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RESEARCH

Dietary BergaPur® supplementation enhanced the hematological, immune, biochemical, and antioxidative reactions of Nile tilapia, *Oreochromis niloticus*

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Abstract

The existing trial investigated the effects of supplementing the diet of Nile tilapia with BergaPur® on the hematology, immune response, biochemical factors, and antioxidative capacity. Four groups of fish with an average weight of 10.0 ± 0.5 g received BergaPur®, de-oiled phospholipid complex at a rate of 0 (control), 1.0, 1.5, and 2.0 g/kg diet for 9 weeks. Sampling included blood, serum, and liver samples from the four groups at the end of each 3 weeks for a 9 weeks total experimental period. Hematologic readings, serum biochemical, and oxidative parameters were investigated. Findings included a significant ($P < 0.05$) hematology augmentation (corpuscular Hb, packed cell volume, leukocytic, monocytic, and lymphocytic counts), and a remarked increased ($P < 0.05$) serum proteins (albumin, globulin, and total) were recorded in BergaPur® groups. Also, AST and ALT levels decreased significantly ($P < 0.05$), while glucose levels increased significantly ($P < 0.05$) in BergaPur® groups at all sampling points. Immune (phagocytosis and lysozyme activity) and antioxidant (SOD, GPx, and CAT) parameters increased significantly ($P < 0.05$) in BergaPur® supplemented groups, compared to the control, while MDA level decreased obviously in the incorporated groups over the control. Our findings verified the positive effects of BergaPur® on the hematological, immune, and biochemical parameters of *O Oreochromis niloticus*, with a suggestive concentration of 2 g/kg diet.

Keywords BergaPur® · Biochemical · Hematology · Immune response · Nile tilapia

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Introduction

The aquaculture intensification faces the challenge of infections including bacterial and parasitic conditions, which lead to the usage of antibiotics and chemical control (Laktuka et al. 2023; Martínez Cruz et al. 2012). The unwise use of antibiotics unsurprisingly led to the advance of mutagenic strains, resistance, and harmful effects for both fish and humans (Jahangiri and Esteban 2018; Salma et al. 2022). Hence, it is imperious to switch to eco-friendly alternatives as natural herbal preparations and prebiotics that can enhance fish performance, health, and immunity skipping the drawbacks to fish and humans (Abbass and Elabd 2022; Dawood and Koshio 2016; Rohani et al. 2022). Moreover, fish intensification and stocking densities commonly accompany increased fish stress and the beginning of an adaptive response with detrimental effects on fish (Okeke et al. 2022; Shi et al. 2023). The Nile tilapia *Oreochromis niloticus* is mainly distributed all over the world (Hassaan et al. 2021), and it is one of the world's most cultivated fish accounting for more than 75% of the global tilapia output (FAO 2022).

Recently, investigations have been directed toward enhancing the immune response as a protective and occasionally a fish disease control plan. Feed additives dietary inclusion has been recognized for their growth-promoting activities in Nile tilapia which is a significant cultured species all over the world (FAO 2020). The important role of phospholipids in sustaining fish's different functions may be due to that the other sources of energy aside from carbohydrates need to be easily digested, and outstanding soy phospholipids decrease the fatty acid synthesis of associated enzymes, which reduces triglycerides levels in the liver (Tan et al. 2022; Wee et al. 2023). BergaPur® principal active ingredients are phosphatidylinositol (PI), phosphatidylcholine (PC), and phosphatidylethanolamine (PE). BergaPur® is the concentrated active component of powder and granular form crude soy phospholipids (PLs). BergaPur® incorporation is expected to enhance overall growth, metabolism, and performance with the help of its content of active phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol (Kari et al. 2022; Tan et al. 2022; Wee et al. 2023). PLs, and their unsaturated fatty acids, can enhance the cell membrane permeability and thus hasten the fish's physiological functions including immune and inflammatory responses (Wang et al. 2022).

Therefore, the present study was directed to assess the possible useful effects of BergaPur® (essential phospholipid complex) on hematobiochemical, immune, and antioxidant responses of Nile tilapia.

Material and methods

Experimental fish and conditions

A total of 360 fish 10.0 ± 0.5 g were acquired and transported to 900-L fiberglass tanks at the unit (1) for fish production affiliated with the Egyptian military veterinary administration and acclimatization lasted for 14 days. Water parameters were recorded as (temperature 25.0 ± 0.5 °C and dissolved oxygen 6.1 ± 0.4 mg/L). Fish received a basal diet at 3% body weight twice/day at 08:00 AM and 03:00 PM. Throughout the experiment, daily checks on mortalities and fish health status were performed. Water exchange was performed twice/week at a rate of 10%, and excreta and uneaten food were siphoned.

