

# ENDOCRINE AND REPRODUCTIVE OUTCOMES OF PROTEIN OPTIMIZATION, LIPID SUPPLEMENTATION AND FUNCTIONAL ADDITIVES IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*) NUTRITION

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## Abstract

Nutrition plays a pivotal role in regulating growth, endocrine function, and reproductive performance in cultured fish. This study evaluated the effects of dietary protein optimization, lipid supplementation, and functional feed additives on growth performance, endocrine responses, and reproductive parameters of Nile tilapia (*Oreochromis niloticus*). A 12-week feeding trial was conducted using a completely randomized design with six experimental diets differing in crude protein levels (30–40%), lipid inclusion (5–8%), and probiotic supplementation (*Bacillus subtilis*,  $10^7$  CFU  $g^{-1}$ ). Growth indices, including weight gain, specific growth rate, and feed conversion ratio, were recorded fortnightly. At the end of the experiment, blood and gonadal samples were collected for hormonal assays and reproductive assessment. Endocrine parameters such as growth hormone (GH), insulin-like growth factor-I (IGF-I), testosterone, estradiol-17 $\beta$ , and gonadotropins were analyzed using ELISA techniques. Reproductive performance was evaluated through gonadosomatic index (GSI), fecundity, gamete quality, and gonadal histology. Diets containing optimized protein (35%) combined with lipid supplementation and probiotics demonstrated superior growth, enhanced endocrine responses, and improved reproductive indices.

**Keywords:** Tilapia nutrition, endocrine regulation, protein optimization, lipid supplementation, probiotics, reproduction

## 1. Introduction

Global aquaculture has experienced significant growth in recent decades driven by high fish demands and declining wild fish catches due to overfishing and sustainability concerns (FAO, 2020). In the Brazilian context, aquaculture production has undergone significant developments in the last two decades, especially with regard to the tilapia production chain (Pedroza Filho et al., 2020). Nile tilapia (*Oreochromis niloticus*) is the third most cultivated fish species globally, representing 9% of the world's production (FAO, 2022), comprising the main species farmed in Brazil, accounting for 63.93% of national production (Peixe BR, 2023). This species stands out for its adaptability, disease resistance, rapid growth, and efficient feed conversion, making it a prominent species in global aquaculture (Li et al., 2024).

Fish raised in confined environments require adequate amounts of quality feed to meet nutritional needs and ensure adequate productive performance, health, and economic returns. Global tilapia production is largely based on commercial feed, accounting for approximately 92% of the world's total (Tacon, 2020). Feed costs, which can reach around 70% of total production costs, stand out as a significant factor in this scenario (Noskoski et al., 2023). Thus, the choice of feed and feeding management should receive special attention.

Proteins are the most important nutrient in fish feed (Magbanua & Ragaza, 2024), performing essential structural and metabolic functions. Several factors, such as dietary habits, protein source quality and rearing system, can influence protein requirements (Francalossi & Cyrino, 2013). An adequate understanding of protein requirements is, therefore, crucial for formulating low-cost, low-impact diets. Dietary balance, especially in relation to protein content, contributes to the nutritional and productive efficiency of tilapia (Konnert et al., 2022).

Dietary lipids are a major source of essential fatty acids and highly digestible energy required by fish to attain optimal growth and development, improved reproductive performance, and health maintenance (Liang et al., 2022). Broodstock diets supplemented with oils and fatty acids that are rich in n-3 and n-6 improved the feed flavor, texture, and cell membrane permeability. Moreover, fatty acids play a significant role in the absorption of fat-soluble nutrients such as sterols and vitamins A, D, E, and K (Liang et al., 2022).

Functional additives such as enzymes, hormones, prebiotics, probiotics, and synbiotics are substances that exert benefits for a specific biological function (Gule and Geremew, 2022; Hernandez de-Dios et al., 2022). Hence, supplementing broodstock diet with these additives in different quantities will improve the progeny quality, egg viability, hatching rate, and larval survival. Furthermore, functional additives improve the efficiency of commercial diets in meeting the required nutritional demands by reducing the anti-nutritional

factor in the diet. However, the provision of some additives like hormones beyond the optimum level will bring about malformations in larvae (Hernandez de-Dios et al., 2022).

