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Role of Phytase Enzyme to Immunity Responses, Hematology and Histopathology in *Cyprinus carpio* L. Against *Saprolegnia* spp. Challenge

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ABSTRACT
This study aimed to investigate the effects of feeding common carp (*Cyprinus carpio* L.) challenged with *Saprolegnia* spp. on their hematological and immunological systems, using phytase enzyme supplementation, and to evaluate histological alterations. A total of 120 *C. carpio* (20.05–20.35 g initial body weight) were divided into six groups. The control group received a basal diet without phytase supplementation, while group T4 was fed a basal diet supplemented with β-glucan (8.5 g/kg diet). Groups T1, T2, and T3 received the basal diet plus phytase enzyme at 1000, 2000, and 4000 IU/kg diet, respectively. In addition, two control groups (positive and negative) were included for the infection test at the end of the 60-day trial. For the challenge, fish in the positive and negative control groups were exposed to a viable *Saprolegnia* spp. suspension (2×10^4 live spores/ml). After 60 days of feeding, the inclusion of phytase enzyme significantly ($P \leq 0.05$) affected hemoglobin concentration (g/dl), WBC count, and other hematological parameters, particularly in group T3. Compared with the control group, T2 also showed a significant ($P \leq 0.05$) increase in these parameters. Furthermore, T3 exhibited significantly higher albumin and total protein levels compared with the control. The WBC count was significantly ($P < 0.05$) higher in T2 and T3 compared with T1, T4, and the control. In comparison with the positive and negative control groups, all phytase-supplemented diets showed a significant increase in respiratory burst activity (NBT reduction). Histopathological examination revealed that gills of fish infected with *Saprolegnia* spp. (positive control) displayed severe tissue alterations, including epithelial lifting, necrosis, mononuclear cell infiltration, and hyperplasia. In contrast, these changes were less pronounced in the gills and skin of fish fed phytase-supplemented diets. The skin of fish in the positive control group showed ulcerative epidermis, edema, mononuclear cell infiltration, and an increase in alarm cells. Liver tissue in both positive and negative control groups appeared normal, whereas fish fed phytase or β-glucan diets showed hepatocyte lipid vacuolation, fatty degeneration, and nuclear pyknosis. Overall, supplementation with phytase (T1, T2, and T3) demonstrated beneficial and protective effects against *Saprolegnia* infection in *C. carpio*, improving hematological and immunological responses while reducing the severity of histopathological damage.

INTRODUCTION

Saprolegniasis, commonly known as water mold, is a widely distributed freshwater disease and represents one of the most significant infections affecting fish as well as wild and cultured shellfish **(Ghiasi *et al.*, 2010)**. *Saprolegnia* naturally occurs in freshwater habitats **(Alejandro *et al.*, 2015; Ashour, 2017)**. In aquaculture, infection can affect the host or eggs, with severe cases covering 20–80% of the skin surface area, leading to lethargy due to damage of the epidermis and underlying tissues **(Hussein *et al.*, 2013; Alloul *et al.*, 2025)**. Mortalities in infected fish typically range from 10 to 30% **(Beakes *et al.*, 1994)**.

Because of strict regulations limiting the use of chemotherapeutics and antibiotics in aquafeeds due to risks of bioaccumulation **(Lim *et al.*, 2013; Al-Shammari & Al-Niaeem, 2025)**, feed additives are gaining increasing importance in aquaculture. These additives serve multiple purposes: some, such as antioxidants, feed preservatives, and pellet binders with antimold or antibacterial properties, are designed to improve feed quality **(Soosean *et al.*, 2010; Salam *et al.*, 2020)**. While others are used to enhance fish productivity, stimulate immune responses, and reduce mortality by improving leukocyte profiles **(Lin & Shiau, 2005; Carnevali *et al.*, 2006)**.

Among functional additives, phytase has attracted particular attention. Its use has been evaluated for improving growth and nutrient utilization in common carp and other aquaculture species **(Schafer *et al.*, 1995; Najem *et al.*, 2020a)**, including *Oreochromis niloticus* **(Liebert & Portze, 2005)**. However, the effectiveness of phytase supplementation has varied depending on the enzyme source and conditions **(Adeola & Cowieson, 2011; Najem *et al.*, 2020b)**, and some published studies have reported conflicting outcomes **(Castillo & Gatlin, 2015)**.

To date, little research has assessed the effects of phytase supplementation on the immunological responses, disease resistance, histopathology, and overall health status of common carp. Therefore, the present study aimed to examine the effects of dietary phytase on the hematological and immunological responses, as well as histological alterations, in *Cyprinus carpio* challenged with *Saprolegnia* spp.

MATERIALS AND METHODS

From January to March 2019, the experiment was conducted at the Fish Diseases Laboratory, College of Veterinary Medicine, University of Baghdad. A total of 120 healthy *Cyprinus carpio* (average body weight: 20.05–20.35 g) were obtained from a commercial farm in Al-Musayyib, Babylon. Fish were acclimated for two weeks prior to the experiment and were examined for health status. Following acclimation, they were immersed in 37% formalin (15 ml/100 L) for 30 minutes, or until stress symptoms

appeared, to eliminate external parasites. During acclimation, fish were housed in two fiberglass tanks (20 × 40 × 150 cm).

