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Potential role of dietary white poplar (*Populus alba* L.) in stimulating growth, digestion, and antioxidant/immune status of Nile tilapia (*Oreochromis niloticus*)

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Abstract

The potential use of feed supplements as immune-stimulant and growth-promoting agents in fish diets has drawn much attention. The current research investigated the effects of feeding Nile tilapia (*Oreochromis niloticus*) on white poplar (*Populus alba* L.) (WP) leaves powder as a dietary supplement on the growth, digestive functions, immune, and oxidant-antioxidant parameters. In addition, the fish resistance against the *Aeromonas sobria* (*A. sobria*) challenge was investigated. For 60 days, fish ($n = 160$; 34.61 ± 0.16 g) were divided equally into four groups, each had four replicates. Fish were fed on four isonitrogenous and isolipidic diets supplemented with varying levels of WP; 0 g/kg (WP0, crude protein (CP) = 37.18%; crude lipid (CL) = 9.98%), 2 g/kg (WP2, CP = 37.22%; CL = 9.56%), 4 g/kg (WP4, CP = 36.95%; CL = 9.47%), and 6 g/kg (WP6, CP = 36.88%; CL = 9.33%), where WP0 was the control diet. The results revealed that WP diets substantially boosted the growth (final body weight, weight gain, and specific growth rate) with an improvement of feed conversion ratio of Nile tilapia in a level-dependent manner with the WP6 group attaining the best outcomes. WP diets improved the amylase (4–6 g/kg level) and lipase (2–6 g/kg level) activity and the intestinal morphometric measures (2–6 g/kg level), where the WP6 group recorded the highest values. WP diets increased the growth hormone (2–6 g/kg level) and reduced leptin hormone and glucose levels (2–6 g/kg level). WP diets boosted the immune-antioxidant indices (total protein, albumin, globulin, complement 3, lysozyme, nitric oxide, total antioxidant capacity, glutathione peroxidase, and catalase) in a level-dependent manner and the WP6 group attained the highest values. All experimental groups exhibited 100% survival at the end of the feeding trial. During the *A. sobria* challenge, the survival of fish was improved in a level-dependent manner (2–6 g/kg) (80%, 85%, 95%, respectively) compared to the control (70%), where the WP6 group recorded the highest survival. Noteworthy, WP diets especially at a level of 6 g/kg can be used as a feed supplement for improving the health, growth, immune-antioxidant functions, and disease resistance of Nile tilapia.

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Introduction

Future protein consumption is a major worry because of the large number of people who are still undernourished and the predicted growth in the population. Consequently, the potential for increased production from aquaculture is a must [1]. Aquaculture is one of the most dynamic industries in the world's food system [2, 3].

Nile tilapia (*Oreochromis niloticus*) is a freshwater species native to Africa, but they have been introduced to numerous countries worldwide [4]. In aquaculture, Nile tilapia is the third-most cultured fish species. Its production on farms has increased significantly over the past 20 years. In many developing nations, it is one of the most popular aquaculture species for addressing the issue of food security [5]. Egypt is currently a major global aquaculture powerhouse, accounting for 71% of the continent's output, and the top producer of aquaculture in Africa [6]. Significantly, Egypt is the world's third-largest producer of tilapia (following China and Indonesia), and the country's economy and food security greatly benefit from tilapia aquaculture [7].

Aquaculture intensification practices are among the industrial policies for increasing aquatic food [8]. Such practices lead to the development of disease problems especially the microbial ones [9]. Among the bacterial diseases, motile *Aeromonas* species is economically significant because it can cause epidemic outbreaks that result in high global mortality rates [10]. According to Shayo et al. [11], *Aeromonas sobria* (*A. sobria*) is a Gram-negative member of the motile *Aeromonas* species which is an opportunistic pathogen, and a secondary invader to damaged skin caused by toxins, other skin infections, or physical causes. Its toxins (hemolysin, aerolysin, and enterotoxin) induce bacterial hemorrhagic septicemia in stressed fish and are associated with numerous outbreaks, financial losses, and health risks to humans [12, 13].

Using medicinal plants as dietary supplements has gained great attention in aquaculture [14]. Medicinal plant-fortified diets improved growth, feeding performance, biochemical variables, immune/antioxidant parameters, and disease resistance against various fish pathogens [15, 16]. Because some medicinal plants have sufficient amounts of nutrients (such as unsaturated fatty acids, proteins, essential amino acids, vitamins, and minerals) to meet the nutritional requirements of the common fish species, they are regarded as appropriate feed additives in aquafeeds [17]. Some have been found to include bioactive substances that can enhance the functioning of digestive enzymes [18]. According to Pu et al. [19], some bioactive constituents have a positive effect on metabolic processes and can boost protein synthesis and trigger digestive enzymes. So, medicinal plants may be able to improve fish growth performance.

White poplar (*Populus alba* L.) (WP) is a prevalent tree in the region of the Mediterranean region and a member of the family *Salicaceae*, genus *Populus*. Active ingredients from the genus *Populus* are used in conventional medicine for a variety of biological purposes, such as antiviral, antifungal, antioxidant, and antitumor properties [20]. These biological activities are due to many secondary metabolites that were extracted from *P. alba*, such as flavonoids, derivatives of salicin, phenolic acids, anthocyanins, and polysaccharides [21]. Their active constituents are present in their leaves, bark, and buds [22]. To our knowledge, there is no data about using WP leaves powder as a feed supplement in fish diet. Consequently, this work represented the first attempt to investigate the potential effect of dietary WP leaf powder on the performance, digestive functions, immune responses, oxidant-antioxidant indices, and intestinal morphology of Nile tilapia.

Materials and methods

Preparation of the plant and gas chromatography/mass spectrometry assay (GC-MS)

Fresh WP leaves were obtained from the Desert Research Center, Egypt. The leaves were air-dried at room temperature (25 °C) for 20 days. Dried WP leaves were ground into a powder with milling. The WP was subjected to GC-MS analysis to determine its phytochemical components according to the Abo El-Fadl et al. [23] protocol. The GC-MS analysis of WP was performed using 1310 TRACE GC Ultra Gas Chromatographs (Thermo Fisher Scientific Inc., MA, USA). The carrier gas (helium) flowed at a steady rate of 1 mL/min.

Diets preparation

According to NRC [24], four isonitrogenous and isolipidic diets were created to meet the nutrient needs of Nile tilapia. WP0, WP2, WP4, and WP6 were basal diets supplemented with 0, 2, 4, and 6 g/kg WP, respectively. The crude protein (CP) was 37.18%, 37.22%, 36.95%, and 36.88%, while the crude lipid was 9.98%, 9.56%, 9.47%, and 9.33% in the WP0, WP2, WP4, and WP6 diets, respectively. The feed ingredients were mixed mechanically and then pelleted using a 1.5 mm meat mincer. The pellets were not extruded to avoid the destruction of

Table 1 Ingredients and chemical composition of the experimental diet content (% on a dry matter basis)

Ingredients	WP0 (basal)	WP2	WP4	WP6
WP	0.00	0.20	0.40	0.60
Ground yellow corn	24.30	24.10	23.90	23.70
Soybean meal	25.50	25.50	25.50	25.50
Corn gluten	11.00	11.00	11.00	11.00
Fish meal	18.00	18.00	18.00	18.00
Wheat	5.00	5.00	5.00	5.00
Wheat bran	9.00	9.00	9.00	9.00
Fish oil	6.00	6.00	6.00	6.00
Premix	1.20	1.20	1.20	1.20
Chemical analysis (%)				
DE (Kcal kg^{-1})*	2950.12	2977.40	2968.6	2955.10
Crude protein	37.18	37.22	36.95	36.88
Nitrogen free extract	42.53	41.91	41.66	41.90
Crude fiber	3.82	4.01	4.16	3.99
Fat	9.98	9.56	9.47	9.33
Ash	6.46	6.04	6.34	6.50
Lysine	2.01	2.18	2.00	2.07
Methionine	0.78	0.78	0.79	0.77

