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Evaluation of dietary rosemary leaf powder on growth, carcass composition and hemato-biochemical profiles of common carp (*Cyprinus carpio*) reared in cage system

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ABSTRACT

This study was conducted to evaluate the effect of rosemary *(Rosmarinus officinalis)* leaf powder (RLP) at 0.0% (control), 0.5%, 1% and 2% as a dietary supplement for eight weeks, on the growth performance, carcass composition and haemato-biochemical parameters. One hundred twenty common carp (*Cyprinus carpio*) (21.11±0.17g) were randomly distributed to 12 cages (0.2 m^3) with three replicates for 56 days. The results showed that RLP-supplemented diets achieved significant enhancement of growth performance (FBW, SGR and WG) and feed utilization (FCR, FCE and PI) compared with a control group. In addition, TFI and PI were significantly improved in 0.5 and 2% RLP-supplemented groups. RLP had no significant effects on body composition except moisture content which was significantly increased in fish fed with a 1% supplemented diet and crude protein which was significantly decreased in fish fed with a 1% supplemented diet compared to the control diet. The results showed a significant increase in RLP-supplemented diets in RBC counts, LYM, MCV, hematocrit, total protein, albumin and globulin compared with the control group. WBC counts significantly increased in fish fed the 0.5 and 2% RLP diets, and hemoglobin significantly elevated in fish fed the 1% RLP diet. Glucose, GRA, MCH, MCHC and MPV were significantly decreased in the group fed the RLP diets compared with the control group. The AST, ALT, LDL, VLDL, CHO and TG levels showed a significant decrease in RLP compared to the control diet ($P < 0.05$), while ALP level fluctuated ($P > 0.05$). The urea and creatinine levels were higher in the RLP groups compared with the control ($P < 0.05$). Dietary RLP 1% increased lipase when compared to the control group. However, amylase levels in fish fed 0.5 and 1% RLP has significantly improved, while fish fed 2% RLP has significantly decreased when compared to the control diet. Overall, the results demonstrated that dietary RLP supplementation enhanced the growth and health status of common carp.

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Introduction

In 2020, it was estimated that there would be 8 million farmed fish used as food, indicating that aquaculture continues to be an important global source of animal protein. The aquaculture industry accounted for 46% of the total production and 52% of the fish consumed by humans (1). Therefore, aquaculture has become a prominent alternative food production source to meet the protein needs of an ever-growing global population due to a decrease in natural fish stocks and an escalating price of food and aquatic products (2). The common carp is an important reared fish species in many parts of the world, contributing 83.4% of freshwater production and 9.2% of total finfish aquaculture production (1). Common carp (*Cyprinus carpio*) is widely distributed in eutrophic freshwater bodies in Europe and Asia. The species is a highly valuable food source for the ever-growing human population with desirable aquaculture capabilities including high growth rate, better feed conversion ratio, higher capability of using carbohydrates and plant protein sources along with relatively high resistance to variable environmental conditions and diseases (3). Due to the growing demand for this species, intensive culture has become increasingly popular, but high stocking density can create stressful conditions and lead to infection outbreaks (4). However, since intensive aquaculture activities have become more widespread, pollution and disease outbreaks have become more prevalent, which has constrained fish growth and health status, exposing them to stressors, diseases, and infectious agents (5,6).

A modulatory action of herbal plants is to promote growth, haematological and biochemical parameters, growth promoters enhance the secretion of digestive enzymes, increasing survival and growth rates of aquatic organisms, particularly fish (7) has become a topic of focus. To control disease and improve the innate and acquired immune response of fish because some medicinal plants have numerous beneficial properties, including stimulation and strengthening the immune system (8). A variety of medicinal plants and their bioactive compounds, including alkaloids, phenolics, and steroids, have been used successfully in aquatic organisms to increase appetite, growth performance, stress responses, and immunity (9,10). Previous studies have demonstrated the positive effects of herbal plants, such as *Taraxacum officinale* (11), *Rosmarinus officinale* (12), *Olea europaea Leecino* (13), *Centella asiatica* (14) on fish health and production.

