

EFFECTS OF DIETARY PHYTASE AND ORGANIC ACIDS ON NUTRIENT UTILIZATION AND ANTIOXIDANT STATUS IN *Clarias gariepinus*

Asma Batool¹, Neelam Arshad², Ayesha Shahid³, Ali Hassan⁴, Bisma Younas⁵, Kainat Shabbir⁴

¹Department of Biological and Environmental Science, Emerson University Multan, Punjab, Pakistan.

²Department of Zoology, Wildlife and Fisheries, University of Agriculture, Faisalabad-38040, Punjab, Pakistan.

³Department of Zoology, Government College University, Lahore, Pakistan.

⁴Department of Zoology, Ghazi University, Dera Ghazi Khan, Pakistan.

⁵Department of Zoology, Government College University, Faisalabad, Punjab, Pakistan.

* Corresponding author. E-mail: khanasma6038@gmail.com (Tel.: +923007039111)

ABSTRACT

Received:
25, Oct, 2025

Accepted:
22, Nov, 2025

Published:
6, Jan, 2026

The present study was conducted to examine the effect of phytase and organic acids (OA) on growth parameters such as final body weight(g), weight gain (%) Specific growth rate (%/day), FCR, Survival (%), nutrient digestibility, body composition, and oxidative stress biomarkers in African catfish (*Clarias gariepinus*). The six dietary treatments for 60 days were designed: control (0% OA, 0 phytase), 2% OA, 4% OA, 0% OA + phytase, 2% OA + phytase, and 4% OA + phytase, each with three replicates. The significant ($p < 0.05$) results were found on the inclusion of phytase and OA on growth and nutrient utilization. The highest final body weight (7.4 ± 0.3 g), weight gain ($460.6 \pm 31\%$), specific growth rate ($3.3 \pm 0.3\%/day$), and the lowest feed conversion ratio (1.5 ± 0.2) were found in the 2% OA + phytase group. Apparent digestibility coefficients for dry matter, protein, and phosphorus were maximized at $46.7 \pm 1.3\%$, $77.2 \pm 1.4\%$, and $48.0 \pm 1.4\%$, respectively ($p = 0.007$), while fecal phosphorus significantly decreased (0.8 ± 0.04 g/kg). Vertebral phosphorus and calcium contents were enhanced significantly ($p < 0.05$) in the combined supplement group. Oxidative stress markers revealed lower ROS (66.5 ± 1.8), SOD (144 ± 2.6 U/mL), and MDA (5.25 ± 0.14 nmol/mg) levels in the 2% OA + phytase group, indicating enhanced antioxidant defense ($p = 0.005$). These outcomes indicate that 2 OA inclusion with phytase improves growth, nutrient digestibility, mineral retention, and antioxidant balance in *C. gariepinus*.

KEYWORDS: *Clarias gariepinus*, Phosphorus Digestibility, Nutrient digestibility, Antioxidant Response

1. INTRODUCTION

Aquaculture is recognized worldwide as an increasingly pivotal sector to reduce overfishing of wild stocks and to meet rising demand for animal protein (Froehlich *et al.*, 2023). Among the various aquaculture fish species, African catfish (*Clarias gariepinus*) is the most cultured species in Asia due to its ability to thrive under a variety of environmental conditions, high feed conversion efficiency (Langi *et al.*, 2024). However, the

intensification of *C. gariepinus* production also carries various challenges, such as inefficiencies in nutrient utilization, which result in economic loss (Besson *et al.*, 2014). Feed constitutes the largest cost item, and environmental degradation through excess excreta, chiefly of nitrogen and phosphorus (Nathanailides *et al.*, 2023). The main nutritional constraint in numerous aquafeeds is the presence of plant protein components, which are more reasonable and maintainable than fishmeal but contain anti-nutritional factors (Ondiba *et al.*, 2022).

Access this article online



<https://doi.org/10.25271/sjuoz.2026.14.1.1825>

Science Journal of University of Zakho
Vol. 14, No. 01, pp. 24–32 January-2026

Printed ISSN 2663-628X;
Electronic ISSN 2663-6298

This is an open access under a CC BY-NC-SA 4.0 license
(<https://creativecommons.org/licenses/by-nc-sa/4.0/>)

Phytic acid (phytate) forms phytate-mineral complexes, which bind with phosphorus and other minerals such as calcium, zinc, and iron that are poorly assimilated by fish due to inadequate fundamental phytase activity (Pujol *et al.*, 2023). The consequence is mineral deficiencies, reduced phosphorus bioavailability, inferior growth, and enlarged phosphorus discharge into the atmosphere, which can lead to eutrophication (Chen *et al.*, 2025). Exogenous phytase supplementation thereby advances growth performance, increasing phosphorus and mineral digestibility and reducing phosphorus excretion by resolving the phytate molecule, phytase proclamations inorganic phosphorus, and diminishing phytate's chelating properties (Selim *et al.*, 2022). Microbial phytase supplemented with graded levels of phytase increases the digestibility of phosphorus, growth, and enhancements in feed conversion ratios in *C. gariepinus* (Adeshina *et al.*, 2023). high-soybean meal diets with phytase enhanced antioxidant status, immunity, disease resistance, and growth in the *C. gariepinus* (Elaigwu *et al.*, 2024).

The rice protein concentrates with phytase supplementation in *Labeo rohita* fingerlings showed nutrient digestibility, improved growth, and positive changes in body chemical characteristics (Iqbal *et al.*, 2021). The microalgae with the phytase in European seabass (*Dicentrarchus labrax*) improve gut microbiota profiles and growth performance (Yadav *et al.*, 2025). The organic acids as feed additives with the enzyme supplementation can inhibit pathogenic bacteria, progress the solubility and stability of minerals, lower the gut pH, and enhance enzyme activity that contributes to better digestion of nutrients (Liang *et al.*, 2022). Organic acid in the diet improves the immune parameters, growth performance, and digestibility of crude protein, feed conversion ratio, and protein efficiency ratio in *C. gariepinus* (Hussein *et al.*, 2023).