WP white poplar leave powder

Soybean meal and corn gluten of Transglobe, USA. Fish meal of Argentinean (Coomarpes Ltd. Mar del Plata, Argentina. The dry matter, crude protein, crude lipid, and crude ash were 90%, 44%, 0.5%, and 6% in soybean meal; 88%, 6.43%, 3%, and 1.06% in corn gluten; 94.17%, 67.25%, 11%, and 12.9% in fish meal

Each kg of premix contains: vitamin D (110,000 IU); vitamin E (11,000 mg); vitamin A (550,000 IU); vitamin C (50 g); vitamin K (484 mg); vitamin B1 (440 mg); vitamin B2 (660 mg); vitamin B3 (13,200 mg); vitamin B5 (1100 mg); vitamin B6 (1045 mg); vitamin B9 (55 mg); copper (330 mg); iodine (110 mg); selenium (44 mg); choline (110,000 mg); biotin (6.6 mg); zinc (6.6 g); manganese (1320 mg); iron (6.6 g)

* DE, digestible energy was determined according to Hepher et al. [26]

some heat-labile ingredients. The pellets were then air-dried at 25 °C for 24 h, rotating frequently to guarantee even drying, and kept refrigerated at 4 °C until needed.

As indicated in Table 1, the proximate chemical analysis of the basal diet was performed, according to the AOAC [25].

Fish, rearing conditions and experimental setup

El-Abbassa fish farms in Sharkia Province, Egypt provided the Nile tilapia (34.61 ± 0.16 g). According to CCoA [27], a routine assessment of the fish's health status was carried out before the trial. The fish were put in 100-liter well-aerated tanks with 10 fish each, filled with chlorine-free tap water. Before the experiment's commencement, fish were fed a basal diet until they reached satiation and were allowed to acclimate to the study's environment for 2 weeks. Any excretory wastes were siphoned each day, and all water in tanks was completely evacuated and replaced with fresh water every three days. The water quality indices were assayed in compliance with APHA [28] guidelines and maintained within the best-recommended ranges throughout the trials. The levels of dissolved oxygen (6.50 ± 0.40 mg/L), water temperature (26.4 ± 2.10 °C), pH (6.9 ± 0.20), ammonia (0.019 ± 0.003 mg/L), and nitrite (0.02 ± 0.013 mg/L) were maintained.

The Institutional Animal Care and Use Committee at Zagazig University reviewed and approved the experimental protocol (ZU-IACUC/2/F/2/2024). For 60 days, 160 fish were divided into four groups in four replicates (40 fish per group and 10 fish per replicate). Three times a day (9 a.m., 12 p.m., and 3 p.m.), the fish were given the experimental diets up to satiation. The unconsumed feed was gathered, dried, and weighed to calculate the accurate feed intake (FI). Fish were continuously observed throughout the experiment to look for any indications of disease or mortality.

Growth performance and survival rate percentage

At the onset and outset of the trial, the initial body weight (IBW) and final body weight (FBW) were assessed. In addition, the FI was determined. The weight gain (WG), feed conversion ratio (FCR), specific growth rate (SGR), and survival rate percentage (SR %) were calculated as follows:

$$\mathrm{WG(g)} = \mathrm{FBW(g)} - \mathrm{IBW(g)}$$

$$\mathrm{FCR} = \mathrm{FI(g)}/\mathrm{WG(g)}$$

$$\mathrm{SGR} = \left[\left(\log \mathrm{FBW} - \log \mathrm{IBW}\right)/\mathrm{time(days)}\right] \times 100$$

$$\mathrm{SR\%} = \left(\text{number of fish in each group remaining after the 60-day feeding period/initial number of fish}\right) \times 100$$

Sampling

After being starved for 24 h, fish were given a 100 mg/L benzocaine solution [29] to sedate them for sample collection at the end of the feeding trial (60 days). Blood samples were taken from the caudal vessels of 12 fish from each group using a 1 mL plastic syringe. The blood samples were centrifuged at 1750 ×*g* for 10 min to separate the serum. After that, the serum was stored at −20 °C until it was used for the biochemical and immunological analysis. Liver samples ($n = 12$) were collected immediately after the fish were dissected to be used in the hepatic oxidant/antioxidant assays. In addition, entire

intestinal samples ($n=12$) were taken on ice for digestive enzyme analysis. Other intestinal (foregut; $n=3$/group) samples were taken in 10% neutral buffered formalin for intestinal morphometric assay.

Digestive enzymes analysis

The entire intestinal samples were weighed and homogenized using a plastic pestle at a ratio of 1:10 in phosphate buffer saline (PBS) solution to measure the activity of digestive enzymes [30]. Following this procedure, each sample was centrifuged for three minutes at 4 °C at 13,000 ×*g* (Hettich Universal company, UK). After that, the supernatant was transferred to ice-filled microtubes to measure the activity of amylase and lipase enzymes. To determine the enzyme activity per g of protein, the total protein (TP) amount of each sample was determined following the Kruger [31] methodology.

The methodology of Bernfeld [32] was utilized to determine the amylase activity. Briefly, 1 mL of the substrate was mixed with 1 mL of enzyme and then incubated for 3 min at 20 ℃. After that, the enzyme reaction was stopped by adding 2 mL of dinitrosalicylic acid reagent. The tube containing the mixture was heated in water for 5 min followed by cooling using running tap water. Finally, 20 mL of water was added and the optical density (OD) of the brown reduction product was measured photometrically and an enzyme-free blank was assessed in the same manner. The Worthington [33] protocol was utilized to determine the lipase activity. Briefly, 5 mL of tributyrin was added to 5 mL of calcium chloride (0.05 M) and polyvinyl alcohol (PVA) (0.1 M) and then incubated at 37 °C for 1 h. Three mL of enzyme was added to the previous mixture followed by adding 20 mL ethanol (80%) to stop the reaction. Finally, titration with NaOH (0.1 M) was done using thymolphthalein as an indicator to estimate lipase activity.

Biochemical assays

Using Cusabio (Wuhan, Hubei, China) kits, Fish growth hormone (GH) (CSB-E12121Fh) and leptin hormone (LEP) (CSB-EL012870FI) were assessed. Purified fish GH was coated in Microelisa Stripplate wells with GH antibody to create a solid-phase antibody, the wells were filled with GH-containing material and GH antibody that had been tagged with horseradish peroxidase (HRP). Following thorough washing, the reactants formed an antibody-antigen-antibody-enzyme complex. 3, 3', 5, 5'–Tetramethylbenzidine substrate solution was then added, and when the HRP enzyme was present, it became blue. The reaction was then stopped by adding a solution of sulfuric acid. To identify LEP antigen targets in fish samples, LEP-ELISA Kits were developed using an HRP colorimetric detection technology and LEP antibody-LEP antigen interactions (immunosorbency). GH and LEP were measured using spectrophotometry at 450 nm. The OD of the samples was then compared to the standard curve to ascertain the levels of GH and LEP in the samples.

