Effect of chitosan nanoparticles and vitamin E on the growth, reproductive, and stress-hormones in *Ompok pabda* (Hamilton, 1822)

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Abstract. This research was conducted to explore the dietary effects of chitosan nanoparticles (CNP), Vitamin E (VE), and their mixture supplementation on the growth, reproductive, and stress-hormonal profiles of Pabdah catfish, *Ompok pabda* (Hamilton, 1822). Fish were allocated to four treatments in triplicate and fed the four supplemented diets: basal diet (control, G1), 5 g CNP kg^{-1} (G2), 500 mg VE kg^{-1} (G3), and 5 g CNP kg^{-1} + 500 mg VE kg^{-1} (G4). The experiment was conducted over 60 days. The stocking density was 20 fish (13.56±0.33g) per 500 L. The growth hormone (GH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH), testosterone (TST), and estradiol (E2) demonstrated synergistic properties in G4. Nevertheless, triiodothyronine (T3) decreased in both G2 and G4, but increased thyroxine (T4) was observed in both G2 and G4. When the fish were supplemented, the stress hormones cortisol and adrenocorticotropic hormone (ACTH) decreased. The inclusion of CNP and VE at 5 g kg^{-1} and 500 mg kg^{-1}, respectively, in the fish diet seems to have a synergistic effect that enhances reproductive hormone levels and reduces stress in Pabdah catfish under intensive culture.
Key Words: growth hormone, chitosan nanoparticles, vitamin E, synergistic, hormones, *Ompok pabda.*

Introduction. In fish, physiological development, reproduction, and adaptation to environmental changes are fundamentally regulated by hormones. Hormones act as chemical messengers produced by endocrine glands and are crucial in modulating growth, maturation, and stress responses in fish. Growth hormone (GH) in fish influences the insulin-like growth factor pathway, enhancing cell proliferation and muscle development (Fuentes et al 2013). Insulin indirectly regulates fish growth by modulating nutrient utilization, metabolism, and endocrine signaling. It enhances glucose uptake and glycogen synthesis, thereby sparing amino acids for protein accretion and the growth process (Mommsen 1991; Won & Borski 2013). The reproductive success of fish in captivity is heavily influenced by follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH promotes the early phase of testicular development by stimulating Leydig cells to produce 11-ketotestosterone, which activates Sertoli cells to release activin B, thereby promoting spermatogonial mitosis and initiating spermatogenesis (Hatef & Unniappan 2019). During spawning, LH facilitates sperm maturation by inducing the synthesis of the maturation-inducing steroid in most fish species (Zohar et al 2010). Thyroid hormones-triiodothyronine (T3) and thyroxine (T4), generated by the hypothalamic-pituitary-thyroid axis, influence many physiological functions in fish and are highly responsive to environmental and internal factors. Likewise, cortisol and adrenaline, as principal stress hormones derived from the product of hypothalamic-pituitary-interrenal and Brain-Sympathetic-Chromaffin axes, regulate osmotic and metabolic regulation at various levels, while thyroid hormones synergistically fine-tune these responses to maintain physiological homeostasis under stress conditions (Peter 2011). Nonetheless, elevation of stress hormones can suppress reproductive hormones, impair immune responses, reduce feeding, and slow growth (Pickering et al 1991). Therefore, it is crucial to clearly understand the hormonal mechanism in fish for sustainable development in aquaculture.

The aquaculture industry has undergone rapid expansion in recent decades, driven by the rising global demand for high-quality fish protein, growing annually by 4.5% and valued at USD 243.26 billion (FAO 2018). Within this context, cultivation of indigenous species like Pabdah catfish has emerged as a promising avenue, especially in South Asian countries. Pabdah catfish, a non-air-breathing, potamodromous catfish species endemic to the Indian subcontinent (Alam et al 2016; Biswas et al 2023; Patra et al 2025) and listed as threatened by the IUCN (Mallon et al 2011). Due to rich lipoprotein content and soft bony structure, this fish species is considered delicious and nutritious to people, and has gained promising attention for commercial culture. Every 100 grams of this fish provides approximately 19.2% protein, 2.0% fat, 0.2 g of iron, 402 mg of calcium, 216 mg of phosphorus, and 79.3% water content (Hossain et al 2019). Although its popularity is growing, Pabdah catfish culture is still in a developing phase due to several biological and environmental constraints. Among them, lower larval survival, reduced reproductive efficiency, and high sensitivity to stress under captive conditions have already been reported (Rawat et al 2019). Despite its current challenges, the cultivation of Pabdah catfish holds considerable potential for improvement through the application of an efficient nutrient delivery system, which collectively can lead to increased productivity in aquaculture operations.

