

Influence of tulsi *Ocimum sanctum* extract on fish health: Growth, hematology, serum immune parameters, and antioxidant status in Common Carp

Syed Sikandar Habib¹, Saima Majeed², Khalid Hussain Rind³, Saira Naz⁴, Ümit Acar^{5,6,*}, Cristina Cravana⁷, Mujeeb Ullah⁸, Khalid Khan⁸, Muhammad Zahid⁸, Mohamed Mohany⁹, and Francesco Fazio^{7,*}

¹Department of Zoology, University of Sargodha, Sargodha, Punjab, Pakistan

²Department of Maritime Sciences, Bahria University Karachi Campus, Karachi, Sindh, Pakistan

³Department of Molecular Biology and Genetics, Shaheed Benazir Bhutto University, Shaheed Benazirabad, Sindh, Pakistan

⁴Department of Zoology, University of Lahore Sargodha Campus, Sargodha, Punjab, Pakistan

⁵Department of Forestry, Bayramiç Vocational School, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye

⁶Faculty of Marine Sciences and Technology, Department of Fisheries Industry Engineering, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye

⁷Department of Veterinary Sciences, University of Messina, Messina, Italy

⁸Department of Zoology, Islamia College University, Peshawar, Khyber Pakhtunkhwa, Pakistan

⁹Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

*Corresponding authors: Ümit Acar. Email: umitacar@comu.edu.tr, Francesco Fazio. Email: ffazio@unime.it.

ABSTRACT

Objective: In aquaculture, the trend is shifting towards using plant-derived alternatives that are abundant in phytochemicals as effective replacements for traditional antibiotics and synthetic feed additives. In the present study, the effects of tulsi *Ocimum sanctum* extract on growth performance, hemato-biochemical indices, serum immune parameters, and antioxidant parameters in Common Carp *Cyprinus carpio* were investigated.

Methods: Common Carp (mean body weight \pm SD = 10.6 \pm 0.13 g) were fed experimental diets that contained tulsi leaf extract at 0.0 (control), 0.5, 1.0, and 1.5% for 60 d (25 fish/treatment).

Results: The findings revealed a considerable enhancement in growth performance and a decreased feed conversion ratio, especially for the 1.0% tulsi-based diet. Additionally, weight gain and feed conversion ratio exhibited significance at both the linear and quadratic levels, as indicated by polynomial contrasts. The hematological and biochemical profiles exhibited improvements in groups receiving tulsi-enriched diets. The antioxidant status of fish serum exhibited a notable increase, as evidenced by elevated activities of total antioxidant capacity, superoxide dismutase, and catalase in fish that received the 1.0% and 1.5% tulsi-based diets. Tulsi-supplemented diets led to remarkable enhancements in serum lysozyme activity, alternative complement activity, and total immunoglobulin content. Moreover, tulsi supplementation at 1.0% and 1.5% showcased a significant reduction in serum glucose and cortisol levels compared to the other groups.

Conclusions: In conclusion, tulsi extract emerged as a valuable asset, positively influencing growth, blood parameters, antioxidant balance, and serum immune response in Common Carp, particularly at supplementation levels ranging from 1.0% to 1.5% in the diet.

KEYWORDS: aquaculture, Common Carp, immunostimulant, phytotherapy, tulsi

LAY SUMMARY

This research provides compelling evidence of the remarkable benefits of tulsi extract in Common Carp aquaculture. By enhancing growth performance, feed conversion efficiency, and key hematological and biochemical parameters, tulsi emerges as a powerful natural supplement. Additionally, its significant role in boosting antioxidant status and immune responses further underscores its potential in promoting overall fish health. These findings open new avenues for sustainable and natural approaches in aquaculture, reinforcing the value of tulsi as a functional feed additive.

Received: May 20, 2024. Revised: February 10, 2025. Editorial decision: February 19, 2025

© The Author(s) 2025. Published by Oxford University Press on behalf of American Fisheries Society.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<https://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact reprints@oup.com for reprints and translation rights for reprints. All other permissions can be obtained through our RightsLink service via the Permissions link on the article page on our site—for further information please contact journals.permissions@oup.com.

INTRODUCTION

Aquaculture plays a crucial role in meeting the ever-growing demand for protein-rich food sources, and the health and performance of cultivated fish species are paramount to sustainable aquaculture practices (Boyd et al., 2022). The Common Carp *Cyprinus carpio* stands out as one of the most widely cultivated freshwater fish globally. In recent years, there has been a growing interest in exploring natural additives to enhance the overall well-being and productivity of cultured fish (Kuebutornye et al., 2024).

The use of antibiotics and chemotherapeutics in aquaculture has faced significant criticism due to adverse effects, such as drug accumulation in tissues, the development of drug resistance, and immunosuppression (Hamed et al., 2022). In response to these concerns, immunostimulants have emerged as a preventive measure (Abdel-Tawwab, Abdulrahman, et al., 2022). Immunostimulants derived from plant sources have gained attention, as they exhibit none of the detrimental side effects associated with antibiotics, making them an attractive alternative for disease treatment in fish and environmental safety (Abdel-Tawwab et al., 2021). Tulsi *Ocimum sanctum* (also known as *O. tenuiflorum*) has a nutritional profile that is rich in essential components, such as protein, fat, carbohydrates, iron, calcium, and vitamins A and C (Parveen et al., 2023). Beyond its nutrient content, tulsi plays a pivotal role in promoting efficient digestion and enhancing the absorption of vital nutrients by the body (Islam et al., 2023). The stem and leaves of tulsi contain a diverse array of compounds that exhibit potential biological activities; these include saponins, flavonoids, triterpenoids, and tannins (Borah & Biswas, 2018).

In Asia, where traditional herbal remedies have been employed for promoting health and preventing diseases for centuries (Yuan et al., 2016), tulsi (also known as “holy basil”) has gained prominence (Parveen et al., 2023). Referred to as the “queen of plants” and the “mother medicine of nature,” tulsi is famous for its extensive medicinal properties, including anti-asthmatic effects (Singh & Agrawal, 1991), anti-inflammatory effects (Mirje & Ramabhimaiah, 2014), hypoglycemic activity (Sethi et al., 2004), antipyretic activity (Pushpam et al., 2017), endocrinological effects (Sethi et al., 2010), antimicrobial activity (Yamani et al., 2016), anti-stress activity (Jothie Richard et al., 2016), anti-tubercular activity (Jayapal et al., 2021), antioxidant activity (Kaur et al., 2018), and immunomodulatory activity (Mondal et al., 2011). Das et al. (2015) documented the immunostimulatory impact of tulsi extract (0.2%) in Rohu *Labeo rohita*, while Bhatnagar and Mann (2023) revealed a notable enhancement in both growth and immunity of *Cirrhinus mrigala* with a tulsi-based supplemented diet.

Despite the proven efficacy of tulsi in various medicinal applications, its potential in fish health has been underexplored. Limited studies have been conducted on the growth and immunomodulatory effects of tulsi in fish, and no extensive study has been reported on the effects of tulsi in Common Carp. In light of the scarcity and scattered nature of research in this area, this study aims to comprehensively explore the growth, hemato-biochemical parameters, serum antioxidant status, and immunomodulatory effects of tulsi on Common Carp.

METHODS

Plant preparation

We purchased fresh tulsi leaves from a local farmer in Pakistan, and the Sargodha University botanical laboratory in Pakistan verified the plant species and the health of the leaves. After being washed with sterile water, the tulsi leaves were dried in the shade at room temperature. Next, they were crushed using an electrical mixer–grinder. The powdered leaves of tulsi were subjected to extraction using distilled water in a Soxhlet apparatus for four cycles following the method described by Paech and Tracey (1956). Subsequently, the extracted material underwent filtration through sterile muslin cloth with a pore size of approximately 100 μm . The resulting filtrate was then evaporated to dryness using a rotary evaporator at a temperature below 40°C to ensure that the bioactive compounds were preserved. The yield, relative to the dry powdered material of tulsi, was found to be 10.39% weight/weight. The resulting dried powder, which constituted the crude aqueous extract of tulsi leaf, was weighed and subsequently stored at –20°C until further use. A study on the phytochemical characteristics of tulsi leaves was carried out using the method described by Panchal and Parvez (2019; Table 1).

Diet preparation

The ingredients for the diet (Table 2) were purchased and individually ground into a powder using a laboratory-scale grinder and then were sifted through a sieve with a mesh size of 250 μm to ensure a uniform particle size. The ingredients were then weighed to approximately 0.01 g and thoroughly blended using a mixer. Four experimental diets were formulated to be isocaloric and isoproteic. These diets included tulsi extract at varying inclusion rates: 0.0 (control), 0.5, 1.0, and 1.5%. The inclusion levels of tulsi were established from previous research conducted on other fish species (Das et al., 2015; Logambal et al., 2000; Venkatalakshmi & Michael, 2001). All ingredients were combined in the required quantities and mixed with distilled water to form a paste. A calculated amount of fish oil sourced from menhaden (Daybrook Fisheries, United States) was then incorporated into the paste before cooking at 85°C for 10 min. Once cooled, the paste was blended with vitamins and mineral mixtures according to the respective treatment protocols. The resulting mixture was passed through a meat grinder to produce uniform-sized (2-mm) pellets. The pellets were air-dried at room temperature for 48 h. After drying, the pellets were packed into polyethylene bags, sealed airtight, and labeled according to the different treatment concentrations for further use. The nutritional content of the analyzed feed was evaluated using the protocol described by the Association of Official Analytical Chemists (2000).

