Beneficial alterations in growth performance, blood biochemicals, immune responses, and antioxidant capacity of common carp (Cyprinus carpio) fed a blend of Thymus vulgaris, Origanum majorana, and Sat

by Mohammad Rudiansyah

Submission date: 05-Jan-2023 11:19AM (UTC+0700)
Submission ID: 1988725486
File name: lterations_in_growth_performance,_blood_biochemicals,_immune.pdf (1.34M)
Word count: 9713
Character count: 52898

Aquaculture 555 (2022) 738254

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Beneficial alterations in growth performance, blood biochemicals, immune responses, and antioxidant capacity of common carp (*Cyprinus carpio*) fed a blend of *Thymus vulgaris, Origanum majorana*, and *Satureja hortensis* extracts

Mohammad Rudiansyah [a], Walid Kamal Abdelbasset [b,c], Saade Abdalkareem Jasim [d], Ghasem Mohammadi [e,f], Santhi Muttipoll Dharmaraju [g], Chairun Nasirin [h], Abduladheem Turki Jalil [i], Maria Jade Catalan Opulencia [j], Mohammed Kadhem Abid [k], Saeid Shahbazi Naserabad [f,*]

[a] Department of Internal Medicine, Faculty of Medicine, Universitas Lambung Mangkurat, Banjarmasin, Indonesia
[b] Department of Health and Rehabilitation Sciences, College of Applied Medical Sciences, Prince Sattam bin Abdulaziz University, Al Kharj, Saudi Arabia
[c] Department of Physical Therapy, Kasr Al-Aini Hospital, Cairo University, Giza, Egypt
[d] Al-maarif University College, Medical Laboratory Techniques Department, Al-anbar, Ramadi, Iraq
[e] Persian Gulf and Oman Sea Ecological Research Center, Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research, Education and Extension Organization (AREEO), Bandar Abbas, Iran
[f] Department of Fisheries, Faculty of Natural Resources, University of Tehran, Karaj, Iran
[g] College of Nursing, Jazan University, Jazan, Saudi Arabia
[h] College of Health Sciences (STIKES) Mataram, Indonesia
[i] Medical Laboratories Techniques Department, Al-Mustaqbal University College, Babylon, Hilla, 51001, Iraq.
[j] College of Business Administration, Ajman University, Ajman, United Arab Emirates.
[k] Department of Anesthesia, College of Health & Medical Technology, Al-Ayen University, Thi-Qar, Iraq

ARTICLE INFO

Keywords:
Aquaculture
Phytogenic
Lamiaceae
Production
Immunity
Common carp

ABSTRACT

A blend of medicinal herbs extracts (BHE) obtained from *Thymus vulgaris*, *Origanum majorana*, and *Satureja hortensis* fed to common carp (20.57 ± 0.66 g; mean ± S.D.) at five incremental levels of 0 (control), 0.5%, 1%, 2%, and 3% of diet for 60 days. At the end of the study, final body weight, body weight increment, specific growth rate, survival rate, and feed conversion ratio were significantly better in the groups fed 1 and 2% BHE relative to the control ($P \leq 0.05$), and the best values were recorded for fish fed the diet containing 1% BHE. According to the results, serum total protein and albumin values showed significant enhancements by 1–3% BHE compared with the control ($P \leq 0.05$). However, globulin concentrations were not affected by BHE inclusion ($P \geq 0.05$). Interestingly, all experimental groups fed with BHE-supplemented diets presented profound declines in terms of serum cortisol, glucose, triglycerides, cholesterol, aspartate aminotransferase, and alkaline phosphatase concentrations as compared to the control ($P \leq 0.05$). Further, incorporating BHE at levels higher than 1% reduced alanine aminotransferase activity ($P \leq 0.05$). The superoxide dismutase, catalase, and glutathione peroxidase activities were significantly intensified in serum by diets supplemented with 1–3% BHE than the control ($P \leq 0.05$). In comparison, malondialdehyde contents were dose-dependently diminished upon BHE additive ($P \leq 0.05$). Significantly higher levels of serum lysozyme (LYZ) activity and mucosal immunoglobulins (Ig) were found in the 2% BHE group relative to the control, 0.5, or 3% BHE treatments ($P \leq 0.05$). The highest levels of serum total Ig and alternative complement activity (ACH_{50}) activity as well as skin mucus LYZ activity were observed in 1% BHE treatment. However, no remarkable differences were detected among treatments for mucus ACH_{50} activity ($P \geq 0.05$). Alkaline phosphatase (ALP) activity showed significant improvements in BHE-supplemented groups as compared to the control ($P \leq 0.05$), with the 2% BHE had notably higher ALP activity than 3% BHE ($P \leq 0.05$). Meanwhile, 1 and 2% BHE resulted in significantly enhanced skin mucus protease activities than the control and 0.5% BHE ($P \leq 0.05$). In conclusion, 1% BHE supplementation was the most optimum dosage favorably improved feed efficiency, growth performance, immunological responses, and antioxidant status of common carp.

* Corresponding author.
E-mail address: saeid.shahbazi@ut.ac.ir (S. Shahbazi Naserabad).

https://doi.org/10.1016/j.aquaculture.2022.738254
Received 21 October 2021; Received in revised form 5 April 2022; Accepted 9 April 2022
Available online 13 April 2022

1. Introduction

Aquaculture is one of the main sectors in food production industry. It provides consumers with safe and high-value protein sources (Dawood et al., 2020). The common carp (*Cyprinus carpio*) has worldwide cultivation and is the most preferred farm fish species in many regions (Harikrishnan et al., 2021; Yousefi et al., 2021a). The recent rapid expansion of aquaculture has brought farming stress conditions and numerous disease outbreaks that lead to reduced productivity and considerable economic loss (Dawood et al., 2018; Ghafarifarsani et al., 2021a; Raissy et al., 2022). Currently, antibiotics, as the traditional way of prevention and treatment of fish diseases, are prohibited in many countries because of consequential adverse effects, including residues in fish and the environment, weakened natural host immunity, higher price, and pathogenic resistance (Awad et al., 2013; Mugwanya et al., 2022; Yılmaz et al., 2016; Brown et al., 2021). Thus, friendly alternative substances such as medicinal herbs are widely practiced in aquaculture to induce beneficial effects on cultured animals and displace antibiotics (El-Saadony et al., 2021; Raissy et al., 2022; Reverter et al., 2021; Yilmaz et al., 2022).

In this context, *Thymus vulgaris, Origanum majorana*, and *Satureja hortensis* and their derivatives have proven cost-effective feed supplements to maximize productivity and boost host health status. These medical plants are native to the Mediterranean region and currently have worldwide distribution and application (Fierascu et al., 2018; Patil et al., 2021). *Thymus, Origanum*, and *Satureja* are the most relevant genera in the *Lamiaceae* family (Morales, 2003).

T. vulgaris contain special levels of different chemotypes namely terpenoids (thymol, carvacrol, linalool, and terpinen), phenolics (quinic, rosmaric, and caffeic acids), and flavonoids (apigenin, luteolin, and cirsimaritin,). These are responsible for *T. vulgaris* growth-promoting, antimicrobial, antioxidative, immunomodulatory, hepatoprotective, anti-hyperlipidemic, and anti-inflammatory effects (Patil et al., 2021). For example, Yousefi et al. (2022); Ghafarifarsani et al. (2021b) and Hoseini and Yousefi (2019) illustrated that *T. vulgaris* supplementation positively affected growth indices and ameliorated the deteriorative consequences of stress responses on intestinal health, immune, antioxidant, and enzymatic activities of rainbow trout (*Oncorhynchus mykiss*). Further, *O. majorana* incorporation in aquafeed increased growth, feeding efficiency, antioxidant capacity, immune status, and resistance against infection. Many compounds of biomedical values such as terpenoids and phenolic compounds also are found in *O. majorana* (Charai et al., 1996; Komaitis et al., 1992). A recent dietary experiment confirmed and encouraged the multiple potential roles of *O. majorana* in growth performance, immune response, and disease resistance (Yousefi et al., 2021a). *S. hortensis* generally contains some metabolites like terpenoids, phenolics flavonoids (Chambre et al., 2020), which positively alter fish growth and immune system responses.

Nevertheless, the medical herbs are not always efficient in enhancing fish growth performance or health status. This can be attributed to seasonal variation, agronomic procedure, climatic factors, genetics, extraction methods, etc., leading to a different composition of plants and obtained extracts in terms of discussed biomolecules (Harikrishnan et al., 2020; Katar et al., 2017). The problem may partly be solved by combining herbs of different compositions to meet the deficiencies.

To our knowledge, there are limited investigations on combining herbal extracts as a novel strategy to enhance the productivity and health status of aquatic animals. Therefore, we examined the synergistic effect of dietary incorporation of *T. vulgaris, O. majorana*, and *S. hortensis* mixture on the growth, blood biochemistry, antioxidant, and immune response of common carp, *C. carpio*.

2. Material and method

2.1. Extraction and diets

S. hortensis, *O. majorana*, and *T. vulgaris* dried leaves were procured from a local medical plants store and washed with deionized water to remove possible impurities. The fan-dried leaves were then separately powdered and added with ethanol (80% *v*/*v*) at a portion of 1:3 (*w*/*v*). The solutions were regularly agitated for three days. Each solution passed through a Whatman filter paper to obtain alcoholic extract. The extract was concentrated by removing alcohol at 40 °C in a rotary evaporator and kept at −70 °C (Yousefi et al., 2021a).

