



## Effects of rosemary enriched diet on physiological parameters in common carp *Cyprinus carpio* L. reared in cages culture

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### Abstract

The current study investigated at how rosemary leaf powder supplementation influenced the growth performance, carcass composition, hematological and biochemical parameters, stress hormones, and heat shock proteins of cage-reared common carp. Nine cylindrical cages of 0.2 m<sup>3</sup> (radius = 25 cm, height = 100 cm) were randomly allocated to hold 54 juvenile common carp *C. carpio* (mean weight=18.18±0.08 g). Three groups of fish were fed a control diet (C) as well as diets containing 1.5% and 3% rosemary powder, respectively. The addition of 3% rosemary resulted in a substantial enhancement in growth parameters and feed consumption. The levels of WBC, Hb, MCHC, RBC, and Hct were significantly elevated by the addition of rosemary to experimental fish. A rosemary-supplemented diet resulted in higher levels of TG, urea, total protein, albumin, lipase, amylase, ferrum, and globulin. However, the addition of substantially reduced the levels of ALT cholesterol, creatinine, and glucose. The value of cortisol, T3, and T4 were considerably reduced with the addition of rosemary to diets of experimental fish. HSP70 and HSP90 levels in fish feed diets administered rosemary increased significantly as supplementation amount increased. According to the findings of this investigation, adding rosemary leaf powder, particularly at a concentration of 3%, in the diet improved growth parameters, hematological and biochemical parameters, and be able to reduce stress responses, thyroid hormone levels, and other stressors that fish exposed in cage culture system.

**Keywords:** *Cyprinus carpio*, rosemary (*Rosmarinus officinale*), physiological parameters.

### Introduction

Fish reared in cage culture may be affected by many factors, such as temperature fluctuations and water pollution from air pollution through dust or waste [1]. When fish are raised in cages rather than ponds, most producers increase stocking density [2] Fish under high stocking densities suffer stress, which compromises their immune systems

[3]. If adequate water exchange is not supplied, unused feed and metabolic waste created by cages will cause eutrophication of the site [4]. Fish have been shown to benefit from a variety of medicinal plants and their bioactive ingredients, including growth-promoting, antimicrobial, immunostimulant, antioxidant, and anti-stress characteristics for instance rosemary [5], curcuma longa (*Curcuma longa*) [6,7], *Eruca sativa* [8], and thyme [9].

Rosemary leaf has long been used as a natural medicine plant owing to its anti-inflammatory, antioxidant, immunostimulatory, and antibacterial effects [10]. Fish fed a rosemary-enriched diet had considerably higher growth rates and feed efficiency than those in the control group in Nile tilapia (*Oreochromis niloticus*). Additionally, given an aflatoxin B-contaminated diet had significantly better innate immunity and antioxidant status after supplementing with 0.5% RLP [5]. Rosemary leaf powder as a dietary supplement enhanced the growth, feed consumption, antioxidant activity, immune response, and disease resistance in those fish [11].

The common carp *C. carpio* found in eutrophic rivers and lakes all throughout Europe and Asia [11]. The species is a highly valuable food source for the world's ever-increasing human population due to its fast growth rate, higher feed conversion ratio, increased ability to use carbohydrates and plant protein sources, and relatively high resistance to a variety of environmental conditions and diseases [12].

Dietary supplements, such as medicinal plants and herbs, might be an effective strategy to alleviate stress in cage-reared fish. However, little is known about the impact of dietary addition of rosemary (*Rosmarinus officinalis*) on growth performance, stress hormones, and heat shock proteins in cage-reared fish. Therefore, the present investigation aims to assess the impact of rosemary leaf powder supplementation on growth performance, feed consumption, approximate carcass composition, haematological and biochemical parameters, stress hormones, and heat shock proteins in cage-reared common carp *C. carpio*.

## Materials and Methods

The present investigation was carried out between 2<sup>nd</sup> of April and 28<sup>th</sup> May, 2022. The aquaculture unit (cage system), Grdarasha station, Salahaddin University-Erbil, Kurdistan Region-Iraq, was the site of research project.

