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DIETARY CURCUMIN NANOPARTICLES IMPROVE GROWTH PERFORMANCE, OXIDATIVE STATUS AND IMMUNE RESPONSE OF EUROPEAN SEABASS (*DICENTRARCHUS LABRAX*)

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Abstract

A 90 days of feeding trial was employed for evaluating the effects of dietary supplementation with curcumin nanoparticles (CURNPs) on growth performance, hematological and biochemical parameters, immune response, antioxidant status, and intestinal and hepatic architecture in European seabass (*Dicentrarchus labrax*) fingerlings. Fish (n = 300, initial body weight = 10.02 g ± 0.11 g) received supplementary CURNPs at rates of 0 (control; CURNPs0), 50 (CURNPs50), 100 (CURNPs100) and 150 (CURNPs150) mg/kg diet for 90 days. Growth performance (final body weight (FBW), daily weight gain (DWG), weight gain (WG), relative growth rate (RGR), and protein efficiency ratio (PER)) and feed uptake (feed conversion rate (FCR)) were improved significantly in CURNPs (100 and 150 mg/kg)-supplemented groups. The hematological parameters red blood corpuscles (RBCs), hemoglobin (Hb), and packed cell volume (PCV) were improved significantly (P<0.001) in CURNPs150 and CURNPs100 groups, while CURNPs150 had the lowest values of monocytes (P = 0.019) and heterophils (P<0.005) when compared with other groups. Glucose was higher and lipid profile (total cholesterol and triglycerides) were lower in all CURNPs-supplemented groups as compared to the control group. Fish fed with CURNPS at various levels exhibited remarkable increase in digestive enzymes (amylase and lipase), immune markers (immunoglobulin G (IgG), lysozyme and phagocytic indices), and antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)). A noticeable decrease (P<0.05) in malondialdehyde (MDA) and considerable upregulation of hepatic insulin-like growth factor 1 (*IGF1*) and interleukin-1beta (*IL-1β*) mRNA levels were found in CURNPs-supplemented fish in a dose-dependent manner. The down regulation of hepatic heat shock protein 70 (*HSP70*) was observed in CURNPs150. Intestinal and hepatic architectures were improved significantly by CURNPs. In conclusion, CURNPs supplementation improved growth performance, feed consumption and digestion, hematological and biochemical markers, immune response, and redox homeostasis in *D. labrax*. Therefore, CURNPs could be supplemented in the diets of *D. labrax* as a natural alternative to promote overall performance.

Key words: curcumin, *Dicentrarchus labrax*, nanoparticles, antioxidants, immune response

Aquaculture is an important source of animal protein and one of the fastest growing and diverse forms of food production globally. The expansion of the industry has provided different approaches to mitigate socio-ecological impacts related to aquaculture production systems (Farag et al., 2021). However, the intensification of fish production needs a special strategy for improving the health and productivity of fish under different environmental issues (Abdelnour et al., 2023; Adenaya et al., 2023). In this context, the antibiotics have been applied extensively for controlling diseases outbreaks in fish (Bondad-Reantaso et al., 2023). Due to the abuse of antibiotics, the bacteria are more resistant and antibiotic-resistant infections represent a major threat not only to humans, but also for terrestrial and aquatic animals (Bondad-Reantaso et al., 2023). Herbs and their constituents are considered an incredible source of therapeutically essential substances that may be utilized to treat a variety of diseases and provide health benefits (Mahmoud et al., 2021). Thousands of functional bioactive secondary substances from herbs were discovered via extensive publications published in the last century (Heidari et al., 2023; Swelum et al., 2021).

Curcumin (CUR) is a water-insoluble polyphenol extracted from the rhizome of *Curcuma longa* (Abdelnour et al., 2020). CUR and its derivatives exhibited antimicro-

bial, antioxidant, appetite enhancing, anti-inflammatory, immune-modulatory and gastro-protective influences on animal and fish health (Alagawany et al., 2021; Heidari et al., 2023). For instance, dietary fortification of CUR in Nile tilapia (*Oreochromis niloticus*) diets enhanced the growth, immunological variables, antioxidant status, feed utilization and the disease resistance and other environmental issues as evidenced by multi authors (Abdel-Ghany et al., 2023; Midhun et al., 2016; Sruthi et al., 2018). The same positive effects of CUR were observed in other fish species such as the crucian carp (*Carassius auratus*) (Jiang et al., 2016), large yellow croaker (*Larimichthys crocea*) (Ji et al., 2021), and rainbow trout (*Oncorhynchus mykiss*) (Yonar et al., 2019). Moreover, this molecule has shown protective effects against toxic elements such as silver nanoparticles toxicity in common carp (*Cyprinus carpio*) (Pirani et al., 2021) and hexavalent chromium in *O. niloticus* (Mohamed et al., 2020). However, the low oral bioavailability, poor aqueous solubility and chemical instability are the major factors that limit the use of CUR (Flora et al., 2013).

Several approaches, including nano-formulation, have been developed to improve the solubility and intracellular delivery of CUR (Abdelnour et al., 2021). Nowadays, the application of nanotechnology is a very promising advancement in the aquaculture industry (George et al., 2023; Yazdani et al., 2023). Nanoparticles (NPs) are small particles that range from 1 to 100 nm in size and can exhibit different physical and chemical properties to their larger material counterparts. Owing to their exceptional features, NPs span across a wide variety of applications and industries (Baig et al., 2021). CUR nanoparticles (CURNPs) have been recently used as a growth promoter in *O. niloticus* to improve the growth, feed efficiency, immunity, and the redox status (Abdel-Ghany et al., 2023; Abdel-Tawwab et al., 2022; Alhawas et al., 2023). These effects could be related to the enhanced availability and aqueous solubility of CUR. In addition, CURNPs have well-acknowledged immunomodulation efficacy in both animals and humans. It has beneficial biological and pharmacological properties, such as anti-inflammatory, antioxidant, cardio-protective, neuroprotective and antimicrobial (Alhawas et al., 2023; Eissa et al., 2023; Elabd et al., 2023; Gad et al., 2022; Sarawi et al., 2021 a, b). Despite the European sea bass (*Dicentrarchus labrax*) is one of the leading and widespread intensively farmed marine fish in the world (Shafique et al., 2021), the influences of dietary administration of CURNPs on its performance and health have not been examined yet. Therefore, this study investigated the potential impact of CURNPs on *D. labrax* growth performance, intestinal and hepatic architecture, and immunological, hematological, and antioxidant biomarkers.

Material and methods

Preparation of CURNPs

CURNPs were kindly gifted by Dr El-Sayed Hemdan Eissa (Fish Research Center, Arish University, Egypt). The supplemented CURNPs were prepared according to Kakran et al. (2012) using a syringe pump procedure employing dichloromethane (DCM) as an organic antisolvent. CUR was dissolved in DCM (5 mg/mL) and then transferred to a 20-mL syringe. The syringe, secured onto a pump, was filled with 20 mL solution that was quickly injected at a fixed flow rate (10 mL/min) into deionized water under magnetic stirring (1000 *x*g) for 2 h. Following filtration, the formed CURNPs were vacuum dried, and the preparation was evaluated using a Zeta sizer (Malvern Instruments, Zeta sizer nano series Nano-s, UK) and the mean diameter of the particles was determined (10–50 nm) as previously reported (Eissa et al., 2022; Elabd et al., 2023).

Diets and experimental conditions

D. labrax fingerlings were obtained from a commercial hatchery (Damietta, Egypt) and transported into the farm to conduct the investigation. The experiment was approved by the animal ethical committee at Zagazig University (No. ZU-IACUC/2/F/110e/2022). The fish were adapted to an indoor recirculating aquaculture system and fed a commercial diet (Table 1) for 15 days. After acclimatization, a total of 300 fingerlings (10.02 g ± 0.11) were stocked equally in 20 hapas (1 × 2 × 1.25 m^3). Fish were randomly divided into four equal groups; each group had five replicates (15 fish/hapa). The formulation of four experimental diets includes four distinct concentrations of CURNPs at 0.0 mg/kg (CURNPs0), 50 mg/kg (CURNPs50), 100 mg/kg (CURNPs100), and 150 mg/kg (CURNPs150). The baseline diet (45% crude protein) was fully blended with CURNPs. The experimental diets were made by properly combining the dry ingredients of each diet followed by the addition of 200 mL/kg water. The mixture was blended to create a paste that was pelleted by passing it through a laboratory pellet machine with a 1 mm diameter. The pellets were dried at room temperature and then kept in plastic bags at 4°C. The chemical composition of the diet was determined using AOAC procedures. For 90 days, the daily ratio was 3% of total biomass thrice/day (09:00, 13:00, and 15:00 h). The water quality measurements such as pH, temperature, dissolved oxygen, unionized ammonia (NH_3), and salinity were 7.16±0.09, 21.1±0.6°C, 5.1±0.3 mg/L, 0.036±0.01 mg/L, and 27.2±0.1 ppt, respectively.