All three herbal extracts were combined in equivalent weights, producing a mixture that supplemented in the percentage proportion of the basal diet at incremental levels of 0% (T1, control), 0.5% (T2), 1% (T3), 2% (T4), and 3% (T5). In brief, the blend of medicinal herbs extracts (BHE) added to the well-grounded basal diets, and the combination passed through a grinder's openings and cut into pellets. The resultant pellets were dried in an oven at 40 °C until moisture content reached less than 10%. The experimental diets were tightly packed into plastic bags and stored in a fridge at −20 °C until being fed to fish.

2.2. Fish and experimental condition

Common carp (*C. carpio*) were obtained from a private fish farm (Gilan, Rasht, Iran) and conveyed to a private farm (Alborz, Karaj, Iran) where the experiment was conducted. At first, fish were stocked in a 1000-L polyethylene tank for acclimation to the new condition. The tank was continuously aerated and the growing water was daily replaced with fresh dechlorinated water to remove uneaten feed and fecal matter. During ten days of the acclimation period, fish were fed on commercial pellets containing 35–37% crude protein (Faradaneh Co., Shahrekord, Iran) to satiation twice daily (at 08:00 and 18:00). Then, 300 common carp juveniles (20.57 ± 0.66 g; mean ± S.D.) were distributed in 12, 300-L polyethylene tanks in triplicates at the rate of 20 fish/tank. In tanks, feeding was conducted twice a day (at 08:00 and 18:00) up to satiation for 60 days. The tanks were supplied with continuous aeration and 50% of water in each tank was exchanged daily with fresh dechlorinated water 1-h after morning feeding. The major water quality parameters values were checked and recorded every day, including water temperature (25.4 ± 1.1 °C), pH (7.7 ± 0.6), and dissolve oxygen (6.45 ± 0.35 mg/L). Total ammonia nitrogen (<0.2 mg/L) was estimated colorimetrically using a commercial kit (Shenasa Co., Iran).

2.3. Growth performance

At the end of the experiment, feeding was ceased for 24 h, fish were anesthetized using clove powder (300 mg/L), and then all fish were weighed and counted to evaluate the following parameters (Hajiahmadiani et al., 2012; Mani and Ebrahimi, 2021):

Weight gain (WG) = [mean final weight (g) − mean initial weight (g)] ÷ mean initial weight (g); Specific growth rate (SGR; %) = Ln [mean final weight (g)] − Ln [mean initial weight (g)] ÷ no. of days × 100; Feed conversion ratio (FCR) = weight gain (g) ÷ feed intake (g); Survival (%) = 100 × (no. of fish harvested ÷ no. of fish stocked).

2.4. Serum preparation

Three fish were collected from each replicate tank (9 fish/treatment) and anesthetized using clove powder (300 mg/L). The blood was obtained from the caudal vein using sterile, non-heparinized syringes, poured into microcentrifuge tubes, and allowed to clot for 3 h at room temperature. Afterward, samples were centrifuged at 2000 ×*g* for 10 min at 4 °C, and supernatant (serum) was stored at −70 °C until analysis.

2.5. Mucus collection

Four sedated fish from each replicate (12 fish/treatment) were randomly collected for skin mucus collection according to (Yousefi et al., 2022). In brief, the body surface of fish was rinsed with an amount of sterile saline solution (NaCl 50 mM) individually, and then mucus was collected through gently stacking and rubbing fish by hand in polyethylene bags containing 10 mL of sterile saline solution. The collected materials were transfered into sterile test tubes, wastes were separated by centrifugation at 3000 ×*g* for 10 min at 4 °C, and the supernatant (mucus) was stored at −70 °C for further analysis.

2.6. Serum biochemistry

The serum chemical parameters were analyzed spectrometrically using commercially available kits following the manufacturer's instructions (Pars Azmun Co., Iran). In brief, the selected parameters and the methods of analysis included: alkaline phosphatase (ALP) by *p*-nitrophenyl phosphate conversion method at 405 nm (Yousefi et al., 2021b), alanine aminotransferase (ALT), aspartate aminotransferase (AST) by Brady's reagent at 505 nm (Reitman and Frankel, 1957), total protein (TP) by biuret method at 540 nm (Lubran, 1978), albumin (ALB) by bromocresol green dye at 630 nm (Doumas et al., 1997), glucose (GLU) by glucose oxidase method at 500 nm (Burtis and Ashwood, 1999), cholesterol (CHO) by cholesterol oxidase/peroxidase enzymatic method at 510 nm (Burtis and Ashwood, 1999), and triglyceride (TRI) by glycerol-3-phosphate oxidase enzymatic method at 546 nm (Burtis and Ashwood, 1999).

The globulin (GLO) content was calculated by subtracting the value of albumin from total protein (Bayunova et al., 2002). Cortisol (COR) concentrations were detected at 450 nm using an enzyme-linked immunosorbent assay kit (Zellbio Co., Germany) following kit's manual (Hajirezaee et al., 2020).

2.7. Serum immune responses

The serum total immunoglobulin (Ig) was measured following the descriptions of Siwicki and Anderson, 1993. In brief, serum samples were analyzed for TP levels using a commercial kit (Pars Azmun Co., Iran). Then, samples were added to 12% polyethylene glycol (PEG; 10,000 MW; Sigma) and TP contents were measured again. The difference between TP levels of the sample before and after PEG treatment represents the serum Ig concentration.

The lysozyme (LYZ) activity of serum was quantified by a turbidimetric assay described by (Ellis, 1990). Briefly, serum (20 μL) was added to 70 μL of *Micrococcus lysodeikticus* suspension (75 μg/mL of phosphate buffer saline; Sigma) in wells of a 96-well plate and incubated at room temperature, with continuous gentle shaking. The absorbance was detected at 450 nm after 1 and 5 min. The unit of enzyme activity was defined as the concentration that causes a decline in absorbance at a rate of 0.001/min. Lysozyme from hen's egg white (Sigma) was used to develop the standard curve (Yousefi et al., 2021b).

The serum alternative complement activity (ACH_{50}) was revealed based on the hemolysis of rabbit red blood cells (RaRBC) and the absorbance was determined at 414 nm. The amount of serum sample giving 50% hemolysis was recorded and ACH_{50} activity was computed following Yano, 1992.

2.8. Serum antioxidant status

Glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) levels in serum samples were quantified using diagnostic reagent kits (ZellBio Co., Lonsee, Germany), following the manufacturer's manual.

Glutathione peroxidase was measured by monitoring the formation of 2-Nitro-5-thiobenzoic acid at 412 nm (Beutler et al., 1963). Superoxide dismutase activity was determined by the inhibition of nitro-blue-tetrazolium reduction and reading the absorbance at 420 nm (Marklund and Marklund, 1974). Catalase activity was determined based on the rate of H_2O_2 disintegration into water and oxygen at 405 nm (Beutler, 1984). Malondialdehyde levels were evaluated using colorimetric thiobarbituric acid-reactive substances (TBARs) microplate assay at 535 nm (Dawn-Linsley et al., 2005).

2.9. Mucus immune responses

The mucus ALP activity analysis was performed using a commercial kit (Pars Azmun Co, Tehran, Iran). The rate of *p*-nitrophenol production is measured at 405 nm and used to quantify ALP activity. Mucus samples were checked for protease activity using the azocasein hydrolysis assay detailed by (Ross et al., 2000). The mucus LYZ and ACH_{50} activities, as well as Ig concentrations, were enumerated as per the given instructions for serum samples.

2.10. Ethical approval

The welfare of fish was taken into consideration and all the experimental procedures followed the ARRIVE guidelines and were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) to minimize fish suffering.

2.11. Statistical analysis

The statistical analysis was conducted using SPSS version 22 (SPSS Inc., IL, USA). The data were confirmed in terms of normal distribution by the Shapiro-Wilk test and homogeneity of variances. The results of one-way ANOVA and Duncan's multiple range test were used to determine the significant differences among treatments with the significance level set at $p < 0.05$.

3. Results

3.1. Growth performance

According to Table 1, the dietary inclusion of a blend of medicinal herbs extract (BHE) significantly affected the growth performances of common carp. The final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) values were significantly better in the groups fed 1 and 2% BHE relative to the control ($P \leq 0.05$). The highest levels of FBW, WG, and SGR and the lowest values of FCR

Table 1
Growth parameters of common carp (*Cyprinus carpio*) fed a mixture of medicinal herbs extracts at graded levels of 0% (T1, control), 0.5% (T2), 1% (T3), 2% (T4), and 3% (T5).

Parameter	T1	T2	T3	T4	T5
IBW (g)	20.59 ± 0.69^{a}	20.46 ± 0.93^{a}	20.61 ± 0.77^{a}	20.18 ± 0.66^{a}	21.02 ± 0.41^{a}
FBW (g)	38.84 ± 0.81^{c}	41.12 ± 0.92^{bc}	45.71 ± 0.88^{a}	43.51 ± 1.00^{ab}	41.90 ± 1.31^{b}
WG (g)	18.25 ± 0.23^{c}	20.65 ± 1.67^{bc}	25.10 ± 0.57^{a}	23.32 ± 1.15^{ab}	20.88 ± 1.52^{bc}
SGR (% d^{-1})	1.05 ± 0.02^{b}	1.16 ± 0.10^{ab}	1.32 ± 0.04^{a}	1.28 ± 0.06^{a}	1.14 ± 0.07^{ab}
FCR	2.80 ± 0.03^{a}	2.48 ± 0.20^{ab}	2.03 ± 0.04^{c}	2.19 ± 0.10^{bc}	2.45 ± 0.17^{ab}
Survival rate (%)	98.33 ± 2.88^{a}	100^{a}	100^{a}	100^{a}	100^{a}

IBW: Initial body weight, FBW: Final body weight, BWI: Body weight increment, SGR: Specific growth rate, FCR: Feed conversion ratio, SR: Survival rate. Values are expressed as means ± S.D. ($n = 3$). Means in the same row bearing different superscript are significantly different at ($P \leq 0.05$).

were obtained by 1% BHE. Fish survival was found statistically similar among study treatments ($P \geq 0.05$).