Chitosan, a naturally occurring cationic polysaccharide formed by removing acetyl groups from chitin, and the properties of chitosan depend on its molecular weight (MW) and degree of acetylation (DA) (Luo & Wang 2013; Collado-Gonzalez & Esteban 2022). MW influences the polymer's chain length, while DA determines the density of positively charged amine groups, which are related to chitosan aqueous solubility (Islam et al 2017; Collado-Gonzalez & Esteban 2022). Nanotechnology utilizes nanoscale materials that show unique properties due to their high surface-to-volume ratio (Collado-Gonzalez & Esteban 2022). These features enable the development of nanoformulations that enhance bioavailability, protect sensitive compounds, and allow targeted delivery of biomolecules. Chitosan nanoparticles (CNP) possess unique characteristics like biocompatibility, biodegradability, and non-toxicity (Divya & Jisha 2018). Their pH-dependent solubility, longer shelf life, and higher drug-loading capacity make them a promising alternative for transferring vitamins, peptides, proteins, antigens, oligonucleotides, and genes (Janes et al 2001). Remarkably, CNP boosts the bioavailability of essential compounds, enables efficient uptake by body cells, and deeply penetrates the targeted sites (Alishahi et al 2011). Therefore, its exceptional properties have been positioned as a highly suitable material for diverse applications in the aqua sector. CNP has previously been shown to have immune-boosting, anti-microbial, anti-oxidant, and anti-mutagenic properties (Wen et al 2011; Abd El-Naby et al 2019). It has been reported that CNP enhances the growth, immunity, and meat quality of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), and Rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792) (Abd El-Naby et al 2019; Abdel-Tawwab et al 2019; Saleh et al 2022). CNP were reported to alleviate endocrine disruptions in female African catfish (*Clarias gariepinus*) exposed to bisphenol by restoring the reproductive hormone concentrations (Hamed et al 2021). Dietary supplementation with chitosan-vitamin C (Ibrahim et al 2021) and chitosan nanoparticles-vitamin E (CNP + VE) (Ahmed et al 2021) nanocomposites has been shown to elevate GH levels in Nile tilapia, improve intestinal microvilli size, and expand the absorptive surface of the intestine, ultimately enhancing nutrient digestibility.

VE, a vital fat-soluble antioxidant, plays a critical role in maintaining physiological balance and promoting health in aquatic organisms, particularly fish (Ibrahim et al 2020, 2021). Its biological functions extend beyond cellular protection from oxidative damage, influencing key hormonal pathways that regulate growth, reproduction, and stress responses. In aquaculture and fisheries science, optimizing the hormonal health of fish is essential for improving productivity, reproductive efficiency, and resilience to environmental stressors. Studies have shown that dietary supplementation of VE enhances the secretion and regulation of growth-related hormones such as GH and insulin-like growth factor-I, contributing to improved somatic development. Additionally, VE supports reproductive performance by modulating sex hormones like TST and E2, which are critical for gametogenesis and spawning behavior (Canyurt & Akhan 2008; Ahmed et al 2021). Furthermore, its antioxidative properties help mitigate stress-induced hormonal imbalances, particularly in lowering cortisol levels, thereby promoting better immune function and overall well-being (Udo & Afia 2013; Ahmed et al 2021). These multifaceted benefits make VE a key nutritional component in advancing sustainable and healthy fish farming practices.

Although studies exist on other fish species, investigations into the hormonal responses of Pabdah catfish following CNP and VE treatment are still unexplored. A holistic understanding of hormonal regulation is essential for advancing health performance and stress management in fish species. Thus, the present study aimed to broaden knowledge on the impacts of dietary CNP, VE, and their combination (CNP-VE) on growth, reproductive, and stress-hormones in Pabdah Catfish.

