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Effects of phytase enzyme supplementation on growth performance, intestinal morphology and metabolism in Nile tilapia (*Oreochromis niloticus*)

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Abstract

Phytase is crucial in enhancing the bioavailability and release of phosphorus and other nutrients bound to phytic acid, making them more bioavailable for animal absorption. This study was carried out to inspect the effect of supplementing low phosphorus (P) diet with di-calcium phosphate (DCP) and liquid phytase enzyme (LP), which contains 1500 FTU/kg, on growth performance, intestinal morphometry, proximate body chemical composition, blood profile, immunity status, liver mitochondrial enzyme activities, the expression response and economic returns of Nile tilapia (*Oreochromis niloticus*). Three triplicate groups of fish (initial weight 5.405 ± 0.045 g, $N = 90$) were fed on three different diets for 90 days. The first was a control diet with zero DCP; the second was a control diet supplemented with 0.71% DCP; the third was a control diet supplemented with 0.03% LP. The groups were designated as CG, DCP and LP, respectively. Results showed that LP induced considerable improvements ($p < 0.05$) in FBW, body weight gain, weight gain rate, specific growth rate, HIS, viscero-somatic index, spleen-somatic index, feed

conversion ratio, blood parameters and the histomorphometry assessment of intestinal villi absorptive capacity, compared with the other groups. Also, whole-body protein and lipid contents pointedly ($p < 0.05$) increased by LP, compared with the DCP group. A positive response ($p < 0.05$) to the phytase enzyme was noted in complexes I, III and IV of the mitochondrial liver complex enzyme activity. Likewise, the relative gene expression levels of (*GHr-1*, *IGF-1*, *FAS* and *LPL*) were notably ($p < 0.05$) upregulated by phytase enzyme, associated with DCP and control groups. Further, phytase recorded the highest total return and profit percentage. It can be concluded that Nile tilapia benefits from using phytase enzyme 1500 FTU/kg at 0.03% without adding DCP in terms of good performance and profits.

KEYWORDS

di-calcium phosphate, gene expression, immune response, intestinal morphometry, liquid phytase enzyme, mitochondrial activity, Nile tilapia

1 | INTRODUCTION

Aquatic organisms have distinct physiological mechanisms that allow mineral absorption from their water and diets (Goff, 2018). While skeletal abnormalities, anorexia and decreased bone mineralization are indicators of a mineral deficit in fish (Lall & Kaushik, 2021), toxicity results from consuming too much mineral from the food or by gill uptake (Lall, 2022); therefore, aquatic species must balance toxicity and mineral shortage to preserve their homoeostasis. It has been established that fish require macro-minerals such as phosphorus, calcium, magnesium, potassium, sodium and chloride (Council, 1993; Jobling, 2012). In skeletal tissue, calcium and phosphorus are first deposited as tricalcium phosphate. Subsequently, it undergoes further crystallographic changes to transform into hydroxyapatite, accumulating in the organic matrix throughout mineralization (Trinkūnaitė-Felsen, 2014).

Phosphorus is essential for developing bone structure and several other physiological processes, such as preserving acid-base balance (Tzadik et al., 2017). Additionally, freshwater fish can absorb phosphorus from their surrounding aquatic environment through their gills or diets (Lall, 2022). According to Komoroske et al. (2016), freshwater fish can survive in habitats with less than 0.5 ppt salinities. However, plant ingredients' anti-nutritional components, for instance, phytate, can assume phosphorus bioavailability (Debnath, Sahu, et al., 2005; Zentek & Goodarzi Boroojeni, 2020), consequently affecting fish performance and health (van Krimpen et al., 2016).

As a result, a particular enzyme is required to breakdown the phytate and reduce the difficulties with fish digestion. Myoinositol (1,2,3,4,5,6)-hexaphosphate phosphohydrolase, also known as phytase, catalysers phytate hydrolysis to release P for absorption (Godoy et al., 2018). Although phytase activity is thought to be confined to the small intestinal brush border membrane, Nile tilapia can only digest around half of the phytate-phosphorus consumed in their diet (Pragya et al., 2021). Subsequently, the phytase enzyme can be added to fish diets to enhance nutrient uptake and growth rate, lowering production costs and improving husbandry outcomes (Kumar et al., 2012).

There has been a lot of recent focus on how adding phytase to the diets of popular aquaculture species, including rainbow trout, affects their growth and nutrient consumption (Wang et al., 2009), channel catfish (*I. punctatus*) (Yan et al., 2002), common carp (*C. carpio*) (Nwanna & Schwarz, 2007), African catfish (*C. gariepinus*) (Kemigabo et al., 2018), Stripped bass (*M. saxatilis*) (Papatryphon & Soares, 2001), Atlantic salmon (*S. salar*) (Carter & Sajjadi, 2011) and Nile tilapia (*Oreochromis niloticus*) (Maas et al., 2018). Nevertheless, until now, no previous research has examined how phytase supplementation affects Nile tilapia's liver mitochondrial enzyme activities and some blood parameters.

Consequently, our research intended to compare the results of phytase supplementation with and without food and di-calcium phosphate (DCP) supplementations on Nile tilapia growth performance, intestinal morphometry, haemato-biochemical profile, body composition, mitochondrial activity, lipogenesis and growth-related-gene expressions, and returns.

2 | MATERIALS AND METHODS

2.1 | Ethical validation

The Institutional Aquatic Animal Care and Use Committee of Egypt's Kafrelsheikh University's Faculty of Aquatic and Fisheries Sciences approved the KFS-IACUC/95/2020 study.

2.2 | Phytase enzyme

A microbial enzyme called 'OptiPhos®PLUS 5000L', produced by the Huvepharma Company in [illegible] was purchased. The density of the

product ranges from 1.10 to 1.20 g per mL. It contains various components, including 6-phytase (4.33% volume/volume), hydrochloric acid (0.02% volume/volume), glycerine (45% volume/volume), sodium benzoate (0.25% weight/volume) and filtered water (up to 100% volume). The active ingredient, 6-phytase (Enzyme Commission Number 3.1.3.26), is obtained from *E. coli* using a genetically modified strain of *K. phaffii* (DSM 32854), formerly Pichia. The commercial *E. coli* phytase was stored in a controlled environment with a constant temperature of 25 ± 2°C and humidity of 60 ± 5% (Additives & Feed, 2015)

2.3 | Experiment design

A group of 90 Nile tilapia fries (*O. niloticus*) with an average weight of 5.405 ± 0.045 g (initial weight ± SE) was obtained from a private fish hatchery in Kafrelsheikh. These healthy fish were then transported to the laboratory of the aquaculture department at Kafrelsheikh University. They were given the basal diet for two weeks while acclimating to temperatures of (28.60 ± 0.45°C), as stated in Table 1.

2.4 | Diet preparation

The fish were assigned randomly to three equal groups, with each group comprising 30 fish (three replicates/group), they were placed in nine glass aquariums (80 cm × 40 cm × 95 cm) with 10 fish per aquarium. The tap water used to fill these aquariums was devoid of chlorine, and an electric pump kept the aquariums regularly aerated. Every day, half the water was replaced.

Three distinct experimental diets were used to supply the essential nutrients for Nile tilapia. These diets were controlled for digestible calorie and protein content (Jobling, 2012); Group 1 (Control group 'CG') was a non-supplemented diet (low P diet) with no addition of DCP and phytase enzyme. Group 2 was a controlled diet with 0.71% 'DCP' (high P diet) following Norag et al. (2018). Group 3 was a control diet (low P diet) + 0.03% of liquid phytase enzyme (LP) (OptiPhos® PLUS 5000L) to give 1500 phytase units/kg diet 'LP' (see Table 1).

All the feed ingredients were mixed, 400 mL/kg of warm water was included to make the mixture moist, and finally, the mixture was cold pressed to form 1 mm pellets. A 45°C air convection oven was used to dry the diets, and they were then stored in airtight pouches until needed (Norag et al., 2018). The total experimental period was 90 days. The fish received feedings at 9 AM and 2 PM throughout the first two weeks, each meal constituting 3% of their total body mass. Commencing from the third week and for the remainder of the study, the feed was reduced to 2% of the fish's body mass. These feeding portions were recalculated biweekly with the collective fish weight in each tank. The experimental conditions included a cycle of 12 h of light monitored by 12 h of darkness. Water quality parameters were routinely monitored to ensure an optimal environment for Nile tilapia, with the temperature maintained at 28.50 ± 0.45°C, pH levels at 8.0 ± 0.02 and dissolved O_2 at 6.30 ± 0.21 mg/L. These measures were recorded using a multi-parameter probe metre (HI9829-03042-HANNA® instruments). Total

TABLE 1 Composition and chemical analysis of the control and experimental diets.