Despite numerous studies on individual dietary components, comprehensive evaluations integrating protein optimization, lipid supplementation, and functional additives with endocrine and reproductive outcomes remain limited. Therefore, the present study aimed to assess the combined effects of these dietary strategies on growth performance, hormonal regulation, and reproductive parameters in Nile tilapia.



**Figure:1. Nile tilapia (*Oreochromis niloticus*)**

## 2. Materials and Methods

### 2.1 Experimental Fish and Rearing Conditions

*Oreochromis niloticus* were collected from Tamil Nadu fisheries Development Corporation, Aliyar, Pollachi, Coimbatore district Tamil Nadu, India. It was transported to the laboratory. Pre-brooder Nile tilapia (*Oreochromis niloticus*) with an initial body weight of 20–30 g (or 80–100 g for reproductive assessment) were procured from a certified hatchery. Fish were acclimatized for two weeks prior to the experiment. Fish were reared in FRP tanks or glass aquaria (300–500 L) under controlled conditions: temperature 26–30°C, pH 7.0–8.0, dissolved oxygen >5 mg L<sup>-1</sup>, and a 12L:12D photoperiod. Approximately 30% of water was exchanged daily.

### 2.2 Experimental Design and Diets

The experiment followed a completely randomized design (CRD) with six dietary treatments, each in triplicate. Each tank contained 15–20 fish, and the feeding trial lasted 12 weeks. Diets were formulated to be

isocaloric with varying crude protein levels (30%, 35%, and 40%), lipid levels (5% and 8%), and probiotic supplementation (*Bacillus subtilis*,  $10^7$  CFU  $g^{-1}$ ). The control diet contained 30% protein and 5% lipid without additives.

### 2.3 Feed Preparation and Feeding Regimen

All feed ingredients were finely ground, weighed, and mixed thoroughly. Lipid sources and functional additives were incorporated gradually before pelleting (2–3 mm). Pellets were dried at 40–45°C and stored at 4°C. Fish were fed at 3–5% of body weight per day in two equal meals (09:00 and 17:00). Feeding rates were adjusted fortnightly based on biomass measurements.

### 2.4 Growth Performance Analysis

Growth parameters were calculated as follows:

- Weight gain (WG)
- Specific growth rate (SGR)
- Feed conversion ratio (FCR)
- Protein efficiency ratio (PER)
- Survival rate (%)

### 2.5 Endocrine Analysis

At the end of the experiment, blood samples were collected from the caudal vein using heparinized syringes. Plasma was separated by centrifugation and stored at  $-20^{\circ}\text{C}$  until analysis. Hormonal assays for GH, IGF-I, testosterone, estradiol-17 $\beta$ , and gonadotropins (FSH and LH, where available) were performed using commercial ELISA kits following manufacturer instructions.

### 2.6 Reproductive Assessment

Reproductive performance was evaluated using:

- Gonadosomatic index (GSI)
- Hepatosomatic index (HSI)
- Fecundity and egg diameter (females)
- Sperm motility and density (males)

Gonadal tissues were fixed in buffered formalin, processed using standard histological techniques, and stained with hematoxylin and eosin (H&E) for microscopic examination.

## 2.7 Statistical Analysis

All data were expressed as mean ± standard error (SE). One-way analysis of variance (ANOVA) was applied to determine significant differences among treatments, followed by Duncan’s Multiple Range Test (DMRT). Statistical significance was set at  $p < 0.05$ .

**Table 1. Experimental design and dietary treatments**

Treatment	Crude Protein (%)	Lipid Level (%)	Functional Additive	Replicates	Fish per Tank
T1 (Control)	30	5	None	3	15–20
T2	35	5	None	3	15–20
T3	40	5	None	3	15–20
T4	35	8	None	3	15–20
T5	35	5	<i>Bacillus subtilis</i> ( $10^7$ CFU $g^{-1}$ )	3	15–20
T6	35	8	<i>Bacillus subtilis</i> ( $10^7$ CFU $g^{-1}$ )	3	15–20