Material and Method

Experimental diets. The CNP was obtained from NANOSHELTM, Intelligent Materials Pvt. Ltd. (Punjab, India) in the form of powder. The degree of deacetylation and average MW were found to be 161 g mol^{-1}, assay > 99%, and average particle size (APS) 80-100 nm. According to Du et al (2009), based on the ionotropic gelation of chitosan and sodium tripolyphosphate, CNP were formulated (Sigma, USA). Using reagent-grade water (Milli-Q SP ultrapure water system, Nihon Millipore Ltd., Tokyo), the CNP underwent rigorous purification before being frozen (Virtis Advantage EL, SP Industries Company, USA). VE (DL-α-tocopherol acetate) was purchased from Sigma-Aldrich. A commercial fish feed (Narish Fish Feed Ltd., protein: 30%, lipid: 5%, crude fibre: 8%, moisture: 12%) was used as a basal diet. To make a firm dough, water was added after the supplements (CNP or VE) were mixed with the basal diet. The diet of the CNP group was supplemented with 5 mg kg^{-1} diet, and the VE group received 500 mg kg^{-1} diet (Naderi et al 2019). The conjugate (CNP + VE) was supplemented with 5 g CNP and 500 mg VE kg^{-1} diet. Following a feed mill to pelletize the grain, room temperature air drying was done, and it was kept refrigerated at 4°C until feeding time. The basal diet without any supplementation was used as the control diet.

Experimental design. A total of 240 Pabdah Catfish were acquired from the local hatchery in Mymensingh and brought to the Genetics and Fish Breeding Laboratory of Gazipur Agricultural University, Bangladesh. Upon arrival at the lab, the fish were housed in rectangular cement tanks (size: 2.17 x 1.55 x 0.64 m) for 21 days of acclimatization at room temperature and fed diets similar to the hatchery. Following acclimatization, fish were moved fish were migrated to twelve circular tanks (500 L) each containing 20 fish. The following four treatment groups were designed: G1- the control group fed with basal diet; G2- basal diet with 5 mg kg^{-1} CNP; G3- basal diet with 500 mg kg^{-1} VE, and G4- the CNP-VE nanocomposite group, consisting of 5 mg kg^{-1} CNP + 500 mg kg^{-1} VE. Each treatment consisted of three replications. The fish were kept in a flow-through system for 60 days with continuous aeration. The fish were fed twice daily (09:00 and 16:00) until apparent satiation at a rate of 2-3% of their body weight. Satiation was determined as the point at which fish ceased active feeding, and uneaten feed remained in the tank for more than 2 minutes. The leftover feed was collected, dried, and weighed (with the dry weight representing approximately 71% of the wet weight), and total feed consumption was calculated accordingly (Mazumder et al 2018; Debi et al 2024). The population from each tank was measured and checked every alternate week.

Hydrobiological parameters. During the experimental period, temperature and pH were monitored on every alternate day, while dissolved oxygen (DO), total ammonia-nitrite (NH_3-N), total dissolved solids (TDS), nitrate (NO_3), and total hardness were measured weekly. A YSI 59 multiparameter water quality sampler (Yellow Springs Instrument Company, OH, USA) was used to measure the parameters.

Hormone analysis. Fish were kept starving for 24 hours after the experiment ended. They were subsequently taken out of the experimental tank and immersed in an anaesthetic solution (MS 222) until ventilator movements ceased. The fish were then transferred to a surgical table. A non-lethal blood sample was collected from the caudal vein of the experimental fish. After allowing the blood to coagulate, it was centrifuged at 12,000 x g for fifteen minutes at 4°C (Mazumder et al 2019). The serum was then kept for analysis at -20°C.

Growth hormone analysis. GH analysis was conducted following the protocol outlined by Khan et al (2016). Serum GH concentrations in all experimental groups of Pabdah catfish were quantified using a Micro Elisa HGH kit (Amgenix MicroLISATM, USA), with all samples analyzed in duplicate to ensure measurement accuracy. The validity of the assay was confirmed through serial dilution of serum samples (0%, 20%, 40%, 60%, and 80%), demonstrating a high degree of correlation with the standard curve. The intra-assay coefficients of variation were below 12%, indicating acceptable levels of precision and reproducibility.