Experimental conditions

About 320 healthy Common Carp fingerlings were procured from a nearby fish farm and underwent a 2-week acclimatization period to adjust to laboratory conditions. The health of the fish was determined by observing their physical and behavioral characteristics. The fish exhibited active swimming and rapid movement; they had shiny and smooth scales, clear corneas, and gills with a normal red color. There were no

Table 1. Phytochemical profile of tulsi leaves (+ = available but low concentration; ++ = medium concentration; +++ = high concentration).

Phytochemical compound	Test	Tulsi leaves
Flavonoids	Alkaline reagent test	++
Alkaloids	Mayer's test	++
Glycosides	Legal's test	+
Tannins	Gelatin test	+
Saponins	Foam test	+++
Anthraquinones	Sulfuric acid test	Absent
Terpenoids	Salkowski's test	Absent
Steroids	Sulfuric acid test	++
Proteins and amino acids	Xanthoproteic test	++
Reducing sugars	Fehling's test	+
Carbohydrates	Molisch's test	+
Fats and fixed oil	Filter paper press test	++
Phytosterols	Liebermann–Burchard's test	+
Phenols	Litmus test	+++

visible wounds, parasites, or fungal infections. Their appetites and feeding behaviors were normal, and they did not show any signs of labored respiration or lethargy. Additionally, there were no other observed indications of disease or stress. During this acclimation phase, the fish were fed with a basal diet comprising 36% crude protein (sourced from Aqua Feed Suppliers, Pakistan) provided in carefully measured quantities to support their nutritional requirements and promote a smooth transition to laboratory conditions. Upon completion of the acclimatization period, 300 physically healthy fish with an average weight (\pm SD) of 10.6 ± 0.13 g were randomly allocated to 12 static fiberglass tank systems. Each tank held 360 L of dechlorinated and aerated tap water, with 25 fish/tank (tank size: $120 \times 50 \times 60$ cm). This experimental setup was maintained for a duration of 60 d; the fish were regularly fed with their respective diets, including the control group and those supplemented with 0.5, 1.0, and 1.5% tulsi. The fish were manually fed three times per day, with each feeding amounting to 3% of the biomass. Feed quantities were adjusted every 2 weeks. Any uneaten feed and fish feces were removed daily using a siphon.

The experiment was conducted in triplicate, with a 50% exchange of rearing water with fresh water weekly. Throughout the experiment, a photoperiod of 12 h light : 12 h dark was maintained. Water quality parameters were monitored daily. The measured values were recorded as follows: Temperature was $27.42 \pm 0.52^\circ\text{C}$ (mean \pm SD), pH was 7.59 ± 0.26 , dissolved oxygen was 7.04 ± 0.57 mg/L, ammonia was 0.002 mg/L, nitrite was 0.005 mg/L, and nitrate was 1.04 mg/L. Temperature, pH, and dissolved oxygen were assessed utilizing a multiprobe water quality system (YSI, Inc.). Chemical parameters of the water, including ammonia, nitrite, and nitrate, were analyzed spectrophotometrically on a weekly basis using HACH kits.

Growth performance

The assessment of growth performance was conducted according to Bulut et al. (2014). In brief, fish were individually weighed at the start and end of the experiment. Additionally, every 2 weeks, the fish were weighed in groups to evaluate their growth

performance. The following formulas were used to determine growth parameters:

$$\text{Weight gain (WG, g)} = \text{final body weight (g)} - \text{initial body weight (g)}$$

$$\text{Weight gain (\%)} = 100 \times (\text{weight gain}/\text{initial weight})$$

$$\text{Feed intake (FI)} = \text{feed consumed}/\text{number of surviving fish}$$

$$\text{Specific growth rate (SGR, \%)} = [(\log_e \text{ final weight} - \log_e \text{ initial weight})/\text{number of days}] \times 100$$

$$\text{Feed conversion ratio (FCR)} = \text{feed given (dry weight)}/\text{body WG (wet weight)}$$

$$\text{Survival rate (SR, \%)} = \text{total number of fishes harvested}/\text{total number of fishes stocked} \times 100.$$

Blood sampling

Upon completion of the experiment, a random selection of five fish was collected from each tank, totaling 15 specimens for each treatment group. The selected fish were sedated using clove oil at a concentration of 80 mg/L (Hajek et al., 2006). Blood samples were then collected from the caudal vein using a 2-mL syringe. The obtained samples were promptly transferred to nonheparinized tubes and subjected to immediate centrifugation at $1,500 \times g$ for 5 min at 4°C to separate the serum. For the analysis of hematological indices, blood was carefully transferred to heparinized tubes and allowed to clot at room temperature for 40 min.

Hematological and serological assays

To count white blood cells (WBCs) and red blood cells (RBCs), heparinized blood was collected to prevent clotting. The blood sample was then appropriately diluted in phosphate-buffered saline to achieve a suitable cell concentration for counting. Cells were counted using a hemocytometer (Sarder et al., 2001). Hemoglobin (Hb) levels were determined by mixing blood samples with Drabkin's reagent. The absorbance of the resulting mixture was then measured at a wavelength of 540 nm using a spectrophotometer. The absorbance values were used to calculate the Hb concentration in the blood samples (Blaxhall & Daisley, 1973). The concentration of Hb was reported in grams per deciliter (g/dL). Hematocrit (Hct) values were assessed via the methodology described by Cyriac et al. (1989). Heparinized blood was loaded into microhematocrit tubes and subjected to centrifugation at $111.8 \times g$ for 5 min. The calculation of Hct involved multiplying the length of packed red cells (mm) by 100 and then dividing it by the total length of the blood sample (mm) in the tube. The resulting value was reported as the Hct level.

Spectrophotometric analysis was used to investigate serum albumin (ALB) and total protein (TP), adhering to the methodologies delineated by Reinhold (1953) for ALB and

Table 2. Ingredient analysis in the experimental diet (fish meal: Fazal Fish Meal, Karachi, Pakistan; soybean meal: Hi-Tech Feed, Lahore, Pakistan; poultry meal: Pak Feed, Lahore, Pakistan).

Basic ingredient (% of total)	Concentration of tulsi extract (%)			
	0.0 (control)	0.5	1.0	1.5
Fish meal (70.5% crude protein)	14	14	14	14
Soybean meal (45% crude protein)	20	20	20	20
Poultry meal (65.5% crude protein)	14	14	14	14
Wheat flour	35	34.5	34	33.5
Corn flour	12	12	12	12
Soybean oil	1	1	1	1
Fish oil	2	2	2	2
Vitamin premix ^a	1	1	1	1
Mineral premix ^b	1	1	1	1
Tulsi extract	0.0	0.5	1.0	1.5
Chemical examination (%)				
Dry matter	90.4	90.3	89.5	90.0
Crude protein	37.3	37.3	36.8	37
Crude fiber	5.4	5.3	5.6	5.5
Crude lipid	8.49	8.35	8.48	8.57
Ash	8.88	8.76	8.63	9.12
Nitrogen-free extract ^c	30.33	30.59	29.99	29.81
Energy (kJ/g) ^d	17.84	17.93	17.47	17.88

^aVitamin premix (per kg of diet): vitamin A (2,000 IU), vitamin B1 (thiamin; 5 mg), vitamin B2 (riboflavin; 5 mg), vitamin B6 (5 mg), vitamin B12 (0.025 mg), vitamin D3 (1,200 IU), vitamin E (63 mg), vitamin K3 (2.5 mg), folic acid (1.3 mg), biotin (0.05 mg), calcium d-pantothenate (20 mg), inositol (60 mg), and choline chloride (2,000 mg).

^bMineral premix (per kg of diet): iron (75.3 mg), copper (12.2 mg), manganese (206 mg), zinc (85 mg), iodine (3 mg), selenium (0.350 mg), and cobalt (1 mg).

^cNitrogen-free extract (dry matter) = (crude lipid + crude ash + crude protein + crude fiber).

^dEnergy was calculated according to 23.6 kJ/g protein, 39.5 kJ/g lipid, and 17.0 kJ/g nitrogen-free extract.

Henry (1964) for TP. Serum ALB was determined by adding a specific volume of the serum sample to bromocresol green reagent and allowing it to react at room temperature for a few minutes. The resulting color change was measured spectrophotometrically at approximately 630 nm. Total protein was determined based on the biuret reaction, with the resulting color change measured spectrophotometrically at 540 nm. The quantification of serum globulin (GLOB) was conducted according to the procedure outlined by Coles (1986). The concentration of serum GLOB was calculated by subtracting the serum ALB value from the serum TP value. The determination of serum glucose (GLU) content was performed using the glucose oxidase reaction at 37°C for 20 min. The absorbance of the colored compound was measured at 500 nm using a spectrophotometer.

Serum cortisol (CORT) content was quantified using a commercial enzyme-linked immunosorbent assay kit (ZellBio, Germany). All tests were conducted in accordance with the guidelines provided by the respective suppliers.

