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EFFECTS OF DIETARY SUPPLEMENTATION OF SAGE (SALVIA OFFICINALIS) ON PHYSIOLOGICAL PERFORMANCE IN JUVENILE COMMON CARP (CYPRINUS CARPIO)

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Abstract

The present study investigated the effects of dietary supplemented with sage leaf powder on growth performance, feed utilization, carcass composition, blood parameters and stress hormones in common carp *Cyprinus carpio* reared in cages in Grdarasha station, College of Agriculture Engineering Sciences, Salahaddin University-Erbil, Kurdistan Region -Iraq. A total of 54 common carp (25.5±0.5g fish^{-1}) were randomly distributed into 9 cylindrical cages 0.2 m³ (Radius=25cm, Height=100cm). After two weeks of adaptation, eight weeks feeding trial was conducted from 17 September to 12 November 2022. There were four groups of fish fed control diet (T1) and fish fed diets supplemented with 0.5 (T2), 1 (T3), 1.5(T4) of sage powder. The results showed a significant increase in growth parameters and feed utilization with addition of sage powder. The level of LYM, MON, Hb, MCHC, RBC and Hct were significantly elevated by the addition of sage to experimental fish. In contrast, dietary supplementation of sage (T2, T3, T4) to common carp diet result in significant reduction in the level of WBC, GRA and MCV. The level of TG, total protein, ALP, albumin, lipase, HDL and globulin contents was increased using sage (*Salvia officinalis*) supplemented diet. However, level of AST, ALT cholesterol, LDL and glucose was significantly decreased with the addition of sage to the diets. The present results showed that dietary supplementation of sage led to a significant decrease in cortisol, T3, and T4 and significant increase acetylcholinesterase (AChE). In conclusion, sage can be used as feed additives enhanced growth, feed efficacy, blood indices and reduce stress.

Keywords: Sage (Salvia officinalis), Cyprinus carpio, Growth performance, Haematological parameters, Biochemical parameters, Stress hormones.

تأثيرات المكملات الغذائية للميرمية (SALVIA OFFICINALIS) على الأداء الفسيولوجي في أسماك الكارب الشائع (CYPRINUS CARPIO)

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الخلاصة

تناولت الدراسة الحالية تأثير التغذية المكملة بمسحوق أوراق المريمية على أداء النمو، الاستفادة من العلف، تكوين الذبيحة، مؤشرات الدم وهرمونات الإجهاد في أسماك الكارب الشائع *Cyprinus carpio* المرباة في الأقفاص في محطة غردارشا، كلية العلوم الهندسية الزراعية، جامعة صلاح الدين – أربيل، اقليم كوردستان – العراق. تم توزيع 54 سمكة من الكارب الشائع (25.5±0.5 غم سمكة$^{-1}$) عشوائيًا على 9 أقفاص أسطوانية سعة 0.2 م^3 (نصف القطر = 25 سم، الارتفاع = 100 سم). بعد أسبوعين من التكيف، تم إجراء تجربة تغذية لمدة ثمانية أسابيع في الفترة من 17 سبتمبر إلى 12 نوفمبر 2022. كانت هناك أربع مجموعات من نظام غذائي متحكم يتغذى على الأسماك (T1) وأنظمة غذائية تتغذى على الأسماك مكملة بـ 0.5 (T2)، (T3) 1، (T4) 1.5 من مسحوق الميرمية. أظهرت النتائج زيادة معنوية في مؤشرات النمو والاستفادة من العلف مع إضافة مسحوق الميرمية. تم رفع مستوى LYM وMON وHb وMCHC وRBC وHct بشكل ملحوظ عن طريق إضافة المريمية إلى الأسماك التجريبية .في المقابل، فإن إضافة المريمية (T2، T3، T4) إلى غذاء الكارب الشائع يؤدي إلى انخفاض كبير في مستوى WBC، GRA و MCV. تم زيادة مستوى TG، البروتين الكلي، ALP، الألبومين، الليباز، HDL والجلوبيولين باستخدام النظام الغذائي المكمل للمريمية (Salvia officinalis). ومع ذلك، انخفض مستوى كوليسترول AST وALT وLDL والجلوكوز بشكل ملحوظ مع إضافة الميرمية إلى النظام الغذائي. أظهرت النتائج الحالية أن المكملات الغذائية للمريمية أدت إلى انخفاض كبير في الكورتيزول وT3 وT4 وزيادة كبيرة في إنزيم الأسيتيل كولينستريز .(AChE) في الختام، يمكن استخدام الميرمية كإضافات غذائية لتعزيز النمو وفعالية التغذية ومؤشرات الدم وتقليل الإجهاد.