Reproductive hormones analysis. Reproductive hormonal concentrations, including FSH, LH, TST, and E2, were measured according to the method outlined by Aizen et al (2007), using commercial ELISA test kits (BioCheck, Inc., San Francisco, California, USA).

Stress hormones analysis. The levels of T3, T4, cortisol, and ACTH in fish serum samples were also measured using competitive enzyme-linked immunosorbent assays (ELISA). Using ultra-pure water, all buffers and reagents were produced and combined in accordance with the instructions provided by each kit. Every test was carried out on a microplate in accordance with the guidelines provided by the manufacturer. Every ELISA plate was subjected to the standard curve. Every sample and every standard underwent duplicate analysis. Using a microplate spectrophotometer (Epoch, Biotek, USA), absorbance values were taken.

Statistical analysis. Before statistical analysis, data sets have been analyzed for homogeneity of variations using the Bartlett test (Sokal & Hohlf 1995), and normality using the Kolmogorov-Smirnov test (Zar 1999). A student's t-test was used in a one-way ANOVA analysis of the data with Origin^{TM} version 2016 and Minitab version 17 computer software. Significant results were defined as $p < 0.05$, and the data were presented as means ± standard error (SE).

Results

Hydrobiological parameters. The physico-chemical properties of the culture conditions determined in the experimental tanks over the study period are shown in Table 1. No significant change was found between treatments when treatment doses were nominal, and parameters stayed the same. These numbers fall within the acceptable range for the culture of this species (Dulić et al 2009).

Table 1

Hydrological parameters of the culture environment during the study period

*Parameters**	*Diet groups*			
	G1	*G2*	*G3*	*G4*
Temperature (°C)	26.57±0.71	26.68±0.98	26.88±0.74	26.60±0.95
pH	7.70±0.19	7.65±0.20	7.61±0.27	7.58±0.23
DO (mg L^{-1})	5.74±0.41	5.60±0.44	5.77±0.43	5.74±0.49
NH_3-N (mg L^{-1})	0.22±0.02	0.21±0.03	0.22±0.04	0.22±0.05
TDS (mg L^{-1})	167.50±12.80	164.17±9.32	171.67±7.45	172.5±11.46
NO_3 (mg L^{-1})	17.5±7.5	11.83±2.85	20.50±3.50	17.5±3.95
TH (mg L^{-1})	133.00±10.92	137.50±13.15	135.33±9.52	130.00±6.46

DO: dissolved oxygen, NH_3-N: ammoniacal nitrogen, TDS: total dissolved solids, NO_3: Nitrate, and TH: total hardness.

Effects on GH. Fish diet supplementation with CNP and/or VE upregulated the level of GH in Pabdah catfish compared to a diet without supplementation. Individually, the level of GH in G4 was higher ($p < 0.05$) than in the other two treatments and control fish groups. Nevertheless, no significant difference was observed between G2 and G3 (Figure 1).

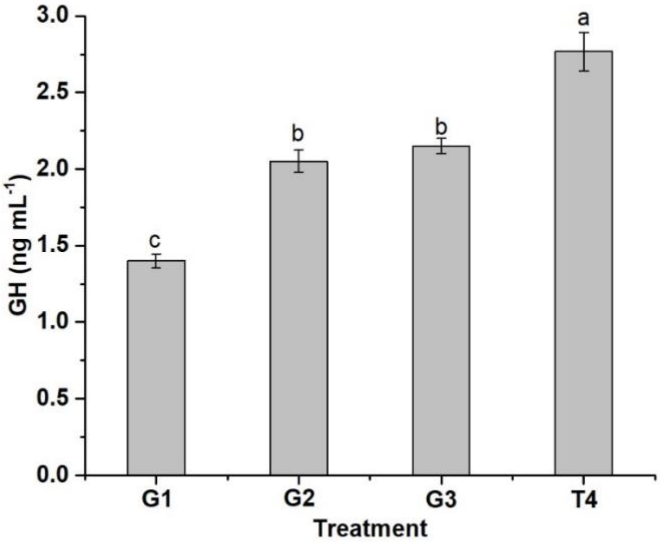

Figure 1. Effects of CNP, VE, and their conjugates on GH in Pabdah catfish. The groups indicated by the same letters do not differ significantly ($p > 0.05$); the data are shown as mean ± SE.