The low concentrations of organic acids such as butyric acid, propionic acid, and formic acetic in *C. gariepinus* enhance the relative growth rate, and condition factor (El-Dakar *et al.*, 2022). Moreover, various organic acids such as malic, citric, formic, and lactic influence antioxidant responses, potentially alleviating oxidative stress in silver carp fingerlings (Reda *et al.*, 2022). Intensive farming causes the oxidative stress imbalance, which harms lipids, health, proteins, nucleic acids, reduces growth, and increases disease susceptibility (Naiel *et al.*, 2023).

The oxidative stress is generated due to an imbalance of the capacity of antioxidant defenses and reactive oxygen species (Afzal *et al.*, 2023). Therefore, integrating feed additives that decrease oxidative impairment or reinforce antioxidant responses is progressively seen as central for aquaculture sustainability (Aluta *et al.*, 2021; Antache *et al.*, 2025).

2. MATERIALS AND METHODS

Experimental Design:

This study was designed to assess the effects of dietary phytase and organic acids (OA) on growth parameters, nutrient digestibility, oxidative stress biomarkers, and body composition of *Clarias gariepinus*. The six dietary treatments with three replicates were designed, such as T0; 0% OA, 0 phytase (Control), T2; 2% OA, no phytase, T3; 4% OA, no phytase, T4; 0% OA, + phytase, T5; 2% OA, + phytase, and 4% OA, + phytase. The 900 juvenile *C. gariepinus* with an average initial weight: 13.0 ± 0.09 g were purchased from the Multan government hatchery Punjab, Pakistan and acclimated for 14 days in fiberglass tanks before the 60-day feeding trial. A basal diet (Table 1) was given during the acclimation period of one week. After the acclimation, fish were randomly distributed at a density of 50 fish per tank into 18 fiberglass tanks (395 L each), with each dietary treatment replicated three times. Water quality parameters such as $26.3 \pm 0.3^\circ\text{C}$ temperature, dissolved oxygen at 7.09 ± 0.05 mg, and pH at 8.07 ± 0.01 L⁻¹ was maintained, and Fish were fed twice daily (08:30 and 15:30) to apparent satiation, and uneaten feed was siphoned after one hour, dried at 60°C , and weighed to fix accurate feed intake for FCR calculations (Owais *et al.*, 2023).

Dietary Preparation:

Experimental diets were formulated for African catfish juveniles. All dry ingredients were thoroughly mixed before oil and water were added to form a homogenous dough. The basal diet contained fish meal, soybean meal, maize gluten, and wheat bran as primary protein and energy sources. Phytase (commercial grade, 10,000 U/g activity) was incorporated at a rate of 1,000 FTU/kg of feed. Organic acids were included at 2% and 4% levels as per treatment design, using a blend of formic, citric, and propionic acids in powder form (Fazal *et al.*, 2025). The dough was pelletized through a 2-mm die, air-dried at room temperature, and stored in airtight containers at 4°C until use. (Table 1).

Table 1: Composition and nutrient content of experimental diets for *Clarias gariepinus* juveniles (g/kg dry matter basis)

Ingredients (g/kg)	0% OA, 0 Phytase	2% OA, 0 Phytase	4% OA, 0 Phytase	0% OA, + Phytase	2% OA, + Phytase	4% OA, + Phytase
Fish meal	180	180	180	180	180	180
Soybean meal	320	320	320	320	320	320
Maize gluten	120	120	120	120	120	120
Vegetable oil	60	60	60	60	60	60
Premix	30	30	30	30	30	30
Dicalcium phos.	25	25	25	25	25	25
Chromic oxide	5	5	5	5	5	5
Organic acid	0	20	40	0	20	40
Phytase	0	0	0	0.1	0.1	0.1
Wheat bran	260	240	220	259.9	239.9	219.9
TOTAL	1000	1000	1000	1000	1000	1000

Growth Performance Metrics:

At the start and end of the feeding period, fish were group-weighed after a 24-hour fasting period. Growth parameters were calculated using standard formulae:

$$\text{Weight gain (WG, \%)} = [(\text{Final body weight} - \text{Initial body weight}) / \text{Initial body weight}] \times 100.$$

$$\text{SGR\%} = (\text{Ln of the final weight (g)} - \text{Ln of the initial weight (g)}) / (\text{Experimental duration (days)})$$

$$\text{Feed conversion ratio (FCR)} = \text{Feed intake (g)} / \text{Weight gain (g)}$$

$$\text{Survival rate (\%)} = (\text{Initial number of fish stocked} - \text{mortality}) / (\text{Initial number of fish}) \times 100. \text{ (Ahmed, 2023)}$$

Nutrient Digestibility Analysis:

Apparent digestibility coefficients (ADC) were determined by means of chromic oxide (0.5%) as an inert marker in the diets. Feces were collected by siphoning the bottom of each tank 1 hour after feeding, collected per replicate, and directly cooled at -20°C until examination. Samples were oven-dried at 60°C , pulverized, and examined for dry matter, crude protein, total phosphorus, and chromic oxide content. Digestibility coefficients were calculated following the method of Sales and Britz (2001).

$$\text{ADC (\%)} = 100 - (100 \times \% \text{ marker in feces} / \% \text{ marker in feed})$$

$$= 100 - (100 \times \% \text{ marker in feces} / \% \text{ marker in feed})$$

Body Composition and Mineralization:

The body proximate composition was examined for crude protein via the Kjeldahl method, and ash by combustion at 550°C for 18 h. Vertebral samples

collected were from three fish per replicate and prepared with distilled water, oven-dried at 60°C , and crushed to fine powder. Phosphorus absorption was determined by the colorimetric molybdovanadate method, while calcium content was determined by atomic absorption spectrophotometry following McCleary's (2013) procedures.