كلمات مفتاحية: المريمية (Salvia officinalis)، Cyprinus carpio، أداء النمو، العوامل الدموية، العوامل البيوكيميائية، هرمونات التوتر.

Introduction

Aquaculture importantly participates in global protein supply and suppresses the catch pressure on natural resources of aquatic organisms. Sustainable aquaculture activity directly correlates to fish farm profitability, which could be augmented by increasing growth rate (31). Modern aquaculture addresses two primary concerns: the reduction of water used for culture; and increasing production output per unit. To meet market demands, farmers may increase stock densities, which can pose stress to fish cultures in intensive operations (16).

Fish reared in cage culture may be affected by many factors such as temperature fluctuation, water pollution by air pollution through dust or wastes, when fish grown in cages instead of ponds, most farmers optimal for a high stocking density. A high stocking density creates a stressful environment for the fish and stress damages the immune system. If proper water exchange is not there, the uneaten feed and metabolic waste released from cages will lead to eutrophication of the site (14).

Medicinal herbs are becoming more popular than ever before as far as the possible adverse effects of synthetic drugs are concerned (29).

The common sage, *Salvia officinalis* is a plant endemic in Mediterranean countries with great medical importance (9)

Common carp (*Cyprinus carpio*) is widely distributed in eutrophic freshwater and is one of the most important cultured species in the cyprinid family, following grass carp, (*Ctenopharyngodon idellus*), and silver carp. This species also is the fourth most important cultivated species in the aquaculture industry and is mainly produced in many Asian and some European countries (2). The species is a highly valuable food source for the ever-growing human population with desirable aquaculture capabilities including a high growth rate, better feed conversion ratio, the higher capability of using carbohydrates and plant protein sources, along with relatively high resistance to variable environmental conditions, and diseases (30).

Dietary supplementation sage, *Salvia officinalis* could be one effective way to reduce negative effects of the stress that fish faced when reared in cage system. Salvia officinalis L. (common sage) is an aromatic perennial evergreen subshrub. It belongs to the Lamiaceae family and is native to the Mediterranean region. Since ancient times, it has been used in folk medicines for the treatment of all kinds of diseases and in a variety of food preparations. Today, it is cultivated in several countries mainly to obtain the dried leaves to be used as raw material in medicine, perfumery, food industry (33). However, limited information is available about the evaluation of the effects of dietary supplementation of sage, *Salvia officinalis* on growth performances, haematological and biochemical, oxidative stress and stress hormones of fish reared in cage culture. Therefore, the present study aims to evaluate the effects of dietary supplemented with on common sage, *Salvia officinalis*, growth performance, feed utilization, haematological and biochemical, oxidative stress, and stress hormones in common carp (*Cyprinus carpio*), reared in cage culture system.

Materials and Methods

Experimental fish: Common carp (*Cyprinus carpio L.*) was obtained from Ankawa hatchery station, Erbil, Kurdistan Region, Iraq. Fish were transported to the aquaculture unit (cage system), Grdarasha station, College of Agriculture Engineering Sciences, Salahaddin University- Erbil, Kurdistan Region -Iraq. Fish acclimated to the cage system for 14 days before the feeding trial. During that time, fish were fed on a maintenance diet (34% protein and 7 % lipid). Fish were graded and randomly distributed into the cages (n=6, 25.86±0.27). Experimental diets were given three times daily at a ration level of ~3% of the fish body weight. Fish were weighed weekly after feeds were withheld for 24h to allow gut clearance, and the amount of diet was adjusted accordingly to the weight.

Diet formulation: Experimental diets were formulated to the (23) guidelines on the nutritional requirements for carp. Four experimental diets were formulated with the same composition with the addition of 0, 0.5, 1 and 1.5 % leave sage powder for the second, third and fourth diet respectively. The diets were formulated to be isonitrogenous 35% and isolipidic 7%. Diets were manufactured by initially dry mixing ingredients before homogenising through a commercial food mixer. Diet pellets were extruded through a cold press extruder (SUNRRY, model: SYMM12, China) using a 2mm aperture die. Feeds were subsequently dried in a dehumidifying oven for 24h at 40°C. Test diet formulations and proximate composition are presented in Table 1.