Effects on reproductive hormones. Supplementation of CNP and VE in the fish diet increases the level of FSH and LH compared to the fish in the control group (Figure 2). The level of FSH in *O. pabda* was significantly higher in G4 compared to the other two individual treatments and control ($p < 0.05$). However, no difference was noticed between G2 and G3, but it increased substantially from G1 (Figure 2a). Similarly, dietary CNP (G2) and VE (G3) and their combination (G4) significantly increased plasma LH concentrations, compared to G1. However, there was no difference among the supplemented treatment groups ($p < 0.05$, Figure 2b).

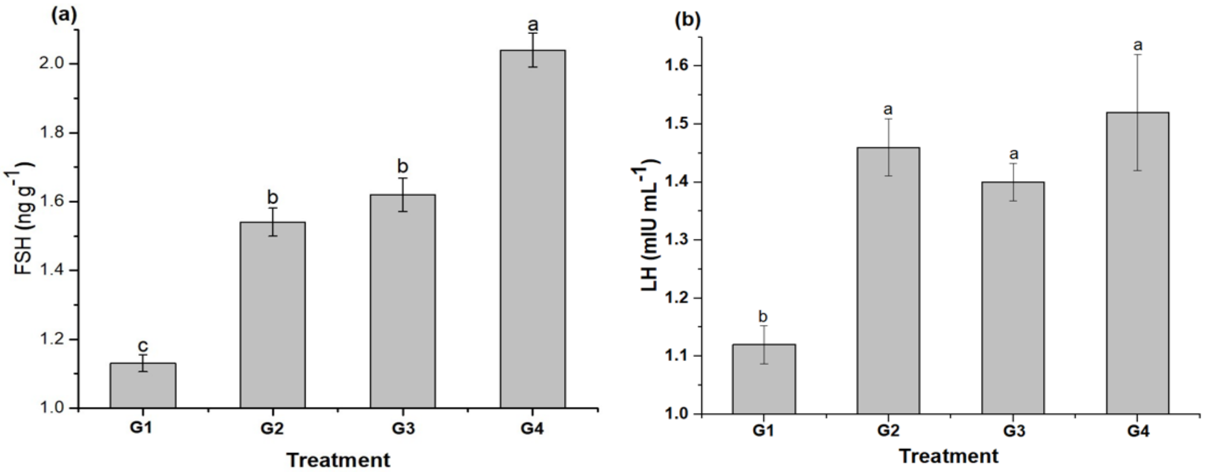

Figure 2. Effects of CNP, VE, and their conjugates on the level of a) FSH, and b) LH in Pabdah catfish. Data are presented as mean ± SE; groups denoted by the same letters do not differ significantly ($p > 0.05$).

The level of serum TST and E2 of the tested fish was improved in all the treatments compared to the control group G1 (Figure 3). The testosterone concentration increased significantly in G4 and G3 com*p*ared to G1. Similarly, the TST level in G2 doesn't differ significantly from the control diet implemented. Nevertheless, there was no significant difference observed between G3 and G4 ($p > 0.05$, Figure 3a). In the same way, the E2 was increased in all the treatments with supplemented groups (Figure 3b). The highest level was recorded in G4, followed by G2, G3, and G1. In G4, the E2 level increased significantly compared to the control group.