	Experimental treatments		
	CG	DCP	LP
Feed ingredients (%)			
Maize	13.76	12.95	13.76
Corn gluten 60	5.00	4.00	5.00
Wheat middlings	30.00	30.00	30.00
Rice bran CF11%	7.50	7.50	7.50
Soya bean meal 46%CP	41.12	41.23	41.12
Fishmeal 65%	1.50	2.50	1.50
DL methionine	0.22	0.22	0.22
Salt	0.50	0.50	0.50
Choline chloride 60	0.10	0.09	0.10
Fish vit & min premix	0.30	0.30	0.30
Di-calcium phosphate	0.00	0.71	0.00
Optiphose plus 5000 L	0.00	0.00	0.03
Proximate composition			
Dry matter (%)	88.79	88.89	88.79
Crude protein (%)	30.00	30.00	30.00
Ether extract (%)	4.29	4.33	4.29
Crude fibre (%)	5.74	5.71	5.74
Ash (%)	5.49	6.19	5.49
Calcium	0.25	0.47	0.25
Phosphorus	0.71	0.86	0.71
Calcium: Phosphorus	0.35	0.55	0.35
Growth energy[a] (kcal)	4399.46	4373.27	4399.46
Nitrogen free extract (%)	54.48	53.77	54.48
Each diet contains (%)			
Vit A	1.05	0.89	1.05
Mg	0.29	0.29	0.29
K	1.33	1.33	1.33
Na	0.24	0.26	0.24
Cl	0.38	0.39	0.38
Lysine	1.57	1.61	1.57
Threonine	1.10	1.10	1.10
Methionine	0.70	0.71	0.70
Cysteine	0.72	0.71	0.72
Methionine + cysteine	1.20	1.20	1.20
Tryptophan	0.37	0.38	0.37
Isoleucine	1.27	1.27	1.27
Valine	1.43	1.43	1.43

(Continues)

TABLE 1 (Continued)

	Experimental treatments		
	CG	DCP	LP
Leucine	2.49	2.43	2.49
Phenylalanine	2.14	2.13	2.14
Phenylalanine+ tryptophan	2.52	2.49	2.52
Histidine	0.78	0.78	0.78
Arginine	1.97	1.99	1.97
Linoleic acid	1.12	1.10	1.12
Linolenic acid	0.36	0.35	0.36
Eicosapentaenoic acid	0.01	0.02	0.01
Docosahexaenoic acid	0.01	0.01	0.01
Lysine	1.57	1.61	1.57

Abbreviations: DCP, di-calcium phosphate; LP, liquid phytase.

[a]Growth energy was calculated by using factors of 5.65, 9.45 and 4.22 Kcal per g of protein, lipid and carbohydrate, respectively. While, nitrogen free extract was equal to: 100−(protein + lipid + ash + fibre) (Abo-Raya et al., 2021).

ammonia nitrogen levels remained low, averaging 0.04 ± 0.002 mg/L, as assessed using a portable colorimeter (Martini MI 405), with all values reported as mean ± standard error.

2.5 | Growth parameters

Six fish were randomly chosen from each aquarium, 18 for each treatment group. To ascertain the final body weight, each fish was anesthetized with tricaine methanesulfonate (MS222) at 25 mg/L concentration (sourced from Argent Laboratories) and weighed individually. The variables for evaluating growth were derived from the subsequent formulas:

$$\text{Body weight gain (BWG): } W1 - W0$$

$$\text{Weight gain rate(WGR\%/day)} : (W1 - W0)/(t \times \log 10(W0/W1))$$

$$\text{Specific growth rate(SGR\%/day)} : (W1 - W0)/(t \times \log 10(W0/W1))$$

$$\text{Feed conversion ratio (FCR)} : \text{Feeding rate(g/day)/BWG(g/day)}$$

$$\text{Hepato-somatic index (HSI):HSI} = (W1 \times \text{liver weight})/(W1 \times \text{final body weight})\%$$

$$\text{Viscero-somatic index (VSI) : VSI} = (W1 \times \text{viscera weight})/(W1 \times \text{final body weight})\%$$

$$\text{Spleen-somatic index (SSI) : SSI} = (W1 \times \text{spleen weight})/(W1 \times \text{final body weight})\%$$

$$\text{Survival rate (SR\%) : SR} = (\text{Final fish count}/\text{Initial fish count})\%$$

Note: 't' represents the experiment duration (days), 'W0' is the initial weight of the fish (g) and 'W1' is the final weight of the fish (g) (Diab et al., 2023).

2.6 | Analysing the intestine morphometrically

Nine fish were chosen for each treatment group to collect tissues from their intestines. After that, the tissues were fixed for three days by preserving them in 10% neutral-buffered formalin. Following fixation, the samples were extensively rinsed with 100% alcohol to eliminate any remaining contaminants. Afterward, a Leica Rotary Microtome was used to embed the tissues in paraffin (RM 2145; Leica Microsystems). After that, the tissue sections were sliced into 5-micrometre-thick pieces and set on glass slides. Then, according to Bancroft and Layton (2013), the slides were subjected to the conventional staining method using hematoxylin and eosin (H&E). Measurements were taken using ImageJ analysis software to determine parameters such as villus height (measured from the tip to the villus-crypt junction), villus width (measured from the midpoint) and inter-villi space. These measurements were expressed in micrometres. Counting the number of goblet cells relative to their surface area allowed us to establish their density (mm^2) (Diab et al., 2023).

2.7 | Whole-body chemical composition

The whole body's chemical makeup was analysed following the feeding session. From each replication, three randomly selected fish were chosen for chemical composition measurement of whole and were kept at −40°C in a deep freezer until required. The whole fish body underwent proximate chemical analysis following the methods described by Arlington (1995); the moisture content of the fish samples was determined by subjecting them to oven drying at 105°C until a constant dry weight was achieved using a Memmert UN110 drying oven from Buchenbach. The crude protein (CP) content was measured using a Micro-Kjeldahl apparatus (Foss Kjeltec 2200). A Soxhlet apparatus assessed the total lipid content, and the samples were extracted with petroleum ether for 16 h. The ash content was calculated based on the weight loss when the samples were incinerated at 550°C for 6 h in a muffle furnace (Heraeus Instruments K1252).

2.8 | Blood sampling

Six fish from each tank had their caudal veins sampled for blood using a sterile syringe that contained EDTA, an anticoagulant. This enabled whole blood collection, which was drawn into plain tubes without

additional anticoagulants. The blood samples were centrifuged for 15 min at 1107 g at 4°C. Following centrifugation, the serum was stored at −20°C until further use. If you require more details or have specific inquiries about this process, please ask for additional information (Feldman et al., 2000).

2.8.1 | Haemato-biochemical analyses

An automatic blood cell counter was used to measure PCV, red blood cell count, Hb, WBCs, monocytes, lymphocytes and neutrophils (Thrall et al., 2004). Total proteins were measured following Doumas et al. (1981) at 540 nm, and serum albumins were calculated calorimetrically following Doumas et al. (1971) at 550 nm. At a wavelength of 540 nm, the activity of the ALT and AST was measured calorimetrically (Reitman & Frankel, 1957). Heinegård and Tiderström (1973), used a calorimetric approach to detect the serum creatinine level. The method outlined by Coulombe and Favreau (1963) was used to estimate the amount of urea. According to the manufacturer's guide outlined in the GPO-PAP and CHOD-PAP commercial clinical kit techniques, serum triglyceride and total cholesterol were measured (Fynn-Aikins et al., 1992). Glucose enzymatic PAP kits from Bio-Merieux, were exploited to determine serum glucose levels (Reitman & Frankel, 1957). The activity of digestive enzymes (lipase and amylase) was determined using the techniques explained by Abdel-Tawwab et al. (2018). The amounts of calcium and phosphorus were determined using commercial kits made by Bio-Diagnostic (Diagnostic and Research Reagents) (Norag et al., 2018).

