

Original article

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

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### ABSTRACT

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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 ( $7.4 \pm 0.3$  g), weight gain ( $460.6 \pm 31\%$ ), specific growth rate ( $3.3 \pm 0.3\%/\text{day}$ ), and the lowest feed conversion ratio ( $1.5 \pm 0.2$ ) 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 ( $0.8 \pm 0.04$  g/kg). Vertebral phosphorus and calcium contents were enhanced significantly ( $p < 0.05$ ) in the combined supplement group. Oxidative stress markers revealed lower ROS ( $66.5 \pm 1.8$ ), SOD ( $144 \pm 2.6$  U/mL), and MDA ( $5.25 \pm 0.14$  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*.

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**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).

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

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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).

temperature, dissolved oxygen at  $7.09 \pm 0.05$  mg, and pH at  $8.07 \pm 0.01$  L<sup>-1</sup> 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).

## 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:  $13.0 \pm 0.09$  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  $26.3 \pm 0.3$  °C

**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
<b>Fish meal</b>	180	180	180	180	180	180
<b>Soybean meal</b>	320	320	320	320	320	320
<b>Maize gluten</b>	120	120	120	120	120	120
<b>Vegetable oil</b>	60	60	60	60	60	60
<b>Premix</b>	30	30	30	30	30	30
<b>Dicalcium phos.</b>	25	25	25	25	25	25
<b>Chromic oxide</b>	5	5	5	5	5	5
<b>Organic acid</b>	0	20	40	0	20	40
<b>Phytase</b>	0	0	0	0.1	0.1	0.1
<b>Wheat bran</b>	260	240	220	259.9	239.9	219.9
<b>TOTAL</b>	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:

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

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

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

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

### 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).

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

### 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 molybdoavanadate 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  $4.7 \pm 0.2$  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  $2.4 \pm 0.2$  ( $p = 0.005$ ), 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  $6.8 \pm 0.4$  g ( $p = 0.007$ ), WG to  $454.8 \pm 29.4$ % ( $p = 0.007$ ), and SGR to  $3.3 \pm 0.2$ /day ( $p = 0.007$ ). FCR improved to  $1.7 \pm 0.3$  ( $p = 0.007$ ) while survival rose to  $90.1 \pm 2.1$ % ( $p = 0.007$ ). The 2% OA + phytase combination yielded optimal results with FBW of  $7.4 \pm 0.3$  g ( $p = 0.004$ ), WG of  $460.6 \pm 31$ % ( $p = 0.004$ ), SGR of  $3.3 \pm 0.3$ /day ( $p = 0.004$ ), and best FCR ( $1.5 \pm 0.2$ ,  $p = 0.004$ ) and survival ( $91.2 \pm 1.7$ %,  $p = 0.004$ ). The 2% OA treatment (no phytase) resulted in FBW of  $5.4 \pm 0.5$  g ( $p = 0.006$ ), WG of  $296.7 \pm 27.3$ % ( $p = 0.006$ ), and SGR of  $2.3 \pm 0.2$ /day ( $p = 0.006$ ). The 4% OA group (no phytase) showed similar FBW ( $5.4 \pm 0.3$  g,  $p = 0.006$ ) but higher WG ( $330.3 \pm 35.2$ %,  $p = 0.006$ ) and SGR ( $2.5 \pm 0.3$ /day,  $p = 0.006$ ) (Table 2).

**Table 2:** Mean  $\pm$  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	$4.7 \pm 0.2$	$252.2 \pm 54$	$2.3 \pm 0.4$	$2.4 \pm 0.2$	$83.2 \pm 2.5$	0.005
2 OA, 0 Phytase	$5.4 \pm 0.5$	$296.7 \pm 27.3$	$2.3 \pm 0.2$	$2.4 \pm 0.5$	$86.6 \pm 3.4$	0.006
4 OA, 0 Phytase	$5.4 \pm 0.3$	$330.3 \pm 35.2$	$2.5 \pm 0.3$	$2.2 \pm 0.4$	$88.8 \pm 1.4$	0.006
0 OA, + Phytase	$6.8 \pm 0.4$	$454.8 \pm 29.4$	$3.3 \pm 0.2$	$1.7 \pm 0.3$	$90.1 \pm 2.1$	0.007
2 OA, + Phytase	$7.4 \pm 0.3$	$460.6 \pm 31$	$3.3 \pm 0.3$	$1.5 \pm 0.2$	$91.2 \pm 1.7$	0.004
4 OA, + Phytase	$6.4 \pm 0.4$	$410.1 \pm 26.4$	$2.8 \pm 0.2$	$1.8 \pm 0.5$	$88.9 \pm 2.4$	0.005