The lysozyme activity in serum samples was assessed using a turbidimetric test as outlined by Koskela et al. (2004). This method relies on lysis of the bacterium *Micrococcus luteus* (Sigma) when exposed to mucus lysozyme. A unit of lysozyme activity was defined as the quantity of enzyme causing a reduction in absorbance of 0.001 per minute. The assessment of alternative complement pathway hemolytic activities (ACH50) in serum followed the procedure described by Yano (1992). Spectrophotometric measurements at 414 nm determined the serum volume causing 50% hemolysis of rabbit RBCs, with ACH50 reported as units per milliliter. The total immunoglobulin (Ig) content in serum samples was determined using the method outlined by Siwicki and Anderson (1993). This method

involves subtracting the entire protein content of serum before and after treatment with 12% polyethylene glycol (10,000 MW; Sigma). The Ig content of serum samples was expressed in milligrams per milliliter (mg/mL).

Antioxidant status

To assess antioxidant activities in the serum, commercially available colorimetric kits from Shanghai Kehua Bio-Engineering Co., Ltd., were employed. The evaluation included the determination of serum total antioxidant capacity (TAC) using the method described by Koracevic et al. (2001). This method assessed the ability of serum antioxidants to neutralize free radicals. The serum sample was mixed with a chromogenic substrate and a peroxidase enzyme, initiating color change, which was measured spectrophotometrically at around 505 nm. Catalase (CAT) activity was measured according to the procedures outlined by Aebi (1984). Catalase was allowed to react with a known amount of H₂O₂; after precisely 1 min, a CAT inhibitor halted the reaction. The remaining H₂O₂ then reacted with 3,5-dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone in the presence of peroxidase, forming a chromophore. The color intensity of this chromophore, measured at 510 nm, was inversely proportional to the amount of CAT present. Superoxide dismutase (SOD) activity was measured following the method of Nishikimi et al. (1972). The reduction in absorbance at 560 nm over 5 min indicated the inhibition rate, which was directly proportional to SOD activity.

Statistical analysis

The normality of the data was assessed through the Kolmogorov–Smirnov test. All statistical analyses for the data

Table 3. Growth performance (mean \pm SD) of Common Carp fed with tulsi extract for a 60-d study period. Within a given row, values without a letter in common are significantly different ($P < 0.05$). Abbreviations are as follows: IW = initial weight, FCR = feed conversion ratio, FI = feed intake, FW = final weight, SGR = specific growth rate, SR = survival rate, and WG = weight gain.

Parameter	Control	0.5% tulsi	1.0% tulsi	1.5% tulsi	P-value for polynomial contrasts	
					Linear	Quadratic
IW (g)	10.60 \pm 0.24 z	10.91 \pm 0.14 z	11.09 \pm 0.28 z	11.12 \pm 0.17 z	0.34	0.76
FW (g)	32.02 \pm 1.87 y	35.62 \pm 1.29 zy	39.03 \pm 1.52 z	38.54 \pm 1.37 z	0.001	0.24
WG (g)	21.42 \pm 1.68 y	24.71 \pm 1.35 zy	27.94 \pm 1.45 z	27.52 \pm 1.29 z	0.0001	0.0001
WG (%)	202.08 \pm 3.98 y	226.55 \pm 3.68 z	227.97 \pm 3.62 z	246.54 \pm 3.54 z	0.0001	0.0001
FI (g)	51.06 \pm 0.52 x	51.70 \pm 0.27 x	54.84 \pm 0.32 y	57.92 \pm 0.37 z	0.0001	0.0001
SGR (%)	1.76 \pm 0.03 y	1.95 \pm 0.05 z	2.06 \pm 0.04 z	2.04 \pm 0.05 z	0.07	0.001
FCR	1.73 \pm 0.05 z	1.49 \pm 0.07 y	1.34 \pm 0.04 y	1.41 \pm 0.06 y	0.01	0.02
SR (%)	100 z	100 z	100 z	100 z	0.83	0.94

obtained in this study were conducted using SPSS version 20.0. One-way ANOVA was used to compare the effects of different concentrations of tulsi extract. Polynomial contrasts were used in the statistical analysis to examine the relationship between different extract concentrations and the growth, hematology, serum immune parameters, and antioxidant status of Common Carp. A P -value less than 0.05 was considered indicative of a significant difference. Tukey's post hoc test was used to assess differences among means, with linear regression serving as the basis.

RESULTS

Growth performance

The effect of tulsi extract supplementation on the growth performance of Common Carp is illustrated in Table 3. Following the 60-d feeding trial, the inclusion of tulsi extract resulted in a significant ($P < 0.05$) increase in WG only at tulsi levels of 1.0% and 1.5% when compared to the control group. The fish fed the experimental diets exhibited a significantly ($P < 0.05$) higher percent of WG compared to the control group. Additionally, the feed intake was significantly ($P < 0.05$) greater in fish that received the diet supplemented with 1.5% tulsi extract, followed by those receiving 1.0% tulsi extract.

Fish that were fed a tulsi-based fortified diet also exhibited a considerable elevation in SGR compared to fish in the control group. Furthermore, the FCR showed a significant ($P < 0.05$) decrease in fish that were fed the experimental diets. There were no instances of fish mortality during the initial 60-d tulsi feeding phase.

In Table 3, the analysis of polynomial contrasts—specifically linear and quadratic regression—unveiled remarkable findings in relation to fish growth. Notably, the final weight exhibited significance ($P < 0.05$) at the linear level. Furthermore, the responses of WG and FCR demonstrated significance ($P < 0.05$) under both linear and quadratic regressions when the fish were fed with tulsi extract at 1.0% and 1.5%. Additionally, the SGR showed significance ($P < 0.05$) at the quadratic level.

Hematological parameters

The hematological parameters of Common Carp that were fed diets supplemented with tulsi extract are given in Table 4. Fish that received diets containing tulsi extract showed

significantly higher counts of WBCs and RBCs compared to the control group ($P < 0.05$). However, within the experimental groups, there was no dose-dependent response, as varying inclusion levels of tulsi extract did not significantly affect these parameters ($P > 0.05$). Further, the polynomial (linear) for WBCs revealed significant ($P < 0.05$) results in fish that were fed diets containing tulsi extract at 1.0% and 1.5%. The group fed with 1.0% tulsi extract demonstrated the highest Hb values, as shown by linear regression, followed by those fed with 1.5% and 0.5% tulsi extract, with all inclusion levels showing significant differences from the control group. Moreover, fish that were fed diets with 1.0% and 1.5% tulsi extract displayed significantly elevated Hct levels compared to the control group. The polynomial contrast (linear and quadratic) for Hct revealed significant results in fish groups with tulsi extract inclusion levels of 1.0% and 1.5%.

Serum biochemical parameters

Table 5 presents the outcomes of serum biochemical assays for Common Carp after the 60-d feeding trial. Fish that were fed diets with 1.0% and 1.5% tulsi extract exhibited significantly ($P < 0.05$) higher levels of ALB and TP, as revealed by quadratic regression, compared to the control group. However, these parameters were not significantly ($P > 0.05$) different from those of fish fed 0.5% tulsi extract. Additionally, only the groups fed with 1.0% and 1.5% tulsi extract showed a significant increase in GLOB levels compared to the control. Although tulsi extract supplementation did not influence GLU levels in Common Carp relative to GLU in fish that received the control diet, CORT levels tended to decrease in tulsi-fed fish. Notably, significantly lower CORT levels were recorded in fish that were fed 1.0% tulsi extract compared to those receiving 0.5% tulsi extract or the control diet. According to the polynomial contrasts, significant ($P < 0.05$) results were obtained for GLOB and CORT.

Immunological indices

The serum lysozyme activity levels in fish treated with 1.0% and 1.5% tulsi extract were significantly higher ($P < 0.05$), followed by 0.5% tulsi extract, compared to the control, as indicated by polynomial (linear) contrasts (Figure 1).

The results of ACH50 (Figure 2) indicated that at the end of the feeding trial, the fish that were given diets fortified with

Table 4. Hematological indicators (mean \pm SD) of Common Carp fed with tulsi extract for a 60-d study period. Within a given row, values without a letter in common are significantly different ($P < 0.05$). Abbreviations are as follows: Hb = hemoglobin, Hct = hematocrit, RBCs = red blood cells, and WBCs = white blood cells.

Parameter	Control	0.5% tulsi	1.0% tulsi	1.5% tulsi	P-value for polynomial contrasts	
					Linear	Quadratic
WBCs ($\times 10^3/\mu\text{L}$)	2.16 \pm 0.09 y	2.57 \pm 0.08 z	2.73 \pm 0.06 z	2.58 \pm 0.08 z	0.01	0.06
RBCs ($\times 10^6/\mu\text{L}$)	1.12 \pm 0.08 y	1.33 \pm 0.05 z	1.43 \pm 0.04 z	1.35 \pm 0.04 z	0.26	0.35
Hb (g/dL)	4.95 \pm 0.13 x	5.47 \pm 0.29 y	6.03 \pm 0.20 z	5.68 \pm 0.25 zy	0.0001	0.07
Hct (%)	20.38 \pm 1.68 y	22.26 \pm 2.06 y	27.48 \pm 1.47 z	26.47 \pm 1.28 z	0.0001	0.0001

Table 5. Biochemical indices (mean \pm SD) of Common Carp fed with tulsi extract for a 60-d study period. Within a given row, values without a letter in common are significantly different ($P < 0.05$). Abbreviations are as follows: ALB = albumin, CORT = cortisol, GLOB = globulin, GLU = glucose, and TP = total protein.