Oxidative Stress Biomarkers:

Blood samples from the caudal vein were collected from the anesthetized fish, and then centrifuged, and serum was stored at -20°C . By fluorescence assay (DCFH-DA probe), the reactive oxygen species were measured. malondialdehyde (MDA), was studied via the thiobarbituric acid reactive substances (TBARS). Superoxide dismutase and catalase activities were determined using commercial kits, with absorbance read at 550 nm and 405 nm, respectively, these oxidative biomarkers determined by following method of Ohkawa et al. (1979).

Statistical Analysis:

Data (mean \pm SEM) were analyzed using two-way ANOVA (SPSS v16.0), followed by Duncan's post-hoc test ($p < 0.05$).

3. RESULTS

The growth parameters revealed significant changes ($p < 0.05$), viz. control (0% OA, 0 phytase) showed baseline performance with final body weight 4.7 ± 0.2 g ($p = 0.005$), weight gain $252.2 \pm 54\%$ ($p = 0.005$), and specific growth rate $2.3 \pm 0.4\%$ /day ($p = 0.005$). Feed conversion ratio was in this group at 2.4 ± 0.2 ($p = 0.005$), with survival rates $83.2 \pm 2.5\%$ ($p = 0.005$). Phytase supplementation alone (0% OA + phytase) significantly improved all growth metrics: FBW increased to 6.8 ± 0.4 g ($p = 0.007$), WG to

454.8 ± 29.4% (p = 0.007), and SGR to 3.3 ± 0.2%/day (p = 0.007). FCR improved to 1.7 ± 0.3 (p = 0.007) while survival rose to 90.1 ± 2.1% (p = 0.007). The 2% OA + phytase combination yielded optimal results with FBW of 7.4 ± 0.3 g (p = 0.004), WG of 460.6 ± 31% (p = 0.004), SGR of 3.3 ± 0.3%/day (p = 0.004), and best FCR (1.5 ± 0.2, p =

0.004) and survival (91.2 ± 1.7%, p = 0.004). The 2% OA treatment (no phytase) resulted in FBW of 5.4 ± 0.5 g (p = 0.006), WG of 296.7 ± 27.3% (p = 0.006), and SGR of 2.3 ± 0.2%/day (p = 0.006). The 4% OA group (no phytase) showed similar FBW (5.4 ± 0.3 g, p = 0.006) but higher WG (330.3 ± 35.2%, p = 0.006) and SGR (2.5 ± 0.3%/day, p = 0.006) (Table 2).

Table 2: Mean± SE of Growth parameters throughout the 60-day experiment of *C. gariepinus* juveniles fed diets containing phytase and organic acid.

Treatment	FBW (g)	WG (%)	SGR (%/day)	FCR	Survival (%)	P-Value
0 OA, 0 Phytase	4.7 ± 0.2	252.2 ± 54	2.3 ± 0.4	2.4 ± 0.2	83.2 ± 2.5	0.005
2 OA, 0 Phytase	5.4 ± 0.5	296.7 ± 27.3	2.3 ± 0.2	2.4 ± 0.5	86.6 ± 3.4	0.006
4 OA, 0 Phytase	5.4 ± 0.3	330.3 ± 35.2	2.5 ± 0.3	2.2 ± 0.4	88.8 ± 1.4	0.006
0 OA, + Phytase	6.8 ± 0.4	454.8 ± 29.4	3.3 ± 0.2	1.7 ± 0.3	90.1 ± 2.1	0.007
2 OA, + Phytase	7.4 ± 0.3	460.6 ± 31	3.3 ± 0.3	1.5 ± 0.2	91.2 ± 1.7	0.004
4 OA, + Phytase	6.4 ± 0.4	410.1 ± 26.4	2.8 ± 0.2	1.8 ± 0.5	88.9 ± 2.4	0.005

The growth parameters of *C. gariepinus* responded distinctly to dietary alterations. (Control fish (0% OA, 0 phytase) demonstrated baseline digestibility values of 40.7 ± 1.2% for dry matter (p = 0.005), 67.5 ± 1.4% for protein (p = 0.005), and 24.3 ± 0.8% for phosphorus (p = 0.005), with faecal phosphorus excretion at 1.2 ± 0.04 g/kg (p = 0.005). Phytase supplementation alone (0% OA + phytase) significantly enhanced all digestibility parameters: dry matter increased to 45.6 ± 1.1% (p = 0.006), protein to 76.8 ± 1.6% (p = 0.006), and phosphorus to 42.0 ± 1.3% (p = 0.006), while reducing faecal phosphorus to 0.7 ± 0.01 g/kg (p = 0.006). The 2% OA + phytase combination proved particularly effective, yielding 46.7 ± 1.3% dry matter (p = 0.007), 77.2 ± 1.4% protein (p = 0.007), and 48.0 ± 1.4% phosphorus digestibility (p = 0.007), with

faecal phosphorus at 0.8 ± 0.04 g/kg (p = 0.007). The 4% OA + phytase presented the maximum dry matter digestibility (57.0 ± 1.4%, p = 0.005) but slightly lesser protein utilization (76.3 ± 1.2%, p = 0.005) compared to other phytase treatments.

Phosphorus absorption remained high (48.2 ± 1.4%, p = 0.005) with faecal excretion at 0.7 ± 0.05 g/kg (p = 0.005). Organic acids alone exhibited variable effects. The 2% OA treatment (no phytase) showed reduced dry matter digestibility (35.4 ± 0.8%, p = 0.007) but improved phosphorus absorption (29.4 ± 1.2%, p = 0.007) versus control. The 4% OA diet (no phytase) repaid dry matter digestibility to control levels (40.5 ± 1.4%, p = 0.006) while keeping intermediate phosphorus values (25.3 ± 1.1%, p = 0.006) (Table 3).