Blood and tissues sampling

Five fish from each group were taken for blood and tissue analysis at the end of the feeding period. Fish were anesthetized with Tricaine methane-sulfonate (MS-222, Sigma Aldrich, 70 mg/L) (Topic Popovic et al., 2012), and then the blood samples were drawn from the caudal vein via puncturing it. Samples were collected on heparin whereas other samples were left to coagulate, centrifuged and serum was separated. Spinal cord sectioning was employed for scarification and liver and intestine were collected on 10% formalin for histological examination and samples from the liver were snapped frozen in liquid nitrogen, stored in Ambion RNAlater (Waltham, MA, USA) and then stored at −80°C until RNA isolation.

Table 1. Formulation and chemical composition of the basal and experimental diets

Ingredients (%)	CURNPs0	CURNPs50	CURNPs100	CURNPs150
Herring fishmeal (crude protein, 72%)	31	31	31	31
Soybean meal (crude protein, 48%)	36	36	36	36
Extruded wheat (crude protein, 12%)	8	8	8	8
Corn gluten meal (crude protein, 60%)	10	10	10	10
Fish oil	6.6	6.6	6.6	6.6
Soybean oil	6.4	6.35	6.30	6.25
Vitamin and mineral mix[1]	2	2	2	2
CURNPs	0	0.005	0.01	0.015
Total	100	100	100	100
Dry matter	90.1	89.7	89.9	88.9
Crude protein	44.1	44.1	44. 2	43.8
Crude lipid	17.1	16.9	17	17.1
Crude fiber	9.7	9.4	9.8	9.4
Ash	12	12.1	11.8	11.9
NFE	17.1	17.2	49.88	49.76
Gross energy (MJ/kg)[2]	525.141	523.662	524.45	523.146

[1]Vitamin mixture (mg/kg premix, free of vitamin C): vitamin A (3300 IU), vitamin D_3 (410 IU), vitamin E (2660 mg), vitamin B_1 (133 mg), vitamin B_2 (580 mg), vitamin B_6 (410 mg), vitamin B_{12} (50 mg), biotin (150 mg), choline chloride (4000 mg), inositol (330 mg), para-amino benzoic acid (9330 mg), niacin (26.60 mg), pantothenic acid (2000 mg). Mineral mixture (mg/kg premix): manganese (325 mg), iron (200 mg), copper (25 mg), iodine (10 mg), cobalt (5 mg). [2]Gross energy was calculated based on the values of protein, lipid, and carbohydrate as 23.6, 39.5, and 17.2 KJ/g, respectively.

Assessment of growth performance

Length measurements were estimated at the beginning (Initial length, IL) and end (Final length, FL) of the feeding trial period. The total length (TL in cm) from the fish snout to the end of the caudal fin recorded to the closest 0.1 cm employing a normal ruler was confirmed using a Vernier caliper. The fish survived biomass was weighed at the start (initial body weight (IBW) g) and end of the feeding trial (final body weight (FBW) g), along with consumed feed intake (FI) throughout the feeding trial period (T = 90 days) were used to calculate the following parameters:

$$\textit{Weight gain \% (WG,\%)} = (\textit{FBW,g} - \textit{IBW,g}) \times 100$$

$$\textit{Daily weight gain (DWG,g/day)} = \frac{\textit{WG,g}}{\textit{days of feeding trial}}$$

$$\textit{Feed conversion ratio (FCR, g/g)} = \frac{\textit{Total FI,g}}{\textit{WG,g}}$$

$$\textit{Specific growth rate (SGR,\%/day)} = 100 \times \left(\frac{\textit{Ln FBW} - \textit{Ln IBW}}{\textit{feeding trial period in days}} \right)$$

$$\textit{Protein efficiency ratio (PER, g/g)} = \frac{\textit{Wg,g}}{\textit{protein intake}}$$

$$\textit{Relative growth rate (RGR,\%)} = 100 \times \frac{\textit{WG,g}}{\textit{IBW,g}}$$

Hemato-biochemical assessments

The whole blood samples were used for the estimation of red blood cells (RBCs) count, hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total white blood cells (WBCs) count, and differential WBCs (neutrophils, lymphocytes, monocytes, eosinophil, basophils, and immature granulocyte) count using XE 2100 automated hematology analyzer (Advia 2120 & Sysmex, Siemens). Serum samples were used for the determination of total protein, albumin, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, urea, triglycerides, and total cholesterol using Bio-diagnostic commercial kits (Cairo, Egypt) catalogue numbers TP 20 20, AB 10 10, GL 13 20, AS 10 61 (45), AL 10 31 (45), CR 12 50, UR 21 10, TR 20 30, and CH 12 20, respectively; immunoglobulin M (IgM) was assayed using Cusabio (China; cat. no. CSB-E12045Fh) ELISA kit, and cortisol using an ELISA kit supplied by MyBioSource (USA; cat. no. MBS9424415). All assays were carried out strictly following the manufacturers' instructions.

Assay of digestive enzymes activities

To assay the activities of amylase and lipase, the samples from the intestine were homogenized in phosphate-buffered saline (pH 7.4), centrifuged and the supernatant was used to assay the activities of lipase and amylase using colorimetric kits supplied by Spinreact (Spain; Cat. no, MX1001275) and Biodiagnostic (Cairo, Egypt; Cat. no. AY 10 50).

Table 2. The primers used for qRT-PCR

Gene	Sequence (5′-3′)	Genbank accession number
*IL-1*β	F: AGTCCTGCTGATTTGATCTACCT R: CCAGACTGGCTTTGTCCACC	AJ311925.1
HSP70	F: CAGATCAGACCGCAGCAGAA R: GACGCTCGTAGTTGAGTGCT	AY423555.2
IGF-1	F: AACAAGAAGGTGAGCACAGCA R: CAGAGCAAGGCTAGTTGATTCC	GQ924783.1
GAPDH	F: GACCCCGCCAACATCAAATG R: GACGACTCTCTTGGCACCTC	XM_051406155.1

*IL-1*β, interleukin-1beta; *HSP70,* heat shock protein 70; *IGF-1,* insulin-like growth factor 1; *GAPDH*, glyceraldehyde-3-phosphate-dehydrogenase.

Determination of immunological variables

The phagocytic activity and index were estimated following Kawahara et al. (1991) procedure. The following formulas were applied to determine the phagocytic index values by counting phagocytic cells on a test smear: Phagocytic activity = (Total number of macrophages containing yeast/total number of macrophages) × 100, and phagocytic index = phagocytized cell count/total phagocytic cell number. Serum lysozyme (LYZ) activity was assessed by a turbidimetric assay as described by Parry et al. (1965) using a suspension of *Micrococcus lysodeikticus* (EC 3.2.1.17) (Sigma-Aldrich, USA). Then, LYZ activities in serum were calculated employing a standard curve established from LYZ extracted from chicken egg white (Sigma-Aldrich, USA).

Determination of malondialdehyde and antioxidants

Serum glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) activities and malonaldehyde (MDA) levels were quantified using reagent kits supplied by Biodiagnostic (Egypt; Cat. no. GP 2524, SD 25 21; CA 25 17, and MD 25 29, respectively) following the producer's guidelines.

Histological examinations

The fixed intestine and liver samples were dehydrated and cleared before embedding in paraffin followed by sectioning at 5 µm thickness for staining with hematoxylin and eosin (Suvarna et al., 2019). The stained sections were examined under a light microscope (Leica, UK) and the histological changes were reported.