2.8.2 | Immune and oxidative stress activities

The analysis of CAT and SOD activity at 450 nm (Hao et al., 2020) using commercially available Biodiagnostic, Kits. Smears of whole blood were made using the technique described by (Kawahara et al., 1991). To assess phagocytic activity and index, the number of cells that were phagocytosed within the phagocytic cells was tallied. The phagocytic activity was calculated by applying the following: Phagocytic activity = (macrophages containing yeast/total number of macrophages) × 100. The phagocytic index was calculated using the formula: Phagocytic index = (number of cells phagocytized/number of phagocytic cells). According to Demers and Bayne (1997) approach, ELISA was used in conjunction with a microplate ELISA reader to measure the serum lysozyme activity at 450 nm.

2.9 | Mitochondrial activity

2.9.1 | Isolation of liver mitochondria

A solution called medium A, which contains 10 mM potassium hydrogen phosphate (KH_2PO_4), 250 mM sucrose and 5 mM ethylenediaminetetraacetic acid (EDTA) adapted to a pH of 7.4, was used for isolating mitochondria from the fish liver. From each tank, two fish were chosen at random and anesthetized using 50 ppm of tricaine methanesulfonate (MS-222). Liver tissues were quickly excised and placed in cold extraction medium A. To prepare the liver tissue for further analysis, you used a portable Teflon/glass homogenizer (Potter-Elvejhem) to mix 3 g of liver tissue with 10 L of medium A. The homogenate was then centrifuged at 600 times the force of gravity (xg) for 10 min at 4°C, with the upper lipid layer removed. Finally, the remaining supernatant was centrifuged at 9000x*g* for 10 min. Following these steps, you have effectively separated the lipids within the liver tissue for further analysis. Accuracy is key in any scientific procedure, so work with reliable and accurate data. According to Suarez and Hochachka (1981), the sediment obtained after the second centrifugation was subjected to multiple washes using medium A. This solution consists of 10 mM KH_2PO_4, 250 mM sucrose and 5 mM EDTA, with a pH of 7.4. After the washing, the sediment was reconstituted in a small amount of medium A, which was further supplemented with 1 mg mL^{-1} bovine serum albumin (BSA).

Mitochondrial fractions from muscle and gut were isolated and prepared in a solution called medium B. Medium B contained 120 mM potassium chloride (KCl), 20 mM histidine (HEPES), 2 mM magnesium chloride ($MgCl_2$), 1 mM EDTA and 5 mg mL^{-1} BSA, with a pH of 7.4.

Individual muscle and intestinal samples weighing 3–5 g were finely minced and separately homogenized in 10 L of medium B using a Potter-Elvejhem grinder to obtain the mitochondrial fractions. After homogenization, the mixture was centrifuged at 600×*g* for 10 min to eliminate fat and fibrous tissue. The remaining liquid was passed through four cheesecloth layers for filtering. After centrifugation at 7000×*g* for 10 min, the pellet formed, including the mitochondria, was redissolved in 10 L of medium B. The remaining liquid was spun in a centrifuge at 17,000×*g* for 10 min after this step. We resuspended the pellets from the prior centrifugation processes in 10 L of medium C, which was pH-adjusted and contained 300 mM sucrose, 2 mM HEPES and 0.1 mM EDTA. The substance in suspension was subsequently spun at 3500×*g* for 10 min. The last step was resuspending the mitochondrial fraction in a minimal volume of medium C (Birch-Machin & Turnbull, 2001; Jang & Kim, 2019). The mitochondrial suspensions were divided into portions and frozen using liquid nitrogen. These frozen samples were then kept at −80°C. The next step was to measure the activity of each complex I–IV using spectrophotometry; therefore, this was done beforehand.

2.9.2 | Assessments of the enzyme activity in the liver's mitochondria

The mitochondrial suspensions were exposed to two cycles of freezing in liquid nitrogen and succeeding thawing in ice-cold water to disrupt the mitochondrial membrane. The enzyme activities were then assessed at a temperature of 28°C using a SmartSpec spectrophotometer. The evaluation involved measuring the reduction in absorbance caused by [illegible]ion of NADH at a wavelength of

340 nm, with 425 nm. The NADH extinction coefficient, which is 6.81 mM^{-1} cm^{-1}, was used in the calculations. Each measurement was duplicated using a Spectrosil Quartz Cuvette, with a final volume of 1 mL for each reading. The enzyme activities are quantified based on the mitochondrial protein content, expressed as milligrams per millilitre. Either 1 µmol of product or 1 µmol of substrate absorbed per min constituted one unit of enzyme activity (Birch-Machin & Turnbull, 2001; Jang & Kim, 2019).

To assess Complex II (Succinate: Ubiquinone1 Oxidoreductase, EC 1.3.5.1) activity, the reduction in absorbance of DCPIP at 600 nm was monitored using a SmartSpec spectrophotometer at 28°C. Duplicate measurements were achieved in a Spectrosil Quartz Cuvette (Birch-Machin & Turnbull, 2001; Jang & Kim, 2019). Mitochondria (10–50 µg of protein) were preincubated in assay media containing 25 mM potassium phosphate buffer, 5 mM $MgCl_2$ (pH 7.2) and 20 mM sodium succinate for 10 min at 28°C. After the addition of DCPIP (50 µM), KCN (2 mM), rotenone (2 µg/mL) and antimycin A (2 µg/mL), a baseline rate was observed for 3 min. The enzyme-mediated reduction of DCPIP was monitored for 4 min by initiating the reaction rate at 65 µM ubiquinone1. The specific activity of DCPIP was determined using an extinction coefficient of $\varepsilon = 19.1\ mM^{1}\ cm^{-1}$. To assess the activity of Complex III (ubiquinol-cytochrome *c* reductase, EC 1.10.2.2), the monitoring of decyl-ubiquinol at 550 nm (with 580 nm as the reference wavelength) was carried out (Jain et al., 1989). For the assessment of Complex IV (cytochrome *c* oxidase, EC 1.9.3.1) activity, the reduction in absorbance at 550 nm (with 580 nm as the reference wavelength) was measured, indicating the oxidation of reduced cytochrome *c* (Birch-Machin & Turnbull, 2001). A mixture of potassium phosphate (20 mM, pH 7), cytochrome *c* subunit II (15 µM) and dodecyl maltoside (0.45 mM) was prepared, and the non-enzymatic rate. Mitochondria (5–15 µg of protein) were then intensified following the oxidation of cytochrome *c* subunit II by a small amount of potassium hexacyanoferrate. The Complex IV activity was determined as the apparent first-order rate constant, and the precise activity level was determined by an extinction coefficient factor of $\varepsilon = 19.1\ mM^{-1}\ cm^{-1}$.

2.10 | QRT-PCR

After the end of the experiment, total RNA was extracted following the manufacturer's guide using the TRIzol reagent. The resulting RNA was reverse transcribed into cDNA using the MultiScribe RT enzyme kit. The cDNA obtained was subjected to real-time triplication PCR analysis using the Power SYBR Green PCR Master Mix on a 7500 Real-Time PCR System from Applied Biosystems in Foster City. To determine the relative fold changes in mRNA expression of different genes, the C_t values of the target genes were compared to a control sample. The fold change was normalized to the expression of the housekeeping gene β-actin. The $2^{-\Delta\Delta C_t}$ method was employed, which involved normalizing the C_t values of the target gene to those of the β-actin housekeeping gene (Livak & Schmittgen, 2001). Table 2 provides the primer sequences (Biovision).

2.11 | Economic efficiency measurements

Economic efficiency was determined using the equation from Kishawy et al. (2022) and Allam et al. (2020). Feed cost (USD/kg WG) = FCR × feed cost of 1 kg.

Net profit (USD/kg WG) = Total returns of kg WG−total costs of kg WG.

Economic efficiency = profit per kg WG/gross cost per kg WG (see Supporting Information S1: Table 1: The supplementary information about the economic efficiency measurements).

2.12 | Statistical analysis

After verifying the normality and homogeneity of the data, the statistical assessment was conducted using GraphPad Prism 6.01. One-way ANOVA and Tukey's post hoc test were employed to identify significant differences among the evaluated groups.

TABLE 2 Primer used for qRT-PCR amplification.