Experimental conditions: Cylindrical iron cages 0.2 m³ (Radius=25cm, Height=100cm) was used for designing the current cage system project for fish treatment. Flow-through aeration was provided by electrical aerator (Prefix manufacturing, Submersible water pump, Veronella (VR), Italy).

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

Diet Ingredient and Proximate composition	Control Diet (C)	0.5% S	1% S	1.5% S
Diet Ingredient (g kg^{-1})				
Soybean[a]	530	530	530	530
Corn[b]	150	150	150	150
Fishmeal[c]	100	100	100	100
Premix	25	25	25	25
Soya oil	50	50	50	50
Wheat flour	100	100	100	100
Wheat bran[e]	15	15	15	15
Vitamin Premix[f]	11	11	11	11
Enzyme	1	1	1	1
Mineral premix[g]	20	20	20	20
Sage	0	5	10	15
Diet Proximate composition (%)				
Moisture	7.79±0.42	7.4±0.42	7.05±0.42	7.8±0.42
Protein	35.41 ± 2.6	35.62 ± 2.6	35.64 ± 1.9	35.7 ± 2.3
Lipid	6.6±0.45	6.9±0.5	6.5±0.08	6.52±0.12
Ash	6.6±0.30	6.05±0.26	6.15±0.44	6.25±0.42

[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 mater 90%, protein=65%).

[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 consist 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.

C= Control diet, 0.5% S = Diet supplemented 0.5 % of sage, 1% S = Diet supplemented 1 % of sage, 1.5% S Diet supplemented 1.5 % of sage.

Experimental design: A total of 54 juvenile common carp (mean weight= 25.5±0.5g) divided into 9 Cylindrical cages 0.2 m³ (6 fish per cage, 3 cages per group of fish). The experiment was arranged in Completely Ramdomized Design (CRD). There were four groups of fish fed control diet and fish fed diets supplemented 0, 0.5, 1 and 1.5 % sage powder.

Proximate composition: Finished test diets and fish samples were analysed according to (4) standard methods. All samples were analysed in triplicate. The moisture content was determined after drying material at105°C with a fan assisted oven until a constant weight was achieved. Similarly, ash level in the samples was measured by incineration in amuffle furnace at550°C for period 16h. 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).

Fish Growth Performances, Feed Utilization, and Mortality

To calculate the growth parameters, the total fish biomass in every tank was individually weighed at the beginning and end of the trial. The data obtained from each group were used to calculate growth performances, feed utilization, and mortality rates. Growth and feed utilization calculations: The growth performance of the fish and feed utilization were measured according to the formulae; Specific growth rate (SGR %) = (In FBW-In IBW)/ T×100; Feed Conversion Ratio (FCR g) = (Feed intake (g)) / (weight gain (g); Survival Rate (%) = 100 –(Mortality (%) =Final Nb /Initial Nb×100).

Haematological and Biochemical Analysis: At the end of the trial, fish were euthanised and blood collected from 6 fish per treatment. Fish were anaesthetised with buffered tricaine methane sulphate (MS222, Phamaq,Norway) at 200 mgL^{-1} followed by the destruction of the brain. Blood was collected from the caudal vein using a 25-gauge heparinized needle and 1-ml syringe (6). The blood samples 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 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 collected and put in labeled ineppendrof tubes stored at -80 ℃ until for biochemical tests. For haematological analysis 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 (MVC), hematocrit (HCT), platelet (PLT) and mean platelet volume (MPV) were measured using 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

Acetylcholinesterase (AchE): Acetylcholinesterase (AchE) activity was determined by the method of (10). 100 µL supernatant was added to a test tube (1.5 mL) containing 880 µL of phosphate buffer (0.1M, pH 7.5), 10 µL of 100 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), and 10 µL of 0.1M acetylthiocholine chloride. Then, the contents were mixed and the absorbance was read spectrophotometry for 1 min using a StatFax 100 spectrophotometer, USA. Three samples were assayed for each tissue. Enzyme activity was reported as millimoles of product formed per mg protein per minute.

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.