Parameter	Control	0.5% tulsi	1.0% tulsi	1.5% tulsi	P-value for polynomial contrasts	
					Linear	Quadratic
ALB (g/dL)	1.63 \pm 0.38 y	1.98 \pm 0.12 zy	2.25 \pm 0.24 z	2.77 \pm 0.17 z	0.09	0.001
TP (g/dL)	3.54 \pm 0.28 y	3.97 \pm 0.12 zy	4.89 \pm 0.14 z	5.47 \pm 0.14 z	0.22	0.01
GLOB (g/dL)	1.91 \pm 0.05 y	1.99 \pm 0.07 y	2.64 \pm 0.14 z	2.70 \pm 0.08 z	0.0001	0.0001
GLU (g/dL)	62.27 \pm 2.53 z	60.75 \pm 2.28 z	57.19 \pm 2.54 z	58.32 \pm 2.02 z	0.37	0.42
CORT (ng/mL)	34.54 \pm 1.72 z	31.43 \pm 1.25 zy	26.27 \pm 2.13 x	27.54 \pm 2.22 yx	0.0001	0.0001

1.0% and 1.5% tulsi extract consistently showed significantly higher ($P < 0.05$) ACH50 compared to the control group, as determined by linear regression.

The Ig content in the fish serum remained significantly higher (polynomial linear) throughout the study period for the groups that were fed diets containing 1.0% and 1.5% tulsi compared to the control group (Figure 3).

Antioxidant status

Table 6 illustrates the findings of serum antioxidant status in Common Carp following the 60-d feeding trial. The results indicated that fish receiving a 1.0% tulsi-based diet exhibited significantly higher levels ($P < 0.05$) of TAC, CAT, and SOD. This trend was followed by fish fed with 1.5% tulsi extract, showing significantly higher values compared to the 0.5% tulsi and control groups. Polynomial contrasts (linear and quadratic) revealed significant differences between fish groups for SOD, with responses being significant ($P < 0.05$). However, for TAC, significance was observed at the quadratic level, while CAT showed significance at the linear level, as indicated in Table 6.

DISCUSSION

Research has shown that incorporating medicinal plant-based supplements in aquaculture can improve growth rates, immune defenses, and resistance to pathogens and stress (Abdel-Tawwab et al., 2024; Fazio et al., 2022). This study evaluated the effects of tulsi leaf extract at 0.5, 1.0, and 1.5% on Common Carp, focusing on growth, hemato-biochemical parameters, antioxidant status, and immune defense. Tulsi has been linked to enhanced digestive enzyme activity and growth (Awad & Awaad, 2017). Our results showed that tulsi extract improves growth, particularly at 1.0% and 1.5% concentrations, with

similar findings from studies on other plant extracts (Rashidian et al., 2022; Yousefi et al., 2021). Tulsi's benefits include antimicrobial effects, enzyme stimulation, and improved digestion and nutrient absorption (Rani et al., 2014). It also offers antistress, antioxidant, antimicrobial, and immunomodulatory properties (Cohen, 2014; Mondal et al., 2011) along with gastroprotective effects (Eshraghian, 2013). Our findings indicate that tulsi enhances the FCR and SGR, thus suggesting improvements in feed utilization and growth performance, which can optimize feed management and reduce costs in aquaculture (Habib, Batool, et al., 2022; Rind et al., 2023; Tran et al., 2022).

In our study, we observed a significant increase in the counts of WBCs and RBCs in fish that were fed a diet supplemented with tulsi as compared to the control group. This suggests that the tulsi-based diet may have a positive influence on the immune response and overall blood physiology of the fish. Gabriel et al. (2021) reported that incorporating garlic *Allium sativum* and aloe *Aloe vera* improved hematological indices compared to unsupplemented fish. Those authors further noted that higher concentrations of the herbal extracts (e.g., 2% and 4%) yielded better results than lower concentrations.

Tulsi is rich in nutrients, like vitamins and minerals that are essential for blood cell development (Arshad et al., 2021). Higher RBC levels in fish are associated with improved oxygen transport, affecting metabolism and overall health (Habib, Naz, et al., 2022). Increased WBC counts can enhance immune function (Habib, Batool, et al., 2022; Ruan et al., 2023). Reductions in RBCs and Hb are linked to an impaired antioxidant system, with ammonia exposure leading to oxidative stress and anemia (Chen et al., 2023; Hoseini et al., 2019). Tulsi's strong antioxidant properties (Vidhani et al., 2016) may protect RBCs from oxidative damage and extend their life span. This supports the findings of the current study, as fish that

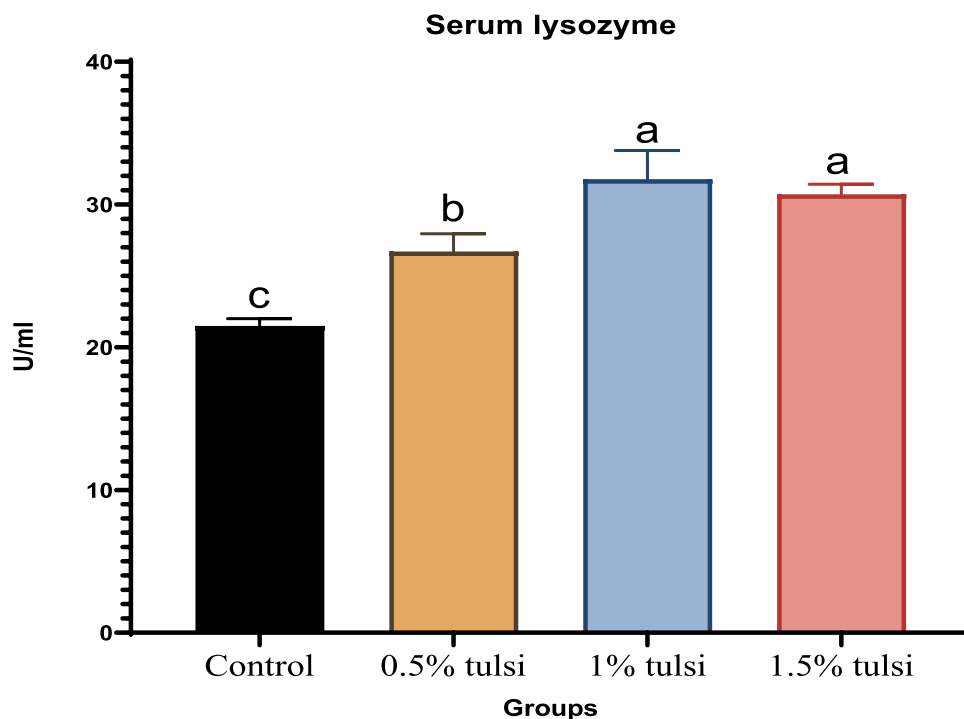


Figure 1. Effect of different concentrations of tulsii extract on the lysozyme activity in the serum of Common Carp. Dissimilar letters signify a significant difference between groups.

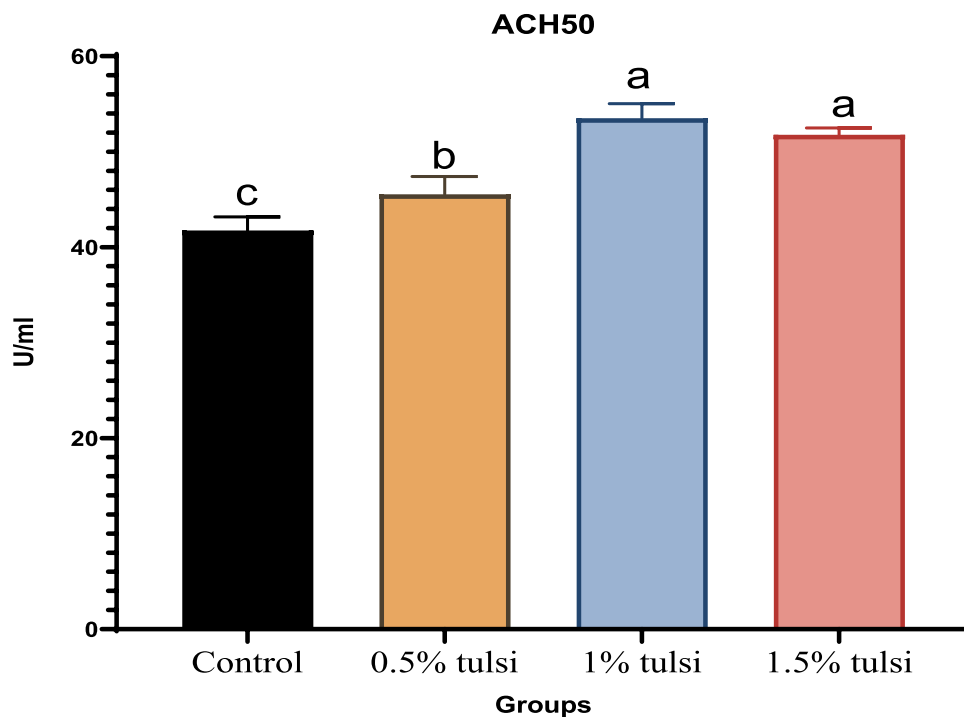


Figure 2. Effect of different concentrations of tulsii extract on the alternative complement pathway hemolytic activity (ACH50) in the serum of Common Carp. Dissimilar letters signify a significant difference between groups.

were fed tulsii extracts showed higher RBC levels compared to control fish. Hematocrit, which reflects RBC quantity and size, also increased. Similar findings have been reported from studies in which fish that were fed with Valonia oak *Quercus aegilops* (Abdel-Tawwab, Abdulrahman, et al., 2022; Abdel-Tawwab,

Eissa, et al., 2022) and *Withania coagulans* (Fazio et al., 2023) showed elevated levels of RBCs, Hb, and Hct.