Subsequently, 10 fish per tank were randomly selected and distributed into 12 aquaria (two replicates per treatment). Six treatments were tested:

- **Control**: basal diet only.
- **T1, T2, T3**: basal diet + phytase enzyme at 1000, 2000, and 4000 IU/kg feed, respectively.
- **T4**: basal diet + β-glucan at 8.5 g/kg feed.

Fish were fed twice daily at 3% of body weight for 60 days. Water in the aquaria was partially replenished daily and tanks were cleaned regularly.

Blood Sampling and Analyses

Blood samples were collected to evaluate:

- **Hematological parameters**: packed cell volume (PCV), hemoglobin (Hb), white blood cell (WBC) count, and red blood cell (RBC) count.
- **Biochemical profile**: total protein, albumin, and globulin.
- **Immunological test**: respiratory burst activity (NBT reduction assay).

Additional blood samples were collected post-challenge with *Saprolegnia* spp. to assess the same parameters. Selected organs (liver, gills, and skin) were examined histopathologically.

Diet preparation

The basal diet consisted of 5 ± 0.4 mm floating pellets purchased from Faradanah Company, Iran. The pellets were ground and weighed according to fish body weight for each treatment. Phytase enzyme was dissolved in warm water (45 °C) and mixed into the basal diet at concentrations of 1000, 2000, and 4000 IU/kg feed, while β-glucan was added at 8.5 g/kg. Formulations were based on **Nwanna and Schwarz (2007)**. The diets were pelletized into 1.5 mm pellets, air-dried at room temperature, and stored in moisture-proof screw-top containers. Diets were prepared weekly. The control group received the commercial basal diet without phytase supplementation.

Isolation of *Saprolegnia* spp.

Saprolegnia spp. was isolated at the Fish Diseases Laboratory, College of Veterinary Medicine, University of Baghdad. Water samples were collected from the Tigris River, Baghdad, and the baiting method was used for fungal isolation **(Rattan *et al.*, 1978)**. Approximately 15–20 ml of river water with chloramphenicol was placed in Petri dishes, and 5–7 *Sesamum indicum* seeds were added as bait. Cultures were incubated at 20 °C for seven days **(Al-Rekabi *et al.*, 1996)**, with hyphae monitored daily until pure cultures were obtained.

Challenge test

After the 60-day feeding trial, 10 fish from each treatment group (in duplicate) were challenged with a *Saprolegnia* spp. suspension (2×10^4 live zoospores/ml). Two control subgroups were established:

- **Positive control**: exposed to viable fungal suspension.
- **Negative control**: not challenged with *Saprolegnia* spp.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA). Results are presented as mean ± standard error (SE). Duncan's Multiple Range Test (MRT) was used to compare treatment means. Statistical significance was accepted at $P< 0.05$.

RESULTS AND DISCUSSION

Hematological parameters

The findings of hematological parameters before and after the *Saprolegnia* spp. challenge are presented in Table (1).

Red blood cells (RBCs)

After 14 days post-challenge, the RBC count of the infected group (positive control) showed a significant reduction ($P\leq 0.05$), reaching $2.46 \pm 0.05 \times 10^6/mm^3$, compared to the negative control ($3.41 \pm 0.03 \times 10^6/mm^3$). In contrast, RBC counts in all treatment groups (T1, T2, T3, and T4) increased significantly ($P\leq 0.05$) after the challenge, reaching 3.29 ± 0.11, 3.06 ± 0.11, 3.58 ± 0.12, and $3.38 \pm 0.11 \times 10^6/mm^3$, respectively, compared with the positive control ($2.46 \pm 0.05 \times 10^6/mm^3$). The negative control ($3.41 \pm 0.08 \times 10^6/mm^3$) also showed significantly higher values ($P \leq 0.05$) than the positive control.

White blood cells (WBCs)

Prior to the challenge, fish fed phytase-supplemented diets (T1, T2, T3, and T4) exhibited significantly higher WBC counts ($P \leq 0.05$) than the control group ($8.67 \times 10^3/mm^3$). Among treatments, T3 showed the highest pre-challenge WBC count ($12.73 \pm 1.09 \times 10^3/mm^3$), followed by T2 ($11.11 \pm 1.12 \times 10^3/mm^3$), T4 ($10.30 \pm 1.11 \times 10^3/mm^3$), and T1 ($10.08 \pm 1.08 \times 10^3/mm^3$).

After the *Saprolegnia* challenge, WBC counts increased significantly ($P\leq 0.05$) in all phytase-supplemented groups compared with their pre-challenge levels. The highest post-challenge WBC counts were observed in T3 ($14.13 \times 10^3/mm^3$) and T2 ($13.95 \times 10^3/mm^3$), followed by T4 and T1. In all cases, WBC counts in treatment groups remained significantly higher ($P \leq 0.05$) than in the control.

Hemoglobin (Hb)

Before the challenge, hemoglobin levels in T1, T2, T3, and T4 were significantly higher ($P\leq 0.05$) at 7.62, 7.36, 8.35, and 8.10 g/dl, respectively, compared with the control group (6.90 g/dl). After infection, Hb levels decreased significantly ($P \leq 0.05$) in all treatment groups, reaching 4.51, 4.82, 6.13, and 5.29 g/dl, respectively, though these values remained comparable to or higher than the positive control (4.62 g/dl).