Table 3: Mean± SE of Nutrient digestibility and faecal phosphorus elimination throughout the 60-day experiment of *C. gariepinus* juveniles fed diets containing phytase and organic acid.

Treatment	ADC-DM (%)	ADC-Protein (%)	ADC-P (%)	Fecal P (g/kg)	P-Value
0 OA, 0 Phytase	40.7 ± 1.2	67.5 ± 1.4	24.3 ± 0.8	1.2 ± 0.04	0.005
2 OA, 0 Phytase	35.4 ± 0.8	67.2 ± 1.1	29.4 ± 1.2	1.0 ± 0.03	0.007
4 OA, 0 Phytase	40.5 ± 1.4	68.7 ± 1.2	25.3 ± 1.1	1.0 ± 0.05	0.006
0 OA, + Phytase	45.6 ± 1.1	76.8 ± 1.6	42.0 ± 1.3	0.7 ± 0.01	0.006

2 OA, + Phytase	46.7 ± 1.3	77.2 ± 1.4	48.0 ± 1.4	0.8 ± 0.04	0.007
4 OA, + Phytase	57.0 ± 1.4	76.3 ± 1.2	48.2 ± 1.4	0.7 ± 0.05	0.005

Dietary alterations significantly changed the body composition (Table 3). The control group (0% OA, 0 phytase) presented 15.4 ± 0.4% body protein and 3.2 ± 0.4% crude ash (p = 0.006). Phytase supplementation (0% OA + phytase) improved body protein 15.8 ± 0.3% (p = 0.003) and ash content to 3.6 ± 0.3% (p = 0.003), representing significant enhancements over control values. The 2% OA treatment (no phytase) yielded 15.2 ± 0.2% protein (p = 0.005) and 3.4 ± 0.2% ash (p = 0.005), while 4% OA showed similar protein (15.4 ± 0.6%, p = 0.004) but identical ash content (3.4 ± 0.2%, p = 0.004).

Phosphorus content increased from 6.05 ± 0.14% in controls (p = 0.006) to 7.42 ± 0.12% with phytase alone (p = 0.003) - a 22.6% enhancement. Calcium levels indicated increasing from 13.17 ± 0.32% to 15.41 ± 0.33% in the same contrast (both p = 0.003). The 2% OA + phytase combination produced intermediate mineralization values (6.97 ± 0.13% P, p = 0.004; 14.64 ± 0.26% Ca, p = 0.004), while 4% OA + phytase showed slightly reduced effects (6.91 ± 0.12% P, p = 0.004; 15.00 ± 0.25% Ca, p = 0.004) (Table 4).

Table 4: Mean± SE of body composition and vertebral mineralization throughout the 60-day experiment of *C. gariepinus* juveniles fed diets containing phytase and organic acid.

Treatment	Body Protein (%)	Crude Ash (%)	Vertebral P (%)	Vertebral Ca (%)	P-value
0 OA, 0 Phytase	15.4 ± 0.4	3.2 ± 0.4	6.05 ± 0.14	13.17 ± 0.32	0.006
2 OA, 0 Phytase	15.2 ± 0.2	3.4 ± 0.2	6.69 ± 0.12	13.60 ± 0.26	0.005
4 OA, 0 Phytase	15.4 ± 0.6	3.4 ± 0.2	6.60 ± 0.11	14.18 ± 0.24	0.004
0 OA, + Phytase	15.8 ± 0.3	3.6 ± 0.3	7.42 ± 0.12	15.41 ± 0.33	0.003
2 OA, + Phytase	15.7 ± 0.1	3.8 ± 0.3	6.97 ± 0.13	14.64 ± 0.26	0.004
4 OA, + Phytase	15.4 ± 0.4	3.5 ± 0.2	6.91 ± 0.12	15.00 ± 0.25	0.004

The oxidative status presented significant treatment effects. Control fish (0% OA, 0 phytase) exhibited baseline oxidative stress markers: ROS at 114.6 ± 2.4 fluorescence units (p = 0.007), SOD activity 236 ± 4.2 U/mL (p = 0.007), CAT activity 30.3 ± 1.2 U/mL (p = 0.007), MDA levels 5.77 ± 0.14 nmol/mg (p = 0.007), and AKP activity 7.51 ± 0.17 U/L (p = 0.007). Phytase supplementation (0% OA + phytase) significantly reduced oxidative stress parameters: ROS decreased to 69.2 ± 1.7 (p = 0.006), CAT activity decreased to 2.4 ± 0.4 U/mL (p = 0.006), while MDA levels exhibited a moderate increase to 6.96 ± 0.13 nmol/mg (p = 0.006). AKP activity declined to 4.17 ± 0.11 U/L (p = 0.006). The

2% OA + phytase combination produced the most pronounced effects: ROS levels fell to 66.5 ± 1.8 (p = 0.005), SOD activity reduced to 144 ± 2.6 U/mL (p = 0.005), CAT activity measured 5.3 ± 0.3 U/mL (p = 0.005), MDA levels dropped to 5.25 ± 0.14 nmol/mg (p = 0.005), and AKP activity reached its lowest point at 3.35 ± 0.07 U/L (p = 0.005). The 2% OA group demonstrated ROS reduction to 83.2 ± 2.6 (p = 0.007) and CAT activity decline to 13.8 ± 0.7 U/mL (p = 0.007), while the 4% OA group showed further ROS reduction to 69.7 ± 2.4 (p = 0.005) but elevated MDA levels (8.24 ± 0.16 nmol/mg, p = 0.005) (Table 5).

Table 5: Mean± SE of oxidative stress biomarkers and alkaline phosphatase activity throughout the 60-day experiment of *C. gariepinus* juveniles fed diets containing phytase and organic acid.