3.2. Serum bio-chemicals

BHE supplemented diets had marked effects on serum biochemicals of common carp (Table 2). Accordingly, serum TP contents registered significant enhancements by 1 and 2% BHE compared with the control ($P \leq 0.05$). Further, ALB values significantly increased by 1–3% BHE supplementation ($P \leq 0.05$). However, GLB was not affected by BHE inclusion ($P \geq 0.05$). Interestingly, all the groups fed with MCH-supplemented diets exhibited significantly declined concentrations of COR, GLU, TRI, CHO, AST, and ALP in the serum when compared to the control ($P \leq 0.05$). The least COR values were detected in the groups of 1 and 2% BHE, showing significant differences from other groups ($P \leq 0.05$). GLU contents were found meaningfully lower in 1% and 3% BHE treatments relative to the control and 0.5% BHE-fed treatments ($P \leq 0.05$). Incorporating fish diet with BHE at 1–3% resulted in significantly lower TRI and ALT levels compared to the control and 0.5% BHE diets ($P \leq 0.05$). 1% BHE supplementation level produced significantly lower CHO concentrations in comparison with the control and 0.5% BHE-fed group ($P \leq 0.05$). AST values were lowest in 1% BHE treatment ($P \leq 0.05$). Significantly lower ALP levels were detected in fish given 1–3% BHE compared to those fish fed the control and 0.5% BHE diets ($P \leq 0.05$).

3.3. Serum immune responses

According to the results (Fig. 1), serum innate responses of common carp were significantly affected by BHE supplementation. At all inclusion levels, the groups treated with BHE presented significant differences relative to control in terms of total Ig concentrations and LYZ activities ($P \leq 0.05$). The highest levels of total Ig were observed in 1% BHE treatment that profoundly differed from the remaining treatments ($P \leq 0.05$). Fish fed 2% BHE had markedly higher LYZ activity than the control or 0.5 and 3% BHE groups ($P \leq 0.05$). However, only the group supplemented with 1% BHE recorded significantly higher levels of ACH_{50} activity relative to the control ($P \leq 0.05$).

Table 2
Serum biochemistry measurements of common carp (*Cyprinus carpio*) fed a mixture of medicinal herbs extracts at graded levels of 0% (T1, control), 0.5% (T2), 1% (T3), 2% (T4), and 3% (T5).

Parameter	T1	T2	T3	T4	T5
TP (g/dL)	2.96 ± 0.12^{b}	3.41 ± 0.26^{ab}	3.65 ± 0.22^{a}	3.52 ± 0.31^{ab}	3.61 ± 0.21^{a}
ALB (g/dL)	1.12 ± 0.08^{b}	1.31 ± 0.09^{b}	1.59 ± 0.12^{a}	1.50 ± 0.18^{a}	1.54 ± 0.13^{a}
GLB (g/dL)	1.83 ± 0.13^{a}	2.10 ± 0.27^{a}	2.06 ± 0.27^{a}	2.02 ± 0.27^{a}	2.07 ± 0.35^{a}
COR (ng/mL)	40.89 ± 1.45^{a}	34.16 ± 1.63^{b}	27.74 ± 1.72^{c}	29.85 ± 1.39^{c}	35.19 ± 4.89^{b}
GLU (mg/dL)	88.57 ± 2.40^{a}	81.68 ± 2.81^{b}	72.85 ± 2.61^{c}	75.86 ± 2.30^{bc}	73.36 ± 1.58^{c}
TRI (mg/dL)	211.39 ± 3.54^{a}	200.41 ± 3.22^{b}	174.10 ± 3.44^{c}	177.94 ± 3.89^{c}	171.76 ± 3.60^{c}
CHO (mg/dL)	91.57 ± 1.93^{a}	84.72 ± 2.45^{b}	76.71 ± 2.84^{c}	82.04 ± 2.23^{bc}	81.29 ± 2.47^{bc}
ALT (U/mL)	25.47 ± 1.14^{a}	24.80 ± 1.41^{a}	17.70 ± 1.12^{b}	17.96 ± 1.13^{b}	20.39 ± 1.15^{b}
AST (U/mL)	72.79 ± 1.18^{a}	63.78 ± 1.75^{bc}	58.89 ± 1.14^{d}	65.06 ± 1.53^{b}	60.49 ± 1.93^{cd}
ALP (U/mL)	54.73 ± 2.29^{a}	46.51 ± 1.57^{b}	40.39 ± 1.66^{c}	40.35 ± 1.66^{c}	41.89 ± 1.29^{bc}

TP, total protein; ALB, albumin; GLB, globulin content; COR, cortisol; GLU, glucose content; TRI, triglycerides; CHO, cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase. Values are expressed as means ± S.D. ($n = 3$). Means in the same row bearing different superscript are significantly different at ($P \leq 0.05$).

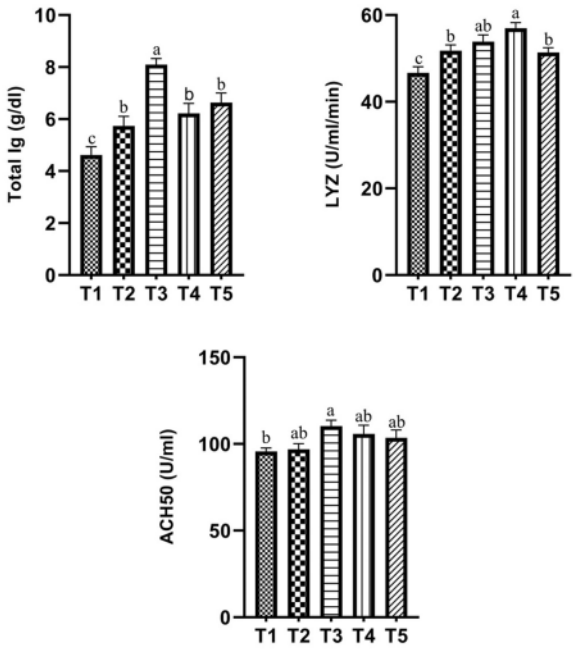

Fig. 1. The effects of experimental diets containing a blend of medicinal herbs extracts (BHE) on the serum immune responses of common carp (*Cyprinus carpio*) at the end of study: total immunoglobulin (total Ig); lysozyme activity (LYZ); alternative complement activity (ACH_{50}). T1, control; T2, 0.5% BHE; T3, 1% BHE; T4, 2% BHE; T5, 3% BHE. Values are expressed as means ± S.D. ($n = 3$). Error bars bearing different superscript are significantly different at ($P \leq 0.05$).

3.4. Serum antioxidants

The results show marked effects of BHE-included diets on the serum antioxidant enzymes of common carp (Fig. 2). The activities of SOD, CAT, and GSH-Px were significantly elevated in fish fed diets supplemented with 1–3% BHE than the control ($P \leq 0.05$). No significant differences were detected among groups of fish fed diets containing 1, 2, and 3% BHE ($P \geq 0.05$). In comparison, MDA levels were meaningfully declined by BHE supplementation in a dose-dependent manner, as follows: 0% BHE > 0.5% BHE > 1, 2, 3% BHE ($P \leq 0.05$).

3.5. Mucus immune responses

According to the results (Fig. 3), BHE incorporation significantly enhanced mucus total Ig contents and LYZ, ALP, and PRO activities of common carp. Especially, significantly higher levels of total Ig were found in 2% BHE group relative to the control or 0.5 and 3% BHE treatments ($P \leq 0.05$). Furthermore, the group of 1% BHE presented meaningfully highest LYZ activity than the remaining treatments ($P \leq 0.05$). Alkaline phosphatase (ALP) activity showed significant improvements in BHE-supplemented groups compared with the control ($P \leq 0.05$). However, there were not any statistical differences in ALP activity among 0.5, 1, and 3% BHE groups or 0.5, 1, and 2% BHE groups ($P \geq 0.05$), though, 2% BHE had notably higher ALP activity than 1% BHE ($P \leq 0.05$). No remarkable differences were detected among treatments in terms of mucus ACH_{50} activity ($P \geq 0.05$). Meanwhile, 1 and 2% BHE resulted in significantly enhanced skin mucus PRO activities than the control and 0.5% BHE ($P \leq 0.05$).