Packed cell volume (PCV%)

Before infection, PCV% was significantly higher ($P\leq 0.05$) in the treatment groups (T1: 24.16%, T2: 26.66%, T3: 24.83%) than in the control (23%). Fourteen days

post-infection, PCV% decreased significantly ($P< 0.05$) across all groups, with values of 16.66, 19.83, and 18.16%, compared to the positive control (15%).

Table 1. Blood parameters in *C. carpio* using β-glucan and phytase supplement and control diet during 60 days (pre- challenge) and 74 days (post challenge)

Blood Parameters / Groups	RBC 10^6/mm^3		Hb g/100ml g/dl		PCV %		WBC 10^3/mm^3	
	Pre-challenge	**Post challenge**	**Pre-challenge**	**Post challenge**	**Pre-challenge**	**Post challenge**	**Pre-challenge**	**Post challenge**
Control (-v)	3.71±0.07 a A	3.41±0.08 a B	7.05±0.25 cd A	6.73±0.20 a A	22.83±0.2.54 c A	21.03±2.51 a A	8.67±0.37 C A	8.87±0.52 b A
Control (+v)	3.8±0.11 aA	2.46±0.05 c B	6.90±0.17 d A	4.62±0.18 c B	23.00±1.58 c A	15.00±0.87 d B	8.9±0.43 c B	12.7±0.74 b A
T1	3.80±0.11 a A	3.29±0.11 ab B	7.62±1.12 b A	4.51±1.15 c B	24.16±1.15 bc A	16.00 ±1.11 cd B	10.00±1.08 bc B	13.32±1.07 ab A
T2	3.92±0.08 a A	3.06±0.11 B B	7.36±1.15 bc A	4.82±1.13 c B	26±1.06 ab A	15.66±1.14 d B	10.3±1.11 bc B	13.95±1.12 ab A
T3	3.95±0.10 a A	3.58±0.12 a A	8.35±1.15 a A	6.13±1.15 a B	27.66±1.12 a A	19.83±1.05 ab B	12.73±1.09 a B	14.13±1.11 a A
T4	3.87±0.11 a A	3.38±0.11 ab B	8.10±1.17 a A	5.29±1.10 bc B	24.83±1.15 bc A	18.16±1.12 bc B	11.11±1.12 ab B	13.49±1.15 ab A
LSD value	0.802 NS	0.388 *	0.417 *	0.544 *	2.207 *	2.166 *	2.194 *	1.902 *

There were substantial differences in the means of having various small characters large letters in the same row and the same column.

Biochemical profile (AG ratio, globulin, albumin and total protein)

The biochemical profile results are summarized in Table (2).

Total protein

Before the *Saprolegnia* challenge, total protein levels were significantly higher ($P\leq 0.05$) in groups T2, T3, and T4 (6.11 ± 0.08, 6.86 ± 0.08, and 5.58 ± 0.02 g/dl, respectively) compared with the positive control (4.90 ± 0.08 g/dl). The highest value was recorded in T3 (6.86 g/dl).

Albumin

Pre-challenge albumin levels did not differ significantly among treatment groups. However, after the challenge, albumin levels decreased significantly ($P \leq 0.05$) in T1, T2, T3, and T4 (2.02, 1.58, 1.71, and 1.60 g/dl, respectively) compared with the positive control (2.37 g/dl). Despite this decline, no significant differences were observed between pre- and post-challenge values within each treatment group.

Globulin

Pre-challenge globulin concentrations were significantly higher ($P \leq 0.05$) in T2, T3, and T4 (4.61, 5.20, and 3.96 g/dl, respectively) compared with the positive control (2.85 g/dl).

Post-challenge, globulin levels further increased significantly ($P \leq 0.05$) in all treatment groups, reaching 4.86, 5.32, 5.95, and 5.32 g/dl in T1, T2, T3, and T4, respectively, compared with the positive control (4.27 g/dl).

Albumin/Globulin (A/G) ratio

At pre-challenge, the A/G ratio was significantly lower ($P \leq 0.05$) in the treatment groups (0.68, 0.32, 0.31, and 0.40% for T1, T2, T3, and T4, respectively) compared with the positive control (0.70%). Following the challenge, a further significant decline ($P \leq 0.05$) was observed in T1, T2, and T3 (0.41, 0.29, and 0.28%, respectively), while T4 maintained a value of 0.30%, all of which were lower than the positive control (0.55%).

Table 2. Biochemical characteristics of *C. carpio* (before and after the challenge) with *Saprolegnia* spp. consisting albumin, albumin-globulin ratio, and total protein, globulin