Rosemary (Rosmarinus officinalis) is a medicinal herb belonging to the Labiatae family whose active extracts are mainly composed of 1,8-cineole, carnosol, carnosic acid, and rosmarinic acid, β- and α-pinene, camphor, flavonoids,

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diterpenes, steroids and triterpenes and camphene (9,15). Several studies have reported that rosemary extract or may have anti-inflammatory, hepatoprotective, antithrombotic, diuretic, antidiabetic, antinociceptive, anticancer and antioxidant activity in experimental animals and humans (16,17). Yousefi et al (2019) has confirmed that RLP oral administration enhances growth production, improves antioxidant status and immune parameters, and limits the adverse effects of high stocking density stress on common carp fingerlings (18). In another study, Naiel et al., (2020) found that rosemary leaf powder RLF improved growth, feed utilization, antioxidant activity, immune response, and disease resistance in Nile Tilapia (*Oreochromis niloticus*) (19). Also, Hassan et al. (2018) showed that Nile tilapia, *Oreochromis niloticus* (L.) fed a diet supplemented with turmeric, rosemary, and thymus, had significantly higher weight gain, specific growth rates, and protein efficiency ratios (PER) than those fed a control diet (20). Moreover, dietary supplementation with 1 and 3 g/kg rosemary extract significantly enhanced performance (SGR, WG, FCR and PER), biochemical indices (total protein, trypsin, amylase, lipase, superoxide dismutase (SOD), catalase (CAT), lysozyme, total immunoglobulin (IG) and white blood cell (WBC)) levels and immunological parameters (glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol and triglyceride) levels of *Oncorhynchus mykiss* fed control diet (21). Most recently, Dezfoulnejad and Molayemraftar (2021) reported that dietary supplementation of rosemary extract, especially 1% to the diet could improve growth performance, feed utilization, hematological and immunological profiles of *Cyprinus carpio* (22). However, rosemary did not significantly alter the sea bass (*Dicentrarchus labrax*) excretion rate of serum urea, uric acid, creatinine, and ammonia after a 45-day feeding trial (23). In addition, rosemary extract did not change growth and feed intake, but it decreased plasma levels of glucose and triglycerides after four weeks, as well as glucose, HDL/LDL cholesterol ratio and plasma alanine aminotransferase after 12 weeks of administration to gilthead seabream (*Sparus aurata*) (24). Therefore, the aim of this study is aimed to assess the effects of rosemary leaf powder RLP (*Rosmarinus officinalis*) on growth performance, carcass composition, haematological and biochemical profiles in common carp (*Cyprinus carpio*) reared in a cage system facility.

Materials and Methods

Preparation of rosemary leaf powder (RLP)

Fresh rosemary leaves (Rosmarinus *officinalis*) were collected from Pirmam mountain (Erbil, Kurdistan Region-Iraq). The leaves were kept in a plastic bag and transferred to the laboratory. Then, they were cleaned and dried in an oven at 40 °C for 24 h and ground to a powder using the grinder and was sieved using a household sifter and the rosemary leaf powder was stored in a plastic bag at room temperature until use. This research has been approved by the Research Committee of the Salahaddin University-Erbil, Iraq with approval no: /AQ3/1/22/R/012 on 22 January 2022.

Diet formulation

Basal diet and three experimental diets were prepared to supplement 0.5%, 1% and 2% RLP/kg diet. Diets were

Table 1. Formulation and proximate analysis of the experimental diets (dry weight).

Ingredient g kg-1	C	RLP 0.5%	RLP1%	RLP 2%
Soybean[a]	55	55	55	55
Corn[b]	13	13	13	13
Fishmeal[c]	12.8	12.8	12.8	12.8
Soya oil	4	4	4	4
Wheat flour [d]	10	9.5	9	8
Wheat bran [e]	2	2	2	2
Vitamin Premix [f]	1.1	1.1	1.1	1.1
Enzyme	0.1	0.1	0.1	0.1
Mineral premix [g]	2	2	2	2
Rosemary leaf powder [h]	-	0.5	1	2
Proximate composition (%)				
Moisture (%)	4.1	4.3	4.2	4.2
Protein (%)	36.59	36.46	36.1	35.8
Lipid (%)	7.6	7.9	7.9	7.7
Ash (%)	7.9	8.1	7.9	8.3