BergaPur® incorporation

Experimental diets were formulated at the factory for feed manufacture affiliated with the Food Industries and Packing Complex (FIPCO), the Egyptian armed forces' logistic authority. Commercial BergaPur® (Berg + Schmidt Asia Pte Ltd., dry matter 66.13, crude protein 31.09, lipid 5.92, and crude fiber 8.2) was incorporated at a rate of 0, 1.0, 1.5, and 2.0 g/kg to the raw ingredients with 15-min mixing and then pass to the cooking line at 85 °C for 40 s, followed by pressing and cooling. Fish were allocated into 4 groups (90 fish/group in triplicates) after acclimatization. The first group received a free control diet; the following groups were incorporated with 1.0-, 1.5-, and 2.0-g BergaPur® de-oiled phospholipid complex/kg diet for a 9-week trial.

Samples collection

This was performed at 3-week intervals. Blood sampling included nine fish/group, and three fish/replicate) was performed using an EDTA syringe through the caudal blood vessels to investigate the hematological parameters (red blood cells (RBCs) and white blood cells (WBCs) counts, packed cell volume (PCV), hemoglobin (Hb), and differential leucocytic count and corpuscular hemoglobin concentrations (MCV, MCH, and MCHC)) and immune response indicators (phagocytic activity and index). Serum was obtained from blood samples without anticoagulant and centrifuged (3000 g for 15 min), and serum was preserved at −20 °C until assaying immune indicators (nitric oxide (NO), and lysozyme), serum proteins and biochemical picture (alanine alanine transaminase (ALT), aspartate transaminase (AST), glucose, and cortisol). For tissue sampling, after careful dissection, liver samples were collected, homogenized, and centrifuged (3000 g for 15 min), and the collected homogenate was preserved at −20 °C for assaying the antioxidants.

Hematologic response

RBCs, WBCs, and PCV counts were investigated according to Camus and Camus (2021); differential leukocyte count was estimated using the Giemsa staining technique following the method reported by Souza and Eren (2022). Corpuscular hemoglobin concentrations (MCH, MCV, and MCHC) were estimated following McNamara (2017). RBCs and WBCs were estimated using Schwa's solution and a hemocytometer following De Briyne (2014). Hemoglobin concentration (g/dL) was estimated with colorimetric Drabkin's reagent kits (Diamond Diagnostics, Egypt) following Thrall et al. (2012), using the formula: Hb (g/dL) = A × 36.8 g/dL; A is the absorbance of the sample. The PCV was calculated using the equation: PCV% = RBCs column height after centrifugation/total blood column height × 100. MCV, MCH, and MCHC were calculated following formulas:

- MCV = (PCV%/RBCs in million) × 100
- MCH pg = (Hb g/100 ml/BCs in millions) × 100
- MCHC% = (Hb g/dL/PCV %) × 100

Immune response indicators

Phagocytosis (index and activity) was estimated according to Silva et al. (2002), by mixing 0.5-ml blood with 0.25 ml of 1×10^6 *Aeromonas hydrophila* suspension to prepare blood smears. The activity was counted by estimating active engulfing cell percentages in relation to the total leukocytic number and PI by dividing total phagocytized cells by phagocytizing cells. Lysozyme activity (μg/ml) was estimated by adding 25-μl undiluted serum to 175-μl *Micrococcus lysodeikticus* lyophilized cells substrate solution (Inova Biotechnology, China) following (Ringwood et al. 2003). Turbidity was assayed (450 nm every 30 s for 5 min) using the ELISA reader via matching with the standard curve. Nitric oxide (NO) was estimated at 450 nm with a NO assay kit (Abcam, USA).

Biochemical and protein profile

Plasma glucose concentration was measured using a commercial kit (SPINREACT, Spain) at 340 nm according to the following formula: Sample absorbance (A)/standard (A) × 100 (standard conc.) = mg/dL glucose in the sample.

Plasma cortisol concentration was measured using a commercial kit (Thermo Fisher, Canada) at 450 nm. A cortisol standard was provided to generate a standard curve for the assay, and all samples were read off a user-generated standard curve.

Hepatic enzymes AST and ALT were measured spectrophotometrically at 450 nm following: alanine aminotransferase U/L = ΔA/min × 1750 and aspartate aminotransferase U/L = ΔA/min × 1750. Serum albumins and globulins were assayed using ready-made kits (Spinreact Co. Spain) following company instructions (Bayer RA-50 chemistry analyzer). Globulin was mathematically estimated. Serum total protein was carried out using available commercial kits (Bio-Chem, Egypt) and measured against a reagent blank spectrophotometrically at wavelength 550 nm, where total protein level g/dL = (sample absorbance/standard absorbance) × 4.