No	Gene	Primer sequence (5′-3′)	Accession number	Reference
1	*β-actin*	For: CCACACAGTGCCCATCTACGA	XM_003455949.2	Qiang et al. (2016)
		Rev: CCACGCTCTGTCAGGATCTTCA		
2	*GHr-1*	For: CAGACTTCTACGCTCAGGTC	AY973232.1	Aanyu et al. (2018)
		Rev: CTGGATTCTGAGTTGCTGTC		
3	*IGF-1*	For: AGTTTGTCTGTGGAGAGCGAG	EU272149.1	Ziková et al. (2010)
		Rev: GTGTGCCGCTGTGAACG		
4	*FAS*	For: TGAAACTGAAGCCTTGTGTGCC	GU433188	Tian et al. (2015)
		Rev: TCCCTGTGAGCGGAGGTGATTA		
5	*LPL*	For: TGCTAATGTGATTGTGGTGGAC	FJ623077	Tian et al. (2015)
		Rev: GCTGATTTTGTGGTTGGTAAGG		

A p-value of <0.05 was considered statistically significant. The results were expressed as the mean ± standard error.

3 | RESULTS

3.1 | Efficiency measures for feeding and growth

Nile tilapia fish that were fed a diet containing the phytase enzyme exhibited improved growth rates ($p < 0.05$) and feed conversion efficiency ($p < 0.05$) compared to fish-fed diets with lower or higher levels of phosphorus; it was noticed that the FCR was 1.411, 1357 and 1.211 for the control, DCP and LP, respectively (Table 3).

In contrast, fish fed a diet containing DCP showed decreased growth rates and feed conversion efficiency ($p < 0.05$) compared to the non-enriched phosphorus control group. Additionally, there was significant improvement in growth performance markers such as weight gain rate (WGR) and specific growth rate (SGR) in diets with reduced phosphorus levels compared to those with enhanced phosphorus content.

3.2 | Intestinal morphometry

The intestinal morphometry of Nile tilapia, following a 90-day consumption of experimental diets, is outlined in Table 4 and illustrated in Figure 1. The villi length and the number of goblet cells in all intestinal segments demonstrated a notable ($p < 0.05$) increase in fish-fed diets with ordinary phosphate (LP) and DCP, respectively, in comparison to those fed the control basal diet. However, the width of the intestinal villi and the inter-villi space did not exhibit significant changes ($p > 0.05$) across the treated groups in the anterior and terminal intestinal sections, with the most favourable outcomes observed in Nile tilapia fed the phytase enzyme. Compared to a low phosphorus diet, high phosphorus feeding and phytase supplementation notably ($p < 0.05$) decreased the inter-villi space in the middle intestine section.

3.3 | Proximate body chemical composition

Here are the chemical components of the fish: Table 5. Dietary supplementation of fish with 1500 phytase units/kg considerably ($p < 0.05$) augmented fish's protein, compared to the Nile tilapia-fed low and high phosphorus diet. Moreover, fish fed on a diet has high phosphorus levels deprived of phytase enzyme significantly ($p < 0.05$) decreased the body's fat content in comparison with Nile tilapia fed the lower phosphorus and phytase diets, with the highest fat content value recorded in the phytase group. Furthermore, enzyme and DCP supplementations resulted in significantly higher body ash contents ($p < 0.05$) than the non-enriched phosphorus diet. However, taking enzyme supplements significantly ($p < 0.05$) reduced the body carbohydrate content compared to Nile tilapia fed the lower and higher phosphorus levels.

3.4 | Biochemical parameters

The addition of 1500 FTU/kg of dietary supplementation to the low phosphorus diet resulted in the most significant effects ($p < 0.05$) on PCV associated with the group of fish fed on diets with low and high phosphorus levels without phytase supplementation (refer to Table 6). Furthermore, the supplementation of the enzyme notably ($p < 0.05$) improved RBCs and Hb levels while showing a

TABLE 3 Growth performance, somatic parameters and survival of fish fed DCP and LP.

Parameters	Experimental treatments			p Value
	CG	DCP	LP	
IW (g)	5.240 ± 0.052^{b}	5.753 ± 0.052^{a}	5.223 ± 0.031^{b}	0.001
FBW (g)	47.75 ± 0.224^{c}	49.97 ± 0.079^{b}	54.75 ± 0.252^{a}	<0.0001
BWG (g)	42.51 ± 0.188^{c}	44.22 ± 0.008^{b}	49.53 ± 0.270^{a}	<0.0001
WGR (%)	811.4 ± 6.640^{b}	768.9 ± 11.56^{c}	948.4 ± 9.868^{a}	<0.0001
SGR (%/day)	2.455 ± 0.008^{b}	2.402 ± 0.014^{c}	2.611 ± 0.010^{a}	<0.0001
FCR	1.411 ± 0.006^{a}	1.357 ± 0.0002^{b}	1.211 ± 0.006^{c}	<0.0001
HSI (%)	1.798 ± 0.026^{c}	2.086 ± 0.026^{b}	2.562 ± 0.007^{a}	<0.0001
VSI (%)	3.280 ± 0.039^{c}	3.529 ± 0.048^{b}	4.846 ± 0.049^{a}	<0.0001
SSI (%)	0.187 ± 0.009^{b}	0.222 ± 0.011^{b}	0.357 ± 0.003^{a}	<0.0001
SR (%)	100	100	100	

Note: Means within each raw that lack common superscripts differ significantly at $p < 0.05$.

Abbreviations: BWG, body weight gain; DCP, di-calcium phosphate; FCR, feed conversion ratio; FW, final body weight; HSI, hepato-somatic index; IW, initial weight; LP, liquid phytase; SGR, specific growth rate; SR, survival rate; SSI, spleen-somatic index; VSI, viscero-somatic index; WGR, weight gain rate.

TABLE 4 Intestinal morphometry of fish fed DCP and LP.

Intestinal portion	variable	Experimental treatments			*p* Value
		CG	DCP	LP	
Anterior part	Villi length (μm)	147.200 ± 2.255^{c}	221.900 ± 6.518^{b}	295.900 ± 18.420^{a}	0.0003
	Villi width (μm)	96.260 ± 8.840	106.300 ± 3.657	119.700 ± 4.857	0.093
	Inter villi space (μm)	92.850 ± 7.919	65.020 ± 14.500	63.200 ± 2.783	0.128
	Goblet cell (no/mm^2)	17.670 ± 1.202^{b}	28.330 ± 2.404^{a}	33.330 ± 1.453^{a}	0.002
Middle part	Villi length (μm)	263.300 ± 13.720^{c}	386.700 ± 14.000^{b}	509.700 ± 9.595^{a}	<0.0001
	Villi width (μm)	51.400 ± 0.952	52.700 ± 10.210	68.350 ± 1.509	0.163
	Inter villi space (μm)	54.480 ± 4.743^{a}	38.410 ± 1.136^{b}	27.390 ± 1.733^{b}	0.002
	Goblet cell (no/mm^2)	21.000 ± 0.577^{c}	34.000 ± 2.082^{b}	45.000 ± 1.155^{a}	<0.0001
Terminal part	Villi length (μm)	104.900 ± 5.302^{b}	155.000 ± 12.400^{ab}	176.800 ± 18.360^{a}	0.021
	Villi width (μm)	84.680 ± 6.382	77.210 ± 8.468	92.090 ± 7.744	0.433
	Inter villi space (μm)	77.510 ± 6.812	51.530 ± 4.097	60.920 ± 7.498	0.068
	Goblet cell (no/mm^2)	9.000 ± 0.577^{b}	13.000 ± 0.577^{b}	18.330 ± 1.453^{a}	0.001

Note: Means within each raw that lack common superscripts differ significantly at $p < 0.05$.

Abbreviations: DCP, di-calcium phosphate; LP, liquid phytase.