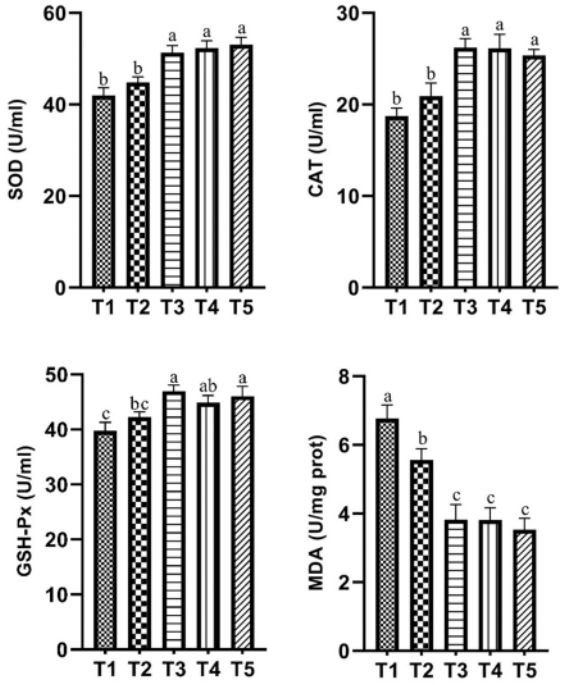

Fig. 2. The effects of experimental diets containing a blend of medicinal herbs extracts (BHE) on the serum antioxidant responses of common carp (*Cyprinus carpio*) at the end of study: malondialdehyde activity (MDA); superoxide dismutase activity (SOD); catalase activity (CAT); glutathione peroxidase activity (GSH-Px). T1, control; T2, 0.5% BHE; T3, 1% BHE; T4, 2% BHE; T5, 3% BHE. Values are expressed as means ± S.D. ($n = 3$). Error bars bearing different superscript are significantly different at ($P \leq 0.05$).

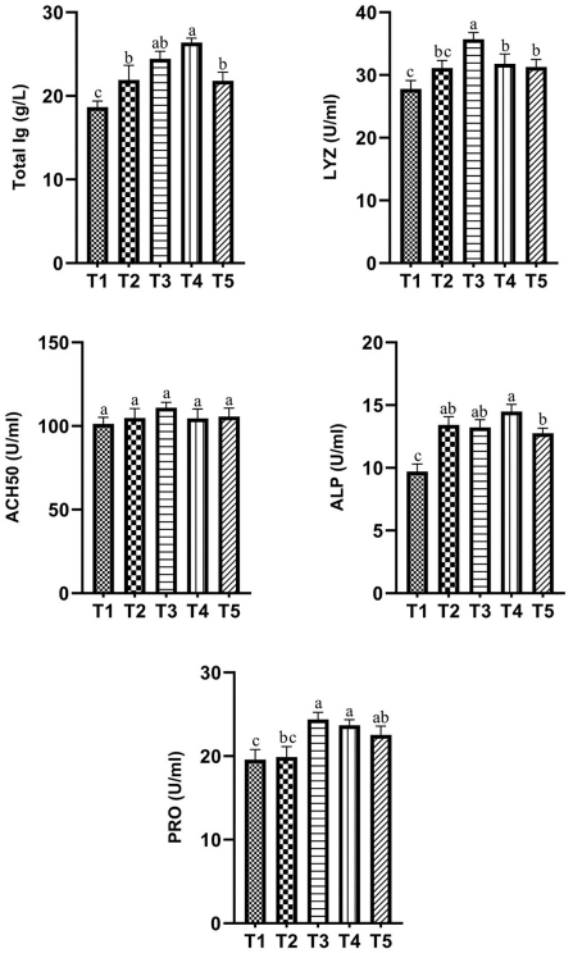

Fig. 3. The effects of experimental diets containing a blend of medicinal herbs extracts (BHE) on the mucus immune responses of common carp (*Cyprinus carpio*) at the end of study: total immunoglobulin (total Ig); lysozyme activity (LYZ); alternative complement activity (ACH_{50}); alkaline phosphatase activity (ALP); Protease activity (PRO). T1, control; T2, 0.5% BHE; T3, 1% BHE; T4, 2% BHE; T5, 3% BHE. Values are expressed as means ± S.D. ($n = 3$). Error bars bearing different superscript are significantly different at ($P \leq 0.05$).

4. Discussion

The present study indicates that the dietary inclusion of a blend of medicinal herbs extract (BHE) significantly improved growth performances of common carp (*C. carpio*) in terms of FBW, WG, SGR, FCR values in the groups fed 1 and 2% BHE relative to the control, with the most optimum levels achieved by the diet containing 1% BHE. No study has evaluated the effects of present BHE on the growth performance of fish. However, the singular administration of *T. vulgaris* was found to beneficially affect intestinal histology and health status (El Euony et al., 2020; Ghafarifarsani et al., 2021b; Di Kong et al., 2021; Yousefi et al., 2022; Ghafarifarsani et al., 2022a) and microbial community as well as digestive enzymes activities (Giannenas et al., 2012; Navarrete et al., 2010), which are associated to improvements in fish growth performance in different fish species. Further, common carp fed diets containing different levels of *O. majorana* extract (0.2–1 g/kg) exhibited better feed utilization, growth indices, and survival (Yousefi et al., 2021a). The number of total lactic acid bacteria was significantly improved by 1% *S. khuzestanica* (Mousavi et al., 2016). Ghafarifarsani et al. (2022b) showed that dietary *S. hortensisis* at an inclusion level of 200 mg/kg improved the growth performance of Caspian roach (*Rutilus caspicus*). A recent study by Magouz et al. (2021) illustrated that a blend of herbal essential oils (0.25–0.5 mL/kg) containing oregano, thymol, carvacrol, 1,8 cineol, pinene β, limonene, pinene, and propylene glycol induced markedly better growth performance, digestive enzymes activity, and intestinal features of Nile tilapia (*Oreochromis niloticus*). The terpenoids, phenolics, and flavonoids improve feed utilization by increasing feed palatability and enhancing beneficial bacteria activity, thereby higher secretion of digestive enzymes (Ahmadifar et al., 2021; Alagawany et al., 2021). Additionally, a better gut health facilitates efficient nutrients absorption (Magouz et al., 2021). Hence, the growth-promoting effect of BHE may be attributed to such bioactive compounds, which are also found in *T. vulgaris*, *O. majorana*, and *S. hortensisis*.

The results evidenced that serum and mucosal total immunoglobulins (Ig) contents can be profoundly affected by BHE treatments. It suggests enhanced immune response of supplemented fish since Igs participate in the complement system activation, opsonization of antigens for phagocytosis, neutralization of toxins, and inhibiting the attachment of the pathogens to body cells or mucosal surfaces (Mashoof and Criscitiello, 2016; Salinas et al., 2011). In agreement with our results, it was reported that individual administration of *O. majorana* in common carp (Yousefi et al., 2021a) and *S. hortensisis* in Caspian roach (Ghafarifarsani et al., 2022b) improved mucus and serum Ig concentrations, respectively. However, serum Ig contests were not affected by dietary supplementation of *T. vulgaris* essential oil in the study of Ghafarifarsani et al. (2021b). The bioactive fraction of medical herbs can damage bacterial cells and release cellular components

(Aragona et al., 2018), thereby enhancing immune responses by affecting B cells that produce Ig in fish.

The results revealed significantly higher LYZ activity in serum and mucus samples of 1 and 2% BHE supplemented treatments in particular, which confirm the improvement of immune responses. LYZ is a principal antimicrobial protein present in body fluids and mucosal surfaces (Raissy et al., 2022). LYZ hydrolysis of peptidoglycan leads to membrane vulnerability and microbial cell death. It also eliminates bacteria through a cationic nature involving the formation of pores on the bacterial cell membrane. At mucosal surfaces, LYZ prevents bacterial overgrowth (Ragland and Criss, 2017). Such beneficial effects of herbal extracts on LYZ activity have been reported by Yousefi et al. (2021a), who showed that the use of 200 mg/kg *O. majorana* ethanolic extract in common carp increased serum and mucus LYZ activities. Further, the elevated activity of serum LYZ was observed following supplementation with 200 mg/kg *S. hortensisis* in Caspian roach (Ghafarifarsani et al., 2022b). Similar results were reported in rainbow trout and snakehead (*Channa argus*) by thymol and *T. vulgaris* supplementation, respectively (Ghafarifarsani et al., 2021b; Yousefi et al., 2022; Di Kong et al., 2021).

ACH_{50} synergizes with lysozyme by the formation of membrane attack complex (MAC) and enhancing the access of lysozyme to periplasmic peptidoglycan. Its attachment also limits bacteria activity and stimulates phagocytosis by opsonizing antigens (Holland and Lambris, 2002). According to the results, the diet containing 1% BHE induced significantly higher levels of ACH_{50} activity in serum as compared to the control. This finding is similar to the previous results obtained by individual supplementation of the same medicinal herbs (Ghafarifarsani et al., 2021a, 2021b; Di Kong et al., 2021; Yousefi et al., 2021a, 2021b; Ghafarifarsani et al., 2022b, 2022c; Raissy et al., 2022; Yousefi et al., 2022).

The physiological roles of ALP may vary depending on the tissue (Smith et al., 2013). In fish, mucosal ALP has antimicrobial effects and contributes to the detoxification of pro-inflammatory microbial compounds present in the water through dephosphorylating, thus protecting the organism and controlling skin inflammation (Lallès, 2019). In our study, 2% BHE treatment in particular improved mucus ALP levels which agrees with a previous study on common carp fed *O. majorana* (Yousefi et al., 2021a). Additionally, other members of the *Lamiaceae* family, such as *O. vulgare*, showed comparable results (Ghafarifarsani et al., 2021a).