[a]Soybean obtained from Kosar local Company and originally sourced in BAF in Turkey and consists of (Dry mater =89%, MEn=2230 kcal/kg, protein=44%, crude lipid= 0.8% crude fiber=7%, total phosphorus 0.65). [b] Corn: (Dry mater=92%, MEn=1525 kcal/kg, protein=19.2%, crude lipid= 2.1%, crude fiber=14.4%, total phosphorus %0.65). [c] Fish meal: (Dry matter 90%, protein=65%). [d] Wheat flour: (Dry mater =87%, MEn=2900 kcal/kg, protein=14.1%, crude lipid= 2.5%, crude fiber=3%, total phosphorus %0.37). [e] Wheat bran: (Dry mater =89%, MEn=1300 kcal/kg, protein=15.7%, crude lipid= 3% crude fiber=11%, total phosphorus %1.15). [f] Vitamin Premix sourced in Kosar Company and originally sourced in BAF in Turkey and consists of Vitamin D3 (300000 IU per kg), Vitamin A (2000000 IU per kg), Vitamin K3 (1600 MG per kg), Vitamin E (40000 MG per kg), Vitamin C (150000 MG per kg), Vitamin B6 (2000 MG per kg), Vitamin B2 (3000 MG per kg), Vitamin B1 (2000 MG per kg), Pantothenic acid B5 (20000 MG per kg), Niacin B3 (8000 MG per kg), Folic acid (800 MG per kg), Cholin (45000 MG per kg), Biotin (2000 MG per kg). [g] Mineral premix consists of 1-trace minerals consisting of selenium (60 MG per kg), manganese (3000 MG per kg), Cobalt (20 MG per kg), Iodine (200 MG per kg), Zinc (6000 MG per kg), Copper (30000 MG per kg)2- calcium carbonate 41% 3- salt 1g per kg limestone 14g per kg. [h] Rosemary leaf powder: obtained in Kurdistan Mountains.

formulated isonitrogenous 36% and isolipidic 7%. All ingredients were homogenized and mixed through a commercial food mixer and then blended with oil, and water was added until a solid dough was formed. Diet pellets were extruded Using a cold press extruder (SUNRRY, model: SYMM12, China) with a 2-mm aperture die and then air-dried at room temperature, stored in the plastic pack for each separate diet until use. The ingredients and analyzed composition of the diets are presented in Table 1.

Experimental fish

Juveniles of common carp (*Cyprinus carpio L.*) were obtained from Ankawa hatchery station, Erbil, Kurdistan Region, Iraq. Fish were transported to the aquaculture unit, Grdarasha station, College of Agriculture Engineering Sciences, Salahaddin University- Erbil, Kurdistan Region -Iraq. The fish were stocked into 12 cages (0.2 m^3), Fish were acclimatized to the cage system for 21 days and fed commercial diets (34% protein and 7% lipid). A total of 120 fish were randomly distributed into the cages (ten fish in a cage, with an initial mean weight of 21.11±0.17g). Each diet was fed to a triplicate cage. Juveniles common carp were fed on one of the experimental diets twice a day at a rate of 3% of live body weight six days a week. Fish biomass was weight weekly and the daily feeding of each experimental group was adjusted weekly. During the experiment, water quality indices including temperature, dissolved oxygen and pH were monitored daily and documented as 24.51 ± 1.29 °C, 67.7%-92.2 saturation and 8.95 ± 0.2, respectively.

Growth performance and feed utilization

The growth performance and feed utilization were assessed by total feed intake (TFI), weight gain (WG), specific growth rate (SGR %), feed conversion ratio (FCR), (FCE) feed conversion efficiency, protein efficiency ratio (PER), protein intake and survival rate (SR). Calculations were conducted as follows;

WG (g/fish) =FBW−IBW;

SGR% = [ln FBW−ln IBW]/t×100, where FBW is final body weight (g); IBW is initial body weight (g); ln = natural logarithmic; t = time in days.

FCR = TFI/WG, where TFI is feed intake (g);

FCE = 100 [Live weight gain (g)/Total Feed Intake, (g);

PER =WG / protein intake (g);

SR =%100 (final number of fish) / (initial number of fish).

Proximate composition

Analyses for test diets and fish samples (nine fish per treatment) were performed according to AOAC (2012) standard methods (25). All samples were analysed in triplicate. The level of moisture was determined after drying material at 105 °C with a fan-assisted oven until a constant weight was achieved. Ash level in the samples was measured by incineration in a muffle furnace at 550°C for a period 24h. Crude protein (N×6.25) was performed by the automated Kjeldhal method after acid digestion (Kjeldahltherm microsystem 40, C.Gerhardt GmbH, KG, Germany). Lipid content was determined through a soxhlet gravimetric method using petroleum ether (1356, Parr Instrument Company, IL, and the USA).