**Experimental fish:** Ankawa hatchery station, Erbil, Kurdistan Region, Iraq, provided the experimental fish. The fish were moved to a cage system. Prior to the feeding trial, the fish acclimated to the experimental cage for 14 days. The fish were fed a maintenance diet (36.59% protein and 7.6% lipid) throughout the acclimated stage. The fish were sorted and placed arbitrarily into the test cages ( $n = 6$ ,  $18.18 \pm 0.08$ g). Three times each day, 3% of the fish's body weight in experiment diets was given. Following a 24-hour period without feeds to allow for gut clearing, fish were weighed once a week, and the amount of diet was modified in accordance with the weight.

**Diet formulation:** According to the [13] recommendations for carp's nutritional requirements, experimental diets were produced. With the exception of the inclusion of

1.5 and 3% rosemary leaf powder for the second and third diets, all three experimental diets had the same composition. The experimental diets were designed to be isonitrogenous (36%), as well as isolipidic (7%). Diets were made by first dry combining ingredients then homogenizing them using electrical mixer. Diet pellets were extruded using a 2mm aperture die using a cold press extruder (SUNRRY, model: SYMM12, China). Feeds were then dried for 24 hours at 40°C in a dehumidifying oven. Table 1 shows the experimental diet formulas and approximate composition

### Experimental conditions

Cylindrical cages 0.2 m<sup>3</sup> (radius = 25 cm, height = 100 cm) was used for designing the current experimental cage system. Electrical aerators by Prefix Manufacturing, a submersible water pump manufacturer in Veronella (VR), Italy, provided flow-through aeration.

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

Ingredient g kg <sup>-1</sup>	R <sub>0</sub>	R <sub>1</sub>	R <sub>2</sub>
Soybean	550	550	550
Corn	130	130	130
Fishmeal	100	100	100
Premix	28	27	28
Soya oil	40	40	40
Wheat flour	100	100	100
Wheat bran	20	15	20
Vitamin Premix	11	11	11
Enzyme	1	1	1
Mineral premix	20	20	20
Rosemary	-	1.5	3
<b>Proximate composition %(DM)</b>			
Moisture (%)	4.1±0.7	3.78±0.3	3.62±0.06
Protein (%)	36.59±0.44	35.53±0.79	35.28±1.02
Lipid (%)	7.6±0.02	7.9±0.15	8.4±1.2
Ash (%)	7.9±0.55	6.9±0.95	7.3±0.45

Mineral premix consists of Mono calcium 5g per kg, salt 1g per kg limestone 14g per kg .

\*R<sub>0</sub>=Control diet

\*R<sub>1</sub>= 1.5% Rosemary

\*R<sub>2</sub>= 3% Rosemary

Rosemary leaf powder: obtained in Kurdistan Mountains



## Experimental design

Nine cylindrical cages measuring 0.2 m<sup>3</sup> each held 54 juvenile common carp (mean weight: 18.18±0.08g; 3 cages per group of fish). A completely randomized design (CRD) was utilized to set up the experiment. There were three groups: control diet supplied to fish and diets supplemented with 1.5 and 3% rosemary leaf powder

## Proximate composition

Fish samples and finished test diets were analyzed using the [14] recommended standards. A consistent weight was achieved after the material had been dried at 105°C with a fan-assisted oven, the moisture content was ascertained. Similar to this, the amount of ash in the samples was determined by incinerating them for 16 hours at 550°C in a muffle furnace. Following acid digestion, crude protein (N\*6.25) was determined using the automated Kjeldhal procedure (Kjeldahltherm microsystem 40, C.Gerhardt GmbH, KG, Germany). Using a soxhlet gravimetric method, petroleum ether (1356, Parr Instrument Company, IL and the USA) was employed to measure lipid content.