Statistical analysis: All data are stated as mean values ± standard error (±SE). Statistical analyses were performed by SPSS statistics version 26 for windows (SPSS Inc., an IBM company, copyright 1989-2019). One-way ANOVA was used to analyze data. To determine where significant differences occurred at the 95% confidence level (associated probability ≤ 0.05), Duncan's multiple ranging ad-hoc LSD test was applied.

Results and Discussion

Growth performances: Table 2 illustrates shows growth performances, feed utilization, and survival of experimented fish. Overall, the growth performance was improved by the addition of sage to common carp diet. The common carp's FW, WG, SGR and survival were significantly ($P \leq 0.05$) increased in the groups that received diets that supplemented by diffrent level of sage compared with fish received control diet. In contrast, the level FCR was significantly ($p \leq 0.05$) reduced by inclusion sage to experimental diets.

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

Parameters	Initial	C	0.5% S	1% S	1.5% S
Moisture (%)	78.39±0.56	77.65±0.23[b]	79.29±0.59[ab]	79.65±0.71[a]	79.98±0.58[a]
Crude protein (%)*	17.44±0.42	14.58±0.21[ab]	13.99±0.38[b]	14.71±0.14[ab]	15.04±0.19[a]
Crude lipid (%) *	1.80±0.08	1.86±0.04[a]	1.78±0.14[a]	2.04±0.24[a]	2.09±0.21[a]
Ash (%) *	1.50±0.05	1.50±0.11[a]	1.73±0.23[a]	1.80±0.15[a]	1.73±0.08[a]

Data are presented as mean ± S.E.
Data in the same row with different subscript are significantly different (P≤0.05).

Carcass composition: According to the body composition results presented in table 3. The levels of moisture and protein were significantly (*P≤0.05*) increased by the addition of different levels of sage to experimental diets. While no significant (*P≥0.05*) difference was found among treatments in the lipid and ash contents.

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

Parameters	C	0.5% S	1% S	1.5% S
IBW (g)	25.88±0.14[a]	25.77±0.29[a]	25.77±0.05[a]	25.99±0.09[a]
FBW (g)	55.83±0.91[c]	57.61±0.11[b]	58.60±0.14[ab]	59.66±0.25[a]
WG (g)	29.94±1.05[b]	31.83±0.38[ab]	32.83±0.16[a]	33.66±0.25[a]
SGR %	1.36±0.03[b]	1.41±0.03[ab]	1.46±0.005[a]	1.48±0.01[a]
FCR	1.92±0.11[a]	1.79±0.02[ab]	1.77±0.006[ab]	1.71±0.02[b]
Survival %	94.44±5.55[a]	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]

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

Hematological parameters: The results of haematological parameters of common carp *Cyprinus carpio* after feeding trail are shown in table 4. Overall, the hamatological prameters was improved by the addition of sage to common carp diet. The level of LYM, MON,Hb, MCHC, RBC and Hct were significantly elevated by the addition of sage to experimental fish. Incontrast, dietary supplementation of sage to common carp resulted in significant reduction in the level of WBC, GRA and MCV. In terms of MCH level, no significant differnce was seen.

Table 4 Hematological parameters in common carp blood fed the experimental diets for 8 weeks (n=6).

Parameters	C	0.5% S	1% S	1.5% S
WBC ($\times 10^9$/L)	96.23±0.44[a]	63.66±0.77[b]	63.00±0.15[b]	62.33±0.63[b]
LYM# ($\times 10^9$/L)	66.41±0.67[c]	71.04±0.27[b]	73.17±0.65[a]	74.17±0.50[a]
MON# ($\times 10^9$/L)	7.50±0.20[a]	4.80±0.08[b]	4.37±0.11[bc]	4.09±0.10[c]
GRA# ($\times 10^9$/L)	21.81±0.36[a]	19.71±0.39[b]	18.71±0.39[bc]	18.05±0.06[c]
Hb g\dL	6.42±0.06[c]	7.38±0.10[b]	7.66±0.12[b]	8.23±0.17[a]
MCH pg	49.76±0.63[a]	49.66±0.29[a]	50.40±0.11[a]	50.70±0.11[a]
MCHC g\dl	21.71±0.34[d]	22.80±0.15[c]	23.80±0.15[b]	24.80±0.15[a]
RBC ($\times 10^6$/L)	2.05±0.11[c]	2.52±0.21[bc]	2.90±0.05[b]	3.50±0.17[a]
MCV fL	202.16±4.17[a]	192.16±4.17[b]	186.83±0.99[b]	183.16±1.28[b]
HCT %	32.13±0.28[c]	33.13±0.28[b]	34.16±0.26[a]	34.83±0.06[a]
MPV fL	8.23±0.08[c]	8.56±0.06[b]	8.73±0.08[ab]	8.90±0.11[a]

Data are presented as mean ± S.E.
Data in the same row with different subscript are significantly different (P≤0.05).