A commercial kit from Bio Diagnostics Company (Cairo, Egypt) (catalog No.; GL 1320) was used to estimate the glucose level. Using an enzymatic method, colorimetric glucose concentration measurement was achieved. Hydrogen peroxide (H_2O_2) was created when the enzyme glucose oxidase catalyzed the conversion of glucose to gluconic acid. Then, in the presence of glucose peroxidase, H_2O_2 combined with the chromogenic oxygen acceptor phenol-aminophenazone to produce a particular shade of pink. The intensity of the color generated determines the glucose concentration. From Stanbio Laboratory (Main St, Boerne, USA), serum proteins electrophoretic distribution, including TP (catalog No.; SB-0250-500) and albumins (ALB) (catalog No.; SB-028-500) were estimated. TP and ALB were assayed calorimetrically using commercial kits (Spinreact company Girona, Spain). The copper ion in the reagent reacted with the peptide bonds in serum to produce a blue-violet color in an alkaline media, which was used for protein estimation. The intensity of the violet color, which was a result of the number of peptide bonds, was used to measure the serum TP. At an acid pH of 4.2, the protein and bromocresol green bind to form an indicator that changes color from yellow-green to green-blue, which was the basis for the ALB measuring method. TP and ALB concentrations were measured at 630 nm and were related to color intensity. While globulins (GLB) were estimated by subtracting ALB from TP [34].

Immune and oxidant/antioxidant assays

Utilizing a spectrophotometric method based on the lysis of freeze-dried *Micrococcus lysodeikticus* particles (Sigma Co., USA), the serum's lysozyme (LYZ) activity was determined [35]. *M. lysodeikticus* suspension (pH = 6.2, 0.2 mg/mL in 0.05 M PBS) was mixed with the serum and then kept for 5 min at 25 °C. The OD was assessed at 540 nm using a 5010 Photometer (BM Co. Germany) every minute for successive 5 min. The calibration curve was done by serial dilutions of lyophilized chicken egg-white LYZ (Sigma Co., USA) to evaluate the LYZ concentration in the serum. Using the spectrophotometry approach, complement 3 (C3) (CSB-E09727s) was performed by utilizing diagnostic kits (Cusabio, Wuhan, Hubei, China) based on the competitive inhibition enzyme immunoassay technique. The antibody initiated the competitive inhibition process with the aid of HRP-labeled C3 and unlabeled C3. The color changed to the sample's C3 content after the substrate solution was

added to the wells. Finally, the intensity of the color was measured. Nitric oxide (NO) was assessed following the Montgomery and Dymock [36] technique through the reaction between 2,6-xylenol and nitrate in the presence of sulphuric acid medium and ammonium chloride. NO was evaluated at an OD of 304 nm spectrophotometry.

Hepatic malondialdehyde (MDA) (catalog No. MD2529), serum total antioxidant capacity (TAC) (catalog No. TA 25 13), hepatic glutathione peroxidase (GPx) (Catalog. No. GP2524), and hepatic catalase (CAT) (Catalog. No. CA2517) were assessed spectrophotometry following the manufacturer's instructions using commercially available Bio-Diagnostic kits (Cairo, Egypt). The MDA was evaluated following the thiobarbituric acid technique. The MDA was calculated in the thiobarbituric acid reactive mixture at 535 nm. TAC was assessed through the reaction of antioxidants in the sample with H_2O_2. The remnant H_2O_2 is evaluated colorimetrically at 505 nm by the enzymatic reaction that includes the conversion of 3, 5, dichloro −2− hydroxy benzensulfonate to a colored endproduct. GPx activity was evaluated as the amount that converts 1 nmol of nicotinamide adenine dinucleotide phosphate (NADPH) to $NADP^+$ per min. GPx was assessed at 25 °C using an absorbance of 340 nm. CAT activity was assessed based on the enzymatic reaction between the H_2O_2, potassium phosphate (pH 7.0), and samples. The molar attenuation coefficient of H_2O_2 was assessed using the UV-VIS spectrophotometer at 240 nm.

Histopathological workup

Intestinal specimens (foregut) were taken, immediately fixed for 24 h in a 10% buffered neutral formalin solution, dehydrated in ethanol gradually (70, 80, 95, 95, and 100%), cleared in xylene, and embedded in paraffin. Paraffin sections of 5 µm thickness were cut with a Leica RM 2155 microtome (England). Following preparation, the sections were routinely stained with hematoxylin and eosin stains and examined under a microscope [37]. Intestinal villi length (VL), intestinal villi width (VW), muscular coat thickness (MCT), and goblet cell count (GCC) were measured. Sections were inspected using a light microscope that was outfitted with a microscopic camera (Leica Microsystem, Germany, full HD and image analysis software).

Bacterial challenge test

The *A. sobria* strain was isolated from an infected *O. niloticus* (Aquatic Animal Medicine Department, Zagazig University, Egypt). The isolate was cultivated in tryptic soy broth (TSB) (Difco, Detroit, MI, USA) and incubated at 25 °C for 24 to 48 h. The isolate was identified using the VITEK® 2 compact (BioMérieux, Missouri, USA). *A. sobria's* median lethal dose 50 (LD_{50}) was determined according to Abdel Rahman et al. [38] using 100 fish divided into five treatments with two replications. Intraperitoneally (IP) injections of 0.1 mL of doses (10^6, 10^7, 10^8, and 10^9 CFU/fish) of 24 h-live bacteria were administered to the first through fourth treatments, while treatment five received an IP injection of 0.1 mL sterile TSB. After that, the mortalities were recorded every day for four days. The Probit Analysis Program version calculated the LD_{50} to be 3×10^7 CFU/fish. To conduct the challenge test, a sub-lethal dose of 1.5×10^7 CFU/fish was employed. After the feeding trial, the fish was fasted for 24-h, and the bacterial isolate was given IP to 20 fish/group (5 fish/ replicate) at a dosage of 0.1 mL (1.5×10^7 CFU/fish). Fish mortalities and clinical abnormalities were noted for 14 days following injection.

Statistical analysis

The data's normality was confirmed using the Shapiro-Wilk test. The data was analyzed using a one-way analysis of variance (ANOVA) using SPSS version 18 (SPSS, Chicago, IL, USA). The significance threshold was set at $P<0.05$, and the data was reported as mean ± standard error (*SE*). Tukey's multiple range tests were used to determine the differences between the means. The survival Kaplan-Meier model was used to determine the fish survival rate during the bacterial challenge. Pearson's correlation was done to determine the relationships between the WP active constituents and the fish performance parameters including the FBW, WG, FI, FCR, and SGR.

Results

WP GC-MS analysis

The major detected constituents in WP were pyrocatechol, trimethylsilyl (TMS) (18.49%), beta-amyrone (13.31%), heptacosane (8.43%), vitamin E (7.46%), γ-sitosterol (7.38%), β-sitosterol, TMS derivative (4.91%), catechol, TMS derivative (6.58%), α-linolenic acid, TMS derivative (5.85%), and phytol (5.47%) (Table 2).

Growth metrics and SR

Table 3 displays the growth metrics of Nile tilapia-fed WP diets. In a level-dependent manner, WP diets substantially (P <0.001) improved the FBW, WG, and SGR when compared to the control (WP0), with WP6 recording the highest values. The FI of fish was not substantially ($P=0.21$) impacted by the WP diets. The WP diets considerably lowered the FCR in a level-dependent manner, with WP6 registering the lowest FCR value. All experimental groups displayed 100% SR at the end of the feeding trial (Table 3).