RNA extraction and gene expression analysis

Total RNA was isolated from the frozen liver samples (10 fish/group) using Trizol (Invitrogen, USA) following the manufacturer's instructions. The isolated RNA was quantified using a NanoDrop and samples with OD260/OD280 nm ratio of ≥1.8 were reverse transcribed into cDNA using Invitrogen (USA) kit. cDNA was amplified using TOPreal™PreMIX SYBR Green qPCR master mix (Enzynomics; cat. no. RT 500) and primers for insulin-like growth factor 1 (*IGF1*), interleukin-1beta (*IL-1β*), and heat shock protein 70 (*HSP70*) (Table 2). The cycling conditions included initial denaturation at 95°C for 5 min followed by 35 cycles of 30 sec at 94°C, 30 sec at 57°C and 60 sec at 7°C. The obtained data were analyzed by employing the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Statistical analysis

All collected and calculated data were checked for normality and homogeneity. To determine the influence of CURNPs dietary supplementation, the data were analyzed using one way-ANOVA. Tukey's range test was applied to analyze variations between means at a 95% confidence level using SPSS software version 22 (SPSS, Richmond, VA, USA), and the data was displayed as means ± standard error of mean (SEM).

Results

Effect of CURNPs on growth performance

After 90 days of feeding trial, results in Table 3 showed the effect of the dietary inclusion of CURNPs on the growth and feed efficiency of *D. labrax* fingerlings. Both groups CURNPs100 and CURNPs150 had the highest values of FBW, DWG and WG (%) ($P<0.001$). The fish given 50 mg/kg diet (CURNPs50) had better FBW, DWG and WG than the control fish ($P<0.001$). Supplementing *D. labrax* diets with CURNPs had no significant effects on IL ($P>0.05$) and FL ($P>0.05$), whereas the values of FCR and PER were improved significantly in CURNPs100 and CURNPs150 followed by CURNPs50 as compared with the CURNPs0 group. All diets improved the SGR ($P<0.001$) and RGR ($P<0.001$) values without inducing any significant difference between CURNPs50 and CURNPs0 groups for RGR ($P>0.05$).

Effect of CURNPs on hematological indices

The effects of CURNPs on hematological variables of *D. labrax* are illustrated in Table 4. RBCs and PCV were improved significantly in fish received CURNPs

at levels 100 or 150 mg/kg diet (P<0.001). CURNPs150 showed the best values of Hb and MCV versus the control and CURNPs50 groups. CURNPs had no significant impact on MCH, MCHC, lymphocytes, eosinophils, and basophils (P>0.05). CURNPs50 group exhibited intermediate values of Hb and PCV and the CURNPs150 fish group had the lowest number of monocytes (P = 0.019) and heterophils (P = 0.005) when compared with other experimental groups. Similar results for heterophils were observed in CURNPs50 and CURNPs100 treated groups (P>0.05).

Effect of CURNPs on serum biochemical parameters

Dietary inclusion of CURNPs had non-significant effects on ALT, AST, urea, creatinine, total protein, albumin, globulin, and cortisol as elucidated in Table 5. Glucose values were greater in all CURNPs-supplemented groups diets in comparison with the control group (P = 0.007). Conversely, fish received 150 mg/kg CURNPs in their diets had lower values of triglyceride (P = 0.002), while total cholesterol was significantly decreased in CURNPs100 and CURNPs150 groups (P = 0.019).

Table 3. Effect of dietary CURNPs on growth performance and feed utilization of European seabass fingerlings

	CURNPs0	CURNPs50	CURNPs100	CURNPs150	P value
IBW (g)	10.02±0.25	10.03±0.21	10.00±0.27	10.04±0.16	0.999
FBW (g)	75.43±0.61 c	82.17±0.47 b	87.50±0.61 a	87.64±0.58 a	<0.001
IL (cm)	14.09±0.12	14.33±0.10	14.11±0.10	14.39±0.08	0.104
FL (cm)	19.24±0.16	19.43±0.19	19.67±0.24	19.88±0.24	0.183
WG (g)	65.41±0.79 c	72.14±0.63 b	77.50±0.79 a	77.60±0.63 a	<0.001
DWG (g/day)	0.58±0.00 c	0.64±0.00 b	0.69±0.00 a	0.69±0.00 a	<0.001
FCR (g/g)	3.04±0.03 a	2.90±0.03 b	2.69±0.03 c	2.71±0.03 c	<0.001
PER	1.37±0.01 a	1.30±0.01 b	1.21±0.01 c	1.21±0.01 c	<0.001
SGR (%/day)	1.80±0.02 b	1.88±0.02 a	1.94±0.02 a	1.93±0.01 a	<0.001
RGR (%/day)	6.64±0.24 b	7.27±0.22 ab	7.89±0.28 a	7.78±0.16 a	0.001

IBW, initial body weight; FBW, final body weight; IL, initial length; FL, final length; WG (%), weight gain percent; DWG, daily weight gain; FCR, feed conversion ratio; PER protein efficiency ratio; SGR, specific growth rate; RGR, relative growth rate. The data were presented as Mean ± SEM. Means in the same row with different letters differ significantly (P<0.05).

Table 4. Effect of dietary CURNPs (mg/kg) on the hematological parameters of European seabass

	CURNPs0	CURNPs50	CURNPs100	CURNPs150	P value
Hb (g/dl)	12.54±0.05 d	12.92±0.06c	13.27±0.02 b	13.50±0.05 a	<0.001
RBCs ($\times 10^6$/mm^3)	4.21±0.04 b	4.17±0.02 b	4.43±0.02 a	4.39±0.01 a	<0.001
PCV (%)	40.00±0.01 c	41.00±0.03 b	43.00±0.05 a	43.00±0.00 a	<0.001
MCV (µm^3)	97.27±0.11 ab	95.93±0.46 b	97.07±0.44 ab	97.95±0.22 a	0.057
MCH (pg)	30.65±0.10	30.48±0.01	30.08±0.01	30.24±0.01	0.097
MCHC (%)	31.51±0.14	31.40±0.12	31.36±0.13	30.87±0.06	0.064
WBCs (10^3/mm^3)	28.21±0.05 a	28.03±0.07 a	27.33±0.12 b	26.06±0.05 b	<0.001
Heterophils (%)	14.00±1.02 a	12.00±0.03 ab	11.00±1.01 ab	10.50±0.50 b	0.005
Lymphocytes (%)	75.50±0.50	75.50±0.50	74.50±1.50	76.00±0.01	0.660
Monocytes (%)	10.50±0.50 a	11.00±0.02 a	10.00±0.02 a	9.00±0.01 b	0.019
Eosinophils (%)	1.00±0.00	1.50±0.50	1.00±0.00	1.50±0.50	0.803
Basophil (%)	1.00±0.00	1.50±0.50	1.50±0.50	1.50±0.50	0.615

Hb, hemoglobin; RBC, red blood corpuscles; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBCs, white blood cells. The data were presented as Mean ± SEM. Means in the same row with different letters differ significantly (P<0.05).

Table 5. Effect of dietary CURNPs (mg/kg) on serum biochemical parameters of European seabass (*D. labrax*)

	CURNPs0	CURNPs50	CURNPs100	CURNPs150	P value
ALT (U/L)	29.51±0.08	28.99±0.75	29.24±0.33	28.48±0.24	0.469
AST (U/L)	19.57±0.14	18.98±0.77	18.73±1.18	18.76±0.94	0.884
Total protein (g/dL)	4.76±0.06	4.87±0.01	4.89±0.06	4.99±0.03	0.104
Albumin (g/dL)	1.29±0.02	1.31±0.01	1.32±0.00	1.32±0.01	0.350
Globulin (g/dL)	3.34±0.04	3.43±0.02	3.44±0.07	3.54±0.05	0.169
Urea (mg/dL)	1.72±0.04	1.66±0.03	1.66±0.02	1.67±0.02	0.463
Creatinine (mg/dL)	0.28±0.01	0.27±0.00	0.28±0.00	0.27±0.01	0.861
Cortisol (ng/mL)	25.08±0.57	25.07±0.36	24.15±0.60	23.58±0.40	0.229
Glucose (mmol/L)	9.65±0.47 b	11.96±0.45 a	12.29±0.87 a	12.97±0.95 a	0.007
Total cholesterol (mg/dL)	88.00±1.01 a	87.71±1.19 a	81.60±2.61 b	81.73±1.48 b	0.019
Triglyceride (mg/dL)	91.73±0.28 a	88.13±0.91 a	89.17±0.43 a	81.78±4.93 b	0.002

ALT, alanine aminotransferase; AST, aspartate aminotransferase. The data were presented as Mean ± SEM. Means in the same row with different letters differ significantly (P<0.05).