In the present study, skin mucus PRO activity was significantly enhanced in 1–3% BHE-supplemented groups. Skin mucus proteases are implicated in the resistance against bacterial and ectoparasite infection since they directly degrade proteins of pathogens or modify chemical properties of the mucosal layer to prevent pathogens attachment/interaction and overgrowth (Fernández-Montero et al., 2021). Thus, higher PRO activities suggest improved immune responses and disease resistance of fish, as shown by singular administration of *O. majorana* in common carp (Yousefi et al., 2021a) and *O. vulgare* in common carp (Ghafarifarsani et al., 2021a).

In the present study, a variety of bioactive compounds contained in the applied medical herbs (*T. vulgaris*, *O. majorana*, and *S. hortensisis*) could act as membrane permeabilizers with cytotoxic effects on bacterial cell structure and function. In addition, they have quorum sensing disrupting properties that limit bacterial biofilm formation (Firmino et al., 2021). Interestingly, the presence of plant-derived biomolecules such as carvacrol and 1,8-cineole in the fish skin mucus is reported by some studies (Mizuno et al., 2018; Zoral et al., 2018). Thus, such an efflux through the skin may also be responsible for the improved immune responses and antimicrobial effects observed in the skin mucosal layer (Firmino et al., 2021).

Low serum total protein levels were reported in malnutrition and disease conditions (Goldstein-Fuchs and LaPierre, 2013). Under such conditions, there is not enough protein for the liver to synthesis albumin. Hence, higher total protein and albumin levels in the current study are most likely signs of more efficient feed utilization, healthy liver, and improved immune responses. Similar results were obtained by previous individual supplementation of medicinal herbs applied in this experiment or other members of the *Lamiaceae* family (Ghafarifarsani et al., 2021a, 2021b; Mohammadi et al., 2020; Yousefi et al., 2021a; Ghafarifarsani et al., 2022a, 2022b, 2022c; Yousefi et al., 2022).

High levels of serum globulin may indicate infection, inflammation, or immune disorders (Smith et al., 2013), whereas its low levels could be a sign of a weakened immune system and liver disease (Hajirezaee et al., 2020). In the present study, the globulins levels were comparable to control, indicating a stable fish health condition and that BHE had no adverse effects on liver and immune responses, supporting supplements safety.

Cortisol and glucose levels in the blood are biomarkers of stress in fish (Hedayati et al., 2019, 2021). The energetic demands of stressed fish lead to increased cortisol levels which is an essential stimulus for liver cells to produce glucose, thereby providing body cells with sufficient energy sources. Liver malfunction, endogenous protein loss, immunosuppression, and disease could be possible outcomes of constantly high cortisol levels in fish (Hajirezaee et al., 2020). Meanwhile, cholesterol is the precursor of some steroid hormones, including cortisol (Wan et al., 2014), and triglycerides are the main source of energy for body cells (Hajirezaee et al., 2020), therefore, the reduced serum cortisol, glucose, cholesterol, and triglycerides levels by BHE supplementation possibly indicate lower stress conditions. Khalil et al. (2020) showed that *T. vulgaris* supplementation attenuated lambda cyhalothrin-induced toxicity through negative influence on mRNA transcription of genes linked to immunity and induction of oxidative injury of the different immune organs. Concurrently lower cortisol, glucose, cholesterol, and triglycerides are reported by other studies on *T. vulgaris* (Hoseini and Yousefi, 2019), *S. hortensis* (Mousavi et al., 2016), and *O. majorana* (Desouky et al., 2015; Toghyani et al., 2010; Wahby et al., 2015) individual supplementation. Indeed, BHE supplementation could lower these compounds through enhancing feed utilization, antioxidant capacity, immune responses, and liver health and function, thereby increasing fish resistance against stress conditions.

Reactive oxygen species (ROS) are strongly associated with many pathological events (Valavanidis et al., 2006; Hoseinifar et al., 2020). They have a very short life span and are thus not easy to detect. Nevertheless, ROS-induced tissue injury may be monitored by measuring the levels of malondialdehyde (MDA) which is formed during lipid peroxidation of polyunsaturated fatty acids (Cherian et al., 2019; Ghafarifarsani et al., 2021c). The present study revealed profound decreases in MDA levels by applying BHE-incorporated diets. Plants and their extracts are proven to effectively lower lipid peroxidation by enhancing antioxidant enzymes activities. Several antioxidant enzymes are found in the body cells to inhibit or heal the damage caused by ROS, as well as to regulate redox-sensitive signaling pathways, among which SOD catalyzes the conversion of superoxide radicals into more stable molecular oxygen and hydrogen peroxide, whereas the CAT converts hydrogen peroxide into water (Weydert and Cullen, 2010; Ghafarifarsani et al., 2021c). GSH-Px catalyzes the reduction of hydrogen peroxide into water (Yousefi et al., 2021a, 2021b). Thus, the enhanced SOD, CAT, and GSH-Px activities in the current study effectively improve cellular oxidative stress defense system in the fish body, which well accord with the findings of the previous studies on the individual administration of *T. vulgaris* (Hoseini and Yousefi, 2019), *O. majorana* (Yousefi et al., 2021a), thymol (Di Kong et al., 2021; Yousefi et al., 2022), and some other species of *Lamiaceae* family (Abdel-Latif et al., 2020; Mohammadi et al., 2020). The various bioactive chemicals namely terpenoids (e.g., thymol, carvacrol, linalool, terpinen), phenolics (e.g., quinic, rosmaric, and caffeic acids), and flavonoids (e.g., apigenin, luteolin, cirsimaritin) contained in medicinal plants and their derivatives are reported to positively enhance SOD, CAT, and GSH-Px but decrease MDA levels in fish (Chambre et al., 2020; Charai et al., 1996; Firmino et al., 2021; Komaitis et al., 1992; Ghafarifarsani et al., 2021a, 2021b; Patil et al., 2021; Ghafarifarsani et al., 2022b, 2022c).

Carvacrol and thymol in particular shown to have strong antioxidant properties relying on their ability to scavenge ROS and other oxygen radicals generated in cells and tissues.

ALT and AST reside in the cytoplasm and play a role in amino acids metabolism and gluconeogenesis. ALP is a membrane-bound metalloenzyme whose activity in sera originates mainly in the liver. These enzymes are well-established biomarkers of fish liver health, which their increased concentrations in blood are a sign of liver damage (Hajirezaee et al., 2020; Abdelhamid et al., 2021; Ghafarifarsani et al., 2021a). The present study demonstrated the considerable declined in ALT, AST, and ALP in BHE-supplemented treatments, indicating the hepatoprotective effect of BHE in carps. Consistent with our results, diets enriched with *T. vulgaris* (Hoseini and Yousefi, 2019), thymol (Di Kong et al., 2021; Yousefi et al., 2022), and *O. vulgare* (Mohammadi et al., 2020) showed similar advantageous effects. These may be attributed to enhancement of antioxidant and immune responses, thus higher liver health status, of fish fed medicinal plants which was also demonstrated by the results of the current study.

5. Conclusion

In conclusion, a blend of *T. vulgaris*, *O. majorana*, and *S. hortensis* extracts (BHE) effectively enhanced the growth performance and feed efficiency in common carp. It also favorably reduced stress conditions and improved fish health status, demonstrated by positive alterations in serum biochemicals, immunological parameters, and antioxidant responses. The diet supplemented with 1% BHE produced the most optimum results in terms of growth performance and health status of common carp.

Author statement

All people who meet authorship criteria are mentioned as authors, and all authors certify that they have taken part sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. In addition, every author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the *Aquaculture*.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Acknowledgements

We are grateful to Dr. Hisham A. Abdelrahman (School of Fisheries, Aquaculture & Aquatic Sciences, Auburn University, USA) for critically reviewing the present manuscript.

References

Abdelhamid, A.F., Ayoub, H.F., Abd El-Gawad, E.A., Abdelghany, M.F., Abdel-Tawwab, M., 2021. Potential effects of dietary seaweeds mixture on the growth performance, antioxidant status, immunity response, and resistance of striped catfish (*Pangasianodon hypophthalmus*) against *Aeromonas hydrophila* infection. Fish Shellfish Immunol. 119, 76–83. https://doi.org/10.1016/j.fsi.2021.09.043.

Abdel-Latif, H.M.R., Abdel-Tawwab, M., Khafaga, A.F., Dawood, M.A.O., 2020. Dietary origanum essential oil improved antioxidative status, immune-related genes, and resistance of common carp (*Cyprinus carpio* L.) to *Aeromonas hydrophila* infection. Fish Shellfish Immunol. 104, 1–7. https://doi.org/10.1016/j.fsi.2020.05.056.

Ahmadifar, E., Yousefi, M., Karimi, M., Fadaei Raieni, R., Dadar, M., Yilmaz, S., Abdel-Latif, H.M.R., 2021. Benefits of dietary polyphenols and polyphenol-rich additives to aquatic animal health: an overview. Rev. Fish. Sci. Aquac. 29 (4), 478–511. https://doi.org/10.1080/23308249.2020.1818689.

Alagawany, M., Farag, M.R., Abdelnour, S.A., Elnesr, S.S., 2021. A review on the beneficial effect of thymol on health and production of fish. Rev. Aquac. 13 (1), 632–641. https://doi.org/10.1111/raq.12490.

Aragona, M., Lauriano, E.R., Pergolizzi, S., Faggio, C., 2018. Opuntia ficus-indica (L.) miller as a source of bioactivity compounds for health and nutrition. Nat. Prod. Res. 32 (17), 2037–2049. https://doi.org/10.1080/14786419.2017.1365073.