The relationships between the fish performance parameters and the most obvious active principles in WP are

Table 2 The findings of GC-MS analysis of white poplar (WP) leaves powder

Peak	Compound	Rt (min)	Area (%)
1	3-Butyn-2-amine, N-methyl-	12.517	2.89
2	Catechol, TMS derivative	17.478	6.58
3	Benzoic Acid, TBDMS derivative	18.52	0.45
4	Pyrocatechol, TMS	19.939	18.49
5	Citronellyl propionate	26.559	1.01
6	Dodecanoic acid, methyl ester	27.423	0.2
7	Stearic acid	27.95	1.25
8	Hexadecanoic acid, ethyl ester	28.093	1.73
9	Palmitic Acid, TMS derivative	28.608	1.33
10	Dihydroergosterol	29.083	0.29
11	Phytol	29.203	5.47
12	methyl tetradecadienoate	29.62	1.08
13	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z,Z)-	29.701	4.74
14	Phosphoric acid, dioctadecyl ester	29.781	0.66
15	α-Linolenic acid, TMS derivative	30.118	5.85
16	Hexacosane	32.367	1.17
17	Dicyclohexyl phthalate	32.768	1.11
18	Heptacosane	33.895	8.43
19	Tetracosane	35.228	1.66
20	1-Hexacosanol, TMS derivative	35.537	0.52
21	Vitamin E	36.825	7.46
22	γ-Sitosterol	38.043	7.38
23	beta-Amyrone	38.101	13.31
24	β-Sitosterol, TMS derivative	38.175	4.91
25	(Z)–4,4-Dimethyl-1,2-epoxy-1-(4-methylphenyl)pentan-3-one	38.238	0.94
26	4-Methylpent-3-en-2-yl benzoate	38.347	1.09

Rt retention time

shown in Figs. 1, 2 and 3. The FBW, WG, and SGR % showed significant strong positive ($P<0.001$) correlations with pyrocatechol TMS ($r=0.95$, 0.95, and 0.97), beta-amyrone ($r=0.94$, 0.94, and 0.95), and heptacosane ($r=0.93$, 0.94, and 0.95), respectively. The FI showed a non-significant ($P>0.05$) weak positive correlation with pyrocatechol ($r=0.37$), TMS, beta-amyrone ($r=0.37$), and heptacosane ($r=0.37$). On the other hand, the FCR value demonstrated a significantly strong negative correlation ($P<0.001$), where the observed r value was 0.99, 0.98, and 0.97 with pyrocatechol, TMS, beta-amyrone, and heptacosane, respectively.

Digestive enzymes

The activity of amylase (Fig. 4A) was significantly improved (P ˂0.001) by WP diets (4–6 g/kg level) comparable to the control, with no notable change between the control and WP2 diets. The activity of lipase (Fig. 4B) was significantly improved (P ˂0.001) by WP diets (2–6 g/kg level) comparable to the control in a level-dependent manner.

Biochemical indices

Table 4 displays the blood biochemical indices of Nile tilapia fed on WP diets. In a level-dependent manner, WP diets (2–6 g/kg level) substantially (P ˂0.001) raised the GH level in comparison to the control. WP diets significantly reduced the LEP level ($P<0.001$) when compared to the control group; however, there was no significant difference observed between the WP4 and WP6 groups. When compared to the control, WP diets (2–6 g/kg level) substantially (P ˂0.001) decreased the blood glucose level. In a level-dependent manner, WP diets (2–6 g/kg level) markedly raised (P ˂0.001) the serum levels of TP and ALB in comparison to the control. GLB level was substantially improved (P ˂0.001) by WP diets (4–6 g/kg level), with no substantial difference between the WP2 and control.

Immune and oxidant-antioxidant indices

Table 5 displays the immune and oxidant/antioxidant indices of Nile tilapia given WP diets. There was no substantial difference ($P=0.47$) in the MDA level by feeding on WP diets. In a level-dependent manner, WP diets (2–6 g/kg level) substantially (P ˂0.001) raised the levels of C3, LYZ,

Table 3 Growth parameters and survival rate (%) of Nile tilapia fed on white poplar (WP) leaves powder diets for 60 days

Parameters	WP0	WP2	WP4	WP6	*P* value
IBW (g/fish)	35.31 ± 0.15	34.96 ± 0.07	34.41 ± 0.45	34.97 ± 0.35	0.29
FBW (g/fish)	56.32 ± 0.65^{d}	61.87 ± 0.84^{c}	73.13 ± 1.43^{b}	96.35 ± 0.59^{a}	< 0.001
WG (g/fish)	21.01 ± 0.80^{d}	26.91 ± 0.79^{c}	38.72 ± 1.17^{b}	61.38 ± 0.69^{a}	< 0.001
FI (g/fish)	51.18 ± 0.55	51.58 ± 0.22	52.26 ± 0.02	51.60 ± 0.26	0.21
FCR	2.43 ± 0.08^{a}	1.91 ± 0.05^{b}	1.35 ± 0.03^{c}	0.84 ± 0.01^{d}	< 0.001
SGR (%/day)	0.35 ± 0.05^{d}	0.41 ± 0.04^{c}	0.55 ± 0.04^{b}	0.73 ± 0.08^{a}	< 0.001
SR (%)	100	100	100	100	-

Values (mean ± *SE*) not sharing the same superscript letter in the same raw are significantly different ($P<0.05$; One-way ANOVA; Tukey's multiple range). *IBW* initial body weight, *FBW* final body weight, *WG* weight gain, *FI* feed intake, *FCR* feed conversion ratio, *SGR* specific growth rate, *SR (%)* survival rate %. WP0, WP2, WP4, and WP6 were fish groups fed on basal diets supplemented with 0, 2, 4, and 6 g/kg WP leaves powder, respectively

