total feed and feed intake rates differed significantly (Table 5). The lowest feed conversion ratio was 1.85, while the highest feed efficiency was 54.11% when fed 30 % protein, which was statistically significantly different.

Table 5. Mean values of total feed intake, rates of feed intake, feed conversion ratio, and feed efficiency of Nile tilapia fed with various levels of protein diets (n=120)

Diet (% protein)	Total feed intake (g)	Rates of feed intake (g/fish/day)	Feed conversion ratio	Feed efficiency
15.5	4,739.99 ± 46.49^{a}	2.26 ± 0.02^{a}	2.81 ± 0.09^{a}	35.73 ± 1.14^{c}
25.0	4,814.28 ± 62.40^{a}	2.29 ± 0.03^{a}	2.34 ± 0.05^{b}	42.74 ± 1.01^{b}
30.0	4,482.66 ± 81.07^{b}	2.13 ± 0.04^{b}	1.85 ± 0.02^{c}	54.11 ± 0.50^{a}
F-test	*	*	**	**
C.V. (%)	2.77	2.78	5.14	4.18

Letter *(s)* in each column indicated statistically significant presented as standard errors of means *(**=p < 0.05; **=p < 0.01*)*. C.V. = coefficient of variation.

The production costs per fish from 4 replication treatments were 185.44, 221.94, and 227.12 baht when fed 15.5, 25, and 30% protein, respectively, which were significantly different (Table 6). When the fish were fed 25 and 15.5% protein, the highest benefit was 43.83 baht, and the lowest use was 12.51 baht, which was significantly different. The benefit-to-cost ratios were 0.07, 0.20, and 0.13 when the fish were fed 15.5, 25, and 30% protein, respectively, and the opportunity cost at 1.35 percent interest was the highest at 1.48 and the lowest at 1.17 when the fish were fed 30 and 15.5 percent protein.

Table 6. Cost, benefit, benefit*:* cost ratio and the opportunity cost of Nile tilapia as affected by various levels of protein diets (n=120)

Diet (% protein)	Cost (Bath)	Benefit (Bath)[1/]	Benefit: Cost	Opportunity cost [2/]
15.5	185.44^{c}	12.51^{c}	0.07	1.17
25.0	221.94^{b}	43.83^{a}	0.20	1.41
30.0	227.12^{a}	30.60^{b}	0.13	1.48
F-test	**	**	-	-
C.V. *(%)*	10.40	7.40	-	-

Letter*(s)* in each column indicated the least significant differences in probability *(***=$p < 0.01$*)*. C.V. = coefficient of variation.

[1/] Sale price of seed Nile tilapia = 0.05 Bath

[2/] The deposit rate reading was 1.35% *(*Bank for Agriculture and Agricultural Cooperatives, 201*9)*

3.2. Discussion

Egg production, egg output, and female productivity were lower than those reported by [14] when bred tilapia with an average weight of 150 g in a 1:1 or 6 fish/pond ratio for 8 weeks. They noted that the average egg production, output, and female productivity were 29,621.1 eggs, 22.73 eggs/m^2/day, and 51.67 eggs/kg of female breeders/day, respectively. Furthermore, [16] reported that tilapia raised in concrete ponds had egg production and female productivity in the range of 19-77 eggs/m^2/day and 72-312 eggs/kg of female breeders/day, respectively. This is likely due to female breeders did not spawn at the same time. This fish can spawn throughout the year, and the spawning cycle lasts about 28-30 days [17]. The egg development was mainly obtained from the female breeder's mouth, with 22.56% and 22.55% of the egg developmental stages in the segmentation and cleavage stages, respectively. This is consistent with previously reported [14] that most eggs found in a female breeder's mouth are in the eyed egg stage and head-tail egg stage. Both are segmentation stages, according to [13].

Tilapia fed with a higher protein diet had a statistically significant increment in the mean fish weight gain and specific growth rate. This result is consistent with a study by [8] on tilapia (*O. niloticus*) by feeding a 25-45 percent protein diet; this could be due to the fish's ability to utilize proteins for adequate growth. On the other hand, the feed conversion rate tends to decrease as the protein level in the diet increases, which is consistent with a study published by [8] in which the feed conversion rate decreased from 2.5 to 1.6 when tilapia were fed an increased protein diet of 25% to 45%, respectively.

The water in the pond had a pH of 8.01-8.04 and a dissolved oxygen concentration of 5.20-5.29 mg/L, both suitable for tilapia culture. The ammonia concentration in the water in the closed system is primarily caused by fish excretion and overfeeding in the pond. The study discovered that when the fish were fed protein diets ranging from 15.5 to 30%, ammonia concentrations tended to increase (T1 to T3). The protein content of the feed caused this increment in ammonia concentration; when the feed had high protein, the fish excreted high ammonia levels. The results are consistent with studies of [18] in rainbow trout (*Oncorhynchus mykiss*) and [19] in grass carp (*Ctenopharyngodon idella*). The ammonia concentrations found in this study are in the range of 0.044-0.104 mg/L, which is a suitable level, that tilapia can survive harmlessly [20]. However, when fed 25 and 30% protein (T2 and T3), the ammonia concentrations were 0.088 and 0.104 mg/L, respectively, which may result in tilapia fertilization, hatching, and embryo toxicities. [21] discovered that increasing the ammonia concentration in water (0.05-0.6 mg/L) would decrease the number of fertilized eggs and hatching rates while increasing the number of deformed eggs.

4. Conclusions

The effects of dietary protein levels on tilapia development and the productive performance of tilapia fry in concrete ponds for 20 weeks were investigated in this study. Dietary protein levels influenced egg development in female breeders' mouths, with 25 percent protein providing enough nutrition for breeders to hatch eggs and produce hatchlings. Furthermore, it has the lowest production cost, feeding 30 and 15.5% protein in second and third place, respectively.

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Author Contributions: Nisarat Tippayadara and Baramee Phungpis participated in data analysis and drafted manuscript. Kanokkan Worawut conceived the study, designed the study, coordinated the study, carried out data analysis, interpreted the results and aided in drafting the manuscript. All authors gave final approval for publication.

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