Antioxidants

The thiobarbituric acid (TBA) method was used to estimate malondialdehyde (MDA) at 540 nm, as TBA reacts with MDA in an acidic medium at 95° for 30 min and yields TBA-reactive product MDA (nmol/g tissue) = abs of sample/abs. of standard × 10/g tissue used. Glutathione peroxidase (Gpx) was assayed at 534 nm. It was measured colorimetrically depending on the reduction of 5,5 dithiobis with glutathione, and a yellow compound was formed, and Gpx was calculated according to the following formula: (absorbance of sample/g tissue used) × 66.66 = mg/g tissue.

Nitro blue reduction method was used to estimate superoxide dismutase SOD at 560 nm for 5 min depending on inhibiting phenazine methosulfate-mediated reduction of nitroblue tetrazolium dye, SOD activity (U/g tissue) = % inhibition × 3.75/g tissue used.

Catalase (CAT) was assayed spectrophotometrically at 510 nm following Abdel Rahman et al. (2022) according to the following equation: CAT (U/g) = (abs. of standard − abs. of sample)/abs. of sample × 1-g tissue.

Statistical analysis

Analysis was performed using one-way ANOVA (SPSS statistical software, v. 22.0) with a general linear model and Duncan's multiple range tests. Values are expressed as mean ± significant error (SE). P-value < 0.05 for means was measured significantly. Different superscript letters indicated significance ($P < 0.05$).

Results

Hematology

BergaPur® significantly improved ($P < 0.05$) total RBC count, Hb, and PCV readings with the most significant elevation for 2-g BergaPur®/kg in the 3rd (3.2 ± 0.00, 9.7 ± 0.01, and 31.7 ± 0.03), 6th (3.3 ± 0.01, 10.0 ± 0.01, and 32.3 ± 0.00), and 9th (3.4 ± 0.02, 10.1 ± 0.02, and 32.7 ± 0.02) weeks. MCH showed the most significant increase for BergaPur® 1 and 1.5 g/kg in the 3rd week (30.1 ± 0.02), for all the BergaPur® concentrations in the 6th week (30.3 ± 0.02, 30.1 ± 0.04, and 30.3 ± 0.01), and for the 1.5 g/kg BergaPur® in the 9th week. MCHC showed the maximum ($P < 0.05$) increase for the BergaPur® 1.5 g/kg in the 6th week (30.6 ± 0.02). However, MCV and MCHC values did not show any significant ($P < 0.05$) differences among the different treatment groups, except for MCHC in the 9th week which gave a significant improvement ($P < 0.05$) in all supplemented groups over the control (Table 1).

Leukogram varied clearly between groups. The heterophils percentage was significantly lower ($P < 0.05$) in BergaPur® groups than in the control. However, WBCs, monocytes, and lymphocytes percentages were significantly ($P < 0.05$) increased in BergaPur® groups than the control. Basophils and eosinophils did not show any significant ($P < 0.05$) differences (Table 2).

Immune parameters

The phagocytic index showed a significant increase ($P < 0.05$) for the 1.5 g/kg BergaPur® group, in the 3rd, 6th, and 9th week, and both the phagocytic activity and index revealed the highest elevation for 2.0 g/kg BergaPur® at the 3 sampling points (Fig. 1).

The lysozyme activity was markedly ($P < 0.05$) improved for the 1.5 g/kg BergaPur® group, in the 3rd week, and for the 1.5 and 2 g/kg BergaPur® group in the 6th and 9th weeks (Fig. 2).

Serum NO showed a significantly ($P < 0.05$) different reading in BergaPur® only in the 3rd-week sampling point, in comparison with the control group (Fig. 3).

Biochemical and serum protein parameters

BergaPur® groups showed significantly ($P < 0.05$) increased serum glucose levels, with the most predominant elevation for 1.5 and 2.0 g/kg BergaPur® in the 3rd (11.8 ± 0.01 and 11.8 ± 0.02), 6th (12.2 ± 0.04 and 12.4 ± 0.01), and 9th (12.7 ± 0.00 and 12.9 ± 0.01)

Table 1 Effect of 1, 1.5, and 2 g/kg diet BergaPur® on RBCs indices in *O. niloticus* at 3, 6, and 9 weeks of feeding period