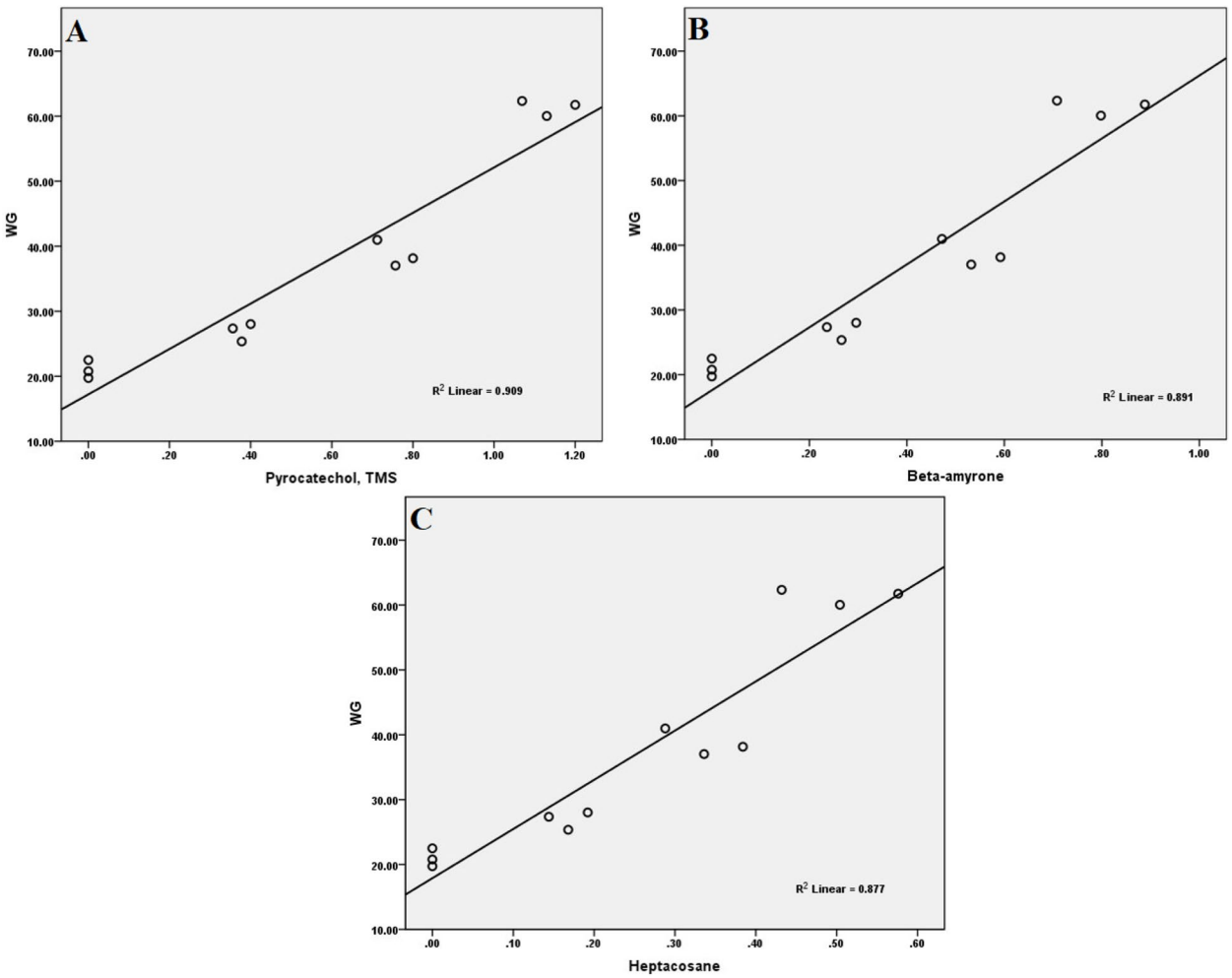

Fig. 1 Relationships (Pearson correlation coefficients) between active principles in white popular (WP) and weight gain (WG) of Nile tilapia. **A** Pyrocatechol, TMS. **B** Beta-amyrone. **C** Heptacosane

NO, TAC, GPx, and CAT in comparison to the control, where the WP6 group recorded the highest values.

Intestinal morphometrics

There is a substantial difference in intestinal histomorphometric measures among the groups, as Table 6; Fig. 5 demonstrate. In all WP groups (2–6 g/kg level), there was a level-dependent improvement ($P < 0.01$) in the VL, VW, MCT, and GCC when compared to the control group, where the WP6 group showed the highest outcomes.

Challenge test

The survival was increased in a level-dependent manner during *A. sobria* challenge (2–6 g/kg level). Compared to the control (WP0) (70%), the survivability was 80%, 85%, and 95% in the WP2, WP4, and WP6 groups (Fig. 6).

Discussion

The current research discussed the potential promoting effect of WP as a feed supplement in Nile tilapia diets. The outcomes of this research indicated that WP dietary supplementation (2–6 g/kg) boosted the growth of Nile tilapia. This result could be attributed to that WP diets improved the GH and suppressed the LEP levels in the experimental fish as investigated in this study. In fish, the primary regulator governing somatic growth is GH through proliferation, hypertrophy, and/or hyperplasia, leading to skeletal muscle growth [39]. One of the main hormones involved in controlling appetite is LEP [40]. The primary physiological function of LEP is related to maintaining energy balance by consuming less food and using more energy. In addition, the LEP hormone can cause appetite suppression [41]. Increasing the GH and

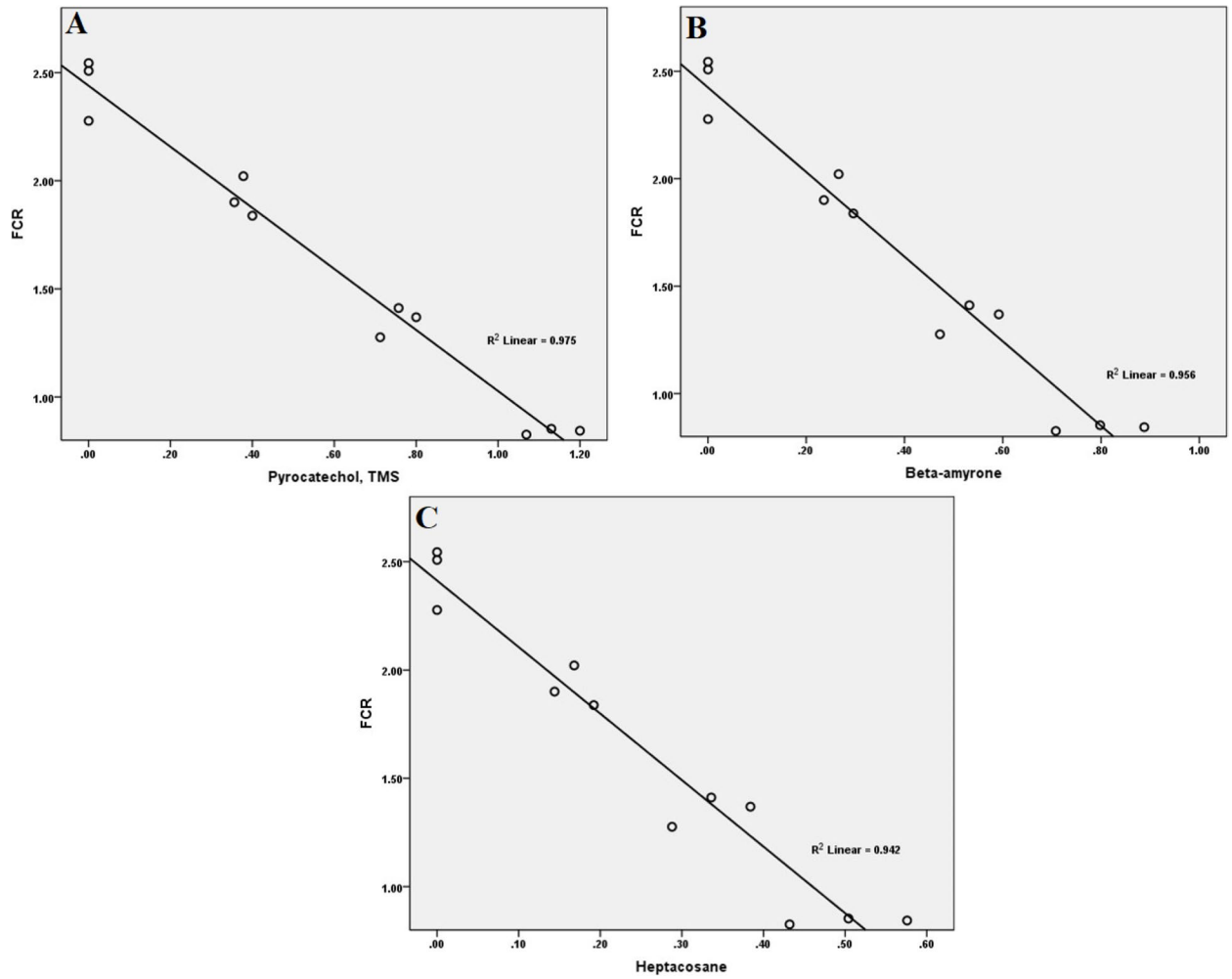

Fig. 2 Relationships (Pearson correlation coefficients) between active principles in white popular (WP) and feed conversion ratio (FCR) of Nile tilapia. **A** Pyrocatechol, TMS. **B** Beta-amyrone. **C** Heptacosane

reducing the LEP were reported in Nile tilapia fed on basil (*Ocimum basilicum*) and red pepper (*Capsicum annuum*) supplemented diets [42].