Biochemical Parameters	Total protein g/dl		Albumin g/dl		Globulin g/dl		A/G %	
Groups	Pre- challenge	Post challenge	Pre- challenge	Post challenge	Pre- challenge	Post challenge	Pre- challenge	Post challenge
Control (-v)	4.86±0.12 d A	4.90±0.15 c B	2.16±0.04 a A	2.16±0.10 a A	2.70±0.07 d A	2.74±0.06 e A	0.80±0.04 a A	0.78±0.04 a A
Control (+v)	4.90±0.08 d B	6.64±0.07 b A	2.01±0.07 a A	2.37±0.05 a A	2.85±0.04 d B	4.27±0.06 d A	0.70±0.06 ab A	0.55±0.02 ab A
T1	5.11±0.05 d B	6.88±1.81 b A	2.07±0.028 a A	2.02±1.75 ab A	3.04±0.19 d B	4.86±0.01 c A	0.68±0.01 abc A	0.41±0.19 ab A
T2	6.11±0.08 b B	6.90±1.89 b A	1.5±0.021 a A	1.58±1.83 c A	4.61±0.06 b B	5.32±0.27 b A	0.32± 0.05 bc A	0.29±0.06 b A
T3	6.86±0.08 a B	7.66±1.98 a A	1.63±0.028 a A	1.71±1.94 bc A	5.20±0.02 a B	5.95±0.02 a A	0.31±0.07 c A	0.28±0.06 b A
T4	5.58±0.02 c B	6.92±2.00 b A	1.62±0.021 a A	1.60±2.02 c A	3.96±0.02 c B	5.32±0.02 b A	0.40±0.27 bc A	0.30±0.02 b A
LSD value	0.242 *	0.251 *	0.242 *	0.242 *	0.251 *	0.437 *	0.081 *	0.110 *
There were substantial differences in the means of having various small characters at same column and huge letters at same row.								

Respiratory burst activity

The respiratory burst activity (NBT reduction) of neutrophils in *C. carpio* following the *Saprolegnia* spp. challenge is presented in Table (3). Dietary phytase supplementation had a significant effect ($P \leq 0.05$) on NBT reduction, with all treatment groups showing higher activity compared with the control group. The greatest increase was observed in T3 (1.68), which was significantly higher than the other treatments and the positive control group (0.52).

Table 3. *C. carpio* neutrophils' respiratory burst activity (NBT decrease) following a challenge with *Saprolegnia* spp.

Treatments	Control (-Ve)	Control (+Ve)	T1	T2	T3	T4	LSD value
Mean ± SE of NBT Post challenge	0.40± 0.04 C	0.52 ± 0.14 C	1.15 ± 0.05 B	1.40 ± 0.10 Ab	1.68 ± 0.09 A	1.52 ± 0.07 Ab	**0.141***

The average of same column with various small letters differed significant at $P \leq 0.05$.

Histopathological study

Gills

Gill sections of the positive control group exhibited several pathological alterations, including epithelial lifting, necrosis, edema, and mononuclear cell infiltration (Fig. 1B, C), whereas the negative control group displayed normal gill architecture (Fig. 1A). In T1, gill sections showed aneurysms at the terminals of secondary lamellae and infiltration of mononuclear inflammatory cells, including lymphocytes and monocytes/macrophages (Fig. 1D, E). T2 gill sections exhibited epithelial cell hyperplasia accompanied by blood congestion (Fig. 1F). Similarly, T3 gill tissues showed epithelial hyperplasia and blood congestion (Fig. 2A, B). In contrast, T4 gill sections demonstrated extensive secondary lamellar fusion, epithelial hyperplasia, and marked infiltration of mononuclear cells (Fig. 2C, D).

Skin

Skin tissues of the negative control group displayed normal structural organization of the epidermal layer, basal layer, and stratum compactum (Fig. 3A). In contrast, the positive control group showed severe histopathological changes, including epidermal erosion and ulceration (Fig. 3B). T1 and T2 skin sections showed aggregation of inflammatory cells (lymphocytes and monocytes/macrophages) (Fig. 3C, D). T3 exhibited marked epidermal hyperplasia, increased mucous and alarm cells, and mononuclear cell infiltration (Fig. 3E). Meanwhile, T4 skin tissues showed edema with infiltration of mononuclear cells (Fig. 3F).

Liver

Liver tissues from both the negative and positive control groups maintained normal histological structures, with no evidence of bleeding, necrosis, or mononuclear cell infiltration (Fig. 4A, B). In contrast, all liver samples from fish fed phytase or β-glucan diets displayed pronounced hepatocyte lipid vacuolation, fatty degeneration, and nuclear pyknosis (Fig. 4C, D).