Haematological and biochemical analysis

At the end of experiment, nine *C. carpio* from each treatment were euthanised and blood samples were collected. Fish were anaesthetised with buffered tricaine methane sulphate (MS222, Phamaq, Norway) at 200mgL-^{1} followed by the destruction of the brain. Fish were not fed for 24h before sampling. Blood samples were taken from the caudal vein using a 25-gauge heparinized needle and 1-ml syringe (26). The blood samples were divided into two halves, the first half of each sample placed in heparinised 2 vials for haematological analysis. The other halves of the blood samples were placed in clot activator and sun-val then put in ice, then immediately placed and centrifuged at 3,500 rpm for 15 minutes and the supernatant serum was collected and put in labeled in Eppendorf tubes stored at -80 ℃ until for biochemical tests. For haematological analysis RBCs, WBC, LYM, MON, GRA, HGB, MCH, MCHC, MCV, HCT, PLT and MPV were measured using a fully-auto hematology analyzer (MCL-3800 made in China). Biochemical tests such as cholesterol, aspartate aminotransferase (AST), alanine transaminase (ALT), triglyceride, alkaline phosphate (ALP), high-density lipids (HDL) and low-density lipids (LDL) were measured using Cobas c111 in the Alpha Medical laboratory for Disease Diagnosis in Erbil city.

Data analysis

The results of this research were expressed as mean ± standard deviations (SD). The hypothesis of normality and homogeneity for data was confirmed. Growth performance, body composition blood indices and serum biochemical test were subjected to a one-way ANOVA test using the SPSS program (Statistical Package for social science, version 26, IBM Company, 2019) to find whether the examined parameters were affected by RLP levels. Duncan's test was used to determine significant differences at 0.05 levels among the treatments.

Results

Growth performance

The effects of three concentrations of RLP in the common carp (*Cyprinus carpio L.*) diets on growth performance for eight weeks are shown in Table 2. All fish readily accepted the experimental diets during the feeding trial. Common carp fed RLP-supplemented diets obtained significantly higher FBW ($P < 0.05$) than unsupplemented diets. As a consequence, the WG and SGR of fish fed RLP supplemented diet were significantly higher ($P < 0.05$), with fish fed 0.5% RLP recording the highest growth, followed by those fed 2.0%, 1.0%, while those fed 0% RLP (control) had the lowest growth ($P < 0.05$) among dietary groups. This is also indicated in the feed utilization (TFI, FCR, FCE, PER and PI), with the best of those parameters achieved in fish fed 0.5%RLP (FCR, FCE and PER), followed by those fed 2%, 1%, while those fed 0% RLP (control) had the highest FCR and lowest FCE and PER. However, fish fed 0.5% and 2%RLP have significantly increased TFI and PI compared to those fed 1% and 0% RLP dietary groups. Furthermore, no significant difference was obtained in the survival rate among different groups at the end of the trial.

Carcass composition

The effect of RLP supplementation in the *C. carpio* diets on whole-body chemical composition is presented

Table 2. Growth performance and feed utilization of common carp (*Cyprinus carpio*) fed the experimental diets for 8 weeks.

Parameters	C	RLP 0.5%	RLP 1%	RLP 2%
IBW (g)	21.06±0.25	21.22±0.10	21.17±0.17	21.00±0.17
FBW (g)	40.50±1.26[a]	51.28±1.68[c]	44.94±0.69[b]	47.94±1.00[b]
WG (g)	19.44±0.9[a]	30.06±1.67[d]	23.78±0.86[b]	26.06±1.11[c]
SGR %	1.17±0.03[a]	1.57±0.06[d]	1.34±0.04[b]	1.44±0.05[c]
TFI	609.37±25.26[a]	684.63±19.58[b]	633.38±1.72[a]	670.63±8.02[b]
FCR	2.09±0.02[a]	1.52±0.04[b]	1.78±0.07[c]	1.72±0.05[c]
FCE	47.85±0.49[a]	65.82±1.96[c]	56.32±2.17[b]	58.26±1.79[b]
PI	222.97±9.24[a]	249.41±7.13[b]	228.65±0.62[a]	240.08±2.87[b]
PER	1.31±0.01[a]	1.81±0.05[c]	1.56±0.06[b]	1.63±0.05[b]
Survival %	100 ±0.00	100 ±0.00	100±0.00	100±0.00

Data are presented as mean ± SD. Data in the same row with different subscripts are significantly different (P≤0.05).

Table 3. Carcass composition of common carp (Cyprinus carpio) fed the experimental diets for 8 weeks.