Treatment	ROS (Fluor.)	SOD (U/mL)	CAT (U/mL)	MDA (nmol/mg)	AKP (U/L)	P-value
0 OA, 0 Phytase	114.6 ± 2.4	236 ± 4.2	30.3 ± 1.2	5.77 ± 0.14	7.51 ± 0.17	0.007
2 OA, 0 Phytase	83.2 ± 2.6	221 ± 3.7	13.8 ± 0.7	6.41 ± 0.14	6.28 ± 0.13	0.007

4 OA, 0 Phytase	69.7± 2.4	174 ± 3.6	12.7 ± 0.6	8.24 ± 0.16	4.94 ± 0.16	0.005
0 OA, + Phytase	69.2± 1.7	188 ± 3.5	2.4 ± 0.4	6.96 ± 0.13	4.17 ± 0.11	0.006
2 OA, + Phytase	66.5± 1.8	144 ± 2.6	5.3± 0.3	5.25 ± 0.14	3.35 ± 0.07	0.005
4 OA, + Phytase	102.7± 2.3	204 ± 4.2	18.3 ± 0.7	4.92± 0.13	5.34 ± 0.3	0.005

4. DISCUSSION

The current study demonstrates the enhanced growth performance, nutrient digestibility, vertebral mineralization, and antioxidant responses of *C. gariepinus* with the dietary inclusion of phytase and organic acids. The consequences of this study enlightening feed utilization effectiveness, reducing environmental phosphorus losses. Phytase supplementation markedly improved growth parameters related to the control diet. The improved feed conversion ratio was recorded in phytase-fed groups, with the greater nutrient assimilation and digestive enzyme activity. Similar findings were described by Adeshina *et al.* (2023), who studied exogenic phytase with the significantly improved feed efficiency, protein maintenance, and growth in *Oreochromis niloticus*. Pragma *et al.* (2023) described that phytase supplementation, improved nutrient bioavailability and discharging bound phosphorus. Grujović *et al.* (2025) studied the organic acid with phytase, with optimal growth, enhancing digestive enzyme secretion, soothing intestinal pH, survival, inhibiting pathogenic bacterial growth, and mutually improving nutrient absorption. Moderate enhancements in growth were also noted in fish getting organic acids alone. This agrees with Libanori *et al.* (2021), who found that dietary organic acids such as formic and citric acids enhanced feed utilization and growth in tilapia by enhancing gut microbiota stability. Nassar *et al.* (2025) described that higher organic acid levels decrease feed utilization and growth in tilapia due to extreme acidification interferes with intestinal enzyme activity and nutrient transporters. The improvement in apparent digestibility coefficients for dry matter, protein, and phosphorus in phytase-supplemented groups aligns with earlier studies in other fish species (Zhang *et al.*, 2024; Tabassum *et al.*, 2025). Rodrigues *et al.* (2022) studied that Phytase not only increases phosphorus bioavailability but also advances protein and mineral acclimatization by discharging amino acids complexed with phytate. The highest phosphorus digestibility and decreased fecal phosphorus recorded in 2% OA + phytase diet group. Similar effects were documented by Bello *et al.* (2022), who quantified that phytase adding in plant-based diets enhanced phosphorus maintenance and compact

phosphorus. Silva *et al.* (2023) described enhancing the catalytic activity of phytase and increasing mineral solubility in the gastrointestinal tract by reducing pH leakage by potentiation of phytase efficiency and ionization of phosphorus. The raised vertebral mineral content, and whole-body protein were significantly found with phytase supplementation. A similar result was noted by Moradi *et al.* (2023), who designated that dietary phytase improvements the bone mineralization by discharging bound phosphorus and enhancing calcium. Singh *et al.* (2024) studied intestinal absorption efficiency and increased mineral solubility with action of organic acid and mineral deposition. Bianucci *et al.* (2025) found that extreme organic acid inclusion slightly condensed phosphorus preservation due to mineral availability at advanced absorptions. 2% OA + phytase combination influenced oxidative stress biomarkers by reducing ROS and MDA levels, signifying a reduction in lipid peroxidation and oxidative impairment. These discoveries are constant with the antioxidant-modulating properties of organic acids and enzymes documented in *Oreochromis niloticus* by de Sire *et al.* (2021), where dietary probiotics and phytase amended immune enzyme activities. Organic acids are designated to improve antioxidant capacity with the mitochondrial energy absorption and reducing free radical generation (Flieger *et al.*, 2021). Remarkably, fish fed 4% organic acids showed advanced MDA levels despite compact ROS, inferring potential oxidative disparity caused by extreme acid inclusion. Doherty *et al.* (2010) and Gao *et al.* (2024) described that concentrated ROS and improved MDA levels in defining the oxidative response of fish.

CONCLUSION

The 2% organic acid plus phytase mixture produced the significant performance, enhancing feed efficiency and reducing phosphorus waste. These consequences reveal a synergistic effect between phytase and organic acids, and concluded that organic acid plus phytase combination is an effective and sustainable dietary approach for enlightening productivity and environmental management in intensive *C. gariepinus* culture systems.

Acknowledgment:

We want to sincerely thank Rana Mehroz Fazal for his valuable comments on this manuscript.

Ethical statement:

The study strictly adhered to national and institutional ethical standards for aquatic research. The university Committee approved all protocols (232-678). All participating researchers provided verbal agreement before experimentation.

Author Contributions:

A.B., N.A. contributed to the concept and design of the work. A.S. were responsible for the statistical analysis and understanding of data. A.H., B.Y. and K.S. drafted the manuscript. All authors reviewed and approved the final version of the manuscript and agreed to be responsible for all aspects of the work.

Funding:

Not applicable

Availability of data and materials:

The corresponding author will provide all data upon reasonable request.

Consent for publication:

Not applicable.

Competing interests:

The authors declare no competing interests.