	Control	1 g/kg BergaPur®	1.5 g/kg BergaPur®	2 g/kg BergaPur®
3 weeks				
RBCs (× 10/mm^6)	2.9 ± 0.00^d	3.1 ± 0.00^c	3.2 ± 0.00^b	3.2 ± 0.00^a
Hb (g/100 ml)	8.7 ± 0.00^d	9.4 ± 0.001^c	9.5 ± 0.01b	9.7 ± 0.01^a
PCV (%)	28.3 ± 0.03^c	31.0 ± 0.00^{ab}	30.7 ± 0.03^b	31.7 ± 0.03^a
MCV	97.7 ± 0.00	99.0 ± 0.00	96.9 ± 0.01	97.9 ± 0.01
MCH	29.9 ± 0.05^b	30.1 ± 0.02^a	30.1 ± 0.02^a	30.0 ± 0.05^{ab}
MCHC	30.6 ± 0.03	30.4 ± 0.04	31.1 ± 0.03	30.7 ± 0.03
6 weeks				
RBCs × (10/mm^6)	2.9 ± 0.00d	3.2 ± 0.00^c	3.3 ± 0.00^b	3.3 ± 0.01^a
Hb (g/100 ml)	8.7 ± 0.04d	9.6 ± 0.00^c	9.8 ± 0.00^b	10.0 ± 0.01^a
PCV (%)	29.0 ± 0.03^c	30.7 ± 0.05^b	31.0 ± 0.03b	32.3 ± 0.00^a
MCV	98.6 ± 0.05	96.5 ± 0.01	95.3 ± 0.01	98.2 ± 0.05
MCH	29.6 ± 0.02^b	30.3 ± 0.02^a	30.1 ± 0.04^a	30.3 ± 0.01^a
MCHC	30.0 ± 0.02^b	31.3 ± 0.03^{ab}	30.9 ± 0.02^b	31.6 ± 0.05^a
9 weeks				
RBCs × (10/mm^6)	3.0 ± 0.02^d	3.2 ± 0.02^c	3.3 ± 0.02^b	3.4 ± 0.02^a
Hb (g/100 ml)	9.2 ± 0.02^c	9.7 ± 0.02^b	10.1 ± 0.02^a	10.1 ± 0.02^a
PCV (%)	29.0 ± 0.02^c	31.3 ± 0.02^b	32.0 ± 0.02^{ab}	32.7 ± 0.02^a
MCV	97.8 ± 0.02	97.3 ± 0.02	97.3 ± 0.02	97.2 ± 0.02
MCH	30.9 ± 0.02	30.2 ± 0.02^b	30.6 ± 0.02^{ab}	30.2 ± 0.02^b
MCHC	31.6 ± 0.02	31.1 ± 0.02	31.4 ± 0.02	31.1 ± 0.02

Values are expressed as mean ± SE. Different superscript letters indicate significance ($P < 0.05$)

weeks. Serum cortisol levels decreased significantly ($P < 0.05$) in BergaPur® groups over the experimental period, with the lowest reading for 2.0 g/kg BergaPur® (32.4 ± 0.04, 30.4 ± 0.03, and 29.3 ± 0.05) throughout the entire experiment (Table 3).

Serum ALT and AST levels decreased significantly ($P < 0.05$) in BergaPur® groups, with the most significant decrease for 2.0 g/kg BergaPur® (26.6 ± 0.01 and 23.4 ± 0.03), (25. ± 0.01 and 23.2 ± 0.01), and (25.0 ± 0.04 and 22.9 ± 0.04) at the three sampling points (Table 3).

BergaPur® significantly elevated ($P < 0.05$) globulin, albumin, and total protein levels, with the most significant increase for 2.0 g/kg BergaPur® (2.00 ± 0.01, 1.29 ± 0.00, and 3.3 ± 0.01), (2.2 ± 0.00, 1.38 ± 0.00, and 3.6 ± 0.00) throughout the entire experiment (Table 3).

Antioxidants

BergaPur® significantly ($P < 0.05$) lowered the MDA level, with the most significant decrease for 2.0 g/kg BergaPur® all over the experimental period (Fig. 4). BergaPur® significantly ($P < 0.05$) increased Gpx and CAT and SOD activities with the highest ($P < 0.05$) result for the 2.0 g/kg BergaPur® group (Figs. 5, 6, 7). CAT level showed the highest ($P < 0.05$) marked elevation for 1.5 and 2.0 g/kg BergaPur® group at the 6th and 9th weeks (Fig. 6).