Awad, E., Austin, D., Lyndon, A.R., 2013. Effect of black cumin seed oil (*Nigella sativa*) and nettle extract (quercetin) on enhancement of immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture. 388–391, 193–197. https://doi.org/10.1016/j.aquaculture.2013.01.008.

Bayunova, L., Barannikova, I., Semenkova, T., 2002. Sturgeon stress reactions in aquaculture. J. Appl. Ichthyol. 18, 397–404. https://doi.org/10.1046/j.1439-0426.2002.00410.x.

Beutler, E., 1984. Catalase in red cell metabolism. A Man. Biochem. methods 105–106.

Beutler, E., Duron, O., Kelly, B.M., 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61, 882–888.

Brown, R., Moore, L., Mani, A., Patel, S., Salinas, I., 2021. Effects of ploidy and salmonid alphavirus infection on the skin and gill microbiome of Atlantic salmon (Salmo salar). Plos One 16 (2), e0243684.

Burtis, C.A., Ashwood, E.R., 1999. Tietz Textbook of Clinical Chemistry, third ed. W. B. Saunders Co., Philadelphia, pp. 29–150.

Chambre, D.R., Moisa, C., Lupitu, A., Copolovici, L., Pop, G., Copolovici, D.M., 2020. Chemical composition, antioxidant capacity, and thermal behavior of *Satureja hortensis* essential oil. Sci. Rep. 10 (1), 1–12. https://doi.org/10.1038/s41598-020-78263-9.

Charai, M., Mosaddak, M., Faid, M., 1996. Chemical composition and antimicrobial activities of two aromatic plants: *Origanum majorana* L. and *O. Compactum* Benth. J. Essent. Oil Res. 8, 657–664. https://doi.org/10.1080/10412905.1996.9701036.

Cherian, D., Peter, T., Narayanan, A., Madhavan, S., Achammada, S., Vynat, G., 2019. Malondialdehyde as a marker of oxidative stress in periodontitis patients. J. Pharm. Bioallied Sci. 11, S297–S300. https://doi.org/10.4103/JPBS.JPBS_17_19.

Dawn-Linsley, M., Ekinci, F.J., Ortiz, D., Rogers, E., Shea, T.B., 2005. Monitoring thiobarbituric acid-reactive substances (TBARs) as an assay for oxidative damage in neuronal cultures and central nervous system. J. Neurosci. Methods 141, 219–222. https://doi.org/10.1016/j.jneumeth.2004.06.010.

Dawood, M.A.O., Koshio, S., Esteban, M.Á., 2018. Beneficial roles of feed additives as immunostimulants in aquaculture: a review. Rev. Aquac. 10 (4), 950–974. https://doi.org/10.1111/raq.12209.

Dawood, M.A.O., Metwally, A.E.S., El-Sharawy, M.E., Atta, A.M., Elbialy, Z.I., Abdel-Latif, H.M.R., Paray, B.A., 2020. The role of β-glucan in the growth, intestinal morphometry, and immune-related gene and heat shock protein expressions of Nile tilapia (*Oreochromis niloticus*) under different stocking densities. Aquaculture. 523, 735205 https://doi.org/10.1016/j.aquaculture.2020.735205.

Desouky, S., Marzouk, M.S., Soliman, A.M., Sayed, A.A., 2015. Modulatory effect of *Origanum majorana* extract against cisplatin-induced dyslipidemia in rats. Citeseer 4, 228–234.

Di Kong, Y., Li, M., Xia, C.G., Zhao, J., Niu, X. Tian, Shan, X.F., Wang, G.Q., 2021. The optimum thymol requirement in diets of *Channa argus*: effects on growth, antioxidant capability, immune response and disease resistance. Aquac. Nutr. 27, 712–722. https://doi.org/10.1111/anu.13217.

Doumas, B.T., Watson, W.A., Biggs, H.G., 1997. Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chim. Acta 258, 21–30. https://doi.org/10.1016/S0009-8981(96)06447-9.

El Euony, O.I., Elblehi, S.S., Abdel-Latif, H.M., Abdel-Daim, M.M., El-Sayed, Y.S., 2020. Modulatory role of dietary *Thymus vulgaris* essential oil and *Bacillus subtilis* against thiamethoxam-induced hepatorenal damage, oxidative stress, and immunotoxicity in African catfish (*Clarias gariepenus*). Environ. Sci. Pollut. Res. 27, 23108–23128. https://doi.org/10.1007/s11356-020-08588-5.

Ellis, A.E., 1990. Lysozyme assays. In: Stolen, J.S., Flecher, T.C., Anderson, D.P., Robertsen, B.S., Van Muiswinkel, W.B. (Eds.), Techniques in Fish Immunology, pp. 101–103.

El-Saadony, M.T., Alagawany, M., Patra, A.K., Kar, I., Tiwari, R., Dawood, M.A.O., Abdel-Latif, H.M.R., 2021. The functionality of probiotics in aquaculture: an overview. Fish Shellfish Immunol. 117, 36–52. https://doi.org/10.1016/j.fsi.2021.07.007.

Fernández-Montero, Á., Torrecillas, S., Montero, D., Acosta, F., Prieto-Álamo, M.J., Abril, N., Jurado, J., 2021. Proteomic profile and protease activity in the skin mucus of greater amberjack (*Seriola dumerili*) infected with the ectoparasite *Neobenedenia girellae*-an immunological approach. Fish Shellfish Immunol. 110, 100–115. https://doi.org/10.1016/j.fsi.2021.01.001.

Fierascu, I., Dinu-Pirvu, C.E., Fierascu, R.C., Velescu, B.S., Anuta, V., Ortan, A., Jinga, V., 2018. Phytochemical profile and biological activities of *satureja hortensis* L.: a review of the last decade. Molecules. 23 (10), 2458. https://doi.org/10.3390/molecules23102458.

Firmino, J.P., Galindo-Villegas, J., Reyes-López, F.E., Gisbert, E., 2021. Phytogenic bioactive compounds shape fish mucosal immunity. Front. Immunol. 12, 1–20. https://doi.org/10.3389/fimmu.2021.695973.

Ghafarifarsani, H., Hoseinifar, S.H., Adorian, T.J., Goulart Ferrigolo, F.R., Raissy, M., Van Doan, H., 2021a. The effects of combined inclusion of Malvae sylvestris, *Origanum vulgare*, and *Allium hirtifolium* boiss for common carp (*Cyprinus carpio*) diet: growth performance, antioxidant defense, and immunological parameters. Fish Shellfish Immunol. 119, 670–677. https://doi.org/10.1016/j.fsi.2021.10.014.

Ghafarifarsani, H., Kachuei, R., Imani, A., 2021b. Dietary supplementation of garden thyme essential oil ameliorated the deteriorative effects of aflatoxin B_1 on growth performance and intestinal inflammatory status of rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 531 https://doi.org/10.1016/j.aquaculture.2020.735928.

Ghafarifarsani, H., Imani, A., Niewold, T.A., Pietsch-Schmied, C., Moghanlou, K.S., 2021c. Synergistic toxicity of dietary aflatoxin B_1 (AFB_1) and zearalenone (ZEN) in rainbow trout (*Oncorhynchus mykiss*) is attenuated by anabolic effects. Aquaculture 541, 736793.

Ghafarifarsani, H., Hoseinifar, S.H., Javahery, S., Yazici, M., Van Doan, H., 2022a. Growth performance, biochemical parameters, and digestive enzymes in common carp (*Cyprinus carpio*) fed experimental diets supplemented with vitamin C, thyme essential oil, and quercetin. Ital. J. Anim. Sci. 21 (1), 291–302.

Ghafarifarsani, H., Hoseinifar, S.H., Aftabgard, M., Van Doan, H., 2022b. The improving role of savory (*Satureja hortensis*) essential oil for Caspian roach (*Rutilus caspicus*) fry: growth, haematological, immunological, and antioxidant parameters and resistance to salinity stress. Aquaculture. 548, 737653 https://doi.org/10.1016/j.aquaculture.2021.737653.

Ghafarifarsani, H., Hoseinifar, S.H., Javahery, S., Van Doan, H., 2022c. Effects of dietary vitamin C, thyme essential oil, and quercetin on the immunological and antioxidant status of common carp (*Cyprinus carpio*). Aquaculture 553, 738053.

Giannenas, I., Triantafillou, E., Stavrakakis, S., Margaroni, M., Mavridis, S., Steiner, T., Karagouni, E., 2012. Assessment of dietary supplementation with carvacrol or thymol containing feed additives on performance, intestinal microbiota and antioxidant status of rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 350–353, 26–32. https://doi.org/10.1016/j.aquaculture.2012.04.027.

Goldstein-Fuchs, D.J., LaPierre, A.F., 2013. Nutrition and kidney disease. In: National Kidney Foundation's Primer on Kidney Diseases, sixth edition, pp. 467–475. https://doi.org/10.1016/B978-1-4557-4617-0.00054-6.

Hajiahmadian, M., Vajargah, M.F., Farsani, H.G., Chorchi, M.M., 2012. Effect of *Spirulina platensis* meal as feed additive on growth performance and survival rate in golden barb fish, *Punius gelius* (Hamilton, 1822). J. Fish. Int. 7 (3–6), 61–64.

Hajirezaee, S., Mohammadi, G., Naserabad, S.S., 2020. The protective effects of vitamin C on common carp (*Cyprinus carpio*) exposed to titanium oxide nanoparticles (TiO2-NPs). Aquaculture. 518, 734734 https://doi.org/10.1016/j.aquaculture.2019.734734.