## Haematological and Biochemical Analysis

Six fish per group were euthanized at the ending of the study, and blood samples were taken. Fish were given a 200mgL<sup>-1</sup> dose of buffered tricaine methane sulphate (MS222, Phamaq, Norway) to anesthetize them before having their brains destroyed. A 25-gauge heparinized needle and a 1-ml syringe were used for drawing blood from the caudal vein [15]. The blood samples were divided into two parts, with the first part of each sample going into two vials that had been heparinized for hematological examination. The other part were put in the clot activator and sun-val, set on ice, and then directly centrifuged at 3,500 rpm for 15 minutes. The supernatant serum was then collected and kept in labeled in eppendorf tubes at -80°C until biochemical testing. A fully automatic hematology analyzer (MCL-3800, made in China) was used to test white blood cell (WBC), lymphocytes (LYM), monocytes (MON), granulocytes GRA, Haemoglobin (HGB), Mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), Red blood cells (RBC), mean corpuscular volume (MCV), hematocrit (HCT), platelet (PLT), and mean platelet volume (MPV). Biochemical tests have been conducted using the Cobas c111, including those for cholesterol, AST, ALT, triglycerides, alkaline phosphatase (ALP), high-density lipids (HDL), and low-density lipids (LDL).

## Stress hormone determinations (ng/ml)

Serum corticosterone (cortisol) levels were determined by ELISA Kit (MBS2700193) for cortisol. Thyroxin (T4) and triiodothyronin (T3) hormones concentration in plasma was determined using ELISA according to the instructions of the kit included in the Cusabio Technology LLC, according to the manufacturer's instruction.

## Data analysis

The data were subjected to statistical analysis one-way ANOVA test using SPSS program (Statistical package for social science, version 26, IBM Company, 2019). All data are presented as mean values  $\pm$  standard error (mean  $\pm$  SE). Duncan test was used to determine significant differences at 0.05 levels among the treatments (Duncan, 1995).

## Results and Discussion

The growth performances and feed utilization measurements (for example, FBW, WG, SGR, FCR, and survival) were significantly ( $P \leq 0.05$ ) greater in the R group, which received a rosemary-supplemented diets, than in the C group, which received a control diet Table 2. This might be because it increases the release of pancreatic enzymes, which are crucial elements in nutritional digestion and absorption [16]. Our results agree with previous studies, Nile tilapia fed diets supplementing with 1% rosemary showed significantly better weight gain and SGR [11]. Furthermore, the addition of rosemary extract to the diets of African catfish (*C. gariepinus*) enhances growth performance and feed intake dramatically [17]. The addition of rosemary leaf powder to the diet enhanced the growth performance of common carp fingerlings [18]. Adding 1 and 3 g/kg rosemary to the diet of rainbow trout increased growth performance markedly [19]. A considerable increase in growth performance was seen in common carp fed diets containing 0.25%, 0.5%, and 1% rosemary leaf extract [20]. In contrast to the current findings, adding 1% rosemary to the diet of sea bass had no obvious effect on the fish's body composition or growth performance [21]. Additionally, the diet enriched with rosemary extract had no influence on gilthead seabream development or feed consumption [22]. The addition of thyme, rosemary, or fenugreek to the diet at 1% did not impact the growth performance of *O. mossambicus* fry [23]. Different fish species, feeding times, rosemary sources, and culture systems could account for this variation.

Adding RLP to common carp diets resulted in no significant ( $P \geq 0.05$ ) effect Table 3. This is consistent with [17] findings, who found that whole-body protein and fat levels in catfish fed diets supplemented with rosemary extract remained constant

To understand the physiological condition of the organs, it is essential to evaluate RBC, Hct, Hb levels, and erythrocyte indices [24]. WBC levels in fish fed 3% RLP were substantially ( $P \leq 0.05$ ) greater than in control groups, although this trend was not significant with inclusion 1.5% Table 4. Lymphocytes, monocytes, and granulocytes did not differ substantially ( $P \geq 0.05$ ) across the three treatments Table 4. The amount of Hb, RBC, and HCT were statically ( $P \leq 0.05$ ) elevated by increasing the inclusion level of RLP compared with control groups of fish Table 4. The level of MCHC was considerably ( $P \leq 0.05$ ) increased by 3% inclusion of rosemary in the diets of experimental fish Table 4. There was no substantial variance ( $P \geq 0.05$ ) among treatments in the levels of MCH, PLT, and MPV in experimental fish. However, the level of MCV showed the opposite trend, which was significantly ( $P \leq 0.05$ ) decreased by the adding of RLP to the experimental fish diet Table 4. Medicinal herbs are beneficial and can be