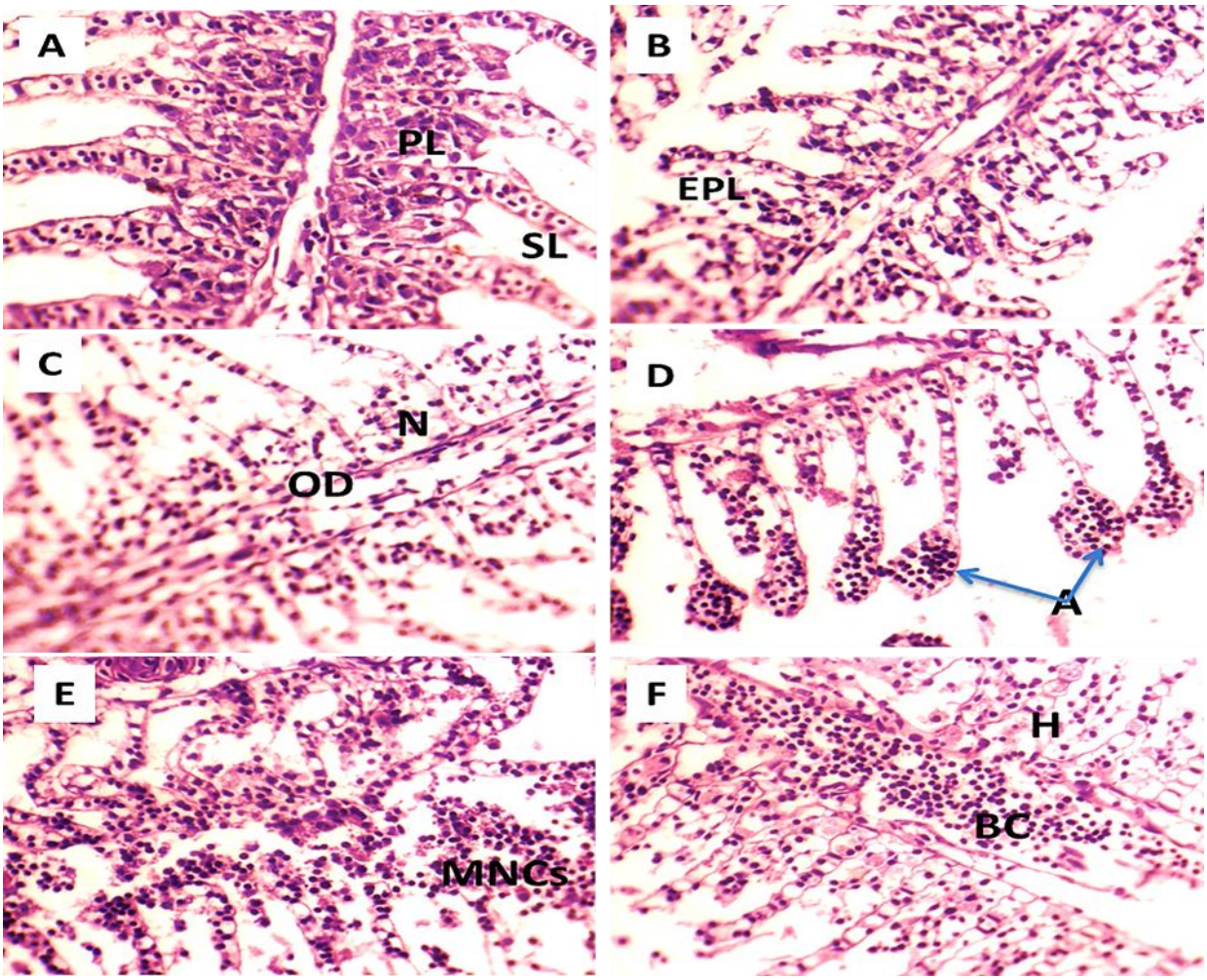

Fig. 1. Photomicrographs of gills of *C. carpio* 60-day phytase-supplemented diet that was challenged with *Saprolegnia* spp. (A). Primary and secondary lamellae in the control gill are normal. (B&C). Positive control gill showing epithelial lifting (EPL) with necrosis (N) and edema (OD) with mononuclear infiltration. (D&E). T1 demonstrating aneurysm (A) the tips of the second lamellae, infiltration of mononuclear cells (MNCs). (F). T2 showing blood congestion (BC) with hyperplasia (H). H&S. X400. Thickness=3-5µm