Table 6. Effect of dietary CURNPs on digestive enzymes, and immunological and oxidative biomarkers of European seabass (*D. labrax*)

	CURNPs0	CURNPs50	CURNPs100	CURNPs150	P value
Digestive enzymes activities					
Lipase (U/mL)	30.68±1.69 b	40.22±1.26 a	40.34±1.64 a	42.67±1.15 a	0.015
Amylase (U/mL)	39.11±0.15 b	41.95±2.22 b	46.96±1.57 a	45.63±3.23 a	0.012
Immune response					
Phagocytic activity (%)	7.65±0.18 b	9.74±0.61 a	10.74±0.06 a	10.73±0.04 a	0.006
Phagocytic index (%)	0.99±0.02 b	1.10±0.01 a	0.99±0.01 b	1.25±0.02 b	0.006
Lysozyme (µg/mL)	6.47±0.12 c	7.62±0.61 bc	8.29±0.10 ab	9.48±0.56 a	0.013
IgM (µg/mL)	2.86±0.33 b	4.12±0.05 a	4.12±0.18 a	4.46±0.35 a	0.042
Oxidative and antioxidant status					
GPx (U/L)	8.53±0.19 b	9.43±0.41 ab	11.69±0.67 a	11.51±1.06 a	0.017
SOD (U/L)	9.04±0.38	9.52±0.05	9.55±0.01	9.65±0.06	0.271
CAT (U/L)	10.71±0.20 b	10.88±0.09 b	10.81±0.15 b	11.54±0.18 a	0.039
MDA (nmol/mL)	13.72±0.06 a	11.06±1.14 b	9.89±0.07 b	9.85±0.33 b	0.028

IgM, immunoglobulin M; GPx, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde. The data were presented as Mean ± SEM. Means in the same row with different letters differ significantly (P<0.05).

Effect of CURNPs on digestive enzymes, immune response and oxidative biomarkers

Data showing the impact of dietary CURNPs on digestive enzymes, blood immunological and oxidative/antioxidant biomarkers are demonstrated in Table 6. Significant higher lipase (P<0.015) and amylase (except for CURNPs50; P<0.012) were detected with increasing the supplemental level of CURNPs in fish diets. Both groups (CURNPs0 and CURNPs50) had similar results for amylase (P>0.05). Groups fed with CURNPs at various levels exhibited a remarkable increase in phagocytic activity (P = 0.006) and IgM (P = 0.042) when compared with CURNPs0 group. Only CURNPs50 had a higher phagocytic index as compared with other groups. The best result of serum LYZ activity was noticed in CURNPs150 group. Notably, no significant difference (P>0.05) in SOD activity was detected among the CURNPs-fed and the non-supplemented groups. A noticeable dose-dependent decrease (P<0.05) in MDA was found in CURNPs-fed fish. A remarkable increase (P<0.05) in CAT activity was detected in the serum of 150 mg/kg diet CURNPs-fed fish. GPx was significantly improved in both CURNPs150 and CURNPs100 groups.

Effect of CURNPs on liver architecture

The impact of CURNPs supplementation on the hepatic architecture of *D. labrax* is presented in Figure 1. Fish fed with basal diets exhibited normal hepatic and hepatopancreatic architecture with slight hepatocytic dissociation (Figure 1 A). Fish fed CURNPs at the levels of 50 (Figure 1 B), 100 (Figure 1 C) and 150 mg (Figure 1 D) showed normal structure of hepatic tissue and hepatopancreatic architecture.

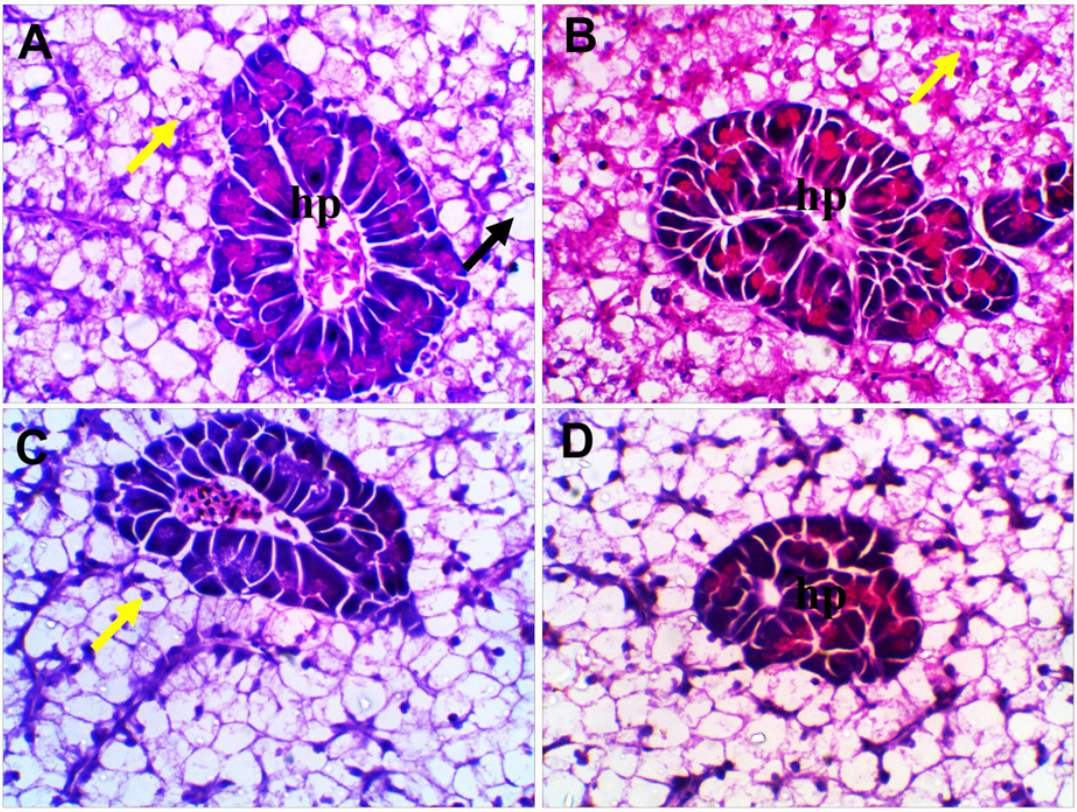

Figure 1. Representative photomicrograph of liver tissue of *D. labrax* fed with various levels of CURNPs. (A) Fish fed the basal diet showing normal hepatic (yellow arrow), hepatopancreatic (hp) architecture with some hepatocytic dissociation (black arrows). (B–D) *D. labrax* fed 50 (B), 100 (C) and 150 mg/kg diet (D) CURNPs showing improvement in the structure of hepatic tissue and hepatopancreatic architecture (H&E, X400)