used instead of antibiotics and chemotherapeutants. Most herbs have the ability to improve both specific and non-specific immune systems by increasing blood cell synthesis and other hematological indices [25]. Erythrocytes and hemoglobin are essential in the transfer of oxygen and carbon dioxide [26]. The majority of fish contain nucleated erythrocytes, which play an essential role in oxygen transport, which is determined by the quantity of hemoglobin in the cell and the gas exchange mechanism. Fish leucocytes, like those of other vertebrates, participate in defensive mechanisms and give protection against infections [26]. In line with the results obtained in the present research, the levels of WBC, RBC, hemoglobin and hematocrit were significantly elevated with the addition of rosemary to the diet of common carp [20].

In aquaculture systems, serum biochemical parameters are key indications of fish health [27, 28]. The improvement of blood parameters, oxidative state, and immune response in farmed fish was observed to strongly correlate with the addition of medicinal plant foods [29, 30]. There was no statistically significant ( $P \geq 0.05$ ) difference in AST, ALP, HDL, LDL, or uric acid levels across fish groups Table 5. The addition of RLP to the common carp's diet considerably ( $P \leq 0.05$ ) lowered the level of ALT Table 5. The inclusion of 3% RLP to the diet of the experimental fish significantly reduced the level of cholesterol. The level of TG was gradually and significantly increased by the addition of RLP Table 5. Dietary addition of rosemary resulted in substantial reduction in ALT and cholesterol levels. Reducing the amount of ALT has been shown to protect the liver of fish [19]. Reducing lipid absorption in the gut, chylomicron, and very-low density lipoprotein secretion, or decreasing intestinal lipoprotein secretion and cellular cholesterol flow in the liver, might result in lower cholesterol and triglyceride levels [31]. In parallel with the current findings, *O. mykiss* given diets containing 1 and 3 g/kg rosemary showed significantly lower levels of ALT, AST, cholesterol, and triglycerides [19]. In the current study, RLP-supplemented diets increased total protein, albumin, and lipase levels, indicating immune system enhancement. Common carp fed with rosemary leaf powder (1.5 and 3 g/kg) showed a rise in digestive enzymes like lipase, which may indicate that the body's digestive enzymes are activated. This is consistent with the studies being conducted, a substantial increase in lipase levels in *O. mykiss* fed diets including 1 and 3 g/kg rosemary was determined [19].

The kidney function indicator urea showed no significant impact in response to RLP supplementation Table 5. This agrees with research by [21], who observed a similar trend in Nile Tilapia. The current study showed significant reduction in creatinine level with dietary supplementation of RLP. Our results supported by [21] who found the same trends with sea bass (*Dicentrarchus labrax*). Urea, uric acid, and creatinine levels are effective indicators of general gill and kidney health in fish [32].

Total protein, albumin, and globulin levels in plasma are all good indicators of fish health [4]. When compared to fish fed a control diet, fish fed RLP had statically greatest amounts of total protein, albumin, lipase, and globulin Table 5. The inclusion of RLP resulted in significantly ( $P \leq 0.05$ ) decreased creatinine levels. The addition of (RLP) causes a substantial ( $P \leq 0.05$ ) and progressive drop in glucose, phosphate, calcium, and

pH values Table 5. The quantity of ferrum was elevated significantly ( $P \leq 0.05$ ) with the addition of RLP Table 5. This is consistent with the results of [11], which showed that supplementing Nile Tilapia diets with RLP significantly increased total protein and albumin levels. Following the same trend, several investigations agree with the findings reported here [33, 34]. The immune-stimulant effects of turmeric, rosemary, or thyme supplementation might explain the increase in globulin levels [11]. The present research showed significant reduction in glucose level with addition rosemary. The finding of current study in glucose level agree with [18] who determined that feeding rosemary leaf powder to common carp fingerlings resulted in a considerable drop in glucose levels.