In the current research, fish that were fed a diet supplemented with tulsii extract exhibited increased levels of ALB, TP, and GLOB in their serum. The elevated levels of these

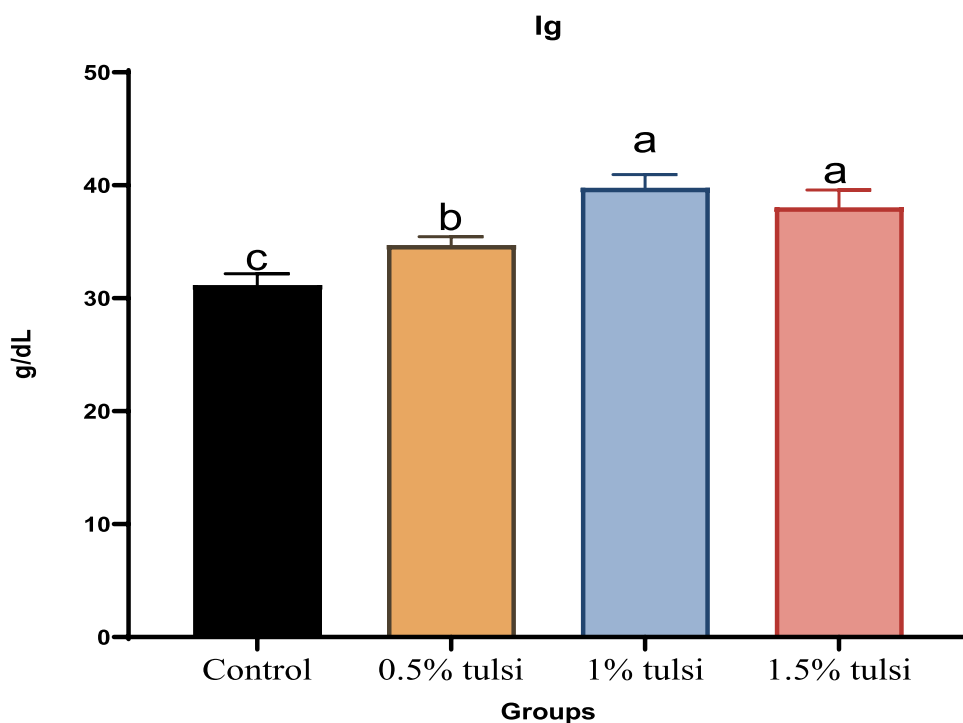


Figure 3. Effect of different concentrations of tulsi extract on the total immunoglobulin (Ig) content in the serum of Common Carp. Dissimilar letters signify a significant difference between groups.

Table 6. Antioxidant status (mean \pm SD) of Common Carp fed with tulsi extract for a 60-d study period. Within a given row, values without a letter in common are significantly different ($P < 0.05$). Abbreviations are as follows: CAT = catalase, SOD = superoxide dismutase, and TAC = total antioxidant capacity.

Parameter	Control	0.5% tulsi	1.0% tulsi	1.5% tulsi	P-value for polynomial contrasts	
					Linear	Quadratic
TAC (mM/L)	1.56 \pm 0.09 x	1.68 \pm 0.08 x	3.14 \pm 0.15 z	2.05 \pm 0.13 y	0.08	0.01
CAT (U/L)	63.83 \pm 2.84 w	71.72 \pm 2.74 x	90.53 \pm 3.02 z	83.56 \pm 2.37 y	0.001	0.09
SOD (U/mL)	5.03 \pm 0.62 x	6.06 \pm 0.35 x	11.06 \pm 0.93 z	8.37 \pm 0.42 y	0.0001	0.0001

parameters observed in our study are consistent with the findings of Mohammadi et al. (2018), who conducted a study involving the administration of date palm *Phoenix dactylifera* seed extract to Common Carp. A study by Amirkhani and Firouzbakhsh (2015) further supports our findings, demonstrating increased levels of ALB, TP, and GLOB in Common Carp that were fed a diet based on basil *Ocimum basilicum*. Basil and tulsi share numerous similarities due to their close relation within the *Ocimum* genus. Both extracts are rich in bioactive compounds, such as eugenol, ursolic acid, rosmarinic acid, and flavonoids, which contribute to their potent antioxidant, antimicrobial, and anti-inflammatory properties. These plants are widely recognized for their immune-boosting and adaptogenic effects (Kamelnia et al., 2023; Srinivas et al., 2016). The higher concentrations of ALB, TP, and GLOB in the serum of fish fed a fortified diet signify improved nutritional status, enhanced growth, and a potentially strengthened immune system (Habib, Batool, et al., 2022; Hoseini et al., 2018). Key indicators of stress in fish involve changes in both serum GLU and CORT levels (Ray

& Sinha, 2014). These alterations are sensitive to variations in environmental conditions or dietary factors (Li et al., 2024; Sopinka et al., 2016). Under normal circumstances, CORT plays a regulatory role in a variety of physiological processes in fish, allowing for quick adjustments in response to stress (Abd El-Naby et al., 2023; Aluru & Vijayan, 2009). Cortisol serves as a stimulator for different components of intermediary energy metabolism, enhancing oxygen absorption, promoting gluconeogenesis, and hindering glycogen synthesis (Sai Kumar, 2023). In the present study, incorporating tulsi into the diet led to reduction in both serum GLU and CORT levels. The significant impact on these biomarkers is attributed to the abundant flavonoids and essential minerals present in tulsi, which actively contribute to the regulation of GLU uptake and lipid metabolism (Borah & Biswas, 2018). Similar outcomes were observed in related research, where the inclusion of nutmeg *Myristica fragrans* extract in the diet resulted in reduced CORT and GLU levels in Common Carp (Rashidian et al., 2022), and supplementation of the diet with ginger *Zingiber officinale* demonstrated

parallel findings of decreased CORT and GLU levels in Common Carp (Fazelan et al., 2020).

Tulsi is recognized for its exceptional pharmacological attributes, including a diverse range of medicinal properties, with notable antibacterial capabilities (Yamani et al., 2016). The inclusion of tulsi extract in fish diets resulted in significant increases in serum lysozyme activity, ACH50, and Ig content. Notably, the most substantial increases were observed in fish that were fed diets containing 1.0% and 1.5% tulsi extract. These results align with the findings of Das et al. (2015), who examined the effects of tulsi leaf extract on the immunity and survival of Rohu. Similar findings were reported in a previous study on Rohu, which investigated the effects of a diet containing garlic (Sahu et al., 2007). A study on Mozambique Tilapia *Oreochromis mossambicus* demonstrated that immunity was enhanced by administering a diet supplemented with *Moringa oleifera* (Mbokane & Moyo, 2018).

Catalase and SOD are essential endogenous antioxidants that play a vital role in protection of organisms from harmful oxygen radicals generated during aerobic processes. The toxic superoxide anion radical (O_2^-) is transformed into nontoxic water (H_2O) and oxygen (O_2) through the coordinated action of SOD and CAT (Sharma et al., 2012). These enzymes contribute significantly to the protection of cells and tissues from oxidative stress, helping maintain cellular health and integrity in the presence of reactive oxygen species (Bratovic, 2020; Yu et al., 2023). In the present research, a notable rise in TAC, CAT activity, and SOD activity in fish serum was observed when the diet was supplemented with tulsi, compared to control levels. This observed effect can be linked to the presence of bioactive compounds in tulsi, such as flavonoids, steroids, terpenoids, glycosides, alkaloids, polyphenols, and vitamins (Borah & Biswas, 2018; Fazio et al., 2022). Other studies have reported similar findings by supplementing fish diets with plant extracts. For instance, in a study conducted by Almarri et al. (2023), Nile Tilapia *Oreochromis niloticus* showed comparable results when fed the leaf extract of sugar apple *Annona squamosa*. Additionally, Yousefi et al. (2021) demonstrated similar outcomes in Common Carp when the diet was supplemented with wormwood *Artemisia absinthium* extract.

In this study, we used an aqueous extract of tulsi to assess its impact on growth, hematology, serum immune parameters, and antioxidant status in Common Carp. It is crucial to recognize that variability in plant extract composition can affect reproducibility and consistency. Factors such as environmental conditions, harvest timing, and extraction methods can influence the chemical profile of tulsi and lead to variations in the concentrations of active compounds, thus impacting the observed biological effects (Habib et al., 2024). Differences in soil quality, temperature, and humidity can also affect phytochemical synthesis (Zhuo et al., 2024). The harvest stage and extraction methods affect the potency and composition of tulsi extracts. To improve reproducibility and standardize use, future research should focus on identifying and quantifying active components. A key limitation of this study is the lack of identification of the specific active compounds in tulsi extract that were responsible for the observed effects. Although we noted benefits in growth, hematology, immune parameters, and antioxidant status, the

precise bioactive constituents remain undetermined. This limits reproducibility and consistency due to variability in plant extract composition.