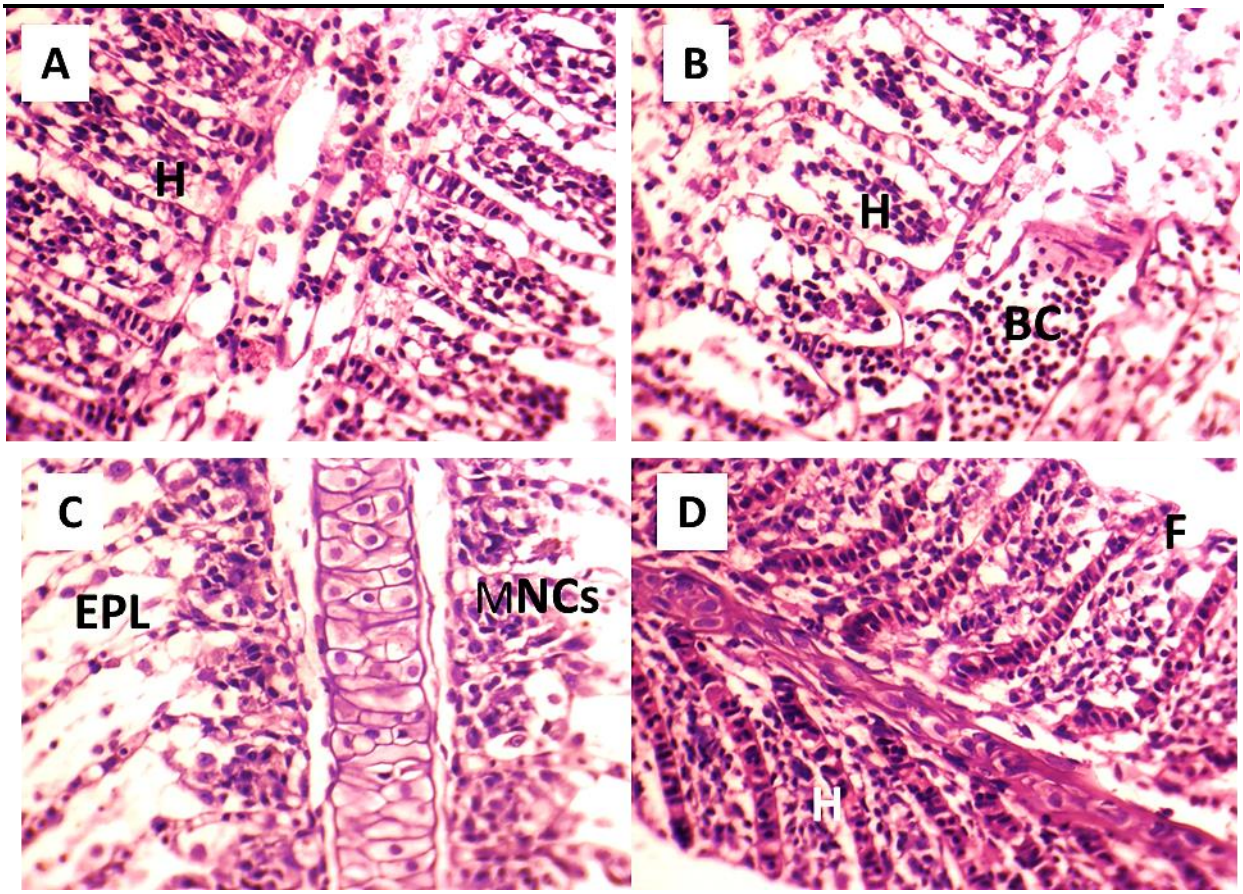

Fig. 2. Photomicrographs of gills of *C. carpio* dietary supplemented with phytase (A&B). T3 showing hyperplasia (H) with blood congestion (BC). (C&D). T4 demonstrating epithelial lifting (EPL), mononuclear cells infiltration (MNCs), hyperplasia (H) with complete fusion of the second lamellae (F). H&S. X400. Thickness=3-5µm