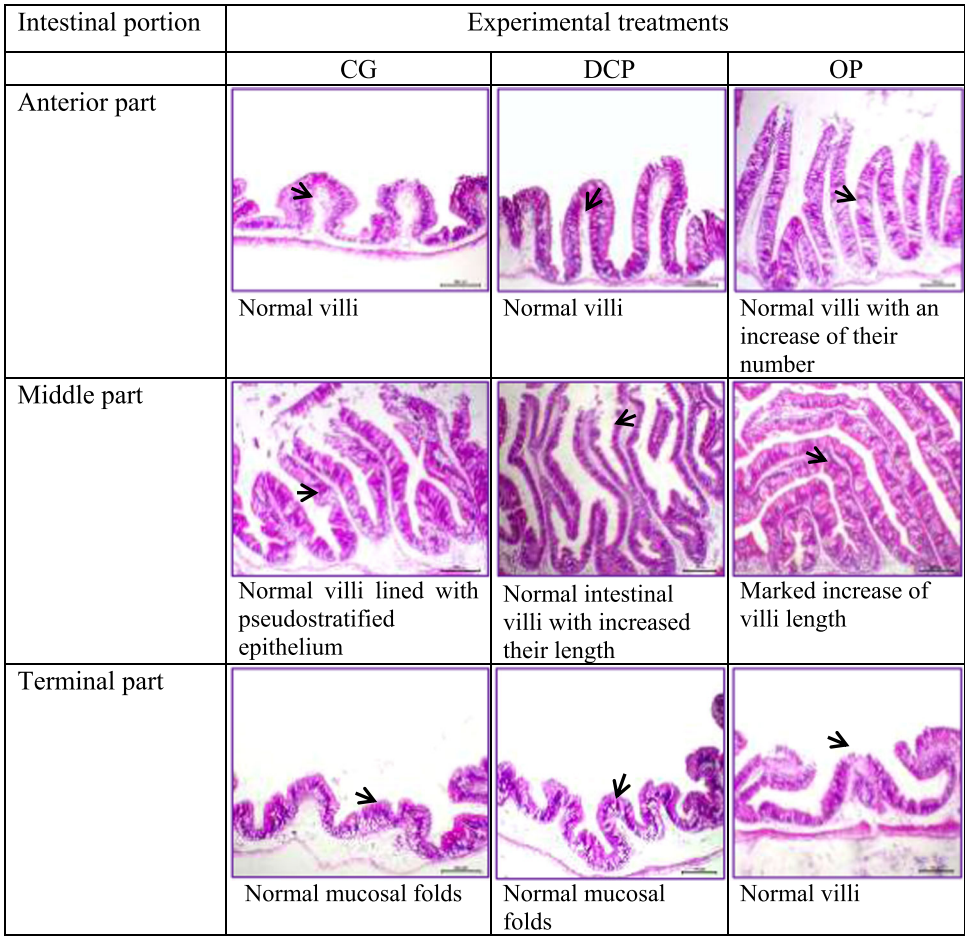

FIGURE 1 Hematoxylin–eosin-stained photomicrograph of the anterior, middle and terminal parts of the intestine of fish fed DCP and LP. DCP, di-calcium phosphate; LP, liquid phytase. [Color figure can be viewed at wileyonlinelibrary.co

TABLE 5 Proximate body chemical composition (%, as DM basis) of fish fed DCP and LP.

Parameters (%)	Experimental treatments			*p* Value
	CG	DCP	LP	
Dry matter	26.720 ± 3.543	26.540 ± 2.152	26.350 ± 2.751	0.667
Crude protein	60.464 ± 0.310^{b}	61.217 ± 3.870^{b}	67.107 ± 1.430^{a}	0.009
Crude fat	7.393 ± 0.625^{a}	5.330 ± 0.060^{b}	7.480 ± 0.740^{a}	0.005
Carbohydrate	9.623 ± 0.995^{a}	8.483 ± 2.925^{a}	0.033 ± 0.015^{b}	0.001
Total ash	22.520 ± 0.700^{b}	24.970 ± 0.950^{a}	25.380 ± 0.595^{a}	0.007

Note: Means within each raw that lack common superscripts differ significantly at $p < 0.05$.
Abbreviations: DCP, di-calcium phosphate; LP, liquid phytase.

TABLE 6 Haemato-biochemical profile of fish fed DCP and LP.

Parameters	Experimental treatments			*p* Value
	CG	DCP	LP	
PCV (%)	18.85 ± 0.650^{c}	23.65 ± 1.150^{b}	28.20 ± 1.700^{a}	0.0003
RBCs (×10/mm^{3})	0.913 ± 0.084^{b}	1.187 ± 0.087^{a,b}	1.570 ± 0.181^{a}	0.028
Hb (g/100 mL)	4.667 ± 0.578^{b}	6.633 ± 0.405^{a,b}	8.667 ± 0.536^{a}	0.004
TP (g/dL)	6.500 ± 0.500^{b}	9.333 ± 1.528^{a}	9.333 ± 0.577^{a}	0.018
Albumin (g/dL)	2.933 ± 0.260^{c}	5.333 ± 0.433^{b}	8.167 ± 0.430^{a}	0.0002
ALT (U/L)	48.67 ± 2.028	51.33 ± 2.333	44.67 ± 3.480	0.284
AST (U/L)	36.00 ± 2.646	39.33 ± 2.333	37.67 ± 3.400	0.725
Creatinine (mg/dL)	0.566 ± 0.290	1.100 ± 0.115	1.067 ± 0.100	0.172
Urea (mg/dL)	40.00 ± 4.041	46.67 ± 1.764	40.33 ± 3.580	0.333
Triglycerides (mg/dL)	124.3 ± 18.32^{a,b}	104.3 ± 10.33^{b}	168.7 ± 10.900^{a}	0.038
Cholesterol (mg/dL)	198.5 ± 1.520^{b}	201.5 ± 3.510^{b}	230.0 ± 4.013^{a}	<0.0001
Glucose (mg/dL)	51.33 ± 2.728^{b}	60.67 ± 3.283^{b}	99.00 ± 0.577^{a}	<0.0001
Amylase (U/L)	211.3 ± 13.420^{b}	262.3 ± 28.990^{a,b}	342.3 ± 25.250^{a}	0.020
Lipase (U/L)	83.33 ± 5.457^{b}	107.3 ± 7.839^{b}	146.0 ± 5.508^{a}	0.001
Calcium	23.23 ± 3.901^{b}	25.10 ± 3.156^{b}	49.77 ± 4.444^{a}	0.004
Phosphorus	16.10 ± 3.677^{b}	21.97 ± 1.876^{b}	46.37 ± 3.028^{a}	0.0008

Note: Means within each raw that lack common superscripts differ significantly at $p < 0.05$.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DCP, di-calcium phosphate; Hb, haemoglobin; LP, liquid phytase; PCV, packed cell volume; RBCs, red blood cells; TP, total protein; WBCs, white blood cells.

nonsignificant ($p > 0.05$) increase in red blood cell and haemoglobin levels linked to the phosphorus-enriched group, in comparison to the fish given the identical diet without phytase treatment.

The biochemical markers of Nile tilapia fed different experimental diets were all within the normal range. The fish that were fed LP had significantly higher blood serum levels of total protein, albumin, globulin, glucose, amylase, lipase, calcium, phosphorus, cholesterol and triglycerides compared to the fish that were fed diets with low and high phosphorus levels ($p < 0.05$). Still, not a single one differed significantly ($p > 0.05$) in the total protein and amylase amounts between LP and DCP groups. Triglycerides did not change substantially ($p > 0.05$) between LP and control group diets. ALT, AST, creatinine and urea, in contrast, failed to demonstrate any noteworthy impacts ($p > 0.05$) across the experimental groups.

3.5 | Functional immune assay

Table 7 displays the results of the functional immunological assays. All immune parameters (phagocytic activity, lysozyme activity and WBCs) substantially ($p < 0.05$) increased by the fish fed with phytase enzyme regarding the control and [illegible]ups. Also, all immune parameters

TABLE 7 Immune response of fish fed DCP and LP.