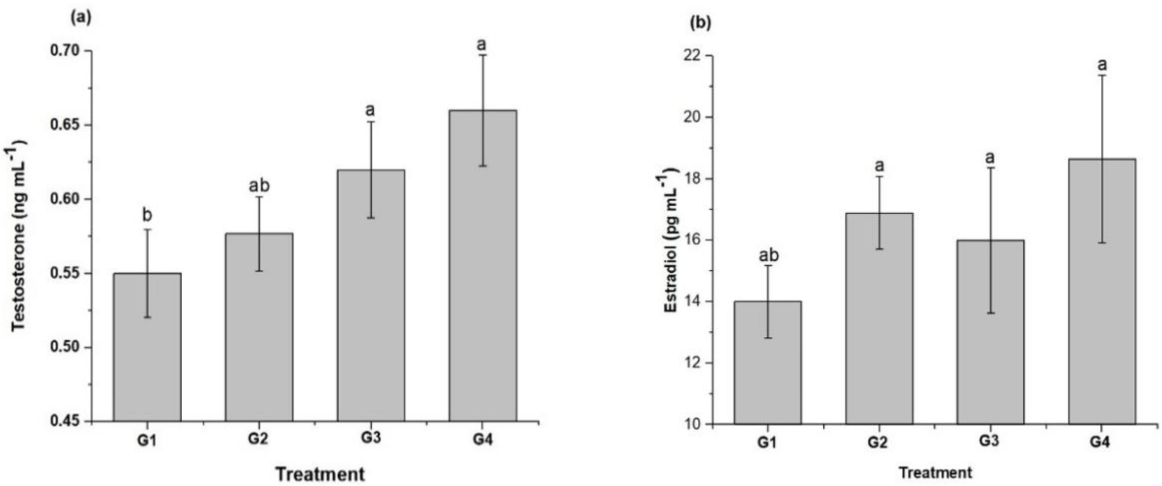

Figure 3. Effects of dietary CNP, VE, and their combination on a) TST, and b) E2 of Pabdah catfish. Data are presented as mean ± SE; groups with different letters differ significantly ($p < 0.05$).

Effects on stress hormones. It was revealed that the level of T3 increased when the fish was fed with CNP and Vit E-supplemented feed (Figure 4a). The T3 secretion was lowest in the control group and increased linearly, followed by G2 > G3 > G4 ($p > 0.05$, Figure 4a). On the other hand, T4 concentration in the combination group was significantly higher than in G1 and G3 supplemented fish groups (Figure 4b). No significant difference was revealed among the individual supplementations (G2 and G3) and control ($p > 0.05$, Figure 4b). Conversely, in the case of both cortisol and ACTH, the highest levels were observed in the G1 group. Cortisol decreased significantly in G2 and G3 compared to G1, while the lowest level was observed in G4 ($p < 0.05$). This suggested a strong stress-attenuating effect (Figure 4c). A similar trend was observed for ACTH, where G1 recorded the highest value, G2 and G3 showed moderate reductions, and G4 displayed a dramatic decline (Figure 4d). The markedly lower values in G4 highlight the superior role of the CNP+VE combination in alleviating stress responses.