Conclusions

In conclusion, integrating tulsi leaf extract into the Common Carp diet at concentrations of 1.0% and 1.5% yielded favorable results, positively influencing various facets of their health and performance. The observed improvements in growth, hemato-biochemical parameters, serum immunity, and antioxidant status suggest that tulsi extract shows potential in enhancing the overall health of Common Carp. These findings emphasize the promise of tulsi as a beneficial dietary supplement for supporting the health and immune response of Common Carp in aquaculture settings. Further exploration of the underlying mechanisms behind these positive effects could contribute to optimizing the use of tulsi in aquaculture practices. Although our study shows positive effects of tulsi extract on Common Carp, variability in plant composition and unidentified active compounds challenge reproducibility. Future research should focus on identifying specific bioactive ingredients and standardizing extraction methods to ensure consistent and reliable results.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The research undertaken received ethical clearance from the Department of Zoology, University of Lahore, with approval granted by the ethical committee. The trial registration number for this study is UOL/ZOO/34, and the initiation date was April 12, 2022. The research adhered strictly to ethical considerations outlined in European Legislation concerning Animal Rights.

FUNDING

This work was funded by the Researchers Supporting Project (RSPD2025R758), King Saud University, Riyadh, Saudi Arabia.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

ACKNOWLEDGMENTS

We extend our appreciation to the Researchers Supporting Project (RSPD2025R758), King Saud University, Riyadh, Saudi Arabia. Author contributions are as follows: S. S. Habib, S. Naz, and M. Zahid conceptualized and designed the study; S. S. Habib, M. Ullah, and S. Naz contributed writing and preparation of the original draft; S. Naz and K. Khan performed the sampling; S. Majeed and Ü. Acar analyzed the data; and K. H. Rind, M. Mohany, F. Fazio, Ü. Acar, and C. Cravana reviewed

and edited the manuscript. All authors read and agreed to the published version of the manuscript.

REFERENCES

- Abd El-Naby, A. S., El Asely, A. M., Hussein, M. N., Fawzy, R. M., & Abdel-Tawwab, M. (2023). Stimulatory effects of dietary chia (*Salvia hispanica*) seeds on performance, antioxidant-immune indices, histopathological architecture, and disease resistance of Nile Tilapia. *Aquaculture*, 563, Article 738889. <https://doi.org/10.1016/j.aquaculture.2022.738889>
- Abdel-Tawwab, M., Abdulrahman, N. M., Ahmad, V. M., Ramzi, D. O. M., & Hassan, B. R. (2022). Effects of dietary oak (*Quercus aegilops* L.) acorn on growth performance, somatic indices, and hemato-biochemical responses of Common Carp, *Cyprinus carpio* L., at different stocking densities. *Journal of Applied Aquaculture*, 34, 877–893. <https://doi.org/10.1080/10454438.2021.1902450>
- 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 Physiology and Biochemistry*, 48, 585–601. <https://doi.org/10.1007/s10695-022-01066-4>
- Abdel-Tawwab, M., El-Ashram, A. M., Tahoun, A.-A., Abdel-Razek, N., & Awad, S. M. M. (2021). Effects of dietary sweet basil (*Ocimum basilicum*) oil on the performance, antioxidants and immunity welfare, and resistance of Indian shrimp (*Penaeus indicus*) against *Vibrio parahaemolyticus* infection. *Aquaculture Nutrition*, 27, 1244–1254. <https://doi.org/10.1111/anu.13265>
- Abdel-Tawwab, M., Hamed, H. S., Monier, M. N., & Amen, R. M. (2024). The ameliorative effects of dietary rosemary (*Rosmarinus officinalis*) against growth retardation, oxidative stress, and immunosuppression induced by waterborne lead toxicity in Nile Tilapia fingerlings. *Annals of Animal Science*, 24, 139–149. <https://doi.org/10.2478/aoas-2023-0057>
- Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology*, 105, 121–126. [https://doi.org/10.1016/s0076-6879\(84\)05016-3](https://doi.org/10.1016/s0076-6879(84)05016-3)
- Almarri, S. H., Khalil, A. A., Mansour, A. T., & El-Houseiny, W. (2023). Antioxidant, immunostimulant, and growth-promoting effects of dietary *Annona squamosa* leaf extract on Nile Tilapia, *Oreochromis niloticus*, and its tolerance to thermal stress and *Aeromonas sobria* infection. *Animals*, 13, Article 746. <https://doi.org/10.3390/ani13040746>
- Aluru, N., & Vijayan, M. M. (2009). Stress transcriptomics in fish: A role for genomic cortisol signaling. *General and Comparative Endocrinology*, 164, 142–150. <https://doi.org/10.1016/j.ygcen.2009.03.020>
- Amirkhani, N., & Firouzabakhsh, F. (2015). Protective effects of basil (*Ocimum basilicum*) ethanolic extract supplementation diets against experimental *Aeromonas hydrophila* infection in Common Carp (*Cyprinus carpio*). *Aquaculture Research*, 46, 716–724. <https://doi.org/10.1111/are.12217>
- Arshad, L., Naqvi, S. Z. H., Hussain, J., Naeem, H., Shahzad, S., Aslam, A., Rehman, A. U., & Ghayas, A. (2021). Effect of neem (*Azadirachta indica*) and tulsi (*Ocimum sanctum*) leaves powder on growth performance, hemato-biochemical profile and carcass traits of Japanese quail (*Coturnix japonica*). *Turkish Journal of Veterinary & Animal Sciences*, 45, 388–395. <https://doi.org/10.3906/vet-2010-113>
- Association of Official Analytical Chemists. (2000). *Official methods of analysis* (17th ed.). Association of Official Analytical Chemists.
- Awad, E., & Awaad, A. (2017). Role of medicinal plants on growth performance and immune status in fish. *Fish & Shellfish Immunology*, 67, 40–54. <https://doi.org/10.1016/j.fsi.2017.05.034>
- Bhatnagar, A., & Mann, D. (2023). The synergic effect of gut-derived probiotic *Bacillus cereus* SL1 and *Ocimum sanctum* on growth, intestinal histopathology, innate immunity, and expression of enzymatic antioxidant genes in fish, *Cirrhinus mrigala* (Hamilton, 1822). *Probiotics and Antimicrobial Proteins*, 17, 271–291. <https://doi.org/10.1007/s12602-023-10143-w>
- Blaxhall, P. C., & Daisley, K. W. (1973). Routine haematological methods for use with fish blood. *Journal of Fish Biology*, 5, 771–781. <https://doi.org/10.1111/j.1095-8649.1973.tb04510.x>
- Borah, R., & Biswas, S. P. (2018). Tulsi (*Ocimum sanctum*), excellent source of phytochemicals. *International Journal of Environment, Agriculture and Biotechnology*, 3, Article 265258. <https://doi.org/10.22161/ijeab/3.5.21>
- Boyd, C. E., McNeven, A. A., & Davis, R. P. (2022). The contribution of fisheries and aquaculture to the global protein supply. *Food Security*, 14, 805–827. <https://doi.org/10.1007/s12571-021-01246-9>
- Bratovic, A. (2020). Antioxidant enzymes and their role in preventing cell damage. *Acta Scientia Nutritional Health*, 4, 132–138. <https://doi.org/10.31080/ASNH.2020.04.0659>
- Bulut, M., Yiğit, M., Ergün, S., Kesbiç, O. S., Acar, Ü., Gültepe, N., Karga, M., Yılmaz, S., & Güroy, D. (2014). Evaluation of dietary protein and lipid requirements of Two-banded Seabream (*Diplodus vulgaris*) cultured in a recirculating aquaculture system. *Aquaculture International*, 22, 965–973. <https://doi.org/10.1007/s10499-013-9720-z>
- Chen, F., Wang, Y., Wang, K., Chen, J., Jin, K., Peng, K., Chen, X., Liu, Z., Ouyang, J., Wang, Y., Zhang, X., Zou, H., Zhou, J., He, B., & Lin, Q. (2023). Effects of *Litsea cubeba* essential oil on growth performance, blood antioxidation, immune function, apparent digestibility of nutrients, and fecal microflora of pigs. *Frontiers in Pharmacology*, 14, Article 1166022. <https://doi.org/10.3389/fphar.2023.1166022>
- Cohen, M. M. (2014). Tulsi–*Ocimum sanctum*: A herb for all reasons. *Journal of Ayurveda and Integrative Medicine*, 5, 251–259. <https://doi.org/10.4103/0975-9476.146554>
- Coles, E. H. (1986). *Veterinary clinical pathology*. W. B. Saunders.
- Cyriac, P. J., Antony, A., & Nambisan, P. N. (1989). Hemoglobin and hematocrit values in the fish *Oreochromis mossambicus* (Peters) after short term exposure to copper and mercury. *Bulletin of Environmental Contamination and Toxicology*, 43, 315–320. <https://doi.org/10.1007/BF01701764>
- Das, R., Raman, R. P., Saha, H., & Singh, R. (2015). Effect of *Ocimum sanctum* Linn. (tulsi) extract on the immunity and survival of *Labeo rohita* (Hamilton) infected with *Aeromonas hydrophila*. *Aquaculture Research*, 46, 1111–1121. <https://doi.org/10.1111/are.12264>
- Eshraghian, A. (2013). Anti-inflammatory, gastrointestinal and hepatoprotective effects of *Ocimum sanctum* Linn: An ancient remedy with new application. *Inflammation & Allergy-Drug Targets*, 12, 378–384. <https://doi.org/10.2174/1871528112666131125110017>
- Fazelan, Z., Vatnikov, Y. A., Kulikov, E. V., Plushikov, V. G., & Yousefi, M. (2020). Effects of dietary ginger (*Zingiber officinale*) administration on growth performance and stress, immunological, and antioxidant responses of Common Carp (*Cyprinus carpio*) reared under high stocking density. *Aquaculture*, 518, Article 734833. <https://doi.org/10.1016/j.aquaculture.2019.734833>
- Fazio, F., Habib, S. S., Naz, S., Filiciotto, F., Cicero, N., Rehman, H. U., Saddozai, S., Rind, K. H., Rind, N. A., & Shar, A. H. (2022). Effect of fortified feed with olive leaves extract on the haematological and biochemical parameters of *Oreochromis niloticus* (Nile Tilapia). *Natural Product Research*, 36, 1575–1580. <https://doi.org/10.1080/14786419.2021.1883606>
- Fazio, F., Habib, S. S., Naz, S., Ullah, M., Nawaz, G., Nava, V., Piccione, G., & Arfuso, F. (2023). *Withania coagulans* fruit extract: A possible useful additive in ameliorating growth and immunity of *Labeo rohita* (Hamilton, 1822). *Natural Product Research*, 37, 803–808. <https://doi.org/10.1080/14786419.2022.2089139>
- Gabriel, N. N., Wilhelm, M. R., Habte-Tsion, H. M., Chimwamurombe, P., & Omorieg, E. (2021). The effects of dietary garlic (*Allium sativum*) and *Aloe vera* crude extract mixtures supplementation on growth performance, feed utilization, hematological parameters, whole body composition, and survival at low pH in African Catfish (*Clarias gariepinus*) juveniles. *Scientific African*, 11, Article e00671. <https://doi.org/10.1016/j.sciaf.2020.e00671>
- Habib, S. S., Batoool, A. I., Rehman, M. F. U., & Naz, S. (2022). Comparative analysis of the haemato-biochemical parameters and growth characteristics of *Oreochromis niloticus* (Nile Tilapia)