Parameters	Experimental treatments			*p* Value
	CG	DCP	LP	
Phagocytic activity (μg mL^{-1})	10.00 ± 0.577^{b}	$11.00 \pm 0.577^{a,b}$	13.670 ± 0.881^{a}	0.023
Phagocytic index	1.145 ± 0.155^{c}	1.950 ± 0.050^{b}	2.500 ± 0.200^{a}	<0.0001
Lysozyme activity (μg Ml^{-1})	0.526 ± 0.139^{b}	0.303 ± 0.104^{b}	1.073 ± 0.047^{a}	0.005
WBCs ($\times 10^{3}/mm^{3}$)	18.87 ± 1.146^{b}	21.73 ± 1.884^{b}	30.87 ± 0.895^{a}	0.002
Heterophils (%)	6.333 ± 1.453^{b}	$9.667 \pm 0.881^{a,b}$	12.67 ± 0.666^{a}	0.015
Lymphocytes (%)	85.33 ± 3.180	83.67 ± 1.764	79.33 ± 1.764	0.250
Monocytes (%)	7.000 ± 0.577^{b}	$8.000 \pm 0.577^{a,b}$	9.333 ± 0.333^{a}	0.047

Note: Means within each raw that lack common superscripts differ significantly at $p < 0.05$.
Abbreviations: DCP, di-calcium phosphate; LP, liquid phytase; WBCs, white blood cells.

non-meaningfully ($p > 0.05$) changed by the fish fed with the high P level, associated with the lower P level without phytase inclusion, except for the phagocytic index, which showed a meaningful raise ($p < 0.05$). However, dietary supplementation of 1500 phytase units/kg to the low P diet-induced non-noteworthy levels ($p > 0.05$) of phagocytic activity, heterophils and monocytes compared with the DCP group.

3.6 | Antioxidant enzymes activity

Assumptions drawn from experiments evaluating the antioxidant enzymes (SOD and CAT) of Nile tilapia fish were reported in Figure 2. Fish fed DCP and LP had significantly increased ($p < 0.05$) SOD and CAT activities, compared with control fish fed the lower phosphorus level without enzyme supplementation, with the highest activity induced by phytase addition ($p < 0.05$).

3.7 | Mitochondrial liver enzyme activities

The activity of complex I hepatic enzymes demonstrated in Figure 3 (ranging from 0.14 to 0.30 units/g mitochondrial protein) afforded a meaningful response to the dietary phytase-enriched diets ($p < 0.05$) associated with the non-enriched P group. However, high P level induced nonsignificant changes ($p > 0.05$) linked to the low P level group deprived of phytase enzyme and the low P level group with phytase inclusion. Complex II (ranging from 0.18 to 0.22 units/g mitochondrial protein) displayed non-noteworthy changes ($p > 0.05$) in comparison to every experimental diet, with the highest and lowest activities reported in the DCP and LP groups, respectively. Dietary supplementation of 1500 phytase units/kg and DCP to the low P diet notably ($p < 0.05$) induced complex III activities related to the control group. In contrast to the highly enriched P group, the dietary phytase-enriched meals increased complex III liver enzyme activity, although this response was not statistically considerable ($p > 0.05$). Complex III ranged from 1.45 to 1.68 units/g mitochondrial protein. The diet containing phytase enzyme and high P levels significantly ($p < 0.05$) induced complex IV liver enzyme activities (ranged from 1.45 to 1.74 units/g mitochondrial protein), compared with non-enriched phosphorus diet, with the highest significant values ($p < 0.05$) were recorded in Nile tilapia fed phytase enzyme.

3.8 | Gene expression analysis

Comparative analysis of growth-related gene mRNA expression (*GHr-1* and *IGF-1*) and lipogenesis-related genes [*fatty acid synthase* (*FAS*) and *LPL*] (Figures 4 and 5) presented that the enrichment of fish fed low P level with phytase enzyme and DCP significantly ($p < 0.05$) induced upregulation levels of all studied genes [*GHr-1* (2.25 and 1.33) fold increase, respectively], [*IGF-1* (3.36 and 1.4) fold increase, respectively], [*FAS* (7.48 and 5.59) fold increase, respectively] and [*LPL* (3.79 and 2.71) fold increase, respectively], compared to control basal diet with the highest expression level was observed in fish received 1500 FTU/kg to the low P diet ($p < 0.05$).

3.9 | Economic evaluation

The study's cost-benefit analysis for several experimental fish is presented in Table 8. The highest total net production value of 1485.81 g fish biomass was observed for fish fed the phytase enzyme. However, records showed the lowest possible value of fish fed the control diet (1275.30 g fish biomass). This was consistent for total return and net profit as well. Following the market prices 2023, the input and output analysis revealed profit % of 78.78, 81.14 and 108.28 for fish-fed diets containing low phosphorus, high phosphorus and low phosphorus levels with phytase enzyme, respectively.

The effects of phytase supplementation were significant ($p < 0.05$) factors on the feed cost, gross cost, net profit and economic efficiency (Table 8). The feed cost was significantly ($p < 0.05$) lower in fish fed the phytase enzyme than in the other groups. There were no significant differences between fish-fed diets containing low and high phosphorus. Similarly, the gross cost of fish production fed on dietary high phosphorus was higher (p [illegible] an the other treated groups. The

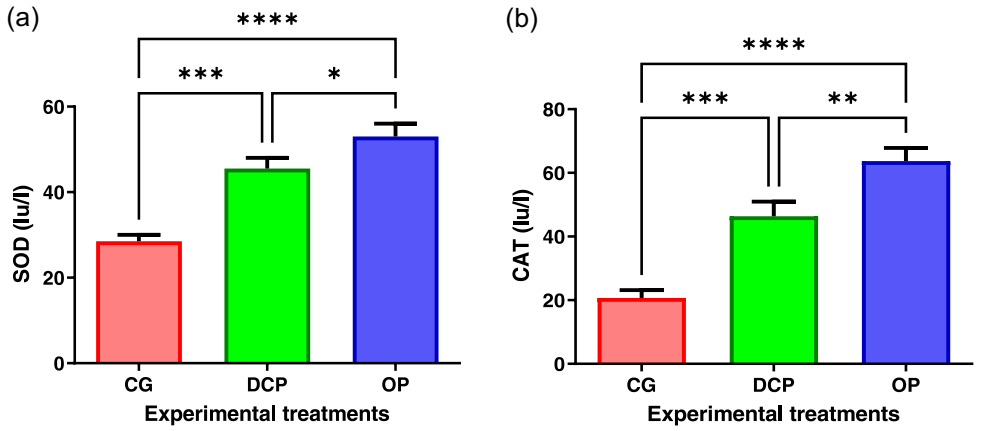

FIGURE 2 Antioxidant enzyme activity of superoxide dismutase 'SOD' (a) and catalase 'CAT' (b) of fish fed DCP and LP. Values are expressed as mean ± SE from triplicate groups. Asterisks on the data bars indicate significant differences between the experimental groups to their control when $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p < 0.0001$. DCP, di-calcium phosphate; LP, liquid phytase. [Color figure can be viewed at wileyonlinelibrary.com]

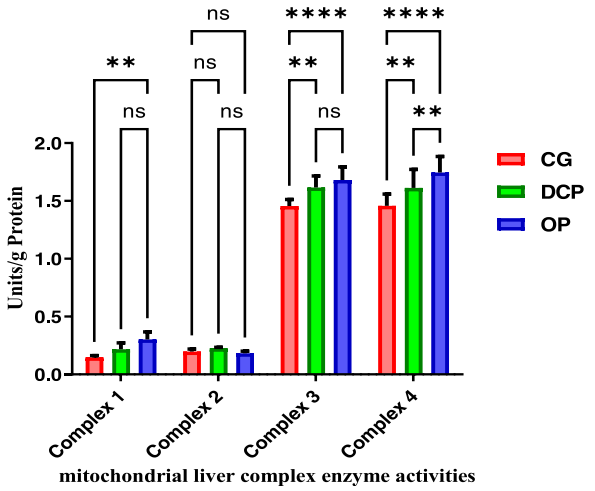

FIGURE 3 Mitochondrial liver enzymes activities of fish fed DCP and LP. Values are expressed as mean ± SE from triplicate groups. Asterisks on the data bars indicate significant differences between the experimental groups to their control when $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p < 0.0001$. Complex I, NADH: ubiquinone oxidoreductase, EC 1.6.5.3; Complex II, succinate: ubiquinone1 oxidoreductase, EC 1.3.5.1; Complex III, ubiquinol: ferricytochrome *c* reductase, EC 1.10.2.2; Complex IV, cytochrome *c* oxidase, EC 1.9.3.1; DCP, di-calcium phosphate; LP, liquid phytase. [Color figure can be viewed at wileyonlinelibrary.com]

net profit and the economic efficiency of the fish diet supplemented with phytase were higher ($p < 0.05$) concerning other treated groups.