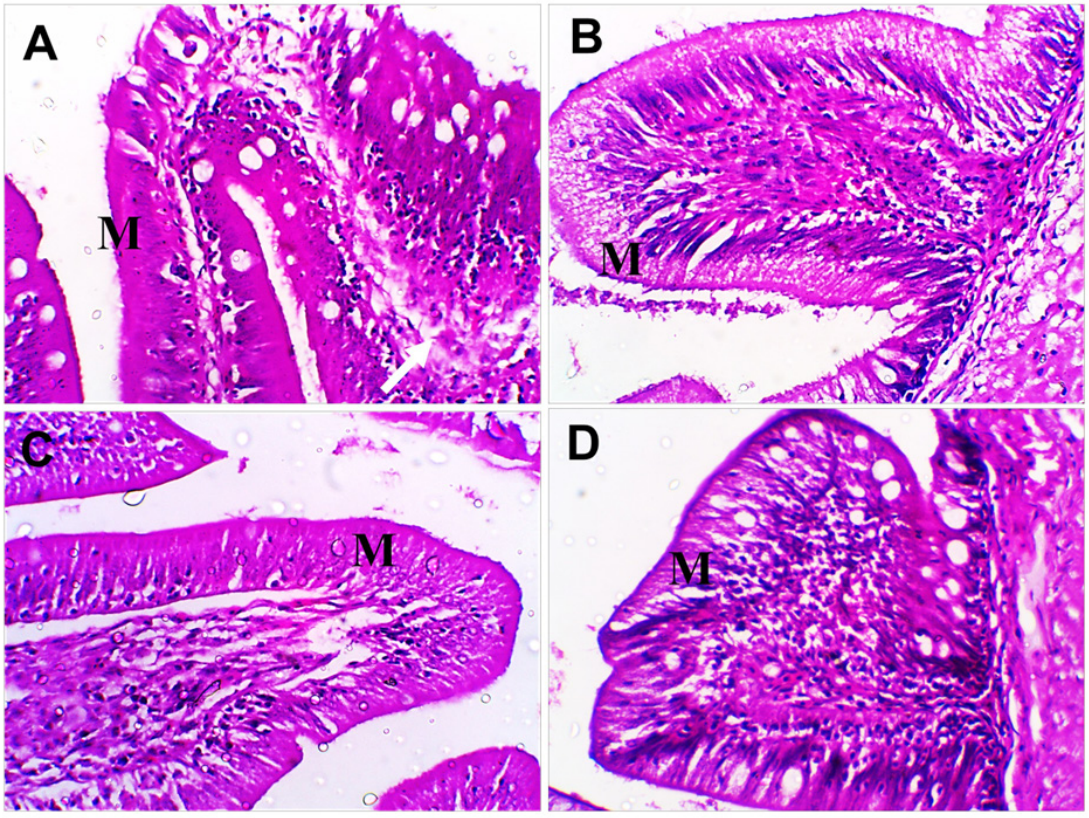

Figure 2. Representative photomicrograph of intestinal tissue of *D. labrax* fed with various levels of CURNPs. The intestine specimen from the CURNPs0 (A), CURNPs50 (B), CURNPs100 (C), and CURNPs150 (D) showing normal structure of the intestinal mucosa (M), villi and lamina propria (H&E, X400)

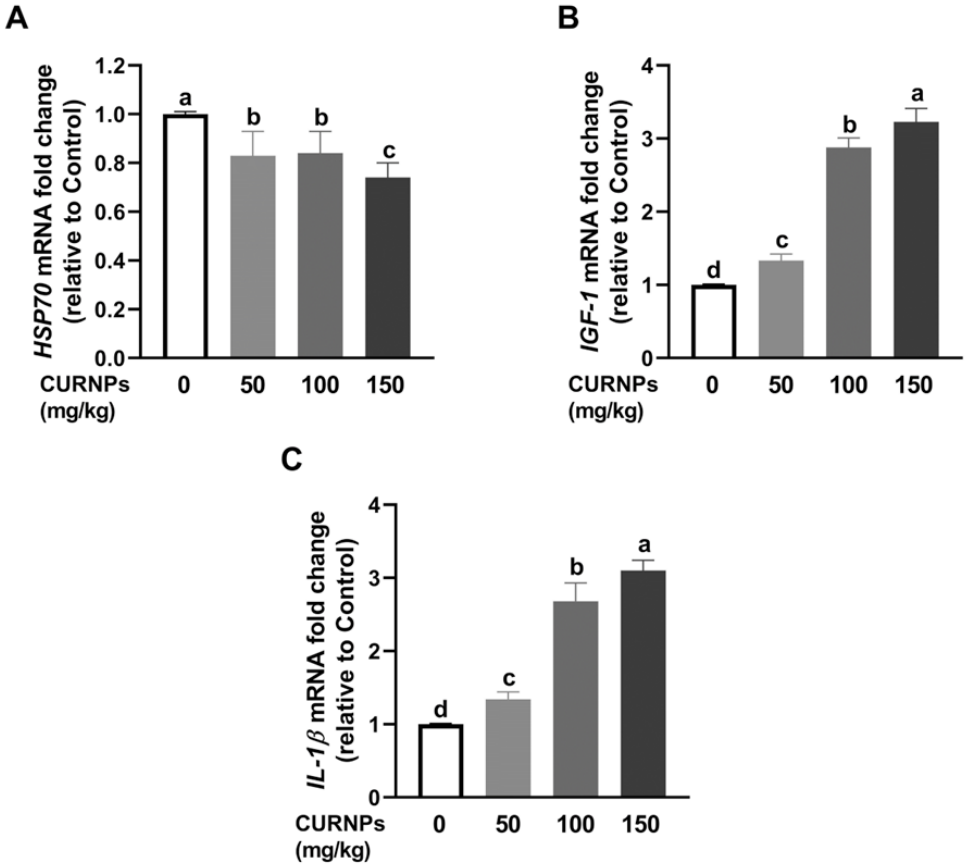

Figure 3. Effect of CURNPs on mRNA abundance of HSP70 (A), IGF-1 (B) and IL-1β (C) in the liver of *D. labrax*. Data are presented as Mean ± SEM. Different symbols indicate significant differences

Effect of CURNPs on intestinal architecture

The impact of CURNPs supplementation on the intestinal architecture of *D. labrax* is presented in Figure 2. The intestine specimen from the CURNPs0 group (Figure 2 A) and fish fed diets containing 50 (Figure 2 B) and 100 mg/kg (Figure 2 C) CURNPs showed normal intestinal architecture with normal intestinal villi, fewer inflammatory cells, and lamina propria involved with the villi bases. As outlined in Figure 2 D, the intestinal tissue of fish supplemented with 150 mg CURNPs exhibited normal intestinal architecture with clear lamina propria and smooth apical portion of intestinal villi.

Effect of CURNPs on *HSP70*, *IGF1* and *IL-1β* expression

As represented in Figure 3 A, a significant down-regulation of hepatic HSP70 mRNA was observed in CURNPs150 (fold change = 0.74±0.08), followed by other CURNPs-supplemented groups. Both groups CURNPs50 and CURNPs100 had similar HSP70 expression (0.83±0.09 and 0.84±0.06, respectively). A noticeable increase ($P<0.05$) in the expression of hepatic IGF1 (fold change = 1.33±0.08, 2.88±0.13 and 3.23±0.10 for CURNPs50, CURNPs100 and CURNPs150 groups, respectively) (Figure 3 B) and IL-1β (fold change = 1.36±0.08, 2.68±0.11 and 3.10±0.13 for CURNPs50, CURNPs100 and CURNPs150 groups, respectively) (Figure 3 C) were noticed. CURNPs150 had higher expression of IGF1 and IL-1β than other groups ($P<0.001$).

Discussion

There is an increasing interest in the fish industry to use novel immuno-stimulatory substances, primarily to prevent the negative effects of intensified culture on fish health and production. Various micronutrients and natural substances have been found to improve fish immune system against a variety of infectious diseases and stressors (Fath El-Bab et al., 2023; Gewida et al., 2023; Naiel et al., 2023). The use of NPs in fish feed increased the digestibility of nutrients in the gastrointestinal tract rather than allowing them to pass through the digestive system without being absorbed (Fajardo et al., 2022). In this regard, the current experiment revealed that CURNPs exerted beneficial effects on *D. labrax* growth matrices, feed efficiency, digestive enzymes, and immunological and oxidative biomarkers.