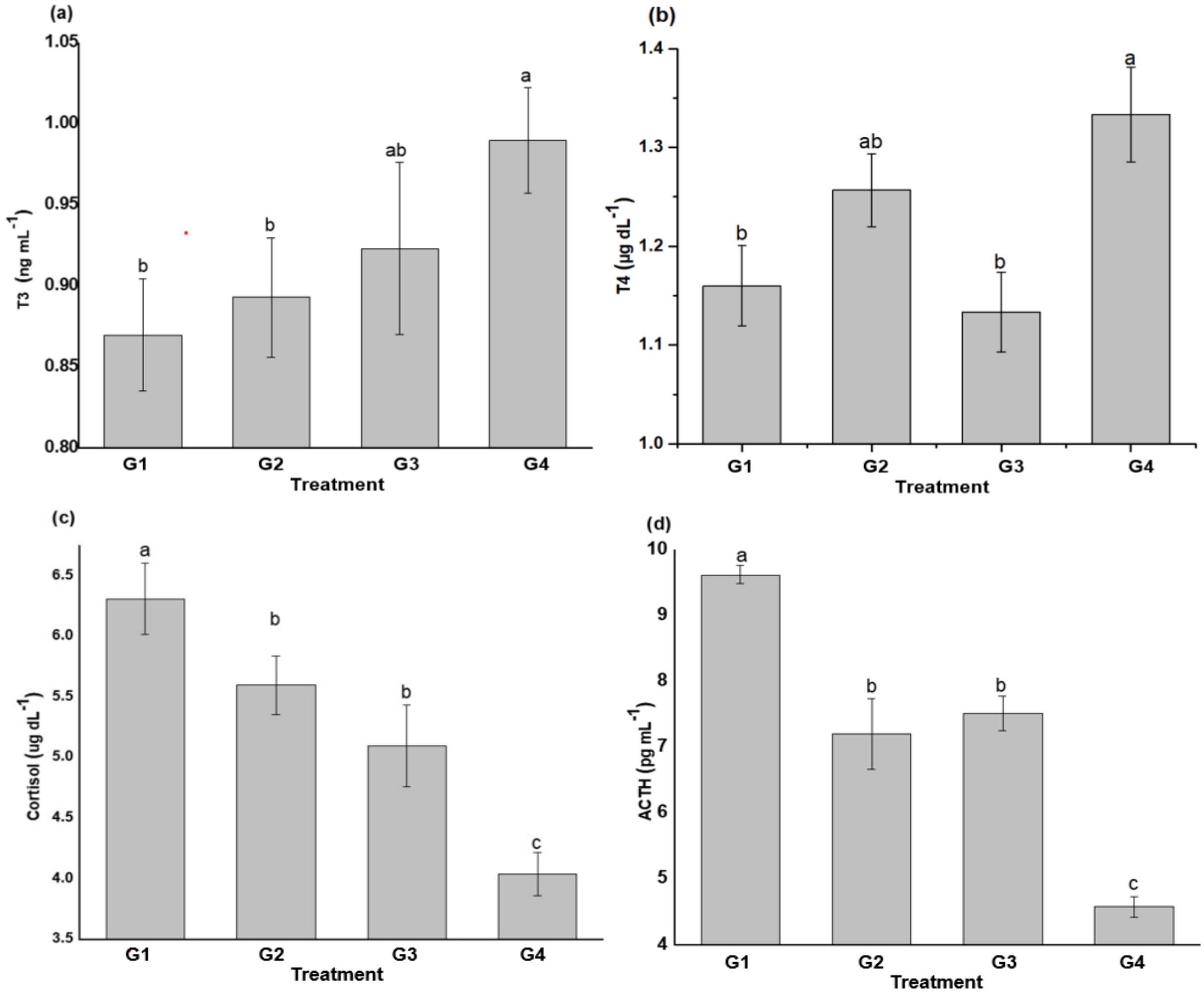

Figure 4. Effects of CNP, vit E, and their combination on a) T3, b) T4, c) cortisol, and d) ACTH concentration of Pabdah catfish. Data are shown as mean ± SE. Significant differences exist between groups with different letters ($p < 0.05$).

Discussion. Despite the distinct biological characteristics of VE and CNP, not much is understood regarding the dietary supplementation of CNP, VE, and their combination in Pabdah catfish. The information at hand has shown that dietary CNP or/and VE positively affects the growth, reproductive, and stress hormones of commercially valuable freshwater Pabdah catfish. GH can stimulate amino acid uptake and protein synthesis in muscles and significantly lower the rate of protein breakdown within muscle fibers. It can also have direct and indirect effects on protein, lipid, and carbohydrate metabolism, which can affect skeletal and muscle growth (Xu et al 2013). In the present study, the level of GH in Pabdah catfish demonstrated synergistic improvement. Similar findings were found by Ibrahim et al (2021), where at all CVCN levels, blood GH levels increased linearly and quadratically compared to the control level. According to Tang et al (2005), chitosan supplementation raised serum GH levels.