4 | DISCUSSION

Phosphorus is crucial for mediating energy metabolism, encoding the genetic material and cell signalling (Hüttemann et al., 2007). Furthermore, dietary P is the primary source of the body's nutrients for skeletal development, growth (Lall, 2022) and bone mineralization (Lall & Kaushik, 2021). Including phytase is thought to be the primary driver of improved P availability (Hirvonen et al., 2019). The impact of phytase enzymes on agricultural fish productivity has been the subject of other studies; however, this one aimed to compare the effects of feeding Nile tilapia DCP and phytase enzymes.

In our study, Nile tilapia considerably outperformed the other groups in terms of growth performance and somatic characteristics after receiving 1500 phytase units/kg of feed, indicating that LP enhances nutrient assimilation. Furthermore, the control group produced the most significant levels of WGR and SGR in contrast to the DCP group, despite the DCP group having the best FBW, BWG, FCR, HIS, VSI and SSI. Similarly, according to Cao et al. (2007), phytase dosages between 250 and 2000 FTU/kg feed are commonly regarded as excellent for the growth performance of many fish species. Additionally, phytase supplementation was found to have positive effects on Rainbow trout (Vielma et al., 2002), Giant yellow croaker (Mai et al., 2006), Nile tilapia (Maas et al., 2018), Common carp (Nwanna et al., 2005) and Rohu (Baruah et al., 2007). Moreover, similar results displayed that raising the Ca:P ratio significantly decreased the WGR (Adeola et al., 2006), suggesting that the high Ca:P ratio hinders P absorption and decreases the efficiency of enzymes (Maltais-Landry, 2015). Also, the positive findings for growth performance parameters in this study by LP could be attributed to the improved liberation of nutrients from meals based on plants by dissolving the bonds between phytate-minerals and phytate-protein (Amer, 2017). Conversely, there was no discernible difference in the growth rates of pond-raised channel catfish on a diet with or without phytase (Robinson et al., 2002), in African catfish (Nwanna et al., 2005) and in Nile tilapia (Riche et al., 2001).

The fish's intestine, an essential digestive organ, is vital in keeping it healthy; moreover, villi length, villi width and goblet cell quantity directly impact fish digestion and absorption (Elsabagh et al., 2018). The histomorphometric evaluation in this study revealed that fish-fed LP exhibited enhanced absorptive capacity in the intestinal villi, as evidenced via an e of the villi in terms of both breadth

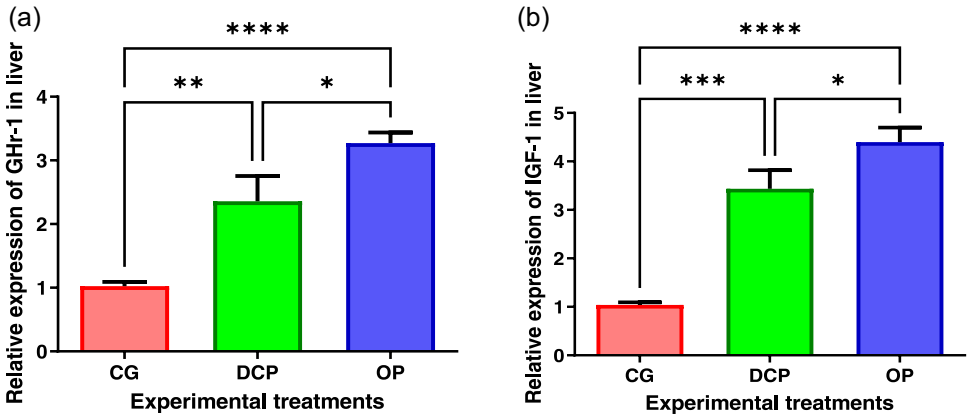

FIGURE 4 The relative expression profile of the growth-related genes, growth hormone receptor-1 '*GHr-1*' (a) and insulin-like growth factors-1 '*IGF-1*' (b) genes of fish fed DCP and LP. Asterisks on the data bars indicate significant differences between the experimental groups to their control when $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p < 0.0001$. DCP, di-calcium phosphate; LP, liquid phytase. [Color figure can be viewed at wileyonlinelibrary.com]

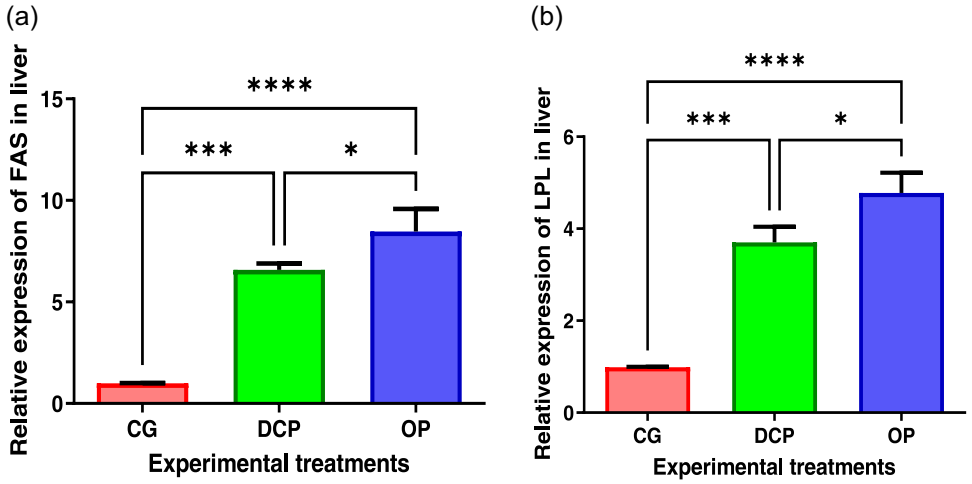

FIGURE 5 The relative expression profile of fatty acid synthase '*FAS*' (a) and lipoprotein lipase '*LPL*' (b) genes of fish fed DCP and LP. Asterisks on the data bars indicate significant differences between the experimental groups to their control when $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and $^{****}p < 0.0001$. DCP, di-calcium phosphate; LP, liquid phytase. [Color figure can be viewed at wileyonlinelibrary.com]

and number of goblet cells, suggesting that phytase contributed to improving the intestine's capacity for absorbing nutrients (Kim et al., 2003). These findings agreed with those related to fish growth performance. According to Adeshina, Akpoilih, Udom, et al. (2023), increased intestinal villi width/height and absorption area were caused by the phytase enzyme (1000 FTU/kg) linked to the control group. Feng et al. (2016) and Liu et al. (2023) found comparable findings. Phytic acid is broken down by phytase to produce inorganic phosphorus and inositol through a process called dephosphorylation, allowing the minerals to be released and then absorbed by the intestine, which can impact its absorptive capacity (Kumar et al., 2012).

Quantitatively estimating food and food substances, such as CP, total carbohydrate, total fat, dietary fibre and moisture, is done using proximate analysis (Ganogpichayagrai & Suksaard, 2020). Our findings revealed that dietary supplementation of fish with 1500 phytase units/kg augmented fish's protein and fat percentages and decreased the body's carbohydrate content, with the DCP group showing the lowest fat level. These outcomes were consistent with (Norag et al., 2018), who noted that P reduction in Nile tilapia feed dramatically enhanced the fat content with or without varying doses of phytase. Comparable findings were also attained by (Sajjadi & Carter, 2004) in Atlantic salmon, (Debnath, Pal, et al., 2005) in Yellow tail catfish and (Liang et al., 2012) in Grass carp. These findings suggested that the liberation of nutrients by LP led to a far more significant amount of lipids and proteins than the other categories (Cowieson et al., 2017).

Additionally, according to Ji et al. (2017), with increasing dietary phosphorus, CP and fat content increased and reduced, respectively, and our findings supported that. One possible explanation for decreased lipid content in DCP-treated fish is a rise in P oxidation of fatty acids (Eya & Lovell, 1997). Conversely, Adeoye et al. (2016) observed that Nile tilapia body moisture, CP, fat and ash contents were unaffected by phyta[illegible]e supplementation.

TABLE 8 Fish production and the economic efficiency of the experimental diets.