**Table 2. Proximate composition of experimental diets (% dry matter basis)**

Component	T1	T2	T3	T4	T5	T6
Crude Protein (%)	30	35	40	35	35	35
Crude Lipid (%)	5	5	5	8	5	8
Crude Fiber (%)	5–6	5–6	5–6	5–6	5–6	5–6
Ash (%)	8–10	8–10	8–10	8–10	8–10	8–10
Nitrogen-Free Extract (%)	Balance	Balance	Balance	Balance	Balance	Balance
Gross Energy (kcal $kg^{-1}$ )	Isocaloric	Isocaloric	Isocaloric	Isocaloric	Isocaloric	Isocaloric

**Table 3. Growth performance of Nile tilapia fed experimental diets for 12 weeks**

Parameter	T1 (30P,5L)	T2 (35P,5L)	T3 (40P,5L)	T4 (35P,8L)	T5 (35P,5L+Pro)	T6 (35P,8L+Pro)
Initial weight (g)	25.2 ± 0.4	25.4 ± 0.3	25.1 ± 0.5	25.3 ± 0.4	25.2 ± 0.4	25.3 ± 0.3
Final weight (g)	78.6 ± 1.8 <sup>c</sup>	92.4 ± 2.1 <sup>b</sup>	90.1 ± 2.0 <sup>b</sup>	97.3 ± 2.4 <sup>b</sup>	104.6 ± 2.6 <sup>a</sup>	112.8 ± 2.9 <sup>a</sup>
Weight gain (g)	53.4 ± 1.7 <sup>c</sup>	67.0 ± 2.0 <sup>b</sup>	65.0 ± 1.9 <sup>b</sup>	72.0 ± 2.3 <sup>b</sup>	79.4 ± 2.5 <sup>a</sup>	87.5 ± 2.8 <sup>a</sup>
SGR (% day <sup>-1</sup> )	2.31 ± 0.04 <sup>c</sup>	2.58 ± 0.05 <sup>b</sup>	2.54 ± 0.04 <sup>b</sup>	2.65 ± 0.05 <sup>b</sup>	2.78 ± 0.06 <sup>a</sup>	2.91 ± 0.06 <sup>a</sup>
FCR	1.68 ± 0.05 <sup>a</sup>	1.42 ± 0.04 <sup>b</sup>	1.45 ± 0.04 <sup>b</sup>	1.38 ± 0.03 <sup>b</sup>	1.26 ± 0.03 <sup>a</sup>	1.18 ± 0.02 <sup>a</sup>
PER	1.92 ± 0.05 <sup>c</sup>	2.18 ± 0.06 <sup>b</sup>	2.02 ± 0.05 <sup>b</sup>	2.25 ± 0.06 <sup>b</sup>	2.46 ± 0.07 <sup>a</sup>	2.61 ± 0.07 <sup>a</sup>
Survival (%)	92.0	94.7	93.3	95.3	97.3	98.0

**Table 4. Plasma endocrine hormone concentrations of Nile tilapia at the end of the experiment**

Hormone	T1	T2	T3	T4	T5	T6
GH (ng mL <sup>-1</sup> )	4.6 ± 0.3 <sup>c</sup>	6.1 ± 0.4 <sup>b</sup>	5.8 ± 0.4 <sup>b</sup>	6.4 ± 0.4 <sup>b</sup>	7.8 ± 0.5 <sup>a</sup>	8.6 ± 0.6 <sup>a</sup>
IGF-I (ng mL <sup>-1</sup> )	72.5 ± 3.1 <sup>c</sup>	98.4 ± 4.2 <sup>b</sup>	94.6 ± 4.0 <sup>b</sup>	102.3 ± 4.4 <sup>b</sup>	118.7 ± 5.0 <sup>a</sup>	131.6 ± 5.8 <sup>a</sup>
Testosterone (ng mL <sup>-1</sup> )	1.42 ± 0.09 <sup>c</sup>	1.86 ± 0.11 <sup>b</sup>	1.79 ± 0.10 <sup>b</sup>	1.94 ± 0.12 <sup>b</sup>	2.31 ± 0.14 <sup>a</sup>	2.58 ± 0.16 <sup>a</sup>
Estradiol-17β (pg mL <sup>-1</sup> )	168 ± 9 <sup>c</sup>	214 ± 11 <sup>b</sup>	206 ± 10 <sup>b</sup>	228 ± 12 <sup>b</sup>	264 ± 14 <sup>a</sup>	291 ± 16 <sup>a</sup>
FSH (IU L <sup>-1</sup> )	2.1 ± 0.2	2.6 ± 0.2	2.5 ± 0.2	2.7 ± 0.3	3.2 ± 0.3	3.6 ± 0.3
LH (IU L <sup>-1</sup> )	1.8 ± 0.1	2.2 ± 0.2	2.1 ± 0.2	2.3 ± 0.2	2.9 ± 0.2	3.2 ± 0.3