Dietary inclusion of CURNPs (100 or 150 mg/kg) enhanced growth indices (FBW, WG%, DWG, RGR) and

improved feed utilization of *D. labrax*. The obtained results coincided with studies reporting the positive effects of CURNPs on the growth metrics and feed utilization in *O. niloticus* (Eissa et al., 2022; Elabd et al., 2023), *C. carpio* (Abdel-Tawwab and Abbass, 2017; Pirani et al., 2021), and African catfish (*Clarias gariepinus*) challenged with *Aeromonas veronii* (Mansour et al., 2023). The group of *D. labrax* that received the high dose of CURNPs exhibited a greater growth performance and feed utilization. It has previously been stated that the above-mentioned activities are mostly due to the appetizing smell of CURNPs, which may increase palatability of fish feed and as a result utilization of consumed feed, leading to better feed efficiency in *Oreochromis* sp. (Eissa et al., 2023). More recently, Alhawas et al. (2023) clarified that the inclusion of 25 and 35 mg/kg diet liposomal CURNPs exerted the most prominent effect on the growth rate and feed conversion ratio in *O. niloticus*. The incorporation of CURNPs also improved the entire digestion pathways, as represented by the increase in the secretions of digestive enzymes amylase and lipase. The enhanced feed efficiency (FI and FCR) in CURNPs-fed fish could be attributed to the improved secretions of the digestive enzymes which have critical roles in the digestion and absorption of nutrients (Midhun et al., 2016). Supplementation of CUR or its nano form has an intrinsic outreach performance on freshwater fish, including crucian carp (*Carassius auratus*) (Jiang et al., 2016), *O. mykiss* (Aqmasjed et al., 2022; Ji et al., 2021), *C. carpio*, *O. niloticus* (Alagawany et al., 2021), and Mozambique tilapia (*Oreochromis mossambicus*) (Sruthi et al., 2018). CUR acts as a growth promoter for beneficial intestinal bacteria and inhibits the growth of pathogenic fungal and microbial species (Alagawany et al., 2021). CURNPs protected *O. niloticus* against *Aspergillus flavus* infection (Eissa et al., 2022, 2023), heat stress (Abdel-Ghany et al., 2023), and fish-borne zoonotic pathogens (Gad et al., 2022). In addition to enhanced release of the digestive enzymes, CUR-mediated improvement of growth parameters and feed consumption was also attributed to boosted activities of Na^+/K^+-ATPase, intestinal alkaline phosphatase, gamma-glutamyl transpeptidase, and creatine kinase, resulting in enhancement of nutrients degradation and assimilation (Jiang et al., 2016; Midhun et al., 2016). Furthermore, CUR exhibits a prebiotic activity reflected through improvement of gut flora and subsequently digestion, absorption, and growth rate in fish (Alhawas et al., 2023; Eissa et al., 2023). This notion is supported by studies showing the positive impact of dietary CUR in *C. auratus* (Jiang et al., 2016), yellow croaker (*Pseudosciaene crocea*) (Naiel et al., 2023), *O. mykiss* (Aqmasjed et al., 2022; Ji et al., 2021) and barramundi (*Lates calcarifer*) (Bao et al., 2022).

Evaluating the hematological indices in fish could reflect the primary changes in health as the response to nutritional intake, pathogenic infection, and capacity to adapt to their environment. Fish fed with CURNPs (100 and 150 mg/kg) had greater erythrogram (Hb, RBCs, PCV, and MCV) and lower values of WBCs, heterophils, monocytes. In accordance with our data, previous studies have revealed improvement of hematological parameters in dietary CURNPs-supplemented *O. niloticus* (Eissa et al., 2022). As per pervious reports (Eissa et al., 2022; Fath El-Bab et al., 2022), the assayed hematological and biochemical parameters in *D. labrax* fall within the normal range. Therefore, anemias and other disorders were excluded in *D. labrax* involved in this investigation. This feature might be attributed to CURNPs-stimulated chemosynthesis and erythropoiesis, and hence improved fish health (Abdel-Tawwab et al., 2022). In this context, investigations revealed improvement of hematological parameters in fish species fed CUR-enriched diet (Mohamed et al., 2020; Yonar et al., 2019). According to our data, there were no significant effects of CURNPs on the majority of blood chemistry of *D. labrax* fish. CURNPs reduced total cholesterol and triglycerides in fish. CUR and its nano form can confer protection for the liver against different insults as shown by their ability to ameliorate circulating transaminases and improve energy homeostasis (Elabd et al., 2023). Elabd et al. (2023) suggested that this property might be linked to the capacity of CURNPs to protect the hepatocyte membrane from oxidative damage and hence prevent the leakage of intracellular enzymes in *O. niloticus*. However, many studies have revealed that CURNPs can decrease the hepatic enzymes in various fish species after feeding trial (Eissa et al., 2022, 2023), an effect that was not observed in our study. Given the normal organ function parameters reported in this study, it could be concluded that CURNPs were safe in *D. labrax* and that the hepatoprotective role of CUR and its nano form could be observed when the fish is challenged with pathogenic microbes, such as *Aspergillus flavus* (Eissa et al., 2022, 2023), *Streptococcus agalactiae* (Alhawas et al., 2023) and *Aeromonas veronii* (Mansour et al., 2023).

It is widely understood that antioxidant biomarkers are an important sign of the general health status of fish. In the current study, dietary CURNPs enhanced CAT and GPx activities in *D. labrax*. All doses of CURNPs exerted positive effects on the activity of antioxidant enzymes and decreased MDA levels. Accordingly, several reports have indicated that the addition of CURNPs to fish diet improved the antioxidant status under normal conditions (Elabd et al., 2023) or after challenge with pathogenic bacteria (Alhawas et al., 2023). The improved antioxidant response might be attributed to the radical-scavenging and metal chelation properties of CURNPs.

Next, we investigated the effect of dietary supplementation of CURNPs on immunological parameters. IgM, phagocytic (index or activity), LYZ activity are the main markers that reflect the status of fish immune system (Alhawas et al., 2023). These markers and processes are among the first defensive lines and play a crucial role in maintaining fish health. CURNPs inclusion in fish diets resulted in considerable enhancement of IgM, LYZ, and phagocytic activity as previously reported (Alhawas et al., 2023; Elabd et al., 2023). CURNPs dietary supple-

mentation enhanced phagocytic activity and IgM in *O. niloticus* (Abdel-Ghany et al., 2023) and *C. gariepinus* (Mansour et al., 2023). The immunostimulatory function of CURNPs might be attributed to its ability to promote LYZ synthesis pathways along with redox homeostasis (Abdel-Ghany et al., 2023). Incorporation CUR (50 and 100 mg kg^{-1}) into fish feed boosted plasma LYZ activity and IgM levels (Mahmoud et al., 2017). It improved both the humoral and innate immune activities in *D. labrax*. Gheytasi et al. (2021) stated that supplementing the diet of *O. mykiss* with lemon essential oil loaded-CURNPs significantly enhanced LYZ activity and complement C3 levels in a dose-dependent manner.

For more insights on the beneficial effects of CURNPs on stress resistance, growth performance, and immunomodulatory events, we determined the mRNA abundance of *IGF1*, *IL-1β* and *HSP70* in the liver of *D. labrax*. The regulation of the *IGF1* reflects performance trends in aquatic animals and this might explain, at least in part, the beneficial role of CURNPs in improving growth performance of *D. labrax*. *IL-1β* is an immune response-modulating cytokine that might be influenced via CURNPs dietary supplementation (Gheytasi et al., 2021). Existing data revealed that CURNPs-based diets upregulated *IL-1β* and *IGF1*, while considerably downregulated the stress indicator *HSP70*. Here, the group supplemented with 150 mg/kg CURNPs exhibited the most prominent effect on the expression of these markers. In accordance, immune-related genes, such as *IL-1β* and complement component 5 (*cc5*), were improved via CUR and/or CURNPs-supplemented diets in *O. niloticus* and *C. carpio* (Alhawas et al., 2023; Pirani et al., 2021), effects that were explained by the immunomodulatory activity of CUR and its nano form. Moreover, treatment with CUR nano-micelle mitigated radiation-induced gastrointestinal toxicity via suppression of inflammatory cells infiltration and protection against villi and crypt shortening in mice (Ghanbarzadeh et al., 2023). In accordance with our data, CURNPs improved intestinal health in *O. niloticus* via increasing villi length, width, and goblet cell count (Bao et al., 2022; Eissa et al., 2023). The enhanced intestine morphometric features might clarify the better growth performance of largemouth bass fed CURNPs-supplemented diets (Bao et al., 2022). Under the influence of radiation and parasitic infection, CURNPs conferred protection mediated via suppression of intestinal inflammation, resulting in enhancement in the nutrient absorption and digestibility (Ghanbarzadeh et al., 2023; Maftuch et al., 2018).

Conclusion

The dietary supplementation of CURNPs exerted beneficial effects on the overall performance and health of *D. labrax*. The findings of this investigation show that nano-CUR could be employed as a dietary supplement to promote growth performance, feed consumption, immune response, and redox balance in *D. labrax*. This supplement could be applied to stimulate digestion process via enhancing the digestive enzymes secretions and intestinal and hepatic architectures, leading to improved nutrient absorption. However, further studies are needed to evaluate the mechanism(s) underlying the immunomodulatory and antioxidant effects of CURNPs in fish for future applications in aquaculture.