- cultured under different feed and habitats (biofloc technology and earthen pond system). *Aquaculture Research*, 53, 6184–6192. <https://doi.org/10.1111/are.16091>
- Habib, S. S., Naz, S., Khalid, S., Kanwal, R., Ameer, I., Khan, S. N. A., Rehman, A. U., Kousar, M., Khan, S. U., & Nazir, N. (2022). Effect of white button mushroom (*Agaricus bisporus*) on immunity and haematological parameters of *Oreochromis niloticus*. *Pakistan Journal of Zoology*, 54, 785–790. <https://doi.org/10.17582/journal.pjz/20201020051009>
- Habib, S. S., Saeed, M. Q., Masud, S., Kesbiç, O. S., Ujan, J. A., Cravana, C., Al-Rejaie, S. S., Mohany, M., & Fazio, F. (2024). Dietary effect of *Withania somnifera* root powder on growth, hematobiochemical parameters, immunity, and disease resistance to *Aeromonas hydrophila* in *Cyprinus carpio*. *Aquaculture Nutrition*, 2024, Article 7321287. <https://doi.org/10.1155/2024/7321287>
- Hajek, G. J., Klyszejko, B., & Dziaman, R. (2006). The anaesthetic effect of clove oil on Common Carp, *Cyprinus carpio* L. *Acta Ichthyologica et Piscatoria*, 36, 93–97. <https://doi.org/10.3750/AIP2006.36.2.01>
- Hamed, H. S., Ismal, S. M., & Abdel-Tawwab, M. (2022). Modulatory effects of dietary cinnamon (*Cinnamomum zeylanicum*) against waterborne lead toxicity in Nile Tilapia fingerlings: Growth performance, haemato-biochemical, innate immunity, and hepatic antioxidant indices. *Aquaculture Reports*, 25, Article 101190. <https://doi.org/10.1016/j.aqrep.2022.101190>
- Henry, R. J. (1964). *Clinical chemistry, principles and technics*. Harper & Row, Hoeber Medical Division.
- Hoseini, S. M., Taheri Mirghaed, A., Iri, Y., & Ghelichpour, M. (2018). Effects of dietary cineole administration on growth performance, hematological and biochemical parameters of Rainbow Trout (*Oncorhynchus mykiss*). *Aquaculture*, 495, 766–772. <https://doi.org/10.1016/j.aquaculture.2018.06.073>
- Hoseini, S. M., Yousefi, M., Hoseinifar, S. H., & Van Doan, H. (2019). Antioxidant, enzymatic and hematological responses of Common Carp (*Cyprinus carpio*) fed with myrcene- or menthol-supplemented diets and exposed to ambient ammonia. *Aquaculture*, 506, 246–255. <https://doi.org/10.1016/j.aquaculture.2019.03.048>
- Islam, R., Sultana, N., Bhakta, S., Haque, Z., Hasan, A., Siddique, M. P., & Islam, M. R. (2023). Modulation of growth performance, gut morphometry, and cecal microbiota in broilers by clove (*Syzygium aromaticum*) and tulsi (*Ocimum sanctum*) supplementation. *Poultry Science*, 102, Article 102266. <https://doi.org/10.1016/j.psj.2022.102266>
- Jayapal, V., Vidya Raj, C. K., Muthaiah, M., Chadha, V. K., Bammachary, U., Selvaraj, S., & Easow, J. M. (2021). In-vitro anti-*Mycobacterium tuberculosis* effect of essential oil of *Ocimum sanctum* L. (tulsi/basil) leaves. *The Indian Journal of Tuberculosis*, 68, 470–473. <https://doi.org/10.1016/j.ijtb.2021.02.009>
- Jothie Richard, E., Illuri, R., Bethapudi, B., Anandhakumar, S., Bhaskar, A., Chinampudur Velusami, C., Mundkinajeddu, D., & Agarwal, A. (2016). Anti-stress activity of *Ocimum sanctum*: Possible effects on hypothalamic–pituitary–adrenal axis. *Phytotherapy Research*, 30, 805–814. <https://doi.org/10.1002/ptr.5584>
- Kamelnia, E., Mohebbati, R., Kamelnia, R., El-Seedi, H. R., & Boskabady, M. H. (2023). Anti-inflammatory, immunomodulatory and anti-oxidant effects of *Ocimum basilicum* L. and its main constituents: A review. *Iranian Journal of Basic Medical Sciences*, 26, 617–627. <https://doi.org/10.22038/IJBMS.2023.67466.14783>
- Kaur, P., Dhull, S. B., Sandhu, K. S., Salar, R. K., & Purewal, S. S. (2018). Tulsi (*Ocimum tenuiflorum*) seeds: In vitro DNA damage protection, bioactive compounds and antioxidant potential. *Journal of Food Measurement and Characterization*, 12, 1530–1538. <https://doi.org/10.1007/s11694-018-9768-6>
- Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S., & Cosic, V. (2001). Method for the measurement of antioxidant activity in human fluids. *Journal of Clinical Pathology*, 54, 356–361. <https://doi.org/10.1136/jcp.54.5.356>
- Koskela, J., Rahkonen, R., Pasternack, M., & Knuutinen, H. (2004). Effect of immunization with two commercial vaccines on feed intake, growth, and lysozyme activity in European Whitefish (*Coregonus lavaretus* L.). *Aquaculture*, 234, 41–50. <https://doi.org/10.1016/j.aquaculture.2003.11.036>
- Kuebutornye, F. K. A., Roy, K., Folorunso, E. A., & Mraz, J. (2024). Plant-based feed additives in *Cyprinus carpio* aquaculture. *Reviews in Aquaculture*, 16, 309–336. <https://doi.org/10.1111/raq.12840>
- Li, Y., He, X., Sun, B., Hu, N., Li, J., You, R., Tao, F., Fang, L., Li, Y., & Zhai, Q. (2024). Combined exposure of beta-cypermethrin and emamectin benzoate interferes with the HPO axis through oxidative stress, causing an imbalance of hormone homeostasis in female rats. *Reproductive Toxicology*, 123, Article 108502. <https://doi.org/10.1016/j.reprotox.2023.108502>
- Logambal, S. M., Venkatalakshmi, S., & Dinakaran Michael, R. (2000). Immunostimulatory effect of leaf extract of *Ocimum sanctum* Linn. in *Oreochromis mossambicus* (Peters). *Hydrobiologia*, 430, 113–120. <https://doi.org/10.1023/A:1004029332114>
- Mbokane, E. M., & Moyo, N. A. (2018). Alterations of haemato-biochemical parameters pre and post-challenge with *Aeromonas hydrophila* and survival of *Oreochromis mossambicus* fed *Moringa oleifera*-based diets. *Fish & Shellfish Immunology*, 83, 213–222. <https://doi.org/10.1016/j.fsi.2018.09.017>
- Mirje, M. M., & Ramabhimaiah, S. (2014). Evaluation of the anti-inflammatory activity of *Ocimum sanctum* Linn (tulsi) in albino rats. *International Journal of Current Microbiology and Applied Sciences*, 3, 198–205.
- Mohammadi, M., Soltani, M., Siahpoosh, A., Hosseini Shekarabi, S. P., Shamsaie Mehrgan, M., & Lymbery, A. (2018). Effect of date palm (*Phoenix dactylifera*) seed extract as a dietary supplementation on growth performance immunological haematological biochemical parameters of Common Carp. *Aquaculture Research*, 49, 2903–2912. <https://doi.org/10.1111/are.13760>
- Mondal, S., Varma, S., Bamola, V. D., Naik, S. N., Mirdha, B. R., Padhi, M. M., Mehta, N., & Mahapatra, S. C. (2011). Double-blinded randomized controlled trial for immunomodulatory effects of tulsi (*Ocimum sanctum* Linn.) leaf extract on healthy volunteers. *Journal of Ethnopharmacology*, 136, 452–456. <https://doi.org/10.1016/j.jep.2011.05.012>
- Nishikimi, M., Rao, N. A., & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical and Biophysical Research Communications*, 46, 849–854. [https://doi.org/10.1016/S0006-291X\(72\)80218-3](https://doi.org/10.1016/S0006-291X(72)80218-3)
- Paech, K., & Tracey, M. V. (1956). *Modern methods of plant analysis*. Springer-Verlag.
- Panchal, P., & Parvez, N. (2019). Phytochemical analysis of medicinal herb (*Ocimum sanctum*). *International Journal of Nanomaterials, Nanotechnology and Nanomedicine*, 5, 008–011. <https://doi.org/10.17352/2455-3492.000029>
- Parveen, A., Perveen, S., Ahmad, M., Naz, F., & Riaz, M. (2023). Tulsi. In M. Zia-Ul-Haq, A. A. Al-Huqail, M. Riaz, & U. F. Gohar (Eds.), *Essentials of medicinal and aromatic crops* (pp. 983–1008). Springer International Publishing.
- Pushpam, M., Durairajan, P., & Arumugam, P. (2017). Comparison of antipyretic effectiveness between standard drugs with different doses of *Ocimum sanctum* Linn (tulsi). *World Journal of Pharmaceutical Sciences*, 5, 162–167.
- Rani, K. U., Latha, C., Pratheeba, M., Dhanasekar, K., Devi, S., Munuswamy, N., & Ramesh, B. (2014). Effect of formulated feeds on growth performance and colour enhancement in the fresh water Gold Fish *Carassius auratus* (Linnaeus, 1758). *World Journal of Pharmacy and Pharmaceutical Sciences*, 3, 1117–1133.
- Rashidian, G., Shahin, K., Elshopakey, G. E., Mahboub, H. H., Fahim, A., Elabd, H., Prokic, M. D., & Faggio, C. (2022). The dietary effects of nutmeg (*Myristica fragrans*) extract on growth, hematological parameters, immunity, antioxidant status, and disease resistance of Common Carp (*Cyprinus carpio*) against *Aeromonas hydrophila*. *Journal of Marine Science and Engineering*, 10, Article 325. <https://doi.org/10.3390/jmse10030325>