Another attribution for the improved growth of Nile tilapia in this research is the activation of digestive enzyme activity (amylase and lipase) by WP diets. In fish, amylase has an important role in digestion through the breakdown of starch and glycogen [43]. Lipase is the fat-digesting enzyme in fish and is essential for the intestine's absorption of lipids [44]. Consequently, activation of the enzyme activity leads to improved digestion [45, 46]. Large molecules are broken down by digestive enzymes into smaller ones that the cells can use to produce energy or other biomolecules. Fish growth is correlated with its ability to digest food, which is regulated by the digestive enzyme activity [47]. In this study, WP diets enhanced the intestinal morphology by increasing the VL, VW, MCT, and GCC. This amelioration in the intestinal morphology could enhance absorption through the intestinal villi [48]. Improved growth in this study could be attributed to the WP's major active principals (pyrocatechol TMS, beta-amyrone, and heptacosane) detected in our GC-MS results. These active principals showed a positive correlation with the fish performance parameters as demonstrated by correlation tests.

Blood parameters, such as glucose, can be used to evaluate the fish's health. The most important factor influencing the blood glucose level is diet formulation [49]. The blood glucose decreased in the WP-included groups, with the lowest value registered in the WP6 group. These outcomes mean that WP inclusion increased the utilization of carbohydrates that beneficially boosted the fish's metabolism [50]. The α-linolenic acid (a major component of WP) had hypoglycemic activity and enhanced insulin resistance [51]. A similar hypoglycemic impact

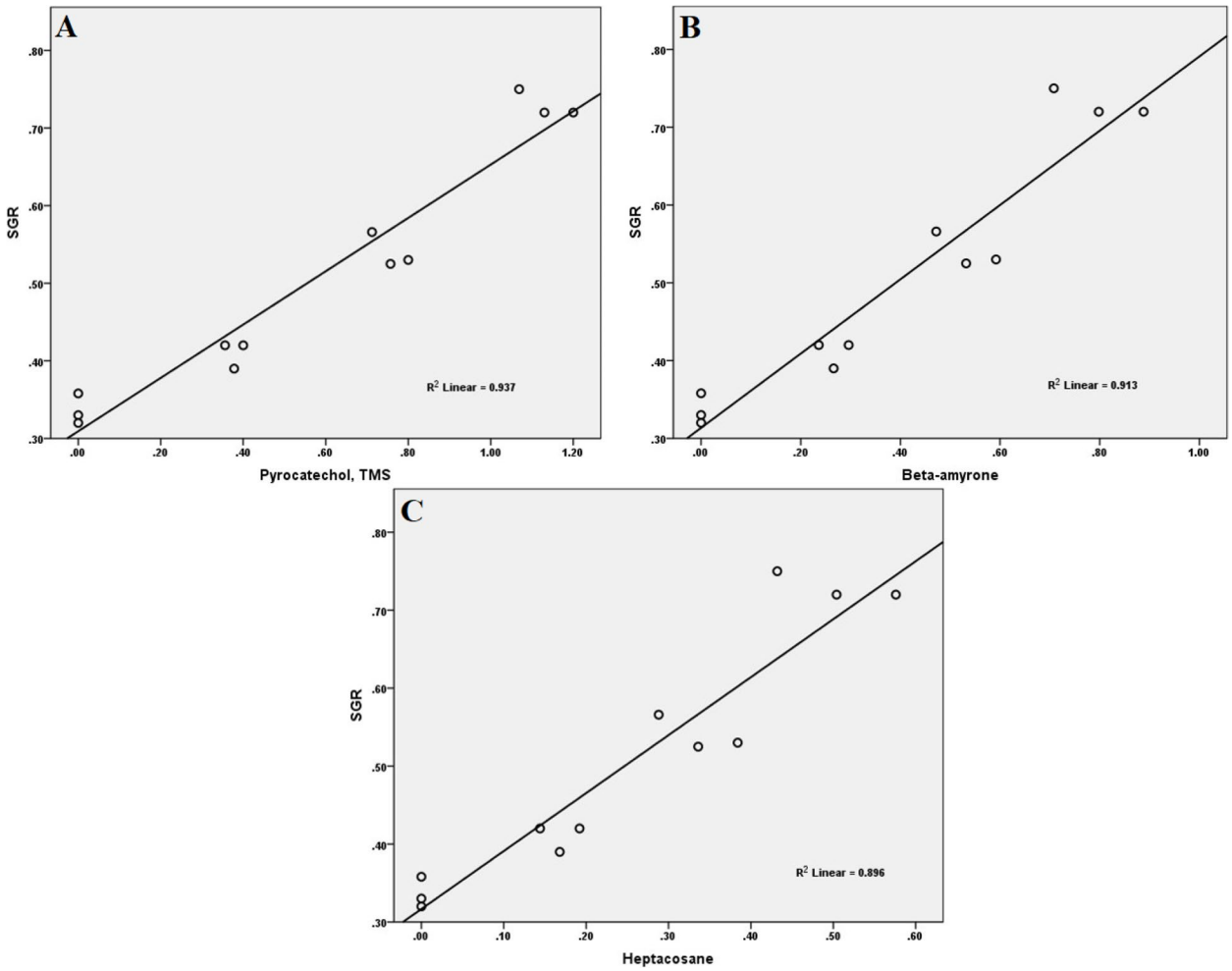

Fig. 3 Relationships (Pearson correlation coefficients) between active principles in white popular (WP) and specific growth rate (SGR) of Nile tilapia. **A** Pyrocatechol, TMS. **B** Beta-amyrone. **C** Heptacosane

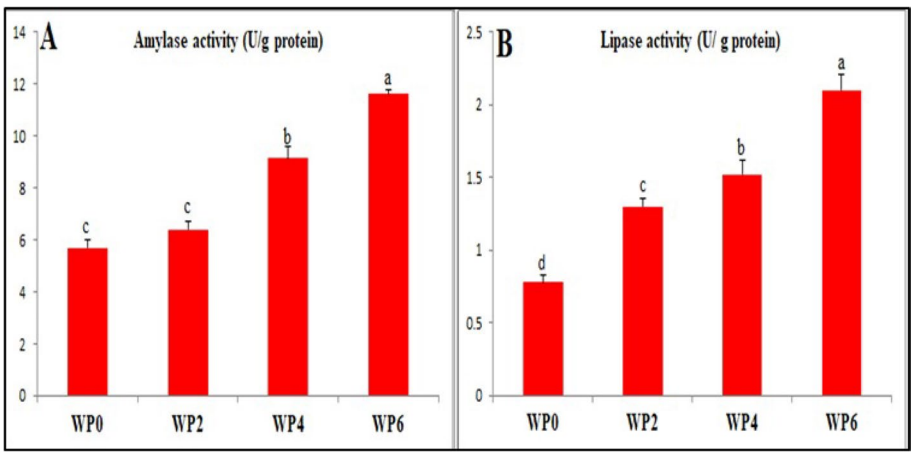

Fig. 4 Digestive enzyme activity of Nile tilapia-fed white poplar (WP) leaves powder for 60 days. (**A**) Amylase. (**B**) Lipase. Values (mean ± *SE*) not sharing the same superscript letter in the same bar are significantly different ($P < 0.05$; One-way ANOVA; Tukey's multiple range). WP0, WP2, WP4, and WP6 were fish groups fed on basal diets supplemented with 0, 2, 4, and 6 g/kg WP leaves powder, respectively

Table 4 Biochemical parameters of Nile tilapia fed on white poplar (WP) leaves powder diets for 60 days.