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

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

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

**Table 3:** Mean $\pm$  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 $\pm$ 1.2	67.5 $\pm$ 1.4	24.3 $\pm$ 0.8	1.2 $\pm$ 0.04	0.005
2 OA, 0 Phytase	35.4 $\pm$ 0.8	67.2 $\pm$ 1.1	29.4 $\pm$ 1.2	1.0 $\pm$ 0.03	0.007
4 OA, 0 Phytase	40.5 $\pm$ 1.4	68.7 $\pm$ 1.2	25.3 $\pm$ 1.1	1.0 $\pm$ 0.05	0.006
0 OA, + Phytase	45.6 $\pm$ 1.1	76.8 $\pm$ 1.6	42.0 $\pm$ 1.3	0.7 $\pm$ 0.01	0.006
2 OA, + Phytase	46.7 $\pm$ 1.3	77.2 $\pm$ 1.4	48.0 $\pm$ 1.4	0.8 $\pm$ 0.04	0.007
4 OA, + Phytase	57.0 $\pm$ 1.4	76.3 $\pm$ 1.2	48.2 $\pm$ 1.4	0.7 $\pm$ 0.05	0.005

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

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

**Table 4:** Mean $\pm$  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 $\pm$ 0.4	3.2 $\pm$ 0.4	6.05 $\pm$ 0.14	13.17 $\pm$ 0.32	0.006
2 OA, 0 Phytase	15.2 $\pm$ 0.2	3.4 $\pm$ 0.2	6.69 $\pm$ 0.12	13.60 $\pm$ 0.26	0.005
4 OA, 0 Phytase	15.4 $\pm$ 0.6	3.4 $\pm$ 0.2	6.60 $\pm$ 0.11	14.18 $\pm$ 0.24	0.004
0 OA, + Phytase	15.8 $\pm$ 0.3	3.6 $\pm$ 0.3	7.42 $\pm$ 0.12	15.41 $\pm$ 0.33	0.003
2 OA, + Phytase	15.7 $\pm$ 0.1	3.8 $\pm$ 0.3	6.97 $\pm$ 0.13	14.64 $\pm$ 0.26	0.004
4 OA, + Phytase	15.4 $\pm$ 0.4	3.5 $\pm$ 0.2	6.91 $\pm$ 0.12	15.00 $\pm$ 0.25	0.004

The oxidative status presented significant treatment effects. Control fish (0% OA, 0 phytase) exhibited baseline oxidative stress markers: ROS at  $114.6 \pm 2.4$  fluorescence units ( $p = 0.007$ ), SOD activity  $236 \pm 4.2$  U/mL ( $p = 0.007$ ), CAT activity  $30.3 \pm 1.2$  U/mL ( $p = 0.007$ ), MDA levels  $5.77 \pm 0.14$  nmol/mg ( $p = 0.007$ ), and AKP activity  $7.51 \pm 0.17$  U/L ( $p = 0.007$ ). Phytase supplementation (0% OA + phytase) significantly reduced oxidative stress parameters: ROS decreased to  $69.2 \pm 1.7$  ( $p = 0.006$ ), CAT activity decreased to  $2.4 \pm 0.4$  U/mL ( $p = 0.006$ ), while MDA levels exhibited a moderate increase to  $6.96 \pm 0.13$  nmol/mg ( $p = 0.006$ ). AKP activity declined to  $4.17 \pm 0.11$  U/L ( $p = 0.006$ ). The 2% OA

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

**Table 5:** Mean $\pm$  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 $\pm$ 2.4	236 $\pm$ 4.2	30.3 $\pm$ 1.2	5.77 $\pm$ 0.14	7.51 $\pm$ 0.17	0.007
2 OA, 0 Phytase	83.2 $\pm$ 2.6	221 $\pm$ 3.7	13.8 $\pm$ 0.7	6.41 $\pm$ 0.14	6.28 $\pm$ 0.13	0.007
4 OA, 0 Phytase	69.7 $\pm$ 2.4	174 $\pm$ 3.6	12.7 $\pm$ 0.6	8.24 $\pm$ 0.16	4.94 $\pm$ 0.16	0.005
0 OA, + Phytase	69.2 $\pm$ 1.7	188 $\pm$ 3.5	2.4 $\pm$ 0.4	6.96 $\pm$ 0.13	4.17 $\pm$ 0.11	0.006

2 OA, + Phytase	66.5 $\pm$ 1.8	144 $\pm$ 2.6	5.3 $\pm$ 0.3	5.25 $\pm$ 0.14	3.35 $\pm$ 0.07	0.005
4 OA, + Phytase	102.7 $\pm$ 2.3	204 $\pm$ 4.2	18.3 $\pm$ 0.7	4.92 $\pm$ 0.13	5.34 $\pm$ 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 exogenous phytase with the significantly improved feed efficiency, protein maintenance, and growth in *Oreochromis niloticus*. Pragya *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.

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#### 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.

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The authors declare no competing interests.

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