Table 2 Effect of 1, 1.5, and 2 g/kg diet Bergapur® on WBCs indices in *O. niloticus* at 3, 6, and 9 weeks of feeding period

BergaPur® g/kg diet	WBCs ($\times 10^3$/mm^3)	Lymphocytes (%)	Heterophils (%)	Monocytes (%)	Basophils (%)	Eosinophils (%)
3 weeks						
0	21.8 ± 0.05^{d}	75.7 ± 0.05^{c}	15.7 ± 0.03^{a}	6.7 ± 0.03	1.0 ± 0.0	0.7 ± 0.03
1	25.7 ± 0.03^{c}	79.7 ± 0.03^{b}	12.7 ± 0.03^{b}	7.3 ± 0.03	0.7 ± 0.03	0.7 ± 0.03
1.5	26.0 ± 0.01^{b}	80.0 ± 0.03^{b}	12.0 ± 0.05^{b}	7.7 ± 0.03	0.3 ± 0.03	0.3 ± 0.03
2	30.2 ± 0.01^{a}	82.0 ± 0.05^{a}	9.7 ± 0.05^{c}	7.3 ± 0.03	0.7 ± 0.03	0.3 ± 0.03
6 weeks						
0	20.3 ± 0.02^{b}	75.3 ± 0.03^{b}	16.3 ± 0.03^{a}	7.0 ± 0.05^{ab}	0.7 ± 0.03	0.7 ± 0.03
1	22.1 ± 0.01^{b}	76.7 ± 0.03^{b}	15.7 ± 0.01^{a}	6.3 ± 0.03^{b}	0.3 ± 0.03	0.3 ± 0.03
1.5	30.2 ± 0.01^{a}	80.7 ± 0.05^{a}	10.7 ± 0.01^{b}	7.3 ± 0.03^{ab}	0.7 ± 0.03	0.7 ± 0.03
2	32.1 ± 0.05^{a}	80.0 ± 0.01^{a}	11.3 ± 0.03^{b}	8.0 ± 0.05^{a}	0.3 ± 0.03	0.3 ± 0.03
9 weeks						
0	24.8 ± 0.03^{d}	75.3 ± 0.01^{b}	15.7 ± 0.02^{a}	7.0 ± 0.00^{b}	1.0 ± 0.05	1.0 ± 0.00
1	30.4 ± 0.02^{c}	79.3 ± 0.03^{a}	11.7 ± 0.02^{a}	7.7 ± 0.03^{ab}	0.7 ± 0.00	0.7 ± 0.03
1.5	32.0 ± 0.02^{b}	81.0 ± 0.03^{a}	9.7 ± 0.03^{b}	8.0 ± 0.02^{ab}	0.7 ± 0.00	0.7 ± 0.03
2	37.4 ± 0.03^{a}	80.0 ± 0.02^{a}	10.0 ± 0.03^{b}	8.7 ± 0.03^{a}	1.0 ± 0.02	0.3 ± 0.03

Values are expressed as mean ± SE. Different superscript letters indicate significance ($P < 0.05$)

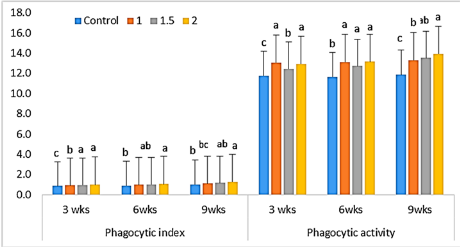

Fig. 1 Phagocytic index and activity of Nile tilapia (*O. niloticus*) supplemented with 1.0, 1.5, and 2.0 g/kg diet BergaPur® at 3, 6, and 9 weeks of feeding period. Values are mean ($n=9$) ± SEM. Mean values with different superscript letters are different significantly ($P<0.05$)

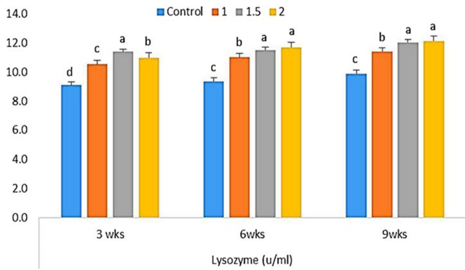

Fig. 2 Serum lysozyme activity of Nile tilapia (*O. niloticus*) supplemented with 1.0, 1.5, and 2.0 g/kg diet BergaPur® at 3, 6, and 9 weeks of feeding period. Values are mean ($n=9$) ± SEM. Mean values with different superscript letters are different significantly ($P<0.05$)

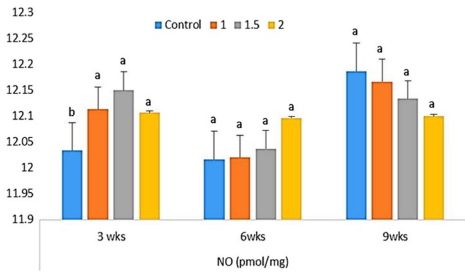

Fig. 3 Serum nitric oxide of Nile tilapia (*O. niloticus*) supplemented with 1.0, 1.5, and 2.0 g/kg diet BergaPur® at 3, 6, and 9 weeks of feeding period. Values are mean ($n=9$) ± SEM. Mean values with different superscript letters are different significantly ($P<0.05$)

Discussion

The current trial was designed to assess the possible BergaPur® (deoiled soy plant-derived lecithin) dietary effects at levels of 1.0, 1.5, and 2.0 g/kg diet on hematology, immune, and biochemical profiles of *Oreochromis niloticus*.