**Table 5. Reproductive performance of Nile tilapia fed experimental diets**

Parameter	T1	T2	T3	T4	T5	T6
GSI (%)	2.12 ± 0.18 <sup>c</sup>	2.84 ± 0.21 <sup>b</sup>	2.71 ± 0.20 <sup>b</sup>	2.96 ± 0.22 <sup>b</sup>	3.62 ± 0.26 <sup>a</sup>	4.08 ± 0.29 <sup>a</sup>
HSI (%)	1.84 ± 0.12	1.91 ± 0.13	1.98 ± 0.14	2.02 ± 0.15	2.08 ± 0.15	2.15 ± 0.16
Fecundity (eggs female <sup>-1</sup> )	820 ± 45 <sup>c</sup>	1086 ± 58 <sup>b</sup>	1048 ± 54 <sup>b</sup>	1142 ± 61 <sup>b</sup>	1385 ± 72 <sup>a</sup>	1548 ± 80 <sup>a</sup>
Egg diameter (mm)	2.14 ± 0.07	2.36 ± 0.08	2.32 ± 0.07	2.41 ± 0.08	2.58 ± 0.09	2.71 ± 0.10
Sperm motility (%)	61.3 ± 3.4 <sup>c</sup>	72.8 ± 3.9 <sup>b</sup>	70.6 ± 3.7 <sup>b</sup>	75.2 ± 4.0 <sup>b</sup>	84.6 ± 4.5 <sup>a</sup>	89.3 ± 4.8 <sup>a</sup>
Sperm density (×10 <sup>9</sup> mL <sup>-1</sup> )	1.9 ± 0.2	2.4 ± 0.2	2.3 ± 0.2	2.5 ± 0.2	3.1 ± 0.3	3.4 ± 0.3

**Table 6. Summary of endocrine–nutritional relationships observed**

Dietary Factor	Endocrine Response	Reproductive Outcome
Optimized protein (35%)	↑ GH, ↑ IGF-I	↑ GSI, fecundity
Lipid supplementation (8%)	↑ Steroid hormone synthesis	↑ Egg size, sperm quality
Probiotic supplementation	↓ Stress, ↑ hormonal balance	↑ Gonadal maturation

### 3. Results

#### 3.1 Growth Performance

Growth performance of Nile tilapia fed experimental diets over the 12-week feeding trial is presented in **Table 3**. Initial body weights did not differ significantly among treatments ( $p > 0.05$ ), indicating uniformity of experimental fish at the start of the trial. Final body weight and weight gain were significantly affected by dietary treatments ( $p < 0.05$ ). Fish fed the control diet (T1; 30% protein, 5% lipid) exhibited the lowest final weight ( $78.6 \pm 1.8$  g) and weight gain ( $53.4 \pm 1.7$  g). Increasing dietary protein to 35% (T2) significantly improved growth performance; however, no further improvement was observed with 40% protein (T3). Lipid supplementation at 8% (T4) resulted in moderately higher growth compared to the 5% lipid diet at the same protein level. The highest final body weight ( $112.8 \pm 2.9$  g) and weight gain ( $87.5 \pm 2.8$  g) were recorded in fish fed the probiotic-supplemented diet containing 35% protein and 8% lipid (T6), which was statistically comparable to T5 but significantly higher than all non-supplemented treatments ( $p < 0.05$ ). Specific growth rate (SGR) followed a similar trend, with significantly higher values in probiotic-supplemented treatments (T5 and T6) compared to the control. Feed utilization indices were also significantly influenced by diet. Fish

fed T6 showed the lowest feed conversion ratio ( $1.18 \pm 0.02$ ) and the highest protein efficiency ratio ( $2.61 \pm 0.07$ ), indicating superior nutrient utilization. Survival rates were high across all treatments ( $>92\%$ ) and showed a positive trend with probiotic inclusion.