Parameters	Initial	C	RLP 0.5%	RLP 1%	RLP 2%
Moisture (%)	78.34±0.09	77.89±0.16[a]	79.27±0.30[a]	80.28±0.39[b]	78.35±1.34[a]
Crude protein (%) *	15.02±0.03	14.46±0.64[a]	13.94±1.00[a]	13.27±0.38[b]	13.42±0.47[a]
Crude lipid (%) *	4.9±0.16	5.05±0.04	4.94±0.06	4.06±0.42	4.03±0.02
Ash (%) *	1.89±0.16	2.03±0.04	1.89±0.13	2.05±0.06	2.51±0.56

Data are presented as mean ± SD. Data in the same row with different subscripts are significantly different (P≤0.05). *Dry matter basis.

in Table 3. The fish-fed diets supplemented with 1% RLP obtained significantly lower ($P<0.05$) crude protein than the other experimental groups. Furthermore, the fish-fed diets supplemented with 1% RLP obtained significantly higher moisture content than the other experimental groups. However, the crude lipid and ash content were not significantly influenced by dietary treatment.

Hematological parameters

Hematological indices (i.e., RBC, WBC, Hb, Hct, MON, LYM, GRA, MCV, MCH, MCHC, PLT and MPV) of common carp fish determined significant effects of dietary treatments Table 4. The highest WBC and MCV levels were observed in fish fed with 0.5% RLP followed by 1% RLP ($p<0.05$). higher level of haemoglobin was obtained in all fish fed RLP supplemented, however, significant differences only achieved in those fish fed with 1% RLP among dietary treatments. Also, RBC, Hct and LYM amounts in the RLP-treated fish had significantly higher levels compared with control fish. On the other hand, the MON, GRA, MCH, MCHC, PLT and MPV values were significantly decreased by different levels of dietary RLP ($p > 0.05$).

Serum biochemical analysis

The findings of the serum biochemical of common carp in different treatments are displayed in Table 5. The total protein, albumin and globulin significantly increased in fish fed RLP supplemented diets compared with the control group ($p < 0.05$). Glucose levels significantly decreased with fish-fed RLP diets compared with the control group ($p < 0.05$). ALT significantly declined with RLP-supplemented diets, however, the fish groups fed the diets supplemented with 1% and 2% RLP exhibited significantly higher levels of ALP than the fish group fed the control diet. The AST was not significantly influenced by the dietary treatment.

Cholesterol, triglyceride and LDL were significantly

Table 4. Hematological parameters in common carp blood fed the experimental diets for 8 weeks (n=9).

Parameters	C	RLP 0.5%	RLP 1%	RLP 2%
WBC ($\times10^9$/L)	98.67±2.15[ab]	103.73±1.40[c]	98.78±2.07[ab]	101.80±1.45[bc]
LYM# ($\times10^9$/L)	72.73±1.16[a]	87.30±1.35[c]	81.53±1.16[b]	87.63±0.75[c]
MON# ($\times10^9$/L)	12.83±0.40[c]	10.20±0.20[b]	12.30±0.40[c]	8.90±0.45[a]
GRA# ($\times10^9$/L)	13.57±0.78[c]	4.27±0.40[a]	6.37±0.40[b]	4.00±0.36[a]
Hb g\Dl	10.33±0.55[a]	10.53±0.40[a]	11.70±0.20[b]	10.63±0.21[a]
MCV fl	210.83±2.40[a]	230.10±2.61[c]	217.27±1.78[b]	213.00±0.1.10[ab]
MCH pg	103.93±2.13[c]	68.27±2.28[a]	70.40±1.97[a]	82.67±1.91[b]
MCHC g\dl	50.27±0.80[c]	30.03±0.1.50[a]	32.27±01.46[a]	39.47±0.80[b]
RBC ($\times10^6$/L)	1.09±0.07[a]	1.50±0.04[c]	1.66±0.05[d]	1.33±0.07[b]
HCT %	22.80±1.05[a]	34.63±0.85[c]	35.63±0.97[c]	27.63±0.64[b]
PLT ($\times10^9$/L)	14.33±1.52[c]	8.33±0.58[a]	10.67±0.58[b]	8.67±0.57[a]
MPV um	6.60±0.26[b]	5.57±0.21[a]	5.63±0.31[a]	5.23±0.15[a]

Data are presented as mean ± SD. Data in the same row with different subscripts are significantly different (P≤0.05).

Table 5. biochemical parameters in the serum of common carp fed the experimental diets (n=9).