Heat shock proteins (HSPs) are present in fish, and research has demonstrated their importance for maintaining cellular integrity and responding to stress [35]. Molecular chaperones, or HSPs, are a class of highly conserved proteins that help other proteins fold, assemble, and degrade; fish generate more HSP when they are exposed to numerous stressors, including high temperatures, heavy metal exposure, oxidative stress, and pathogen infection [36]. Fish fed diets contained showed significant ( $P \leq 0.05$ ) increase in the value of HSP70 and HSP90 Table 6. Our results are supported by other previous studies with different fish species and different dietary additions. This is consistent with the findings of [37] who discovered that Huoxiangzhengqi decoction (HXZQD), Xiaochaihu decoction (XCHD), and Yinchenhao decoction (YCHD) were three traditional Chinese medicines (TCMs) that could enhance physiological response, antioxidant capacity, and the expression of two HSPs.

Cortisol levels in the blood are good indications of fish stress [38]. The control group had the highest significant ( $P \leq 0.05$ ) cortisol value, followed by R 1.5%, and R 3% had the lowest value Table 7. Our findings have also been published in studies including various nutritional supplements and a variety of fish species. Adding *Moringa oleifera* aqueous extracts to the diet of Nile tilapia under hypoxic stress caused a significant decrease in blood cortisol level [39]. Dietary supplementation of cineole significantly decreased serum cortisol level in (*O mykiss*) under crowding stress [25]. Thyroxine (T4) and triiodothyronine (T3), the two primary thyroid hormones (THs) secreted by the hypothalamic-pituitary-thyroid (HPT) axis, have a variety of physiological impacts on fish [40]. Thyroid hormones influence growth, development, behavior, and stress [41]. The T3 and T4 levels were considerably ( $P \leq 0.05$ ) reduced in response to a 3% rosemary dietary supplement. This is consistent with the findings of [42], who observed that adding date palm (*Phoenix dactylifera*) to meals significantly lowered T3 and T4 levels in common carp.



**Table (2): Growth performance and feed utilization of fish fed the experimental diets for 8 weeks. (n=3)**

Parameters	Initial	R <sub>0</sub>	R <sub>1</sub>	R <sub>2</sub>
Moisture (%)	78.14±0.	77.88±0.11 <sup>a</sup>	79.29±1.03 <sup>a</sup>	79.93±1.15 <sup>a</sup>
Crude protein	17.57±0.	14.92±0.25 <sup>a</sup>	14.04±0.66 <sup>a</sup>	14.16±0.73 <sup>a</sup>
Crude lipid (%) *	1.81±0.1	1.87±0.07 <sup>a</sup>	1.79±0.24 <sup>a</sup>	2.12±0.4 <sup>a</sup>
Ash (%) *	1.5±0.1	1.5±0.2 <sup>a</sup>	1.7±0.4 <sup>a</sup>	1.85±0.25 <sup>a</sup>

Data are presented as (Mean ± SE).

Data in the same row with different subscript are significantly different ( $P \leq 0.05$ )

**Table (3): Carcass composition of fish fed the experimental diets for 8 weeks (n=3)**

Parameters	R <sub>0</sub>	R <sub>1</sub>	R <sub>2</sub>
IBW (g)	18.20±0.3 <sup>a</sup>	18.26±0.06 <sup>a</sup>	18.07±0.02 <sup>a</sup>
FBW (g)	41.75±0.25 <sup>b</sup>	44.015±0.18 <sup>a</sup>	45.24±0.58 <sup>a</sup>
WG (g)	23.55±0.55 <sup>b</sup>	25.75±0.12 <sup>a</sup>	27.17±0.56 <sup>a</sup>
SGR %	1.48±0.04 <sup>b</sup>	1.9±0.00 <sup>ab</sup>	1.63±0.02 <sup>a</sup>
FCR	2.02±0.13 <sup>a</sup>	2.16±0.29 <sup>a</sup>	1.70±0.11 <sup>b</sup>
Survival %	91.66±0.13 <sup>b</sup>	91.66±0.29 <sup>b</sup>	100±0.00 <sup>a</sup>

Data are presented (Mean ± SE).

Data in the same row with different subscript are significantly different ( $P \leq 0.05$ )

\*Dry matter basis (DM.).