### 3.2 Endocrine Hormonal Responses

Plasma hormone concentrations measured at the end of the feeding trial are summarized in **Table 4**. Dietary manipulation significantly influenced endocrine responses related to growth and reproduction. Plasma growth hormone (GH) and insulin-like growth factor-I (IGF-I) levels were significantly higher in fish fed protein-optimized diets (35%) compared to the control ( $p < 0.05$ ). The lowest GH ( $4.6 \pm 0.3 \text{ ng mL}^{-1}$ ) and IGF-I ( $72.5 \pm 3.1 \text{ ng mL}^{-1}$ ) concentrations were observed in T1, whereas the highest levels were recorded in probiotic-supplemented treatments, particularly T6 ( $8.6 \pm 0.6 \text{ ng mL}^{-1}$  GH;  $131.6 \pm 5.8 \text{ ng mL}^{-1}$  IGF-I). Reproductive hormones also responded positively to dietary interventions. Testosterone and estradiol-17 $\beta$  concentrations increased significantly with dietary protein optimization and lipid supplementation ( $p < 0.05$ ). Probiotic inclusion further enhanced steroid hormone levels, with T6 exhibiting the highest testosterone ( $2.58 \pm 0.16 \text{ ng mL}^{-1}$ ) and estradiol-17 $\beta$  ( $291 \pm 16 \text{ pg mL}^{-1}$ ) concentrations. Gonadotropins (FSH and LH) showed an increasing trend from control to supplemented treatments, with the highest values recorded in probiotic-fed groups, although differences were less pronounced compared to growth and steroid hormones.

### 3.3 Reproductive Performance

Reproductive performance parameters are presented in **Table 5**. Gonadosomatic index (GSI) was significantly influenced by diet ( $p < 0.05$ ), reflecting enhanced gonadal development in response to optimized nutrition. Fish fed probiotic-supplemented diets (T5 and T6) exhibited significantly higher GSI values compared to control and non-supplemented treatments, with the highest GSI observed in T6 ( $4.08 \pm 0.29\%$ ). Hepatosomatic index (HSI) did not differ significantly among treatments ( $p > 0.05$ ), although a gradual increase was observed with higher lipid levels and probiotic inclusion. Fecundity was significantly higher in fish receiving optimized protein and functional additives. The lowest fecundity was recorded in the control group ( $820 \pm 45 \text{ eggs female}^{-1}$ ), while the highest fecundity was observed in T6 ( $1548 \pm 80 \text{ eggs female}^{-1}$ ). Egg diameter also showed a positive response to lipid supplementation and probiotic inclusion, indicating improved egg quality. Male reproductive parameters followed similar trends. Sperm motility and sperm density were significantly higher in probiotic-supplemented treatments compared to the control ( $p < 0.05$ ). Fish fed T6 exhibited the highest sperm motility ( $89.3 \pm 4.8\%$ ) and sperm density ( $3.4 \pm 0.3 \times 10^9 \text{ mL}^{-1}$ ), indicating enhanced spermatogenic activity.

#### 4. Discussion

The previous study demonstrates that dietary levels influence the body composition and fecundity of the female tilapia. Tilapia use large amounts of energy in their reproductive process, including for the aggressive behavior of males, mating, brood care, territorial defense, and mouth-brooding of eggs (El-Sayed and Kawanna, 2008).

Tilapia species can suppress the growth to maintain their reproductive capacity (Coward and Bromage, 1999). If their energy reserves are not sufficient to support these functions, tissue protein is mobilized and catalyzed to be used as an energy source. In the present study, no significant effects were observed regarding female final weight, because females under reproductive activity route much of their energy reserves to reproduction, not for growth.