Parameters	WP0	WP2	WP4	WP6	*P* value
GH (pg/mL)	421.33 ± 5.23^{d}	561.00 ± 11.26^{c}	773.33 ± 3.84^{b}	916.00 ± 8.02^{a}	< 0.001
LEP (pg/mL)	171.88 ± 11.39^{a}	102.59 ± 1.44^{b}	76.56 ± 1.23^{c}	65.77 ± 0.67^{c}	< 0.001
Glucose (mg/dL)	140.50 ± 0.76^{a}	127.61 ± 1.28^{b}	98.46 ± 1.98^{c}	67.35 ± 1.16^{d}	< 0.001
TP (g/dL)	2.74 ± 0.04^{d}	3.14 ± 0.06^{c}	3.80 ± 0.20^{b}	5.32 ± 0.19^{a}	< 0.001
ALB (g/dL)	1.50 ± 0.03^{d}	1.70 ± 0.05^{c}	1.96 ± 0.06^{b}	3.08 ± 0.08^{a}	< 0.001
GLB (g/dL)	1.24 ± 0.01^{c}	1.44 ± 0.02^{c}	1.84 ± 0.14^{b}	2.24 ± 0.12^{a}	< 0.001

Values (mean ± *SE*) not sharing the same superscript letter in the same raw are significantly different ($P < 0.05$; One-way ANOVA; Tukey's multiple range). *GH* growth hormone, *LEP* leptin hormone, *TP* total protein, *ALB* albumin, *GLB* globulin. WP0, WP2, WP4, and WP6 were fish groups fed on basal diets supplemented with 0, 2, 4, and 6 g/kg WP leaves powder, respectively

Table 5 Immune and oxidant/antioxidant parameters of Nile tilapia fed on white poplar (WP) leaves powder diets for 60 days.

Parameters	WP0	WP2	WP4	WP6	*P* value
C3 (µg/mL)	37.32 ± 0.48^{d}	42.52 ± 1.47^{c}	54.62 ± 2.54^{b}	75.97 ± 0.95^{a}	< 0.001
LYZ (ng/mL)	7.39 ± 0.61^{d}	10.56 ± 0.32^{c}	14.22 ± 0.56^{b}	19.60 ± 0.34^{a}	< 0.001
NO (ng/mL)	2.80 ± 0.38^{d}	5.54 ± 0.35^{c}	11.34 ± 0.66^{b}	16.66 ± 0.30^{a}	< 0.001
MDA (µmol/g tissue)	15.17 ± 0.08	14.58 ± 0.22	14.72 ± 0.21	15.07 ± 0.47	0.47
TAC (mmol/L)	0.28 ± 0.02^{d}	0.49 ± 0.03^{c}	0.86 ± 0.04^{b}	1.25 ± 0.08^{a}	< 0.001
GPx (U/g protein)	15.91 ± 1.76^{d}	27.41 ± 0.59^{c}	31.80 ± 0.39^{b}	43.33 ± 1.61^{a}	< 0.001
CAT (U/g protein)	0.88 ± 0.17^{d}	3.49 ± 0.55^{c}	6.68 ± 0.30^{b}	10.55 ± 0.33^{a}	< 0.001

Values (mean ± *SE*) not sharing the same superscript letter in the same raw are significantly different ($P < 0.05$; One-way ANOVA; Tukey's multiple range). *C3* complement 3, *LYZ* lysozymes, *NO* nitric oxide, *MDA* malondialdehyde, *TAC* total antioxidant capacity, *GPx* glutathione peroxidase, *CAT* catalase. WP0, WP2, WP4, and WP6 were fish groups fed on basal diets supplemented with 0, 2, 4, and 6 g/kg WP leaves powder, respectively

Table 6 Intestinal morpho-metrics of Nile tilapia fed on white poplar (WP) leaves powder diets for 60 days.

Parameters	WP0	WP2	WP4	WP6	*P* value
VL (µm)	196.67 ± 10.06^{d}	265.00 ± 7.50^{c}	353.67 ± 9.47^{b}	457.00 ± 10.80^{a}	0.002
VW(µm)	55.66 ± 4.43^{d}	68.66 ± 1.20^{c}	81.33 ± 6.00^{b}	105.00 ± 7.63^{a}	< 0.001
MCT (µm)	57.35 ± 1.48^{d}	82.12 ± 1.27^{c}	93.33 ± 1.17^{b}	104.68 ± 1.28^{a}	< 0.001
GCC	11.00 ± 0.57^{d}	18.00 ± 0.57^{c}	23.00 ± 0.88^{b}	28.00 ± 0.33^{a}	< 0.001

Values (mean ± *SE*) not sharing the same superscript letter in the same raw are significantly different ($P < 0.05$; One-way ANOVA; Tukey's multiple range). VL, villus length; VW, villus width; MCT, muscular coat thickness; GCC, goblet cells count. WP0, WP2, WP4, and WP6 were fish groups fed on basal diets supplemented with 0, 2, 4, and 6 g/kg WP leaves powder, respectively

was achieved by another member of the family *Salicaceae* (*Populus balsamifera*) in rats [52].

TP, ALB, and GLB are crucial markers for assessing immune functions and denoting an improvement in the innate immune response [53, 54]. Non-specific immunity is the first defense against any infection. Leucocytes produce LYZ, a component that is necessary for phagocytosis and activates the complementary system. Moreover, an essential component known as C3 performs several immunological tasks, such as regulating the inflammatory response and getting rid of invasive infections [55]. Phagocytic cells are vital components of innate immunity that phagocytize pathogens. To help phagocytes fight off infections, the macrophages release NO, a highly reactive oxygen molecule with antibacterial properties [56, 57]. In this study, WP diets improved the immune functions of Nile tilapia by improving the TP, ALB, GLB, LYZ, C3, and NO activity. As well as improving the fish resistance against *A. sobria* by increasing the survival during the challenge. These results could be attributed to the biologically active constituents in WP (vitamin E, β-sitosterol, TMS derivative, α-linolenic acid, and phytol). Vitamin E improved the immune function of Caspian brown trout (*Salmo trutta caspius*) [58] and Nile tilapia [59]. α-linolenic acid improved the non-specific immune functions in Nile tilapia by enhancing