Harikrishnan, R., Thamizharasan, S., Devi, G., Van Doan, H., Ajith Kumar, T.T., Hoseinifar, S.H., Balasundaram, C., 2020. Dried lemon peel enriched diet improves antioxidant activity, immune response and modulates immuno-antioxidant genes in *Labeo rohita* against Aeromonas sorbia. Fish Shellfish Immunol. 106, 675–684. https://doi.org/10.1016/j.fsi.2020.07.040.

Harikrishnan, R., Devi, G., Van Doan, H., Balasundaram, C., Esteban, M.Á., Abdel-Tawwab, M., 2021. Impact of grape pomace flour (GPF) on immunity and immune-antioxidant-anti-inflammatory genes expression in *Labeo rohita* against *Flavobacterium columnaris*. Fish Shellfish Immunol. 111, 69–82. https://doi.org/10.1016/j.fsi.2021.01.011.

Hedayati, S.A., Farsani, H.G., Naserabad, S.S., Hoseinifar, S.H., Van Doan, H., 2019. Protective effect of dietary vitamin E on immunological and biochemical induction through silver nanoparticles (AgNPs) inclusion in diet and silver salt (AgNO3) exposure on zebrafish (*Danio rerio*). Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 222, 100–107. https://doi.org/10.1016/j.cbpc.2019.04.004.

Hedayati, S.A., Sheikh Veisi, R., Hosseini Shekarabi, S.P., Shahbazi Naserabad, S., Bagheri, D., Ghafarifarsani, H., 2021. Effect of dietary *lactobacillus casei* on Physiometabolic responses and liver histopathology in common carp (*Cyprinus carpio*) after exposure to Iron oxide nanoparticles. Biol. Trace Elem. Res. 1-9 https://doi.org/10.1007/s12011-021-02906-9.

Holland, M.C.H., Lambris, J.D., 2002. The complement system in teleosts. Fish Shellfish Immunol. 12, 399–420. https://doi.org/10.1006/fsim.2001.0408.

Hoseini, S.M., Yousefi, M., 2019. Beneficial effects of thyme (*Thymus vulgaris*) extract on oxytetracycline-induced stress response, immunosuppression, oxidative stress and enzymatic changes in rainbow trout (*Oncorhynchus mykiss*). Aquac. Nutr. 25, 298–309. https://doi.org/10.1111/anu.12853.

Hoseinifar, S.H., Yousefi, S., Van Doan, H., Ashouri, G., Gioacchini, G., Maradonna, F., Carnevali, O., 2020. Oxidative stress and antioxidant defense in fish: the implications of probiotic, prebiotic, and Synbiotics. Rev. Fish. Sci. Aquac. 29 (2), 198–217. https://doi.org/10.1080/23308249.2020.1795616.

Katar, D., Kacar, O., Kara, N., Aytaç, Z., Göksu, E., Kara, S., Elmastaş, M., 2017. Ecological variation of yield and aroma components of summer savory (*Satureja hortensis* L.). J. Appl. Res. Med. Aromat. Plants. 7, 131–135. https://doi.org/10.1016/j.jarmap.2017.07.005.

Khalil, S.R., Elhakim, Y.A., Abd El-fattah, A.H., Ragab Farag, M., Abd El-Hameed, N.E., El-Murr, A.E., 2020. Dual immunological and oxidative responses in *Oreochromis niloticus* fish exposed to lambda cyhalothrin and concurrently fed with thyme powder (*Thymus vulgaris* L.): stress and immune encoding gene expression. Fish Shellfish Immunol. 100, 208–218. https://doi.org/10.1016/j.fsi.2020.03.009.

Komaitis, M.E., Ifanti-Papatragianni, N., Melissari-Panagiotou, E., 1992. Composition of the essential oil of marjoram (*Origanum majorana* L.). Food Chem. 45, 117–118. https://doi.org/10.1016/0308-8146(92)90020-3.

Lallès, J.P., 2019. Biology, environmental and nutritional modulation of skin mucus alkaline phosphatase in fish: a review. Fish Shellfish Immunol. 89, 179–186. https://doi.org/10.1016/j.fsi.2019.03.053.

Lubran, M.M., 1978. The measurement of total serum proteins by the biuret method. Ann. Clin. Lab. Sci. 8, 106–110.

Magouz, F.I., Shehab El-Din, M.T., Amer, A.A., Gewaily, M.S., El-Dahdoh, W.A., Dawood, M.A.O., 2021. A blend of herbal essential oils enhanced the growth performance, blood bio-immunology traits, and intestinal health of Nile tilapia (*Oreochromis niloticus*). Ann. Anim. Sci. https://doi.org/10.2478/aoas-2021-0066.

Mani, A., Ebrahimi, E., 2021. Equally weighted multivariate optimization of feeding rate for sub-yearling great sturgeon (*Huso huso*) using desirability function model. J. World Aquac. Soc. 1–10. https://doi.org/10.1111/jwas.12857.

Marklund, S., Marklund, G., 1974. Involvement of the superoxide anion radical in the autoxidation of Pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem. 47, 469–474. https://doi.org/10.1111/j.1432-1033.1974.tb03714.x.

Mashoof, S., Criscitiello, M.F., 2016. Fish immunoglobulins. Biology (Basel). 5 (4), 45. https://doi.org/10.3390/biology5040045.

Mizuno, S., Urawa, S., Miyamoto, M., Hatakeyama, M., Sasaki, Y., Koide, N., Ueda, H., 2018. Effects of dietary supplementation with oregano essential oil on prevention of the ectoparasitic protozoans *Ichthyobodo salmonis* and *Trichodina truttae* in juvenile chum salmon *Oncorhynchus keta*. J. Fish Biol. 93, 528–539. https://doi.org/10.1111/jfb.13681.

Mohammadi, G., Rafiee, G., El Basuini, M.F., Van Doan, H., Ahmed, H.A., Dawood, M.A.O., Abdel-Latif, H.M.R., 2020. Oregano (*Origanum vulgare*), St John's-wort (*Hypericum perforatum*), and lemon balm (*Melissa officinalis*) extracts improved the growth rate, antioxidative, and immunological responses in Nile tilapia (*Oreochromis niloticus*) infected with *Aeromonas hydrophila*. Aquac. Reports. 18, 100445 https://doi.org/10.1016/j.aqrep.2020.100445.

Morales, R., 2003. The history, botany and taxonomy of the genus *Thymus*. In: Thyme: The Genus Thymus. CRC press, pp. 1–43.

Mousavi, E., Mohammadiazarm, H., Mousavi, S.M., Ghatrami, E.R., 2016. Effects of inulin, savory and onion powders in diet of juveniles carp *Cyprinus carpio* (Linnaeus 1758) on gut micro flora, immune response and blood biochemical parameters. Turkish J. Fish. Aquat. Sci. 16, 831–838. https://doi.org/10.4194/1303-2712-v16_4_09.

Mugwanya, M., Dawood, M.A.O., Kimera, F., Sewilam, H., 2022. Updating the role of probiotics, prebiotics, and synbiotics for tilapia aquaculture as leading candidates for food sustainability: a review. Probiotics Antimicrob. Proteins 14, 130–157. https://doi.org/10.1007/s12602-021-09852-x.

Navarrete, P., Toledo, I., Mardones, P., Opazo, R., Espejo, R., Romero, J., 2010. Effect of *Thymus vulgaris* essential oil on intestinal bacterial microbiota of rainbow trout, *Oncorhynchus mykiss* (Walbaum) and bacterial isolates. Aquac. Res. 41 (10), e667–e678. https://doi.org/10.1111/j.1365-2109.2010.02590.x.

Patil, S.M., Ramu, R., Shirahatti, P.S., Shivamallu, C., Amachawadi, R.G., 2021. A systematic review on ethnopharmacology, phytochemistry and pharmacological aspects of *Thymus vulgaris* Linn. Heliyon. 7 (5), e07054 https://doi.org/10.1016/j.heliyon.2021.e07054.

Ragland, S.A., Criss, A.K., 2017. From bacterial killing to immune modulation: recent insights into the functions of lysozyme. PLoS Pathog. 13 (9), e1006512 https://doi.org/10.1371/journal.ppat.1006512.

Raissy, M., Ghafarifarsani, H., Hoseinifar, S.H., El-Haroun, E.R., Shahbazi Naserabad, S., Van Doan, H., 2022. The effect of dietary combined herbs extracts (oak acorn, coriander, and common mallow) on growth, digestive enzymes, antioxidant and immune response, and resistance against *Aeromonas hydrophila* infection in common carp, *Cyprinus carpio*. Aquaculture. 546, 737287 https://doi.org/10.1016/j.aquaculture.2021.737287.

Reitman, S., Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28, 56–63. https://doi.org/10.1093/ajcp/28.1.56.

Reverter, M., Tapissier-Bontemps, N., Sarter, S., Sasal, P., Caruso, D., 2021. Moving towards more sustainable aquaculture practices: a meta-analysis on the potential of plant-enriched diets to improve fish growth, immunity and disease resistance. Rev. Aquac. 13, 537–555. https://doi.org/10.1111/raq.12485.

Ross, N.W., Firth, K.J., Wang, A., Burka, J.F., Johnson, S.C., 2000. Changes in hydrolytic enzyme activities of naive Atlantic salmon *Salmo salar* skin mucus due to infection with the salmon louse *Lepeophtheirus salmonis* and cortisol implantation. Dis. Aquat. Org. 41, 43–51. https://doi.org/10.3354/dao041043.