Parameters	C	RLP 0.5%	RLP 1%	RLP 2%
Total protein g/dL	6.87±0.65[a]	8.57±0.20[b]	9.56±0.33[c]	9.66±0.36[c]
Albumin	1.30±0.01[a]	1.45±0.05[b]	1.61±0.02[c]	1.63±0.03[c]
Globulin mg/dL	6.23±0.08[a]	7.10±0.17[b]	7.88±0.1[c]	8.02±0.1[c]
Glucose mg/dL	87.33±4.51[a]	59.33±4.04[b]	67.00±4.00[c]	55.33±5.03[b]
AST U/L	61.33±.6.2	56.23±2.58	55.34±3.2	55.13±7.2
ALT U\L	73.33±1.79[a]	66.12±2.57[b]	57.39±2.00[c]	60.11±3.21[c]
ALP U\L	128.33±5.69[b]	125.14±8.19[b]	147.07±9.00[c]	92.00±3.00[a]
Cholesterol mg/dl	138.33±2.51[a]	98.23±7.19[c]	104.17±5.23[c]	119.67±4.53[b]
TG mg\dL	178.33±4.51[a]	156.33±3.06[c]	168.00±4.58[b]	165.33±4.04[b]
HDL mg\dL	28.33±1.53[c]	31.00±1.00[b]	34.00±1.00[a]	34.67±1.53[a]
LDL mg\dl	32.3±2.35[a]	31.43±0.90[a]	30.07±1.70[a]	29.20±0.66[b]
VLDL	30.33±0.88[a]	35.00±1.54[b]	34.33±0.33[b]	33.72±1.17[b]
Urea mg/dL	8.00±1.00[a]	12.33±1.53[b]	13.67±1.53[b]	13.66±1.52[b]
Uric acid mg/dL	0.05±0.004	0.05±0.006	0.06±0.006	0.05±0.005
Creatinine mg/dL	1.24±0.02[a]	1.33±0.01[c]	1.29±0.02[b]	1.29±0.02[b]
Lipase U/L	23.90±2.02[a]	22.63±0.80[a]	51.57±1.25[b]	21.40±0.92[a]
Amylase U/L	75.67±6.10[c]	164.67±6.02[a]	133.67±3.06[b]	62.33±4.51[d]

Data are presented as mean ± SD. Data in the same row with different subscripts are significantly different (P≤0.05).

decreased in fish fed with RLP dietary treatments compared with the fish fed control diet. HDL levels significantly increased in fish fed with diets containing RLP than in the fish fed control group. No significant difference was found among fish groups in the level of Uric acid. Also, results obtained from this study showed a significant increase in the levels of urea and creatinine with supplementation of the RLP. The level of lipase was significantly increased by adding of 2% RLP to the diet of experimental fish. Fish fed with 1% and 2% RLP showed the highest level of amylase compared to fish fed the control diet.

Discussion

Natural plant products have been shown to improve fish performance when taken as a dietary supplement (27). The research study provided evidence that the dietary supplementation of RLP significantly enhanced the fish performance and feed utilization in common carp reared in cage culture. The 0.5 % RLP supplementation significantly enhanced weight gain, SGR, FCR, FCE and PER. This may be due to the fact that it promotes appetite and stimulates the secretion of pancreatic enzymes which are important for digesting and assimilating nutrients (28). In line with the current study, Yousefi *et al.* (2019) obtained that the RLP at 1, 2 and 3% supplementation significantly increased growth performance and feed efficacy in common carp (18). Hassan *et al.* (2018) found that the 1% rosemary dietary significantly increased weight gain, SGR and PER in Nile tilapia (20). Also, Turan and Yiğitarslan (2016) showed that supplementing rosemary extract diets for African catfish (*Clarias gariepinus*) can significantly elevate growth performance and feed utilization (29). In addition, Ayoub *et al.* (2019) showed that diet supplementation of rosemary leaf powder improved growth performance in Nile tilapia fingerlings (30). Likewise, Karataş *et al.*(2022)found that the inclusion of rosemary 1 and 3 g/kg to rainbow trout, *Oncorhynchus mykiss* diet significantly improved feed utilization and growth performance for this salmonid species (21). More recently, Dezfoulnejad and Molayemraftar (2021) reported that a rosemary-supplemented diet exhibited a significantly enhanced final weight, weight gain, specific growth rate and feed conversion ratio of common carp juveniles (22). In contrast to the current study, sea bass (*Dicentrachus labrax*), did not show significant growth or feed utilization effects when rosemary was added at 1% (23). Also, gilthead seabream (*Sparus aurata*) growth was markedly influenced by the diet supplemented with rosemary extract (31). Furthermore, in the work of Yılmaz *et al*. (2013) study, the addition of thyme, rosemary, or fenugreek at a level of 1.0% of the diet did not change the growth performance of *O. mossambicus* fry (32). Several factors could explain the different results obtained in different studies on fish growth and feed efficiency, including the fish species, source, level and form of rosemary, duration of the feeding trial and culture system. In general, groups fed diets supplemented with RLP exhibited numerous positive effects with regard to these measured parameters, which may be due to the presence of active components (carnosol and rosmanol) in this plant. Various phytobiotic compounds have been identified as possessing digestion stimulation characteristics, in addition to exhibiting antimicrobial activity against pathogenic bacteria commonly found in the intestine (15,33).