**Table (4): Hematological parameters in fish blood fed the experimental diets for 8 weeks (n=6).**

Parameters	R <sub>0</sub>	R <sub>1</sub>	R <sub>2</sub>
AST U/L	161.33±1.2 <sup>a</sup>	156±4.58 <sup>a</sup>	155±7.2 <sup>a</sup>
ALT U/L	129.66±0.88 <sup>a</sup>	124.66±0.88 <sup>b</sup>	125.33±0.88 <sup>b</sup>
ALP U/L	48.33±2.84 <sup>a</sup>	52.33±1.45 <sup>a</sup>	46.66±0.88 <sup>a</sup>
Cholesterol mg/dl	127±2.64 <sup>a</sup>	122.66±0.88 <sup>a</sup>	110.66±0.88 <sup>b</sup>
TG mg/dL	148±1.15 <sup>c</sup>	173.66±0.88 <sup>b</sup>	185.33±2.07 <sup>a</sup>
HDL mg/dL	24.66±2.84 <sup>a</sup>	30±0.57 <sup>a</sup>	28±3.51 <sup>a</sup>
LDL mg/dl	30.3±0.35 <sup>a</sup>	30.46±0.51 <sup>a</sup>	30.27±0.37 <sup>a</sup>
VLDL	30.33±0.88 <sup>b</sup>	35.00±1.54 <sup>a</sup>	34.33±0.33 <sup>a</sup>
Urea mg/dL	7.66±0.44 <sup>b</sup>	16.16±0.6 <sup>a</sup>	16.66±0.24 <sup>a</sup>
Uric acid mg/dL	0.051±0.004 <sup>a</sup>	0.05±0.0057 <sup>a</sup>	0.055±0.0057 <sup>a</sup>
Total protein g/dL	8.03±0.29 <sup>b</sup>	10.19±0.04 <sup>a</sup>	10.54±0.06 <sup>a</sup>
Albumin	1.30±0.01 <sup>c</sup>	1.60±0.05 <sup>b</sup>	1.8±0.00 <sup>a</sup>
Creatinine mg/dL	0.5±0.008 <sup>c</sup>	0.33±0.008 <sup>b</sup>	0.29±0.008 <sup>a</sup>
Lipase U/L	24.86±0.12 <sup>c</sup>	32.80±0.15 <sup>b</sup>	40.23±0.57 <sup>a</sup>
Amylase U/L	77.00±0.57 <sup>c</sup>	162.33±1.2 <sup>a</sup>	114.33±4.37 <sup>b</sup>
Ferrum ug/dL	83.70±0.52 <sup>c</sup>	126.16±0.18 <sup>b</sup>	142.93±0.81 <sup>a</sup>
Globulin mg/dL	6.23±0.08 <sup>c</sup>	8.40±0.17 <sup>b</sup>	9.30±0.1 <sup>a</sup>
Glucose mg/Dl	83.0±1.15 <sup>a</sup>	60.33±0.88 <sup>b</sup>	46.66±0.88 <sup>c</sup>

Data are presented (Mean ± SE).

Data in the same row with different subscript are significantly different ( $P \leq 0.05$ )