Hematology is one of the important indicators of fish health and may give essential and trustworthy data on nutrient deficiencies, metabolic disorders, stress status, and deviations in response to ecological alterations (Makled et al. 2017; Tabassum et al. 2021; Zakaria et al. 2022). Results showed significant ($P<0.05$) changes in the hematological parameters in support of the enhanced erythrogram and leukogram of Nile tilapia in response to BergaPur® supplementation. Likewise, hematological parameters (Hb%, RBC, MCV, and MCH) were elevated markedly in Nile tilapia received Lysomax (natural bioemulsifier containing soy lecithin) diet (El-Sayed et al. 2021). Alike results were described for dietary lecithin that elevated blood parameters in stellate sturgeon (*Acipenser stellatus*) (Jafari et al. 2018). The elevated RBC and Hb readings might be related to the bigger fish metabolic demand for lecithin-based regimes (Jafari et al. 2018), which was accompanied by a sharp rise in growth performance. In addition, mannan oligosaccharides increased ($P<0.05$) hematocrit readings in pacu (Sado et al.

Table 3 Effect of 1, 1.5, and 2 g/kg BergaPur® on serum glucose, cortisol, ALT, AST, total protein, and albumin and globulin concentrations in *O. niloticus* at 3, 6, and 9 weeks of feeding period

BergaPur® g/kg feed	Cortisol (ng/ml)	Glucose (mg/dl)	ALT (U/l)	AST (U/l)	Total prot. (g/dl)	Albumin (g/dl)	Globulin (g/dl)
3 weeks							
Control	33.1 ± 0.05^{a}	10.4 ± 0.01^{c}	27.4 ± 0.02^{a}	24.4 ± 0.01^{a}	3.2 ± 0.01^{b}	1.29 ± 0.00^{b}	1.86 ± 0.00^{c}
1.0	32.5 ± 0.05^{b}	11.7 ± 0.03^{b}	27.1 ± 0.02^{a}	24.1 ± 0.02^{a}	3.3 ± 0.01^{a}	1.32 ± 0.00^{a}	1.95 ± 0.01^{b}
1.5	32.7 ± 0.02^{b}	11.8 ± 0.01^{a}	26.7 ± 0.01^{b}	23.4 ± 0.05^{b}	3.3 ± 0.01^{a}	1.30 ± 0.00^{b}	1.99 ± 0.00^{ab}
2.0	32.4 ± 0.04^{c}	11.8 ± 0.02^{a}	26.6 ± 0.01^{b}	23.4 ± 0.03^{b}	3.3 ± 0.01^{a}	1.29 ± 0.00^{b}	2.00 ± 0.01^{a}
6 weeks							
Control	31.3 ± 0.01^{a}	10.7 ± 0.01^{d}	27.4 ± 0.03^{a}	24.3 ± 0.02^{a}	3.2 ± 0.01^{d}	1.30 ± 0.00^{c}	1.9 ± 0.00^{c}
1.0	30.2 ± 0.04^{c}	12.0 ± 0.03^{c}	26.3 ± 0.03^{b}	24.0 ± 0.02^{b}	3.5 ± 0.00^{c}	1.34 ± 0.00^{b}	2.1 ± 0.01^{b}
1.5	30.9 ± 0.03^{b}	12.2 ± 0.04^{b}	26.3 ± 0.02^{b}	23.4 ± 0.02^{c}	3.5 ± 0.01^{b}	1.34 ± 0.00^{b}	2.1 ± 0.01^{a}
2.0	30.4 ± 0.03^{c}	12.4 ± 0.01^{a}	25.0 ± 0.01^{b}	23.2 ± 0.01^{d}	3.6 ± 0.00^{a}	1.38 ± 0.00^{a}	2.2 ± 0.00^{a}
9 weeks							
Control	31.4 ± 0.01^{a}	11.0 ± 0.02^{c}	26.7 ± 0.04^{a}	24.3 ± 0.01^{a}	3.2 ± 0.00^{d}	1.2 ± 0.00^{c}	1.9 ± 0.01^{d}
1.0	30.0 ± 0.02^{b}	12.4 ± 0.01^{b}	26.4 ± 0.02^{ab}	23.8 ± 0.02^{b}	3.6 ± 0.00^{c}	1.3 ± 0.00^{b}	2.2 ± 0.01^{c}
1.5	29.7 ± 0.01^{b}	12.7 ± 0.00^{ab}	25.9 ± 0.04^{b}	23.5 ± 0.02^{c}	3.7 ± 0.01^{b}	1.4 ± 0.00^{ab}	2.3 ± 0.00^{b}
2.0	29.3 ± 0.05^{b}	12.9 ± 0.01^{a}	25.0 ± 0.04^{c}	22.9 ± 0.04^{d}	4.0 ± 0.01^{a}	1.4 ± 0.02^{a}	2.5 ± 0.01^{a}