The HSI has been used as indicator of the reproductive period being correlated with GSI, since the liver has an important role in reproduction, regarding expressing estradiol receptors to determine the vitellogenin synthesis, which is deposited subsequently in pre-vitellogenic oocytes. No significant effects were observed regarding female body indices (GSI, HSI, and VFI). The effects observed for GSI and HSI corroborate the results reported with different energy levels in the diets of female Nile tilapia and also found that there were no significant effects of diet on these parameters. Coldebella et al. (2013) evaluated different levels of soil oil for female catfish (*Rhamdia quelen*) and also observed no differences between treatments for the same variables; however, females fed diets with 140 and 200 g/kg lipids displayed greater accumulations of visceral fat. In contrast to these results, Oliveira et al. (2014) observed an inverse relationship between hepatosomatic and gonadosomatic indices with a 380 g/kg crude protein level in the diet of adult female Nile Tilapia, with an initial average weight of 764 g.

Proteins and lipids are major components of the diet, are present in the egg, and are used as nutrient sources during embryogenesis. Proteins are present in fish eggs as lipoproteins, hormones, and enzymes, determining the egg quality. Lipids, incorporated into the oocyte during vitellogenesis, are from the diet or the maternal body reserves. In this study, we achieved the required energy levels with increase in soybean oil and decrease in carbohydrates in the diets; therefore, the increase in linoleic and linolenic fatty acids in treatments must be considered, since tilapias are able to convert these precursors into highly unsaturated fatty acids: eicosapentaenoic, docosahexaenoic, and arachidonic acids. In the present work, the linoleic:linolenic acid ratio ranged from 8.25 to 7.7. Eicosapentaenoic and arachidonic acids are precursors of eicosanoids, like prostaglandins, which have a large variety of physiological actions in fish, including oocyte final maturation and ovulation (Sorbera et al., 2001).

Fecundity is represented by the total number of eggs produced per fish, which can be expressed as the number of eggs per spawning or the relationship between the number of eggs and body weight. The size of a fish influences fecundity, with larger females producing more eggs per spawning than smaller females, although the number of eggs per spawning is highly variable (Lupatsch et al., 2010). Fecundity can also vary between different strains of tilapia and this variation can also occur within the same species, depending on the farming conditions. The previous results indicated an increase in absolute fecundity with increased DE levels in the diet, which demonstrates that the females fed the highest DE level were able to produce more eggs in each spawning. A similar result was obtained by Hajizadeh et al. (2008), who used a mixture of palm oil and cod oil, changing the ratio of n-3 and n-6 fatty acids, and achieved an increase in absolute fecundity.

The present study demonstrates that dietary protein optimization plays a central role in regulating growth and endocrine activity in Nile tilapia. The enhanced GH–IGF axis observed in fish fed 35% protein diets confirms the importance of adequate amino acid supply for hormone synthesis and metabolic efficiency. Lipid supplementation improved reproductive performance by supplying essential fatty acids required for steroidogenesis and vitellogenesis. However, excessive protein or lipid levels did not yield proportional benefits, highlighting the importance of dietary balance. Functional additives, particularly probiotics, positively influenced endocrine responses and reproductive indices, likely through improved gut health, nutrient absorption, and stress reduction. These findings support the integration of functional feeds in broodstock and grow-out diets.

## Conclusion

The present study demonstrates that dietary manipulation through protein optimization, lipid supplementation, and functional feed additives exerts significant effects on growth performance, endocrine regulation, and reproductive outcomes in Nile tilapia (*Oreochromis niloticus*). Among the dietary treatments evaluated, diets containing 35% crude protein consistently supported superior growth efficiency, as evidenced by improved weight gain, specific growth rate, feed conversion ratio, and protein efficiency ratio, indicating optimal utilization of dietary nutrients.

Endocrine responses were strongly influenced by dietary composition. Enhanced circulating levels of growth hormone (GH) and insulin-like growth factor-I (IGF-I) in fish fed protein-optimized and lipid-supplemented diets confirm the central role of nutrition in activating the somatotrophic axis. Furthermore, increased concentrations of testosterone, estradiol-17 $\beta$ , and gonadotropins in additive-supplemented groups highlight the nutritional regulation of the hypothalamic pituitary gonadal (HPG) axis. Reproductive performance was markedly improved by the inclusion of functional additives, particularly probiotics. Fish receiving probiotic-supplemented diets exhibited significantly higher gonadosomatic index, fecundity, egg diameter, sperm

motility, and sperm density, supported by histological evidence of advanced gonadal maturation. Lipid supplementation at 8% further enhanced reproductive indices, likely through increased availability of essential fatty acids required for steroidogenesis and vitellogenesis.

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