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Conflicts of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization: M.A.E.N., A.M.M., A.F.F-E., A.B-A., and A.F.A.; methodology, investigation, data curation and formal analysis: A.B-A., A.F.A., M.A., A.F.F-E., A.A.A., S.A.A, M.M.E., M.A.E.N., and A.M.M.; writing – original draft preparation: M.A.E.N. A.B-A., S.A.A., and A.M.M.; writing – review and editing: A.M.M. and A.F.A.; visualization and supervision, project administration, A.M.M., and M.A.E.N. All authors have read and agreed to the published version of the manuscript.

Data availability statement

All data are available within the manuscript.

References

Abdel-Ghany H.M., El-Sisy D.M., Salem M.E.-S. (2023). A comparative study of effects of curcumin and its nanoparticles on the growth, immunity and heat stress resistance of Nile tilapia (*Oreochromis niloticus*). Sci. Rep., 13: 2523.

Abdel-Tawwab M., Abbass F.E. (2017). Turmeric powder, *Curcuma longa* L., in common carp, *Cyprinus carpio* L., diets: growth performance, innate immunity, and challenge against pathogenic *Aeromonas hydrophila* infection. J. World. Aquacult. Soc., 48: 303–312.

Abdel-Tawwab M., Eissa E.-S.H., Tawfik W.A., Abd Elnabi H.E., Saadony S., Bazina W.K., Ahmed R.A. (2022). Dietary curcumin nanoparticles promoted the performance, antioxidant activity, and humoral immunity, and modulated the hepatic and intestinal histology of Nile tilapia fingerlings. Fish Physiol. Biochem., 48: 585–601.

Abdelnour S.A., Hassan M.A.E., Mohammed A.K., Alhimaidi A.R., Al-Gabri N., Al-Khaldi K.O., Swelum A.A. (2020). The effect of adding different levels of curcumin and its nanoparticles to extender on post-thaw quality of cryopreserved rabbit sperm. Animals, 10: 1508.

Abdelnour S.A., Alagawany M., Hashem N.M., Farag,M.R., Alghamdi E.S., Hassan F.U., Bilal R.M., Elnesr S.S., Dawood M.A.O., Nagadi S.A., Elwan H.A.M., ALmasoudi A.G., Attia Y.A. (2021). Nanominerals: fabrication methods, benefits and hazards, and their applications in ruminants with special reference to selenium and zinc nanoparticles. Animals, 11: 1916.

Abdelnour S.A., Ghazanfar S., Abdel-Hamid M., Abdel-Latif H.M.R., Zhang Z., Naiel M.A.E. (2023). Therapeutic uses and applications of bovine lactoferrin in aquatic animal medicine: an overview. Vet. Res. Commun., 47: 1015–1029.

Adenaya A., Berger M., Brinkhoff T., Ribas-Ribas M., Wurl O. (2023). Usage of antibiotics in aquaculture and the impact on coastal waters. Mar. Pollut. Bull., 188: 114645.

Alagawany M., Farag M.R., Abdelnour S.A., Dawood M.A., Elnesr S.S., Dhama K. (2021). Curcumin and its different forms: A review on fish nutrition. Aquaculture, 532: 736030.

Alhawas B., Abd El-Hamid M.I., Hassan Z., Ibrahim G.A., Neamat-Allah A.N.F., Rizk El-Ghareeb W., Alahmad B.A.-H.Y., Meligy A.M.A., Abdel-Raheem S.M., Abdel-Moez Ahmed Ismail H., Ibrahim D. (2023). Curcumin loaded liposome formulation: Enhanced efficacy on performance, flesh quality, immune response with defense against *Streptococcus agalactiae* in Nile tilapia (*Orechromis niloticus*). Fish Shellfish Immunol., 138: 108776.

Aqmasjed B., Sajjadi M.M., Falahatkar B., Safari R. (2022). Effect of curcumin and ginger extract on growth and biochemical indices in rainbow trout (*Oncorhynchus mykiss*). J. Aquac. Dev., 16: 35–48.

Baig N., Kammakakam I., Falath W. (2021). Nanomaterials: a review of synthesis methods, properties, recent progress, and challenges. Mater. Adv., 2: 1821–1871.

Bao X., Chen M., Yue Y., Liu H., Yang Y., Yu H., Yu Y., Duan N. (2022). Effects of dietary nano-curcumin supplementation on growth performance, glucose metabolism, and endoplasmic reticulum stress in juvenile largemouth bass, *Micropterus salmoides*. Front. Mar. Sci., 9: 924569.

Bondad-Reantaso M.G., MacKinnon B., Karunasagar I., Fridman S., Alday-Sanz V., Brun E., Le Groumellec M., Li A., Surachetpong W., Karunasagar I. (2023). Review of alternatives to antibiotic use in aquaculture. Rev Aquac., 15: 1421–1451.

Eissa E.-S.H., Ezzo O.H., Khalil H.S., Tawfik W.A., El-Badawi A.A., Abd Elghany N.A., Mossa M.I., Hassan M.M., Hassan M.M., Eissa M.E.H., Shafi M.E., Hamouda A.H. (2022). The effect of dietary nanocurcumin on the growth performance, body composition, haemato-biochemical parameters and histopathological scores of the Nile tilapia (*Oreochromis niloticus*) challenged with *Aspergillus flavus*. Aquac. Res., 53: 6098–6111.

Eissa E.-S.H., Alaidaroos B.A., Jastaniah S.D., Munir M.B., Shafi M.E., Abd El-Aziz Y.M., Bazina W.K., Ibrahim S.B., Eissa M.E.H., Paolucci M., Alaryani F.S., El-Hamed N.N.B.A., El-Hack M.E.A., Saadony S. (2023). Dietary effects of nano curcumin on growth performances, body composition, blood parameters and histopathological alternation in red tilapia (*Oreochromis* sp.) challenged with *Aspergillus flavus*. Fishes, 8: 208.

Elabd H., Mahboub H.H., Salem S.M., Abdelwahab A.M., Alwutayd K.M., Shaalan M., Ismail S.H., Abdelfattah A.M., Khalid A., Mansour A.T. (2023). Nano-curcumin/chitosan modulates growth, biochemical, immune, and antioxidative profiles, and the expression of related genes in Nile tilapia, *Oreochromis niloticus*. Fishes, 8: 333.

Fajardo C., Martinez-Rodriguez G., Blasco J., Mancera J.M., Thomas B., De Donato M. (2022). Nanotechnology in aquaculture: Applications, perspectives and regulatory challenges. Aquac. Fish., 7: 185–200.

Farag M.R., Abdelnour S.A., Patra A.K., Dhama K., Dawood M.A.O., Elnesr S.S., Alagawany M. (2021). Propolis: Properties and composition, health benefits and applications in fish nutrition. Fish Shellfish Immunol., 115: 179–188.

Fath El-Bab A.F., Majrashi K.A., Sheikh H.M., Shafi M.E., El-Ratel I.T., Neamat-Allah A.N., El-Raghi A.A., Elazem A.Y.A., Abd-Elghany M.F., Abdelnour S.A. (2022). Dietary supplementation of Nile tilapia (*Oreochromis niloticus*) with β-glucan and/or *Bacillus coagulans*: Synergistic impacts on performance, immune responses, redox status and expression of some related genes. Front. Vet. Sci., 9: 1011715.

Fath El-Bab A.F.F., Amer A.A., El-Nawsany M.M., Ibrahim I.H., Gouda A.H., El-Bahlol A.A., Naiel M.A. (2023). Oregano leaf extract dietary administration modulates performance, redox status, intestinal health, and expression of some related genes of Nile tilapia (*Oreochromis niloticus* L.). Ann. Anim. Sci., 24: 179–190.

Flora G., Gupta D., Tiwari A. (2013). Nanocurcumin: a promising therapeutic advancement over native curcumin. Crit. Rev. Ther. Drug Carrier Syst., 30: 331–368.

Gad H.A., Alshubaily F.A., Alsieni M.A., Tayel A.A., Diab A.M. (2022). Biosynthesis of nano-curcumin/nano-selenium composite and their potentialities as bactericides against fish-borne pathogens. Green Process. Synth., 11: 1098–1107.

George D., Lakshmi S., Sharma A., Prakash S., Siddiqui M., Malavika B., Elumalai P. (2023). Nanotechnology: A novel tool for aquaculture feed development. In: Nanotechnological Approaches to the Advancement of Innovations in Aquaculture. Springer, Cham., pp. 67–88.