FSH and LH are known as pituitary gonadotropins, are essential to maintaining control of reproductive processes in vertebrates by regulating the development of gametes and hormones in the gonads (Mateos et al 2002). FSH is responsible for starting gametogenesis and promoting growth in the gonads, while LH primarily oversees the maturation of the gonads and the release of sperm or eggs (Salam et al 2024). The fact that led to increased gonadotropins (FSH and LH) levels in Pabdah catfish suggests that these dietary additives might have an advantageous influence on the reproductive performance of Pabdah catfish. CNP has been reported to have various biological activities, including antioxidant and immunostimulatory effects. These properties may have aided in the observed increase in LH levels, as oxidative stress and immune system function can affect reproductive processes in fish (Bhat et al 2016). VE, a well-known antioxidant, is also likely to have played a role in enhancing LH production, as reproductive hormones may be adversely affected by oxidative stress. The combination of CNP and VE may have had a synergistic effect on LH levels, further enhancing their reproductive benefits. This supports the idea that using multiple dietary supplements with complementary mechanisms of action can be more effective than using them individually. Similarly, the G4 group exhibited the highest level of FSH, indicating a potential synergistic effect of the two supplements. These findings suggest that dietary supplementation with CNP, VE, or their combination could potentially improve reproductive hormone levels in Pabdah catfish. Elevations of TST and E2 were linked to this increase. The current investigation confirms the physiological increases in blood levels of gonadotropins (FSH, LH), TST, and E2 in Pabdah catfish. This study's findings of elevated TST and E2 during vitellogenic growth and ovulation are in line with previous findings in a number of species (Rather et al 2013). The trend of FSH and LH was also observed in the levels of TST and E2 in fish that were supplemented. This is in line with numerous teleost studies (Goetz 1983; Rather et al 2013) that indicate 17a, 20β-OHP plays a role in the last stages of oocyte maturation.

In the current investigation, the thyroid hormone levels in Pabdah catfish were significantly impacted by CNP or/and VE administration. All vertebrates, including fish, need T3 and T4 in order for the pituitary gland to properly secrete growth hormones (Khan et al 2016). It is generally known that T3 and T4 are necessary for fish, humans, and several other mammalian species to have appropriate pituitary GH secretion (Khan et al 2016; Izadpanah et al 2022). T3 has been shown to raise GH messenger RNA levels in teleost pituitary cells (Farchi-Pisanty et al 1995). In a different study, it was shown that injecting T4 into female *Oreochromis niloticus* significantly boosted the production of activin bA in the larvae's developing tissues (Mousa 2004). These data reveal that CNP and VE supplemented diets can improve thyroid hormone regulation in Pabdah catfish.

The most often used biomarker of stress is cortisol, and increased levels of cortisol cause gluconeogenesis and glycogenolysis to increase in order to satisfy the increased energy demands of stress (Hajirezaee et al 2020). The primary glucocorticoid released by teleost fish intestine tissue is responsible for mediating energy restriction to reestablish homeostatic balance (Sadoul & Vijayan 2016). In the current investigation, Pabdah catfish supplemented with CNP or/and VE showed a progressive decrease in serum cortisol elevation. This suggests that the supplementation may have an anti-stress effect on Pabdah catfish by reducing the amplitude of elevated cortisol, most likely by inhibiting the secretion of ACTH (Haddad et al 2013). VE -fed tilapia and zebrafish exposed to zinc and silver nanoparticles, respectively, showed a comparable drop in serum cortisol, as did Rohu (*Labeo rohita*) exposed to nitrite and supplemented with dietary VE (Farsani et al 2017; Hedayati et al 2019). The primary hormones regulating fish stress conditions are adrenal gland-produced cortisol hormone (the primary corticosteroid in fish) and pituitary-derived ACTH (Montero et al 2015). A variety of pathways, including the standard cAMP/protein kinase A (cAMP/PKA) pathway and other cAMP-independent pathways reliant on protein kinase C activation or acetylcholine release, are stimulated by ACTH release in fish. These pathways also activate a cascade of enzymes belonging to the cytochrome P450 family, which converts 11-desoxicortisol to cortisol, which is then released into the bloodstream after a stressful event (Stocco et al 2005). Supplementing with CNP-VE, either alone or in combination, lowers stress levels as indicated by a notable drop in blood cortisol and ACTH levels.

Conclusions. The findings of the current study demonstrated that the endocrine system, in terms of growth hormone, reproductive hormones, and stress hormone levels, was significantly improved with both CNP and VE. However, the interaction between CNP and VE works synergistically to modulate the hormonal levels of Pabdah catfish to stress. Because of this, supplementing with both CNP and VE was more successful than supplementing with either micronutrient alone in promoting growth, stress responses, and reproductive hormonal responses of Pabdah catfish. As a result, this study suggests combining the addition of CNP to commercial diets to improve the growth, hormonal responses, and overall performance of Pabdah catfish during intense culture.

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Conflict of interest. The authors declare that there is no conflict of interest.

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