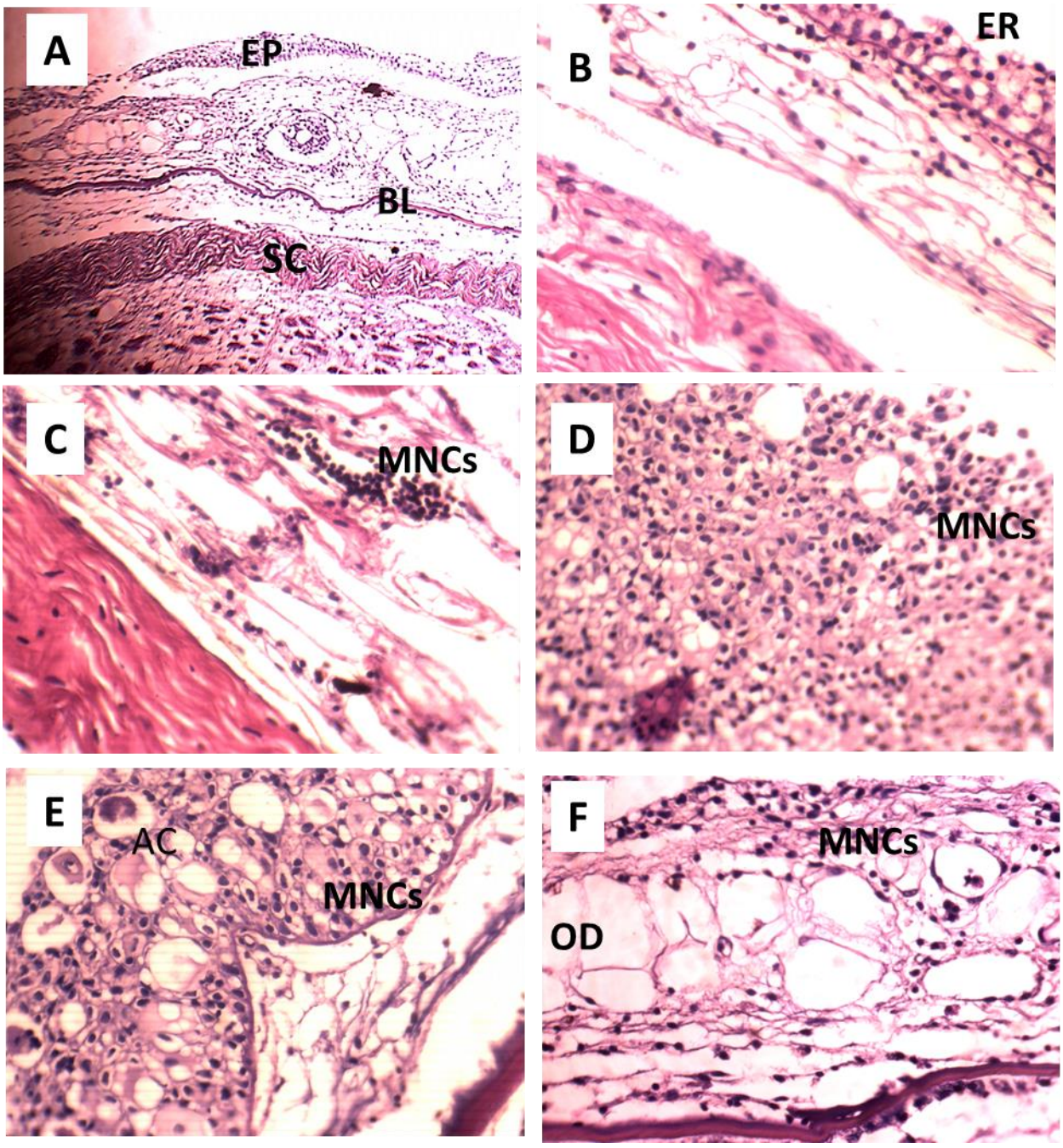

Fig. 3. Photomicrographs displaying histological structures through the skin of *C. carpio* after a 60-day phytase supplement and a *Saprolegnia* spp. challenge. (A): The stratum compactum (SC), basal layer (BL), and epidermal layer (EP) are 10x visible in the negative control skin; the epidermis is degraded and ulcerative in the positive control; (C&D): mononuclear cells (MNCs) infiltration is shown in T1 and T2, D with hyperplasia, E with MNCs infiltration and mild epidermal hyperplasia accompanied by an increase in mucous and alarm cells (AC); (F): MNCs infiltration is seen in T4 with edema (OD). B-F X400 H&S. Thickness: 3-5 µm

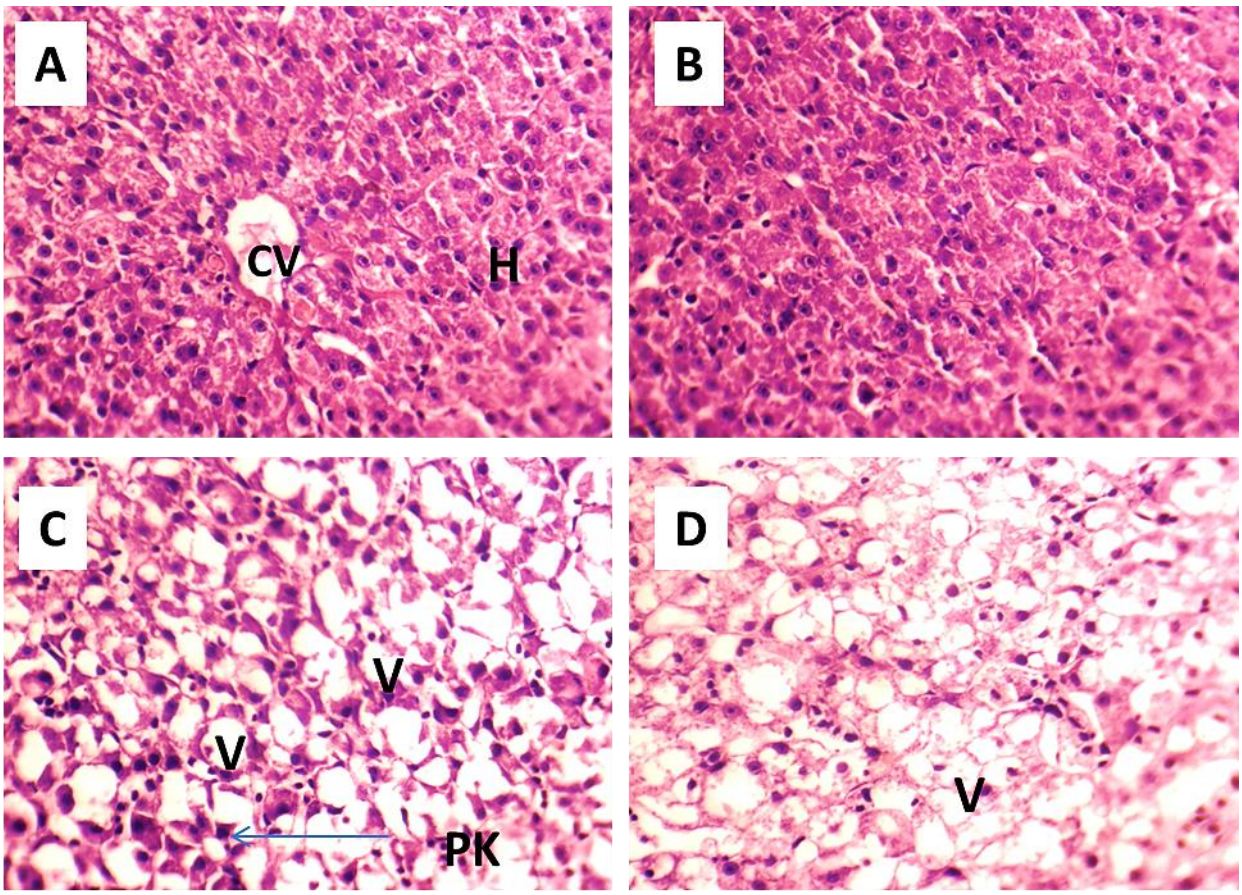

Fig. 4. Photomicrographs of livers of *C. carpio* dietary supplemented with phytase (A&B). Negative and positive control liver showing normal centrally vein (CV) and hepatocytes (H). (C&D) represented T1, T2, T3 and T4 demonstrating cytoplasmic vacuolation (V) in hepatic tissue with nuclear pyknosis (PK). H&S. X400. Thickness=3-5µm

Hematological parameters

Leukocytes (white blood cells) are key components of the fish immune system and serve as one of the body's first lines of defense. Their numbers rise sharply in response to infections **(Akrami *et al.*, 2015; Sabah *et al.*, 2019; Bashar *et al.*, 2025)**. In the present study, higher WBC counts in infected *C. carpio* (particularly in T3) likely reflect a strong cellular immune response to fungal infection. Similar results were reported by **Sardar *et al.* (2007)** and **Jumma (2024)**, who found that supplementing carp diets with microbial phytase (500 FTU/kg) maintained normal hematological parameters, including Hb, PCV, RBC, and WBC counts, even when dietary dicalcium phosphate and trace minerals were reduced.