The findings of our study revealed that dietary administration of RLP significantly decreased the crude protein in the 1% RLP diet compared with the control fish fed. Similarly, Naiel *et al.* (2019) reported that the contaminated diet with aflatoxin and supplemented with 0.1% Rosemary exhibited significantly lower crude protein than the basal diet or contaminated diet groups (34). Also, the moisture content was significantly higher in the 1% RLP-supplemented diet than in the control group. This agrees with the study done by Hassan *et al.* (2018) These authors stated that significant differences in moisture contents of *O. niloticus* occurred when fed with a diet containing rosemary extracts compared with the control group (20). The lipid and ash contents of whole-body composition were not affected by dietary supplementation in *C. carpio.* This result is in line with the results of Yilmaz *et al.* (2019) who also reported no significant difference in the amount of lipid and ash contents of *O. niloticus* fed with a diet containing rosemary extracts compared with the control fish fed (35). Also, Yousefi *et al.* (2019) described that rosemary powder had no significant change in the amount of protein and lipid in *C. carpio* (18).

An indicator of fish health, haematology parameters are mostly reliant on nutritional and non-nutritional factors making them ideal for assessing feed additive effects on fish health in aquaculture (36). Medicinal plants are safe and effective alternatives to antibiotics and chemotherapeutic drugs since they stimulate both specific and non-specific immune systems by producing blood cells and other hematological factors (37). An assessment of RBCs, Hct, Hb and derivative indices such as MCV, MCH, and MCHC can provide valuable evidence on fish erythrocyte status and oxygen-carrying capability in fish (37). The results of the current study observed that RLP supplementation levels significantly increase RBC and Hct levels. Dezfoulnejad and Molayemraftar (2021) reported that dietary rosemary leaf extract administration significantly enhanced RBC, Hct and Hb levels in *C. carpio* (22). Ngugi *et al.* (2017) showed that dietary supplementation of lemon (*Citrus limon*) essential oil significantly increased RBC and Hct levels in Ningu fish *L. victorianus* (38). RLP affected the blood parameters supports the hypothesis that the promoted growth performance is associated to the beneficial effects of rosemary on fish health. There is evidence that rosemary may have positive effects on blood parameters due to its high amounts of vitamins (A, C, and B) and minerals (calcium, potassium, sodium, and iron) (15). Several studies revealed that different can induce herbal plants had increased haematological improvement (27). Consequently, rosemary leaf powder supplementation significantly affected the haematological indices of common carp, according to the results of the current study.

The findings of this study, the amount of WBC was elevated in carp-fed diets containing PLP compared with the control, while the significant increase appeared only in fish fed 0.5% RLP diet. Dezfoulnejad and Molayemraftar (2021) stated that diets supplementing RLP attained significantly higher WBC counts in common carp (22). Consequently, this change in the count of WBC in fish-fed RLP may be associated to bioactive compounds of rosemary such as rosmarinic acid (17,33).

An elevated level of AST, ALT, and ALP enzymes can indicate liver damage, degeneration, necrosis, and destruction as a result of cellular damage. The slight decline in AST approves that 0.5, 1, and 2% of rosemary leaf powder RLP and a significant decrease of ALT was observed in RLP-fed groups compared to the control-fed group suggesting a hepatoprotective effect on the liver of fish. A significant increase of ALP was observed in fish fed 1% RLP, while, a significant decrease was observed in fish fed 2% compared to control fish fed. In other similar investigations, the levels of ALT, AST, and ALP of juvenile beluga *Huso huso* remained unaffected by dietary levels of onion powder except for the AST level of *H. huso* fed 1% dietary onion powder (39). However. Recently, Naeil *et al.* (2020) demonstrated that adding rosemary leaf powder RLP to the diet of Nile tilapia does not affect ALT level, while a significant reduction was observed in fish fed 1% RLP compared to control fish(19). Karataş *et al.* (2020) found that a significant decrease was observed in AST and ALT levels of rainbow trout fed with dietary rosemary extract (21).