Salinas, I., Zhang, Y.A., Sunyer, J.O., 2011. Mucosal immunoglobulins and B cells of teleost fish. Dev. Comp. Immunol. 35, 1346–1365. https://doi.org/10.1016/j.dci.2011.11.009.

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. Tech Fish Immunol. 3, 23–30.

Smith, G.S., Walter, G.L., Walker, R.M., 2013. Clinical pathology in non-clinical toxicology testing. Haschek and Rousseaux's handbook of toxicologic pathology. 565–594. https://doi.org/10.1016/B978-0-12-415759-0.00018-2.

Toghyani, M., Mousavi, S.K., Modaresi, M., 2010. Effect of water extract of marjoram (*Origanum Majorana* L.) as an alternative to antibiotic growth promoter on immunity and serum lipid profile of broiler chicks. In: ICBEE 2010–2010 2nd International Conference on Chemical, Biological and Environmental Engineering, Proceedings, pp. 314–316. https://doi.org/10.1109/ICBEE.2010.5653956.

Valavanidis, A., Vlahogianni, T., Dassenakis, M., Scoullos, M., 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol. Environ. Saf. 64, 178–189. https://doi.org/10.1016/j.ecoenv.2005.03.013.

Wahby, M.M., Yacout, G., Kandeel, K., Awad, D., 2015. LPS-induced oxidative inflammation and hyperlipidemia in male rats: the protective role of *Origanum majorana* extract. Beni-Suef Univ. J. Basic Appl. Sci. 4, 291–298. https://doi.org/10.1016/j.bjbas.2015.11.004.

Wan, J., Ge, X., Liu, B., Xie, J., Cui, S., Zhou, M., Chen, R., 2014. Effect of dietary vitamin C on non-specific immunity and mRNA expression of three heat shock proteins (HSPs) in juvenile *Megalobrama amblycephala* under pH stress. Aquaculture. 434, 325–333. https://doi.org/10.1016/j.aquaculture.2014.08.043.

Weydert, C.J., Cullen, J.J., 2010. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nat. Protoc. 5, 51–66. https://doi.org/10.1038/nprot.2009.197.

Yano, T., 1992. Assays of hemolytic complement activity. Tech. fish Immunol. 2, 131–141.

Yılmaz, S., Ergün, S., Çelik, E.Ş., 2016. Effect of dietary spice supplementations on welfare status of sea bass, *Dicentrarchus labrax* L. Proc. Natl. Acad. Sci. India Sect. B - Biol. Sci. 86, 229–237. https://doi.org/10.1007/s40011-014-0444-2.

Yilmaz, S., Yilmaz, E., Dawood, M.A.O., Ringø, E., Ahmadifar, E., Abdel-Latif, H.M.R., 2022. Probiotics, prebiotics, and synbiotics used to control vibriosis in fish: a review. Aquaculture. 547, 737514 https://doi.org/10.1016/j.aquaculture.2021.737514.

Yousefi, M., Ghafarifarsani, H., Hoseinifar, S.H., Rashidian, G., Van Doan, H., 2021a. Effects of dietary marjoram, *Origanum majorana* extract on growth performance, hematological, antioxidant, humoral and mucosal immune responses, and resistance of common carp, *Cyprinus carpio* against *Aeromonas hydrophila*. Fish Shellfish Immunol. 108, 127–133. https://doi.org/10.1016/j.fsi.2020.11.019.

Yousefi, M., Naderi Farsani, M., Ghafarifarsani, H., Hoseinifar, S.H., Van Doan, H., 2021b. The effects of dietary supplementation of mistletoe (*Viscum album*) extract on the growth performance, antioxidant, and innate, immune responses of rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 536, 736385 https://doi.org/10.1016/j.aquaculture.2021.736385.

Yousefi, M., Ghafarifarsani, H., Hoseini, S.M., Hoseinifar, S.H., Abtahi, B., Vatnikov, Y.A., Van Doan, H., 2022. Effects of dietary thyme essential oil and prebiotic administration on rainbow trout (*Oncorhynchus mykiss*) welfare and performance. Fish Shellfish Immunol. 120, 737–744. https://doi.org/10.1016/j.fsi.2021.12.023.

Zoral, M.A., Ishikawa, Y., Ohshima, T., Futami, K., Endo, M., Maita, M., Katagiri, T., 2018. Toxicological effects and pharmacokinetics of rosemary (*Rosmarinus officinalis*) extract in common carp (*Cyprinus carpio*). Aquaculture. 495, 955–960. https://doi.org/10.1016/j.aquaculture.2018.06.048.

Beneficial alterations in growth performance, blood biochemicals, immune responses, and antioxidant capacity of common carp (Cyprinus carpio) fed a blend of Thymus vulgaris, Origanum majorana, and Sat

ORIGINALITY REPORT

19%	10%	21%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Marwan Mahmood Saleh, Saif Y. Hasan, Sarmad Ghazi Al-Shawi, Muneam Hussein Ali et al. " (IBRC-M 10,711) ameliorates the growth retardation, oxidative stress, and immunosuppression induced by malathion toxicity in goldfish ( ) ", Annals of Animal Science, 2022 Publication	5%
2	Ghasem Mohammadi, Mahmoud Hafezieh, Aliakbar Karimi, Mohamad Nor Azra et al. "The synergistic effects of plant polysaccharide and Pediococcus acidilactici as a synbiotic additive on growth, antioxidant status, immune response, and resistance of Nile tilapia (Oreochromis niloticus) against Aeromonas hydrophila", Fish & Shellfish Immunology, 2021 Publication	2%

3	Hamed Ghafarifarsani, Seyed Hossein Hoseinifar, Susan Javahery, Hien Van Doan. "Effects of dietary vitamin C, thyme essential oil, and quercetin on the immunological and antioxidant status of common carp (Cyprinus carpio)", Aquaculture, 2022 Publication	2%
4	Saeed Hajirezaee, Ghasem Mohammadi, Saeid Shahbazi Naserabad. "The protective effects of vitamin C on common carp (Cyprinus carpio) exposed to titanium oxide nanoparticles (TiO2-NPs)", Aquaculture, 2020 Publication	1%
5	www.researchsquare.com Internet Source	1%
6	Hafsan Hafsan, Dmitry Bokov, Walid Kamal Abdelbasset, MustafaM. Kadhim et al. " Dietary essential oil improved growth, haematology, immunity and resistance to in rainbow trout ( ) ", Aquaculture Research, 2022 Publication	1%
7	Ghasem Mohammadi, Gholamreza Rafiee, Mohammed F. El Basuini, Hien Van Doan et al. "Oregano (Origanum vulgare), St John's-wort (Hypericum perforatum), and lemon balm (Melissa officinalis) extracts improved the growth rate, antioxidative, and immunological	1%

responses in Nile tilapia (Oreochromis niloticus) infected with Aeromonas hydrophila", Aquaculture Reports, 2020
Publication

8 Ghasem Mohammadi, Ali Akbar Karimi, Mahmoud Hafezieh, Mahmoud A.O. Dawood, Haitham G. Abo-Al-Ela. "Pistachio hull polysaccharide protects Nile tilapia against LPS-induced excessive inflammatory responses and oxidative stress, possibly via TLR2 and Nrf2 signaling pathways", Fish & Shellfish Immunology, 2022 1 %
Publication

9 Alexei Valerievich Yumashev, Rumi Iqbal Doewes, Rustem Adamovich Shichiyakh, Shadia Faris ahmad et al. "The Effects of Dietary Gracilaria Corticata (Marine Macroalgae, Rhodophyta) Extraction on Growth Performance, Antioxidant Defence, Plasma and Mucosal Immune Components and Immune-related Gene Expressions in Goldfish, Carassius Auratus", Research Square Platform LLC, 2022 1 %
Publication

10 Hamed Ghafarifarsani, Seyed Hossein Hoseinifar, Maryam Aftabgard, Hien Van Doan. "The improving role of savory (Satureja hortensis) essential oil for Caspian Roach 1 %

(Rutilus caspicus) fry: Growth, haematological, immunological, and antioxidant parameters and resistance to salinity stress", Aquaculture, 2021
Publication

---

11 Hamed Ghafarifarsani, Seyed Hossein Hoseinifar, Taida Juliana Adorian, Fernanda Rodrigues Goulart Ferrigolo et al. "The effects of combined inclusion of Malvae sylvestris, Origanum vulgare, and Allium hirtifolium boiss for common carp (Cyprinus carpio) diet: Growth performance, antioxidant defense, and immunological parameters", Fish & Shellfish Immunology, 2021 1 %
Publication

---

12 Mehdi Raissy, Hamed Ghafarifarsani, Seyed Hossein Hoseinifar, Ehab R. El-Haroun, Saeid Shahbazi Naserabad, Hien Van Doan. "The effect of dietary combined herbs extracts (oak acorn, coriander, and common mallow) on growth, digestive enzymes, antioxidant and immune response, and resistance against Aeromonas hydrophila infection in common carp, Cyprinus carpio", Aquaculture, 2022 1 %
Publication

---

13 Ghasem Mohammadi, Gholamreza Rafiee, Hisham A. Abdelrahman. "Effects of dietary Lactobacillus plantarum (KC426951) in biofloc 1 %

and stagnant-renewal culture systems on growth performance, mucosal parameters, and serum innate responses of Nile tilapia Oreochromis niloticus", Fish Physiology and Biochemistry, 2020

Publication

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		