**Table (5): Biochemical parameters in serum of fish fed the experimental diets (n=4)**

Parameters	R <sub>0</sub>	R <sub>1</sub>	R <sub>2</sub>
AST U/L	161.33±1.2 <sup>a</sup>	156±4.58 <sup>a</sup>	155±7.2 <sup>a</sup>
ALT U/L	129.66±0.88 <sup>a</sup>	124.66±0.88 <sup>b</sup>	125.33±0.88 <sup>b</sup>
ALP U/L	48.33±2.84 <sup>a</sup>	52.33±1.45 <sup>a</sup>	46.66±0.88 <sup>a</sup>
Cholesterol mg/dl	127±2.64 <sup>a</sup>	122.66±0.88 <sup>a</sup>	110.66±0.88 <sup>b</sup>
TG mg/dL	148±1.15 <sup>c</sup>	173.66±0.88 <sup>b</sup>	185.33±2.07 <sup>a</sup>
HDL mg/dL	24.66±2.84 <sup>a</sup>	30±0.57 <sup>a</sup>	28±3.51 <sup>a</sup>
LDL mg/dl	30.3±0.35 <sup>a</sup>	30.46±0.51 <sup>a</sup>	30.27±0.37 <sup>a</sup>
VLDL	30.33±0.88 <sup>b</sup>	35.00±1.54 <sup>a</sup>	34.33±0.33 <sup>a</sup>
Urea mg/dL	7.66±0.44 <sup>b</sup>	16.16±0.6 <sup>a</sup>	16.66±0.24 <sup>a</sup>
Uric acid mg/dL	0.051±0.004 <sup>a</sup>	0.05±0.0057 <sup>a</sup>	0.055±0.0057 <sup>a</sup>
Total protein g/dL	8.03±0.29 <sup>b</sup>	10.19±0.04 <sup>a</sup>	10.54±0.06 <sup>a</sup>
Albumin	1.30±0.01 <sup>c</sup>	1.60±0.05 <sup>b</sup>	1.8±0.00 <sup>a</sup>
Creatinine mg/dL	0.5±0.008 <sup>c</sup>	0.33±0.008 <sup>b</sup>	0.29±0.008 <sup>a</sup>
Lipase U/L	24.86±0.12 <sup>c</sup>	32.80±0.15 <sup>b</sup>	40.23±0.57 <sup>a</sup>
Amylase U/L	77.00±0.57 <sup>c</sup>	162.33±1.2 <sup>a</sup>	114.33±4.37 <sup>b</sup>
Ferrum ug/dL	83.70±0.52 <sup>c</sup>	126.16±0.18 <sup>b</sup>	142.93±0.81 <sup>a</sup>
Globulin mg/dL	6.23±0.08 <sup>c</sup>	8.40±0.17 <sup>b</sup>	9.30±0.1 <sup>a</sup>
Glucose mg/Dl	83.0±1.15 <sup>a</sup>	60.33±0.88 <sup>b</sup>	46.66±0.88 <sup>c</sup>

Data are presented (Mean ± SE).

Data in the same row with different subscript are significantly different ( $P \leq 0.05$ )

**Table (6): Heat shock proteins (HSP70 and HSP90) of common carp fed the experimental diets**

Parameters	R <sub>0</sub>	R <sub>1</sub>	R <sub>2</sub>
HSP70	6.33±0.12 <sup>c</sup>	6.80±0.05 <sup>b</sup>	7.36±0.13 <sup>a</sup>
HSP90	1.70±0.05 <sup>c</sup>	2±0.5 <sup>b</sup>	2.36±0.08 <sup>a</sup>

Data are presented (Mean ± SE).

Data in the same row with different subscript are significantly different ( $P \leq 0.05$ )

**Table (7): Serum stress hormones in fish fed the experimental diets for 8 weeks. (n=3).**

Parameters	R <sub>0</sub>	R <sub>1</sub>	R <sub>2</sub>
Cortisol	56.15±0.54 <sup>a</sup>	54.60±0.25 <sup>a</sup>	52.03±0.52 <sup>b</sup>
T3	1.36±0.07 <sup>a</sup>	1.01±0.01 <sup>b</sup>	0.96±0.008 <sup>b</sup>
T4	0.92±0.01 <sup>a</sup>	0.87±0.008 <sup>b</sup>	0.80±0.005 <sup>c</sup>

Data are presented (Mean ± SE).

Data in the same row with different subscript are significantly different ( $P \leq 0.05$ )

Cortisol ng/ml, T3: triiodothyronine (ng mL<sup>-1</sup>); T4: thyroxine (ng mL<sup>-1</sup>)



The current study concludes by demonstrating how rosemary administration diets can enhance innate immune responses and feed intake, leading to enhanced growth performance. This study also shown the effectiveness of rosemary feed additives as a growth stimulant for common carp, and fish farmers should be advised to increase their use of these feed additives in fish diets. The findings of the current study demonstrate that common carp benefit from dietary rosemary supplementation. Additionally, dietary rosemary supplementation has the ability to reduce stress hormone decline, oxidative stress, and stress reactions that fish under cage culture conditions experienced. Faster fish development would result in shorter production times; nevertheless, more study with several significant aquaculture species and production methods is needed to properly assess the benefits of using rosemary leaf powder at industrial farming levels.

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