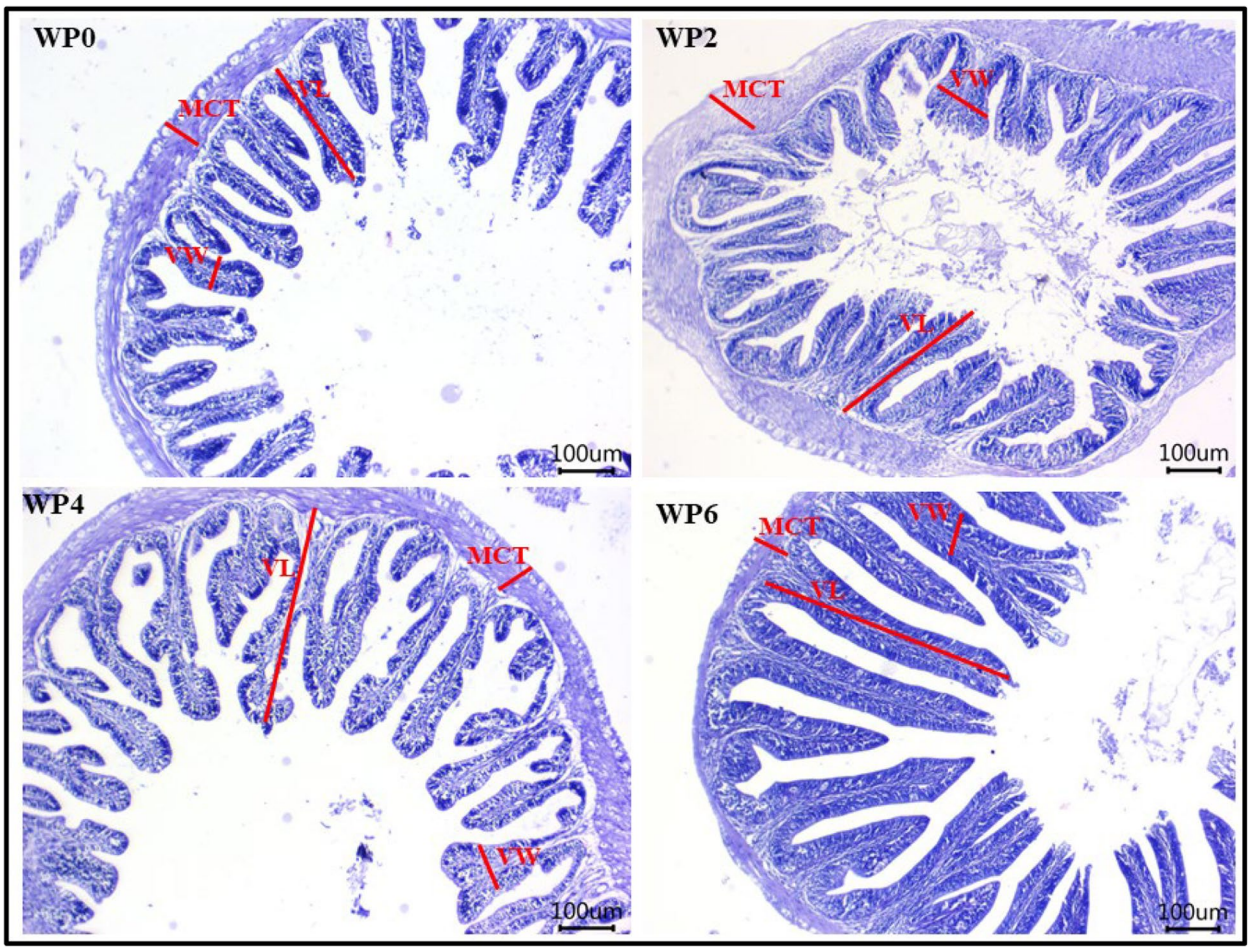

Fig. 5 Showing the effect of dietary supplementation of white poplar (WP) leaves powder for 60 days on the histomorphology of Nile tilapia regarding villous length (VL), villous width (VW), and muscular coat thickness (MCT). WP0, WP2, WP4, and WP6 were fish groups fed on basal diets supplemented with 0, 2, 4, and 6 g/kg WP leaves powder, respectively

the LYZ, NO production, and phagocytic activity [60]. β-sitosterol is a detected compound in our GC-MS which has immunostimulant activity [61]. Phytol activates the immune system by promoting the expression of several chemokines and cytokines [62].

According to Trenzado et al. [63], fish's antioxidant defense system stops the production of reactive oxygen species (ROS) under normal physiological conditions through the use of enzymes like CAT and GPx. In this study, the serum TAC, hepatic CAT, and hepatic GPx were significantly improved by WP diets. These results could be attributed to the antioxidant properties of some active constituents (such as pyrocatechol, TMS, vitamin E, β-sitosterol, TMS derivative, catechol, α-linolenic acid, and phytol) of WP [64], which were detected in our GC-MS. In addition, pyrocatechol and catechol were reported to have powerful antioxidant activities through free radical scavenger capacity [65]. Vitamin E stops the synthesis of peroxides [66]. Li et al. [67] found that there was a correlation between the activity of the fish antioxidant defense system and vitamin E. Similar results were obtained in Caspian trout (*Salmo caspius*), where there was a positive relationship observed between dietary vitamin E and serum GPx activity [68]. The hypothesis that vitamin E is involved in the GPx activity is supported by the observations of this phenomenon in hybrid snakehead (*Channa argus*× *Channa maculata*) [66] and mirror carp (*Cyprinus carpio*) [69]. β-sitosterol has antioxidant potential through ROS scavenger activity [70] and/or activation of the antioxidant enzyme (CAT) [71]. α-Linolenic acid had antioxidant activity in juvenile Russian sturgeon (*Acipenser gueldenstaedti*) [72]. Phytol has potent antioxidant activities through scavenging ROS and/or nitrogen species that are generated by metabolism and cellular stress [62].

Overall, WP diets could be used as feed supplements to improve Nile tilapia health, growth, and resistance to bacterial challenge which help in the sustainable development of aquaculture.

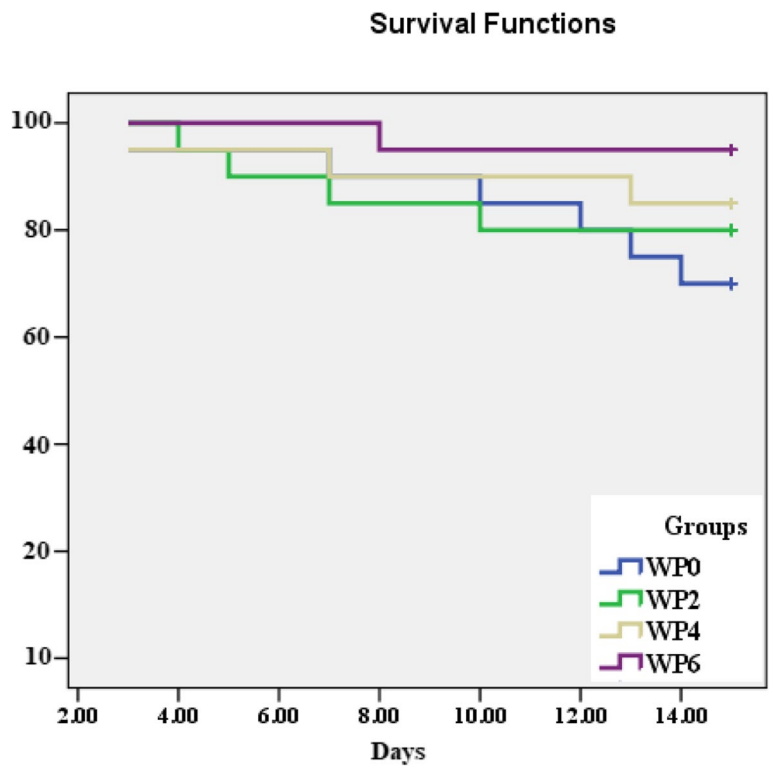

Fig. 6 Survival Kaplan-Meier curves during *A.sobria* challenge of Nile tilapia after feeding on white poplar (WP) leaves powder for 14 days. WP0, WP2, WP4, and WP6 were fish groups fed on basal diets supplemented with 0, 2, 4, and 6 g/kg WP leaves powder, respectively

Conclusions

We can conclude that WP could be used as feed supplements in Nile tilapia diets, especially at 6 g/kg level for improving the growth, digestive functions, immune-antioxidant indices, and intestinal morpho-metrics. Further researches are needed to investigate the higher WP supplementary levels. In addition, investigation of the effects of dietary WP at the transcriptomic levels is also needed.

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Authors' contributions
R.E. I., M. S., M. E., H. A. G., F. E., E. M. Y., A. A. A., M. F. B., S. J. D., A. N. A.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization. R. E. I.: Writing – original draft. R. E. I., M. S. &A. N. A.: Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

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Data availability
All data generated or analyzed during this study are included in this article.

Declarations

Ethics approval and consent to participate
The Institutional Animal Care and Use Committee of Zagazig University, Egypt approved the experimental protocol (ZUIACUC–2-F–3–2024). All methods were performed in accordance with the relevant guidelines and regulations. Informed consent to has been obtained from the private farm owners.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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