Gewida A.G., Abouzed T.K., Abdelghany M.F., Khames D.K., Zayed M.M., Elsawy H.B., AbdEl-Kader M.F., Naiel M.A. (2023). Dietary effect of S-methylmethionine sulfonium chloride on growth, serum biochemical parameters, body composition, and expression of some related gene in *Oreochromis niloticus*. Ann. Anim. Sci., 24: 151–160.

Ghanbarzadeh A., Farhood B., Noodeh F.A., Mosaed R., Hassanzadeh G., Bagheri H., Najafi M. (2023). Histopathological evaluation of nanocurcumin for mitigation of radiation- induced small intestine injury. Curr. Radiopharm., 16: 57–63.

Gheytasi A., Hosseini Shekarabi S.P., Islami H.R., Mehrgan M.S. (2021). Feeding rainbow trout, *Oncorhynchus mykiss*, with lemon essential oil loaded in chitosan nanoparticles: effect on growth performance, serum hemato-immunological parameters, and body composition. Aquac. Int., 29: 2207–2221.

Heidari H., Bagherniya M., Majeed M., Sathyapalan T., Jamialahmadi T., Sahebkar A. (2023). Curcumin-piperine co-supplementation and human health: A comprehensive review of preclinical and clinical studies. Phytother. Res., 37: 1462–1487.

Ji R., Xiang X., Li X., Mai K., Ai Q. (2021). Effects of dietary curcumin on growth, antioxidant capacity, fatty acid composition and expression of lipid metabolism-related genes of large yellow croaker fed a high-fat diet. Br. J. Nutr., 126: 345–354.

Jiang J., Wu X.-Y., Zhou X.-Q., Feng L., Liu Y., Jiang W.-D., Wu P., Zhao Y. (2016). Effects of dietary curcumin supplementation on growth performance, intestinal digestive enzyme activities and antioxidant capacity of crucian carp *Carassius auratus*. Aquaculture, 463: 174–180.

Kakran M., Sahoo N.G., Tan I.-L., Li L. (2012). Preparation of nanoparticles of poorly water-soluble antioxidant curcumin by antisolvent precipitation methods. J. Nanopart. Res., 14: 757.

Kawahara E., Ueda T., Nomura S. (1991). *In vitro* phagocytic activity of white-spotted char blood cells after injection with *Aeromonas salmonicida* extracellular products. Fish Pathol., 26: 213–214.

Livak K.J., Schmittgen T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods, 25: 402–408.

Maftuch M., Sanoesi E., Farichin I., Saputra B.A., Ramdhani L., Hidayati S., Fitriyah N., Prihanto A.A. (2018). Histopathology of gill, muscle, intestine, kidney, and liver on *Myxobolus* sp.-infected Koi carp (*Cyprinus carpio*). J. Parasit. Dis., 42: 137–143.

Mahmoud H.K., Al-Sagheer A.A., Reda F.M., Mahgoub S.A., Ayyat M.S. (2017). Dietary curcumin supplement influence on growth, immunity, antioxidant status, and resistance to *Aeromonas hydrophila* in *Oreochromis niloticus*. Aquaculture, 475: 16–23.

Mahmoud A.M., Bin-Jumah M., Abukhalil M.H. (2021). Antiinflammatory natural products from marine algae. In: Inflammation and natural products, S. Gopi, A. Amalraj, A. Kunnumakkara, S. Thomas (eds). Academic Press, pp. 175–203.

Mansour S., Bakry K.A., Alwaleed E.A., Ahmed H., Al-Amgad Z., Mohammed H.H., Emeish W.F.A. (2023). Dietary nanocurcumin impacts blood biochemical parameters and works synergistically with florfenicol in African catfish challenged with *Aeromonas veronii*. Fishes, 8: 298.

Midhun S.J., Arun D., Edatt L., Sruthi M., Thushara V., Oommen O.V., Sameer Kumar V., Divya L. (2016). Modulation of digestive enzymes, GH, IGF-1 and IGF-2 genes in the teleost, tilapia (*Oreochromis mossambicus*) by dietary curcumin. Aquac. Int., 24: 1277–1286.

Mohamed A.A.-R., El-Houseiny W., Abd Elhakeem E.-M., Ebraheim L.L., Ahmed A.I., Abd El-Hakim Y.M. (2020). Effect of hexavalent chromium exposure on the liver and kidney tissues related to the expression of CYP450 and GST genes of *Oreochromis niloticus* fish: Role of curcumin supplemented diet. Ecotoxicol. Environ. Saf., 188: 109890.

Naiel M.A.E., Ghazanfar S., Negm S.S., Shukry M., Abdel-Latif H.M. (2023). Applications of antimicrobial peptides (AMPs) as an alter-

native to antibiotic use in aquaculture: a mini-review. Ann. Anim. Sci., 23: 691–701.

Parry R.M. Jr, Chandan R.C., Shahani K.M. (1965). A rapid and sensitive assay of muramidase. Proc. Soc. Exp. Biol. Med., 119: 384–386.

Pirani F., Moradi S., Ashouri S., Johari S.A., Ghaderi E., Kim H.P., Yu I.J. (2021). Dietary supplementation with curcumin nanomicelles, curcumin, and turmeric affects growth performance and silver nanoparticle toxicity in *Cyprinus carpio*. Environ. Sci. Pollut. Res., 28: 64706–64718.

Sarawi W.S., Alhusaini A.M., Fadda L.M., Alomar H.A., Albaker A.B., Aljrboa A.S., Alotaibi A.M., Hasan I.H., Mahmoud A.M. (2021 a). Curcumin and nano-curcumin mitigate copper neurotoxicity by modulating oxidative stress, inflammation, and Akt/GSK-3β signaling. Molecules, 26: 5591.

Sarawi W.S., Alhusaini A.M., Fadda L.M., Alomar H.A., Albaker A.B., Aljrboa A.S., Alotaibi A.M., Hasan I.H., Mahmoud A.M. (2021 b). Nano-curcumin prevents cardiac injury, oxidative stress and inflammation, and modulates TLR4/NF-κB and MAPK signaling in copper sulfate-intoxicated rats. Antioxidants, 10: 1414.

Shafique L., Abdel-Latif H.M., Hassan F.-u., Alagawany M., Naiel M.A., Dawood M.A., Yilmaz S., Liu Q. (2021). The feasibility of using yellow mealworms (*Tenebrio molitor*): Towards a sustainable aquafeed industry. Animals, 11: 811.

Sruthi M., Nair A.B., Arun D., Thushara V., Sheeja C., Vijayasree A.S., Oommen O.V., Divya L. (2018). Dietary curcumin influences leptin, growth hormone and hepatic growth factors in tilapia (*Oreochromis mossambicus*). Aquaculture, 496: 105–111.

Suvarna S.K., Layton C., Bancroft J.D. (2019). Theory and practice of histological techniques. 8th Edition. Elsevier Health Sci.

Swelum A.A., Hashem N.M., Abdelnour S.A., Taha A.E., Ohran H., Khafaga A.F., El-Tarabily K.A., Abd El-Hack M.E. (2021). Effects of phytogenic feed additives on the reproductive performance of animals. Saudi J. Biol. Sci., 28: 5816–5822.

Topic Popovic N., Strunjak-Perovic I., Coz-Rakovac R., Barisic J., Jadan M., Persin Berakovic A., Sauerborn Klobucar R. (2012). Tricaine methane-sulfonate (MS-222) application in fish anaesthesia. J. Appl. Ichthyol., 28: 553–564.

Yazdani Z., Mehrgan M.S., Khayatzadeh J., Shekarabi S.P.H., Tabrizi M.H. (2023). Dietary green-synthesized curcumin-mediated zinc oxide nanoparticles promote growth performance, haemato-biochemical profile, antioxidant status, immunity, and carcass quality in Nile tilapia (*Oreochromis niloticus*). Aquac. Rep., 32: 101717.

Yonar M.E., Yonar S.M., İspir Ü., Ural M.Ş. (2019). Effects of curcumin on haematological values, immunity, antioxidant status and resistance of rainbow trout (*Oncorhynchus mykiss*) against *Aeromonas salmonicida* subsp. *achromogenes*. Fish Shellfish Immunol., 89: 83–90.

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