REFERENCES

- Adeshina, I., Akpoilih, B. U., Udom, B. F., Adeniyi, O. V., & Abdel-Tawwab, M. (2023). Interactive effects of dietary phosphorus and microbial phytase on growth performance, intestinal morphometry, and welfare of Nile tilapia (*Oreochromis niloticus*) fed on low-fishmeal diets. *Aquaculture*, 563, 738995. DOI: [10.1016/j.aquaculture.2022.738995](https://doi.org/10.1016/j.aquaculture.2022.738995).
- Afzal, S., Abdul Manap, A. S., Attiq, A., Albokhadaim, I., Kandeel, M., & Alhojaily, S. M. (2023). From imbalance to impairment: the central role of reactive oxygen species in oxidative stress-induced disorders and therapeutic exploration. *Frontiers in pharmacology*, 14, 1269581. DOI: [10.3389/fphar.2023.1269581](https://doi.org/10.3389/fphar.2023.1269581)
- Ahmed, B. S. (2023). Nutritional effects of dietary spirulina (*Arthrospora platensis*) on morphological performance, hematological profile, biochemical parameters of common carp (*Cyprinus carpio L.*). *Egyptian Journal of Veterinary Sciences*, 54(3), 515-524. DOI: [10.3389/ejvs.2023.1276981](https://doi.org/10.3389/ejvs.2023.1276981)
- Aluta, U. P., Aderolu, A. Z., Lawal, M. O., & Olutola, A. A. (2021). Inclusion effect of onion peel powder in the diet of African catfish, *Clarias gariepinus*: Growth, blood chemistry, hepatic antioxidant enzymes activities and SOD mRNA responses. *Scientific African*, 12, e00780. DOI: [10.1016/j.sciaf.2021.e00780](https://doi.org/10.1016/j.sciaf.2021.e00780)
- Antache, A., Simionov, I. A., Petrea, Ş. M., Nica, A., Georgescu, P. L., Oprică, L., & Porocho, V. (2025). Insect–Antioxidants Symbiotic Nexus—Pathway for Sustainable and Resilient Aquaculture: A Case Study for Evaluating Koi Carp Growth and Oxidative Stress Status. *Antioxidants*, 14(4), 371. DOI: [10.3390/antiox14040371](https://doi.org/10.3390/antiox14040371)
- Bello, A., Dersjant-Li, Y., van Eerden, E., Kwakernaak, C., & Marchal, L. (2022). Supplementation of an all-plant-based inorganic phosphate-free diet with a novel phytase maintained tibia ash and performance in broilers under a commercial production setting. *Journal of Applied Poultry Research*, 31(2), 100253. DOI: [10.1016/j.japr.2022.100253](https://doi.org/10.1016/j.japr.2022.100253)
- Besson, M., Komen, H., Aubin, J., De Boer, I. J. M., Poelman, M., Quillet, E., & Van Arendonk, J. A. M. (2014). Economic values of growth and feed efficiency for fish farming in recirculating aquaculture system with density and nitrogen output limitations: a case study with African catfish (*Clarias gariepinus*). *Journal of animal science*, 92(12), 5394-5405. DOI: [10.2527/jas.2014-8266](https://doi.org/10.2527/jas.2014-8266)
- Bianucci, E., Furlan, A. L., Llugany, M., Poschenrieder, C., & Tolrà, R. (2025). Insights into the physiological and biochemical responses of peanut plants under combined arsenic and flooding stress. *Plant Physiology and Biochemistry*, 110266. DOI: [10.1016/j.plaphy.2025.110266](https://doi.org/10.1016/j.plaphy.2025.110266)
- Chen, X., Ma, T., Xie, F., & Tang, Z. (2025). Phosphate source apportionment across the agriculture-urban gradient in Asia's longest river: Combining machine learning and multi-isotope techniques. *Agricultural Water Management*, 320, 109874. DOI: [10.1016/j.agwat.2025.109874](https://doi.org/10.1016/j.agwat.2025.109874)
- de Sire, A., Marotta, N., Marinaro, C., Curci, C., Invernizzi, M., & Ammendolia, A. (2021). Role of physical exercise and nutraceuticals in modulating molecular pathways of osteoarthritis. *International journal of molecular sciences*, 22(11), 5722. DOI: [10.3390/ijms22115722](https://doi.org/10.3390/ijms22115722)
- Doherty, V. F., Ogunkuade, O. O., & Kanife, U. C. (2010). Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in some selected fishes in Lagos, Nigeria. *Am Eurasian J Agric*