Supporting this, **Abo Norag *et al.* (2018)** observed enhanced phagocytic index and activity in fish challenged with *Aeromonas hydrophila* when phytase was added at 500–1000 FTU/kg to low-phosphorus diets. These findings align with the current results, where phytase supplementation boosted leukocyte activity, strengthening innate immunity. In Nile tilapia, dietary nucleotides have similarly been shown to enhance lysozyme activity, non-specific immunity, and leukocyte activation indices **(Ramadan *et al.*, 1994; Shiau *et al.*, 2015)**. Hematological traits are thus valuable markers of fish health and stress response **(Adeoye *et al.*, 2016; Sabah *et al.*, 2024)**. Nonetheless, as highlighted by **Khajepour *et al.* (2012)**, phytase supplementation does not always significantly affect PCV or muscle composition, suggesting context-dependent effects.

Biochemical parameters

Blood protein fractions (total protein, albumin, and globulin) are fundamental indicators of fish health and immune status **(Kumar *et al.*, 2010)**. In this study, phytase-supplemented diets significantly increased total protein and globulin levels, while albumin levels remained stable or decreased after *Saprolegnia* challenge. This supports the role of phytase in improving protein metabolism and immune-related functions.

Similar trends were reported in grass carp, where phytase supplementation significantly increased albumin and total protein concentrations compared with controls **(Liu *et al.*, 2013)**. Likewise, **Kumar *et al.* (2011)** found higher levels of globulin, albumin, and total protein in the Nile tilapia fed phytase diets, reflecting enhanced intestinal nutrient absorption and improved systemic health. However, **Sardar *et al.* (2007)** reported that microbial phytase at 500 FTU/kg reduced plasma lipid, liver glycogen, and some mineral levels, suggesting that phytase responses may vary depending on dietary formulations and nutrient availability.

Respiratory burst activity

Neutrophils are essential immune cells that contribute to phagocytosis and pathogen elimination. In this study, neutrophil and macrophage activity, measured through NBT reduction, was significantly higher in phytase-fed groups compared with the control. These findings are consistent with **Abo Norag *et al.* (2018)**, who reported normalization of immune parameters and increased respiratory burst activity in fish fed phytase-supplemented low-phosphorus diets. Similarly, **Salih and Mustafa (2017)** found that synbiotic-supplemented groups exhibited significantly enhanced respiratory burst activity after a *Saprolegnia* challenge, highlighting the potential of dietary additives in modulating non-specific immunity.

Histopathology

Gill and skin tissues are particularly vulnerable to environmental changes and pathogen invasion due to their direct contact with the external environment **(Camargo & Martinez, 2007; Oday *et al.*, 2024)**. The gill lesions observed in the present study—epithelial lifting, hyperplasia, and lamellar fusion—are typical non-specific responses to infection or irritants, serving as protective adaptations to increase the diffusion distance

between blood and harmful agents **(Mallat, 1985)**. Gills and skin not only act as physical barriers but also produce immune-related molecules such as lysozyme and antibodies **(Bols *et al.*, 2001; Carlson & Zelikoff, 2008)**. The current results coincide with those of **Refai *et al.* (2010)** and **Hussein *et al.* (2013)**, who documented similar pathological responses in fish exposed to fungal infections.

Fish skin, with its high metabolic activity, is known to respond rapidly to environmental stimuli **(Iger *et al.*, 1994)**. Moreover, it is frequently targeted by opportunistic pathogens **(Whitear, 1986)**. In the present study, epidermal ulceration and inflammatory cell infiltration were pronounced in positive controls, but less severe in phytase-fed groups, suggesting a protective effect of supplementation.

Interestingly, liver tissues of fish in the phytase and β-glucan groups exhibited lipid vacuolation and fatty degeneration, which contrasts with the normal liver morphology observed in controls. Similar hepatic alterations were reported in the Nile tilapia challenged with *A. hydrophila* when fed phytase diets, indicating possible metabolic side effects associated with supplementation **(Abo Norag *et al.*, 2018)**.

CONCLUSION

In the present study, the skin of the positive control group exhibited severe histopathological alterations, including ulcerative epidermis, mononuclear cell infiltration, edema, and an increased number of alarm cells. These pathological changes were less pronounced in the gill and skin tissues of fish fed diets supplemented with phytase enzymes, indicating a protective effect. The liver of both positive and negative control groups maintained normal histological structure; however, all liver sections from fish receiving phytase or β-glucan supplementation displayed hepatocyte lipid vacuolation, fatty degeneration, and nuclear pyknosis. Overall, dietary phytase supplementation at different levels (T1, T2, and T3) demonstrated beneficial and protective effects against *C. carpio* challenged with saprolegniasis, enhancing immune responses and reducing the severity of gill and skin lesions.

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