Values are expressed as mean ± SE. Different superscript letters indicate significance ($P < 0.05$)

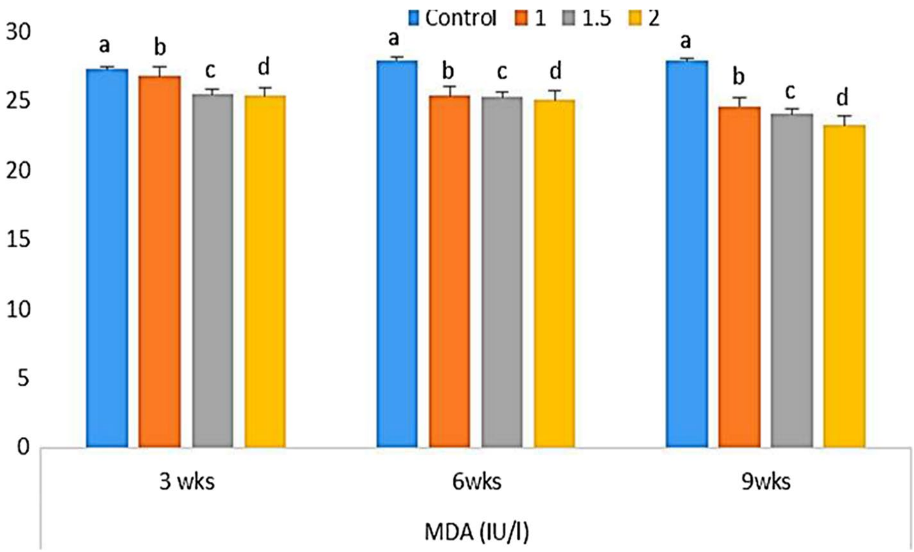

Fig. 4 MDA in liver samples of Nile tilapia (*O. niloticus*) supplemented with 1.0, 1.5, and 2.0 g/kg diet BergaPur® at 3, 6, and 9 weeks of feeding period. Values are mean ($n = 9$) ± SEM. Mean values with different superscript letters are different significantly ($P < 0.05$)

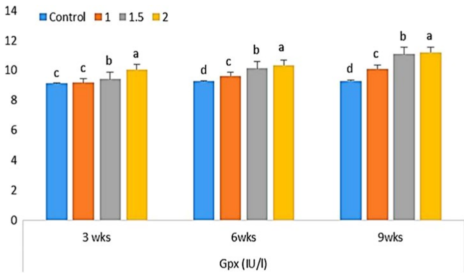

Fig. 5 GPx in liver samples of Nile tilapia (*O. niloticus*) fed on 1.0, 1.5, and 2.0g/kg diet BergaPur® at 3, 6, and 9 weeks of feeding period. Values are mean ($n = 9$) ± SEM. Mean values with different superscript letters are different significantly ($P < 0.05$)

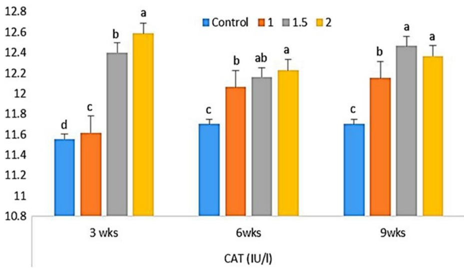

Fig. 6 CAT in liver samples of Nile tilapia (*O. niloticus*) fed on 1.0, 1.5, and 2.0g/kg diet BergaPur® at 3, 6, and 9 weeks of feeding period. Values are mean ($n = 9$) ± SEM. Mean values with different superscript letters are different significantly ($P < 0.05$)

2014). Moreover, 6% dietary phospholipid incorporation improved the hematological parameters of *Pangasianodon hypophthalmus* (Torsabo et al. 2023).

This may be due to hemoglobin synthesis which can provide fish with protection in infectious and stressful conditions through increasing red blood cells production and stimulating the nonspecific immune responses (Macedo et al. 2023; Torsabo et al. 2023).