Items	Experimental treatments		
	CG	DCP	LP
Feed cost (USD/kg WG)	1.551 ± 0.01[a]	1.533 ± 0.02[a]	1.331 ± 0.01[b]
Total returns (USD/kg WG)	3.71 ± 0.21[c]	3.86 ± 0.11[b]	4.32 ± 0.13[a]
Total costs (USD/kg WG)	2.06 ± 0.12[b]	2.12 ± 0.21[a]	2.06 ± 0.14[b]
Net profit (USD/kg WG)	1.64 ± 0.01[b]	1.74 ± 0.02[b]	2.26 ± 0.04[a]
Economic efficiency (%)	79.61 ± 2.3[b]	82.07 ± 1.4[b]	109.70 ± 3.1[a]

Note: Means within each raw that lack common superscripts differ significantly at $p < 0.05$.

Abbreviations: DCP, di-calcium phosphate; LP, liquid phytase.

Fish nutritional and metabolic status, overall health and physiological responses to stress can all be monitored using haematological and biochemical measures (Adeoye et al., 2016). Dietary inclusion of phytase-enriched RBCs, PCV and Hb, linked with the control diet and DCP group, demonstrates the capacity of LP to promote hematopoiesis (Fazio et al., 2014), health status (Adel et al., 2016) and the immune response of fish (Carbone & Faggio, 2016). Fish-fed P and/or phytase-containing diets exhibited considerably higher levels of haemoglobin, PCV, RBCs and WBC counts (Adeshina, Akpoilih, Tiamiyu, et al., 2023). Furthermore, the Nile tilapia biochemical indicators examined in our study were in the range of their average values, indicating that the phytase enzyme causes non-nutritional stress and has no impact on liver function in Nile tilapia. Similar investigations were recorded by (El-Zibdeh et al., 1995; Hlophe-Ginindza et al., 2016; Ji et al., 2017).

The immunological response of fish can be evaluated using conventional methods such as white blood cell count, lysozyme activity, phagocytic index and phagocytic activity (Diab et al., 2023). The current study found that supplementing fish diets with phytase enzymes improved their immunological capacities when assessed against fish-fed DCP and a control diet. A rise in lysozyme activity, phagocytic activity and WBC count signalled this (Chen et al., 2017). Adeshina, Akpoilih, Udom, et al. (2023) and Zarghi et al. (2022) reported similar outcomes. In contrast, Nile tilapia fish differential leucocyte counts were not significantly affected by phytase enzyme supplementation (Norag et al., 2018).

Keeping the equilibrium between hydrogen peroxide production and removal, which is an adaptive immune response, catalase's activity must rise during oxidative injury (Jia et al., 2019; Wang et al., 2013). Additionally, SOD is a crucial enzyme that eliminates ROS, which poses a hazard to cells under stress by inflicting spectacular damage to the cell structure and biomolecular function, promptly or incidentally leading to a variety of disorders (Costa et al., 2010). This research concludes that fish-fed LP had greater SOD and CAT activity than fish fed the control and DCP diets, suggesting that phytase enzyme might modulate the Nile tilapia's antioxidant status (Kapewangolo et al., 2016). These findings corroborate those made by Adeshina, Akpoilih, Udom, et al. (2023), who claimed that phytase enzyme (1000 FTU/kg) heightened catalase activity and superoxide dismutase activity. On the other hand, it was found that organic acid and phytase in the diet significantly reduced blood levels of ROS, catalase activity and serum SOD (Zhu et al., 2014). Following Cheng et al. (2016), replacing NaH_2PO_4 with phytase did not affect the activities of serum CAT, AKP and IgM concentrations when compared to the reference diet. Phytase breaks down phytic acid-protein complexes to increase the digestion of dietary proteins. This may result in a rise in the availability of amino acids necessary to produce antioxidant enzymes and proteins connected to the immune system (Kokou & Fountoulaki, 2018).

The conventional role of mitochondria is oxidative phosphorylation, which generates ATP from the free energy generated when food is oxidized. Growth, motility and homoeostasis are biochemical and physiological processes that rely on ATP as their primary energy source (Brand et al., 2013). The results of the existing investigation displayed that liver mitochondrial enzyme complex activities (I, III and IV) were positively affected by the phytase enzyme. However, complex II did not significantly differ between groups, with the phytase enzyme showing the lowest activity. One Complex II enzyme is involved in the electron transport chain and the citric acid cycle (Iverson et al., 2023). Our results confirmed that LP restricted the enzyme's active site, inhibiting enzyme activity (VanderLinden et al., 2015). These results supported the notion that enhancing mitochondrial function is linked to encouraging development and using feed (El-Nokrashy et al., 2021), and they were associated with our study's significant influence on FCR. The current data are reliable with those in (Eya et al., 2013). The authors state that to reduce the cost of producing energy within cells, connecting the dots between mitochondrial enzyme complexes and enhanced performance and feed efficiency in different animals is essential. The findings were reported by (Dröse et al., 2009; Ragab et al., 2022; Randi et al., 2021).

qRT-PCR assays to assess the expression of genes involved in Nile tilapia growth and lipolysis. It would indicate that the complex system responsible for controlling development, reproduction and differentiation is essential in expressing ILGF-1 and GH during development (Abo-Raya et al., 2021). Furthermore, according to Albalat et al. (2007), a necessary enzyme for the breakdown of lipids in animals is lipoprotein lipase or LPL. Additionally, *Only the FAS enzyme can reductively produce long-chain fatty acids* (Kuhajda, 2006), and it has the power to change the rates of fatty acid production and hydrolysis (Smith et al., 2003). When phytase enzyme and DCP were added to the fish diet, GHr, IGF-1, LPL and FAS expression were associated with the control group, with the maximum activity produced by the 1500 phytase units/kg group. Given that *ILGF-1* and *GH* were upregulated, it might be hypothesized that dietary phytase might stimulate the somatotrophic axis (Abo-Raya et al., 2021). This may be justified by removing the phytate-binding phosphorus complex and [illegible] the amount of protein readily

available in response to dietary phytase enzyme inclusion (Kumar et al., 2012). In addition, increased *FAS* and *LPL* expressions suggested that the phytase enzyme (1500 FTU/kg meal) inhibits fat synthesis and causes fat deposition in Nile tilapia (Qiao et al., 2014). These findings supported our results of the approximate chemical composition. The expression of the lipogenic genes (*LPL* and *FAS*) may be one factor contributing to higher body fat deposition (Bogacka et al., 2004). Similar investigations were stated by Rajan et al. (2021); Safari et al. (2022); and Hassaan et al. (2019). On the other side, increased phosphorus levels (1.32% and 1.59%) dramatically decreased the relative gene expression levels of *FAS*, according to research by Ji et al. (2017). Norag et al. (2018) and Tian (2006) theorized that FAS lacked tissue-specific control and functioned as a negative feedback regulator of fat accumulation; however, our findings contradicted that theory. Little is known about how dietary phytase and phosphorus affect the relative expression of *LPL* and *FAS* in different fish species; consequently, greater research is need to have in order to comprehend the processes by which they alter lipid metabolism.

DCP and phytase enzyme supplements increased the fish yield of Nile tilapia-fed diets in the current study by 4% and 16.5%, respectively, as contrasted with the control diet. In addition, profit percentage rose in the DCP and phytase groups relative to the control group by roughly 2.36% and 29.5%, respectively. If DCP costs $5.74 per ton and LP prices are $0.18 per ton on average, switching to LP instead of DCP will save around $5.56 per ton of feed. The information showed that Nile tilapia utilizes nutrition more effectively when given phytase as a supplement. Similarly, the cost of meat was affected by phytase enzymes at a level of 1500 FTU/kg in birds' diets without adding DCP (Ali, 2021). Al-Harthi et al. (2020) attained that adding phytase to diets made with olive cakes achieved the maximum economic efficiency. Similar results have been published by Coppedge (2012); Scholey et al. (2018); and Selle and Ravindran (2007). Therefore, finding a comparatively less expensive alternative ingredient has been a continuing research objective (El-Nokrashy et al., 2021). Furthermore, the rising feed cost is considered one of the most significant issues restricting profitability in fish production (Abdel Rahman et al., 2010).

5 | CONCLUSION

In summary, the utilization of the phytase enzyme at 0.03% in Nile tilapia diets, without the addition of DCP, may enhance growth, intestinal histomorphology, hemato-biochemical performance and immune-oxidative indices, mitochondrial liver enzymes activities, lipid metabolism and returns; furthermore, improvement in terms of transcriptomic response. In addition, at the level of the phytase enzyme supplementation were benefits on the level of the economic efficiency concerning the DCP and lower phosphorus diet

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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