- Environ Sci, 7(3), 359-365. DOI: [10.1012/j.agwat.2010.105874](https://doi.org/10.1012/j.agwat.2010.105874)
- Elaigwu, A. M., Auta, J., & Onimisi, H. U. (2024). Effect of Phytase-Supplemented Diets on Growth Indices, Nutrient Digestibility and Utilisation, Carcass Composition, and Sustainability of African Catfish (*Clarias gariepinus*). Aquatic Science and Fish Resources (ASFR), 5(1), 111-121. DOI: [10.21608/asfr.2024.312521.1066](https://doi.org/10.21608/asfr.2024.312521.1066)
- El-Dakar, A. Y., Shalaby, S. M., Mohamed, B. K., & Abdel-Aziz, M. F. A. (2022). Improving the growth, feed efficiency and hematological indicators of Nile tilapia fingerlings *Oreochromis niloticus* using dietary lactic acid supplementation with different feeding routines. Mediterranean Aquaculture Journal, 9(1), 25-37. DOI: [10.21608/maj.2022.156031.1012](https://doi.org/10.21608/maj.2022.156031.1012)
- Fazal, R. M., Fatima, A., Al Sulivany, B. S., Hussain, R., Hassan, A., Shahid, A., & Owais, M. (2025). Enhancing growth performance, antioxidant defense, and immune response in striped catfish (*Pangasius hypophthalmus*) through dietary supplementation with aloe vera: a sustainable aquaculture approach. Science Journal of University of Zakho, 13(4), 510-518. DOI: [10.25271/sjuoz.2025.13.4.1691](https://doi.org/10.25271/sjuoz.2025.13.4.1691)
- Flieger, J., Flieger, W., Baj, J., & Maciejewski, R. (2021). Antioxidants: Classification, natural sources, activity/capacity measurements, and usefulness for the synthesis of nanoparticles. Materials, 14(15), 4135. DOI: [10.3390/ma14154135](https://doi.org/10.3390/ma14154135)
- Froehlich, H. E., Montgomery, J. C., Williams, D. R., O'Hara, C., Kuempel, C. D., & Halpern, B. S. (2023). Biological life-history and farming scenarios of marine aquaculture to help reduce wild marine fishing pressure. Fish and Fisheries, 24(6), 1034-1047. DOI: [10.1111/faf.12767](https://doi.org/10.1111/faf.12767)
- Gao, F., Zhao, Y., Shi, X., Qiao, D., Pei, C., & Kong, X. (2024). Signalling regulation of reactive oxygen species in fish inflammation. Reviews in Aquaculture, 16(3), 1266-1285. DOI: [10.1111/raq.12815](https://doi.org/10.1111/raq.12815)
- Grujović, M. Ž., Semedo-Lemsaddek, T., & Marković, K. G. (2025). Application of Probiotics in Foods: A Comprehensive Review of Benefits, Challenges, and Future Perspectives. Foods, 14(17), 3088. DOI: [10.3390/foods14173088](https://doi.org/10.3390/foods14173088)
- Hussein, E. E., Habiba, M. M., Ashry, A. M., Al-Zayat, A. M., Teiba, I. I., Shehata, A. I., & El Basuni, M. F. (2023). Effects of dietary supplementation with organic acids mixture on growth, feed efficiency, hematobiochemical parameters, immunity, and intestinal microbiota of Gilthead seabream (*Sparus aurata*) juveniles. Aquaculture Reports, 33, 101846. DOI: [10.1016/j.aqrep.2023.101846](https://doi.org/10.1016/j.aqrep.2023.101846)
- Iqbal, M., Afzal, M., Yaqub, A., Anjum, K. M., & Tayyab, K. (2021). Combined effects of citric acid and phytase supplementation on growth performance, nutrient digestibility and body composition of labeo rohita fingerlings. Aquaculture Studies, 22(1). DOI: [10.4194/AQUAST656](https://doi.org/10.4194/AQUAST656)
- Langi, S., Maulu, S., Hasimuna, O. J., Kaleinasho Kapula, V., & Tjipute, M. (2024). Nutritional requirements and effect of culture conditions on the performance of the African catfish (*Clarias gariepinus*): a review. Cogent Food & Agriculture, 10(1), 2302642. DOI: [10.1080/23311932.2024.2302642](https://doi.org/10.1080/23311932.2024.2302642)
- Liang, Q., Yuan, M., Xu, L., Lio, E., Zhang, F., Mou, H., & Secundo, F. (2022). Application of enzymes as a feed additive in aquaculture. Marine Life Science & Technology, 4(2), 208-221. DOI: [10.1007/s42995-022-00133-8](https://doi.org/10.1007/s42995-022-00133-8)
- Libanori, M. C. M., Santos, G. G., Pereira, S. A., Lopes, G. R., Owatari, M. S., Soligo, T. A., & Mourinho, J. L. P. (2021). Dietary supplementation with benzoic organic acid improves the growth performance and survival of Nile tilapia (*Oreochromis niloticus*) after challenge with *Streptococcus agalactiae* (Group B). Aquaculture, 545, 737204. DOI: [10.1016/j.aquaculture.2021.737204](https://doi.org/10.1016/j.aquaculture.2021.737204)
- McCleary, B. V., Sloane, N., Draga, A., & Lazewska, I. (2013). Measurement of total dietary fiber using AOAC Method 2009.01 (AACC International Approved Method 32-45.01): evaluation and updates. Cereal Chemistry, 90(4), 396-414. DOI: [10.1094/CCHEM-01-13-0002-R](https://doi.org/10.1094/CCHEM-01-13-0002-R)
- Moradi, S., Abdollahi, M. R., Moradi, A., & Jamshidi, L. (2023). Effect of bacterial phytase on growth performance, nutrient utilization, and bone mineralization in broilers fed pelleted diets. Animals, 13(9), 1450. DOI: [10.3390/ani13091450](https://doi.org/10.3390/ani13091450)
- Naiel, M. A., Negm, S. S., Ghazanfar, S., Shukry, M., & Abdelnour, S. A. (2023). The risk assessment of high-fat diet in farmed fish and its mitigation approaches: A review. Journal of animal physiology and animal nutrition, 107(3), 948-969. DOI: [10.1111/jpn.13777](https://doi.org/10.1111/jpn.13777)
- Nassar, A. A., Gharib, A. A. E. A., Abdelgalil, S. Y., AbdAllah, H. M., & Elmowalid, G. A. (2024). Immunomodulatory, antioxidant, and growth-promoting activities of dietary fermented *Moringa oleifera* in Nile tilapia (*Oreochromis niloticus*) with in-vivo protection against *Aeromonas hydrophila*. BMC Veterinary Research, 20(1), 231. DOI: [10.1186/s12917-024-04045-7](https://doi.org/10.1186/s12917-024-04045-7)
- Nathanailides, C., Kolygas, M., Tsoumani, M., Gouva, E., Mavraganis, T., & Karayanni, H. (2023). Addressing phosphorus waste in open flow freshwater fish farms: Challenges and solutions. Fishes, 8(9), 442. DOI: [10.3390/fishes8090442](https://doi.org/10.3390/fishes8090442)

- Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*, 95(2), 351-358. DOI: [10.1016/0003-2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3)
- Ondiba, R. N., Ogello, E. O., Kembanya, E., Gichana, Z., & Obiero, K. (2022). Future demand and supply of aquafeed ingredients: Outlines to commercialize non-conventional protein ingredients to enhance aquaculture production for food security in sub-Saharan Africa. *Aquatic Ecosystem Health & Management*, 25(4), 75-84. DOI: [10.14321/achm.025.04.75](https://doi.org/10.14321/achm.025.04.75)
- Owais, m., riaz-ud-din, q. U. R. E. S. H. I., irfan, m., jamil, s., yasin, r., ramzan, s., & fazal, r. M. (2023). Effect of Different Salinity Levels on Growth Performance, Hematological Parameters and Proximate Composition of *Cyprinus Carpio*. *University of Sindh Journal of Animal Sciences (USJAS)*, 7(04), 52-60. DOI: [10.1065/10488398.2023.2046236](https://doi.org/10.1065/10488398.2023.2046236)
- Pragya, Sharma, K. K., Kumar, A., Singh, D., Kumar, V., & Singh, B. (2023). Immobilized phytases: an overview of different strategies, support material, and their applications in improving food and feed nutrition. *Critical Reviews in Food Science and Nutrition*, 63(22), 5465-5487. DOI: [10.1080/10408398.2022.2047226](https://doi.org/10.1080/10408398.2022.2047226)
- Pujol, A., Sanchis, P., Grases, F., & Masmiquel, L. (2023). Phytate intake, health and disease: "let thy food be thy medicine and medicine be thy food". *Antioxidants*, 12(1), 146. DOI: [10.3390/antiox12010146](https://doi.org/10.3390/antiox12010146)
- Reda, R. M., El-Murr, A., Abd Elhakim, Y., & El-Shahat, W. (2022). *Aeromonas veronii* detection in Egyptian fish farms with summer tilapia mortality outbreaks and the role of formic acid in limiting its spread. *Aquaculture Research*, 53(3), 940-956. DOI: [10.1111/are.15676](https://doi.org/10.1111/are.15676)
- Rodrigues, E. J. D., Ito, P. I., Ribeiro, L. F. M., de Carvalho, P. L. P. F., Xavier, W. D. S., Guimarães, M. G., & Barros, M. M. (2022). Phytase supplementation under commercially intensive rearing conditions: impacts on Nile Tilapia growth performance and nutrient digestibility. *Animals*, 13(1), 136. DOI: [10.3390/ani13010136](https://doi.org/10.3390/ani13010136)
- Sales, J., & Britz, P. J. (2001). Evaluation of different markers to determine apparent nutrient digestibility coefficients of feed ingredients for South African abalone (*Haliotis midae* L.). *Aquaculture*, 202(1-2), 113-129. DOI: [10.1016/S0044-8486\(01\)00531-2](https://doi.org/10.1016/S0044-8486(01)00531-2)
- Selim, S., Abdel-Megeid, N. S., Khalifa, H. K., Fakiha, K. G., Majrashi, K. A., & Hussein, E. (2022). Efficacy of various feed additives on performance, nutrient digestibility, bone quality, blood constituents, and phosphorus absorption and utilization of broiler chickens fed low phosphorus diet. *Animals*, 12(14), 1742. DOI: [10.3390/ani12141742](https://doi.org/10.3390/ani12141742)
- Silva, L. I. D., Pereira, M. C., Carvalho, A. M. X. D., Buttrós, V. H., Pasqual, M., & Dória, J. (2023). Phosphorus-solubilizing microorganisms: a key to sustainable agriculture. *Agriculture*, 13(2), 462. DOI: [10.3390/agriculture13020462](https://doi.org/10.3390/agriculture13020462)
- Singh, S., Mondal, D., Thakur, M., Habib, M., Jan, K., Dhar, P., & Bashir, K. (2024). Emerging trends in nutraceutical research: Role of minerals. *Functional Foods and Nutraceuticals: Chemistry, Health Benefits and the Way Forward*, 81-112. DOI: [10.1016/j.nchbwf.2024.102627](https://doi.org/10.1016/j.nchbwf.2024.102627)
- Singh, S., Mondal, D., Thakur, M., Habib, M., Jan, K., Dhar, P., & Bashir, K. (2024). Emerging trends in nutraceutical research: Role of minerals. *Functional Foods and Nutraceuticals: Chemistry, Health Benefits and the Way Forward*, 81-112. DOI: [10.1016/j.nchbwf.2024.102627](https://doi.org/10.1016/j.nchbwf.2024.102627)
- promotion, nutrient utilization, and environmental mitigation. *Aquaculture International*, 33(6), 1-31. DOI: [10.1007/s10499-025-01427-3](https://doi.org/10.1007/s10499-025-01427-3)
- Zhang, Q., Walk, C. L., Cowieson, A. J., Stamatopoulos, K., Wu, J. L., & Sorbara, J. O. B. (2024). Efficacy of a novel phytase in response to low and high phytate diets using a short-term digestibility model in broiler chickens at two ages. *Animal Feed Science and Technology*, 307, 115832. DOI: [10.1016/j.anifeedsci.2024.115832](https://doi.org/10.1016/j.anifeedsci.2024.115832)
- Tabassum, S., Hussain, S. M., Ali, S., Sarker, P. K., & Al-Ghanim, K. A. (2025). *Moringa oleifera* seed meal as a sustainable fishmeal substitute: Growth and health implications for *Cirrhinus mrigala*. *Aquaculture Reports*, 40, 102634. DOI: [10.1016/j.aqrep.2025.102634](https://doi.org/10.1016/j.aqrep.2025.102634)
- Yadav, N. K., Patel, A. B., Kashyap, S., Deepti, M., Savaliya, B. D., Singh, Y. R., & Sahu, A. (2025). Phytase as a functional feed additive in aquaculture: growth