Our findings showed that serum levels of total protein, albumin and globulin were markedly enhanced in fish-fed diets containing rosemary leaf powders. The superior levels were also observed at the highest supplementing rosemary leaf powders. Similar to the present results, Yousefi *et al.* (2019) stated that rosemary powder diets enhanced significantly plasma albumin, globulin and total protein concentrations amounts in *C. carpio* (18). Also, Dezfoulnejad and Molayemraftar (2021) reported that serum total protein, albumin and globulin levels in common carp presented an increasing trend with increasing dietary RLP levels (22). Consequently, rosemary leaf powder appears to be capable of improving *C. carpio* adaptive immune responses. In this research, glucose blood levels were considerably suppressed in fish fed different levels of rosemary leaf powder. It is consistent with our findings that Hernández *et al.* (2015) obtained significantly lower glucose levels in gilthead seabream after four weeks of rosemary supplementation, as well as inferior glucose levels after twelve weeks (24). Similarly, Dezfoulnejad and Molayemraftar (2021) showed that diets containing rosemary extract were significantly lowered the serum glucose concentration of *C. carpio* (22). In contrast, Yousefi *et al.* (2019) presented that diets that added rosemary powder significantly increased *C. carpio* serum glucose levels (18). A possible explanation could be the active ingredients found in rosemary have polyglycaemic and antistress effects when administrated orally (9). The results of the current study indicated that using a diet containing rosemary leaf powder reduced cholesterol, triglycerides, and LDL levels in common carp and increased HDL levels. In gilthead seabream, Hernández *et al.* (2015) stated that rosemary extract-supplemented diets resulted in significantly lower triglycerides, and decreased cholesterol after four weeks, as well as a lower HDL/LDL ratio after twelve weeks (24). Also, Karataş *et al.* (2020) reported that 1–3 g/kg doses of rosemary extract displayed a decrease in serum cholesterol and triglyceride of rainbow trout (21). Similarly, Dezfoulnejad and Molayemraftar (2021) confirmed that dietary rosemary leaf powder in diets lowered cholesterol, triglycerides, and LDL status while elevating HDL values in the blood of common carp (22). There is a possibility that these changes are due to rosemary extract's hypolipidemic and hypocholesterolemia effects (40). The result of our study indicated that rosemary leaf powder is capable of modulating health status, including cholesterol, triglycerides, LDL, HDL and glucose levels in *C. carpio*. In line with the obtained results the hypothesis of combination effects of serum biochemical parameters and growth performance in *C. carpio* fed with rosemary supplementation.

Indicators of kidney function urea and creatinine responded significantly to RLP supplementation, while uric acid did not. On the contrary, the present results are not in agreement with the results of Naiel *et al.* (2019) who tested diets contaminated with the addition of aflatoxin with rosemary extract powder in experiments with Nile tilapia (*Oreochromis niloticus*) (34). These authors revealed a significant reduction in urea and creatinine levels in both the control group and those receiving the aflatoxin diets. Also, (23) reported the same trends with sea bass

(*Dicentrarchus labrax*). Similarly, Naiel *et al.* (2020) stated that diets supplemented with different levels of RLP in the diet of Nile tilapia (*Oreochromis niloticus*) yielded no significant effect in plasma urea and creatinine concentrations (19).

This study concludes that dietary supplementation of rosemary leaf powder RLP could positively enhance growth performance, haematological, biochemical and immunological parameters in *C. carpio*. The results showed that dietary administration of rosemary leaf powder, particularly 0.5%, significantly reduced the level of FCR in comparison with control. This result may reduce the cost of feeding for common carp. Optimal levels of RLP can be determined by further studies to determine other contributing factors, such as stress responses to pathogens and environmental factors. The global demand for countermeasures to address antimicrobial resistance and mitigate the use of antibiotics in animal production, including aquaculture warrants further attention. This work on carp presents evidence for the attributes of rosemary leaf powder as a natural feed additive with many attributes for this species. A reduction in chemotherapeutic and antimicrobial medicines for fish using prophylactic nutritional strategies offer sustainable solutions.

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Interest conflict

The author declares that he has no conflict of interest.

Author's contribution

The author did all the work alone.

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