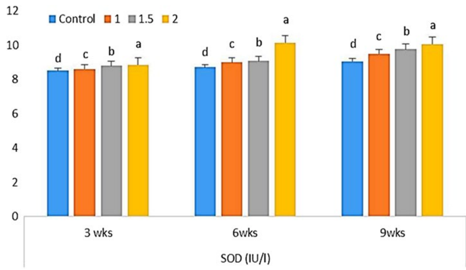

Fig. 7 SOD in liver samples of Nile tilapia (*O. niloticus*) fed on 1.0, 1.5, and 2.0g/kg diet BergaPur® at 3, 6, and 9 weeks of feeding period. Values are mean ($n=9$) ± SEM. Mean values with different superscript letters are different significantly ($P<0.05$)

Immune response parameters are usually used as indicators for the efficiency of the dietary supplement and the health status of the fish (Elabd et al. 2020, 2019; Gad et al. 2024). Here, the immune response was significantly enhanced in BergaPur® groups. Likewise, immune response parameters (phenoloxidase, respiratory burst, lysozyme, and phagocytic activities) were improved significantly in Nile tilapia that received lecithin-incorporated diets (Batista et al. 2023). This may be attributed to improved fatty acid and phospholipids, which are the precursor for eicosanoids that are involved in various physiological functions including inflammatory and immune reactions (Wang et al. 2022). Contradictory, phagocytic capacity, and index did not show any significantly improved readings in response to probiotic, prebiotic, or symbiotic supplements (Cavalcante et al. 2020).

In the present trial, BergaPur® groups showed markedly elevated serum proteins. Correspondingly, Nile tilapia which received soy lecithin (SL) revealed increased lipid and fatty acid levels (Batista et al. 2023). Also, the increased phospholipid levels incorporation elevated the albumin, globulin, and total protein of *P. hypophthalmus* (Torsabo et al. 2023). Those improved levels could be attributed to the production of more proteins to meet the high-energy needs and the stimulating properties of phospholipid (Khan et al. 2020; Torsabo et al. 2023).

Hepatic indicators (AST, ALT, and LDH) are diagnostic measurements for liver functionality (Elabd et al. 2022; Gad et al. 2024). The present trial also revealed decreased AST and ALT levels in BergaPur® groups that reflect healthy liver activity and hepatoprotective action of BergaPur®. Similarly, *P. hypophthalmus* fed phospholipids-rich diets showed hepatoprotective effects in different doses (Torsabo et al. 2023). Furthermore, our work revealed that BergaPur® supplementation increased glucose levels in Nile tilapia. In addition, cortisol levels were reduced significantly in samples obtained in the 3rd week. That indicates the protective and anti-stress effects of BergaPur® as stress resulting from population density associated with cultivation and production management in aquaculture practices can increase circulating cortisol levels and generates a decrease in specific and nonspecific immunity (Brydges et al., 2009; Ramsay et al., 2009). Likewise, phospholipid supplementation decreased ALT and AST levels in comparison to the control *P. hypophthalmus* indicating the hepatic protection properties which could be because of the enhanced immune response and decreased cortisol (Bockus et al. 2021; Torsabo et al. 2023).

Currently, BergaPur® also increased antioxidants SOD, CAT, and Gpx. Not much research concentrated on the modulation of oxidative stress response via BergaPur® dietary supplementation. In the same way, Lysomax significantly increased SOD activity which may be due to the antioxidative properties of the lecithin-containing product that improves Nile tilapia health condition (El-Sayed et al. 2021). In addition, dietary phospholipids are well-known for their enhancing properties of the antioxidant and the lipid peroxidation capacities in fish (Cai et al. 2016; Wee et al. 2023). In the same instance, dietary phospholipid has radical scavenging properties and enhances the activities of SOD, CAT, Gpx, and glutathione-S-transferase

(GST) that have been documented in common carp *Cyprinus carpio* (Adel et al. 2017; Wee et al. 2023), stellate sturgeon, and stellate sturgeon (*Acipenser stellatus*) (Jafari et al. 2018). PI is known to improve lipid metabolism (myoinositol–cholinesterase and transaminase), sustains tissue integrity, and plays a crucial role in stress resistance to adverse conditions of temperature, salinity, and transfers (El-Sayed et al. 2021; Paibulkichakul et al. 1998).

Conclusion

Conclusively, the BergaPur® in the present study revealed enhanced hematology, immune, and antioxidative responses in *Oreochromis niloticus*. This suggests the promising role of BergaPur® as a dietary inclusion for Nile tilapia and as an alternative to the usage of antibiotics for enhancing the health management of aquatic animals. Current findings make us recommend BergaPur® dietary incorporation with a suggestive concentration of 2-g BergaPur®/kg diet in Nile tilapia.

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Declarations

Ethics approval All the experimental procedures were carried out following the guidelines of the Research Ethics Board, Fac. of Vet. Med., Benha University, BUFVTM 02–06-22.

Conflict of interest The authors declare no competing interests.

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