- Ray, S. N. C., & Sinha, R. C. (2014). Serum cortisol and glucose: Reliable bioindicators of stress in the fish *Labeo rohita*. *International Journal of Innovative Science, Engineering & Technology*, 1, 6–17.
- Reinhold, R. R. (1953). Determination of serum albumin. *Clinical Chemistry*, 21, 1370–1372.
- Rind, K. H., Habib, S. S., Ujan, J. A., Fazio, F., Naz, S., Batool, A. I., Ullah, M., Attaullah, S., Khayyam, K., & Khan, K. (2023). The effects of different carbon sources on water quality, growth performance, hematology, immune, and antioxidant status in cultured Nile Tilapia with biofloc technology. *Fishes*, 8, Article 512. <https://doi.org/10.3390/fishes8100512>
- Ruan, Y., Yuan, P.-P., Li, P.-Y., Chen, Y., Fu, Y., Gao, L.-Y., Wei, Y.-X., Zheng, Y.-J., Li, S.-F., Feng, W.-S., & Zheng, X.-K. (2023). Tingli Dazao Xiefei decoction ameliorates asthma in vivo and in vitro from lung to intestine by modifying NO–CO metabolic disorder mediated inflammation, immune imbalance, cellular barrier damage, oxidative stress and intestinal bacterial disorders. *Journal of Ethnopharmacology*, 313, Article 116503. <https://doi.org/10.1016/j.jep.2023.116503>
- Sahu, S., Das, B. K., Mishra, B. K., Pradhan, J., & Sarangi, N. (2007). Effect of *Allium sativum* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. *Journal of Applied Ichthyology*, 23, 80–86. <https://doi.org/10.1111/j.1439-0426.2006.00785.x>
- Sai Kumar, B. A. A. (2023). Hormonal regulation of metabolism, water, and minerals. In P. K. Das, V. Sejian, J. Mukherjee, & D. Banerjee (Eds.), *Textbook of veterinary physiology* (pp. 391–415). Springer Nature.
- Sarder, M. R. I., Thompson, K. D., Penman, D. J., & McAndrew, B. J. (2001). Immune responses of Nile Tilapia (*Oreochromis niloticus* L.) clones: I. Non-specific responses. *Developmental and Comparative Immunology*, 25, 37–46. [https://doi.org/10.1016/S0145-305X\(00\)00040-9](https://doi.org/10.1016/S0145-305X(00)00040-9)
- Sethi, J., Sood, S., Seth, S., & Talwar, A. (2004). Evaluation of hypoglycemic and antioxidant effect of *Ocimum sanctum*. *Indian Journal of Clinical Biochemistry*, 19, 152–155. <https://doi.org/10.1007/BF02894276>
- Sethi, J., Yadav, M., Sood, S., Dahiya, K., & Singh, V. (2010). Effect of tulsi (*Ocimum sanctum* Linn.) on sperm count and reproductive hormones in male albino rabbits. *International Journal of Ayurveda Research*, 1, 208–210. <https://doi.org/10.4103/0974-7788.76782>
- Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany*, 2012, Article 217037. <https://doi.org/10.1155/2012/217037>
- Singh, S., & Agrawal, S. S. (1991). Anti-asthmatic and anti-inflammatory activity of *Ocimum sanctum*. *International Journal of Pharmacognosy*, 29, 306–310. <https://doi.org/10.3109/13880209109082904>
- Siwicki, A. K., & Anderson, D. P. (1993). An easy spectrophotometric assay for determining total protein and immunoglobulin levels in fish sera: Correlation to fish health. *Techniques in Fish Immunology*, 3, 23–30.
- Sopinka, N. M., Donaldson, M. R., O'Connor, C. M., Suski, C. D., & Cooke, S. J. (2016). Stress indicators in fish. In C. B. Schreck, L. Tort, A. P. Farrell, and C. J. Brauner (Eds.), *Biology of stress in fish* (Fish Physiology 35, pp. 405–462). Academic Press. <https://doi.org/10.1016/B978-0-12-802728-8.00011-4>
- Srinivas, N., Sali, K., & Bajoria, A. A. (2016). Therapeutic aspects of tulsi unraveled: A review. *Journal of Indian Academy of Oral Medicine and Radiology*, 28, 17–23. <https://doi.org/10.4103/0972-1363.189984>
- Tran, H. Q., Nguyen, T. T., Prokešová, M., Gebauer, T., Doan, H. V., & Stejskal, V. (2022). Systematic review and meta-analysis of production performance of aquaculture species fed dietary insect meals. *Reviews in Aquaculture*, 14, 1637–1655. <https://doi.org/10.1111/raq.12666>
- Venkatalakshmi, S., & Michael, R. D. (2001). Immunostimulation by leaf extract of *Ocimum sanctum* Linn. in *Oreochromis mossambicus* (Peters). *Journal of Aquaculture in the Tropics*, 16, 1–10.
- Vidhani, S. I., Vyas, V. G., Parmar, H. J., Bhalani, V. M., Hassan, M. M., Gaber, A., & Golakiya, B. A. (2016). Evaluation of some chemical composition, minerals fatty acid profiles, antioxidant and antimicrobial activities of tulsi (*Ocimum sanctum*) from India. *American Journal of Food Science and Technology*, 4, 52–57. <https://doi.org/10.12691/ajfst-4-2-5>
- Yamani, H. A., Pang, E. C., Mantri, N., & Deighton, M. A. (2016). Antimicrobial activity of tulsi (*Ocimum tenuiflorum*) essential oil and their major constituents against three species of bacteria. *Frontiers in Microbiology*, 7, Article 681. <https://doi.org/10.3389/fmicb.2016.00681>
- Yano, T. (1992). Assay of hemolytic complement activity. In J. S. Stolen, T. C. Fletcher, D. P. Anderson, S. L. Kaattari, & A. F. Rowley (Eds.), *Techniques in fish immunology* (pp. 131–141). SOS Publications.
- Yousefi, M., Zahedi, S., Reverter, M., Adineh, H., Hoseini, S. M., Van Doan, H., El-Haroun, E. R., & Hoseinifar, S. H. (2021). Enhanced growth performance, oxidative capacity and immune responses of Common Carp, *Cyprinus carpio* fed with *Artemisia absinthium* extract-supplemented diet. *Aquaculture*, 545, Article 737167. <https://doi.org/10.1016/j.aquaculture.2021.737167>
- Yu, K., Chai, B., Zhuo, T., Tang, Q., Gao, X., Wang, J., He, L., Lei, X., & Chen, B. (2023). Hydrostatic pressure drives microbe-mediated biodegradation of microplastics in surface sediments of deep reservoirs: Novel findings from hydrostatic pressure simulation experiments. *Water Research*, 242, Article 120185. <https://doi.org/10.1016/j.watres.2023.120185>
- Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The traditional medicine and modern medicine from natural products. *Molecules*, 21, Article 559. <https://doi.org/10.3390/molecules21050559>
- Zhuo, T., Yu, K., Chai, B., Tang, Q., Gao, X., Wang, J., He, L., Lei, X., Li, Y., & Meng, Y. (2024). Microplastics increase the microbial functional potential of greenhouse gas emissions and water pollution in a freshwater lake: A metagenomic study. *Environmental Research*, 257, Article 119250. <https://doi.org/10.1016/j.envres.2024.119250>