Serum biochemical analysis: The results of biochemical parameters in serum of common carp fed the experimental diets were shown in table 5. The lowest significant ($P \leq 0.05$) AST value was reported in the group of 1.5% S , followed by 0.5% S and 1% S while the higest value was reported by control diet. The ALT, cholesterol, LDL and glucose levels were significantly ($P \leq 0.05$) decreased, progressively, in response to dietary supplemntation of sage to experimental fish. In contrast, the level of ALP, TG, HDL, total protein, albumin, lipase, amylase and globulin were significantly ($P \leq 0.05$) increased in response to dietary supplemntation of sage to experimental fish.

Table 5 biochemical parameters in serum of common carp fed the experimental diets (n=4).

Parameters	C	0.5% S	1% S	1.5% S
AST U/L	161.33±1.20^{a}	152.66±1.45^{b}	153.00±1.15^{b}	150.66±0.88^{b}
ALT U\L	129.66±0.88^{a}	124.66±0.88^{b}	125.33±0.88^{b}	123.66±0.88^{b}
ALP U\L	47.33±1.20^{b}	52.33±1.45^{a}	46.66±0.88^{b}	46.00±1.52^{b}
Cholesterol mg/dl	127.0±2.64^{a}	122.66±0.88^{a}	110.66±0.88^{b}	108.00±0.57^{b}
TG mg\dL	149.33±0.66^{c}	170.33±0.88^{b}	180.66±0.88^{a}	182.66±1.20^{a}
HDL mg\dL	25.66±1.85^{c}	31.00±0.57^{b}	34.66±0.33^{ab}	36.33±1.76^{a}
LDL mg\dl	30.30±0.35^{a}	29.50±0.23^{ab}	28.63±0.58^{bc}	27.80±0.26^{c}
Total protein g/dL	8.36±0.13^{c}	9.19±0.04^{b}	10.54±0.06^{a}	10.87±0.27^{a}
Albumin	1.35±0.02^{c}	1.60±0.02^{b}	1.75±0.02^{a}	1.82±0.01^{a}
Lipase U/L	25.53±0.21^{c}	32.90±0.17^{b}	38.90±0.35^{a}	39.56±0.31^{a}
Amylase U/L	87.00±0.57^{d}	135.66±2.18^{c}	142.66±0.88^{b}	151.00±1.15^{a}
Globulin mg/dL	1.23±0.08^{d}	1.90±0.11^{c}	2.30±0.100^{b}	2.76±0.08^{a}
Glucose mg/dL	84.66±0.88^{a}	73.66±2.72^{b}	66.66±0.88^{c}	58.00±0.57^{d}

Data are presented as mean ± S.E.
Data in the same row with different subscript are significantly different ($P \leq 0.05$).

Stress Hormones: The level of stress hormones in the serum of common carp fed the experimental diets is presented in Table 6. The levels of cortisol, T3, and T4 were significantly ($P \leq 0.05$) decreased in response to dietary supplementation of sage to experimental fish. Nevertheless, the level of AChE was significantly ($P \leq 0.05$) increased in response to dietary supplementation of sage to experimental fish.

Table 6 Serum hormones in common carp (*Cyprinus carpio*) fed the experimental diets for 8 weeks. (n=3).

Parameters	Control	0.5% S	1% S	1.5% S
(AChE) 10^{-3}	2.05±0.02^{c}	2.47±0.08^{b}	2.80±0.05^{a}	2.93±0.08^{a}
Cortisol	76.39±0.40^{a}	75.70±0.26^{ab}	74.86±0.20^{bc}	74.06±0.29^{c}
T3	0.58±0.00^{a}	0.48±0.01^{b}	0.48±0.04^{b}	0.40±0.00^{c}
T4	0.91±0.005^{a}	0.86±0.008^{b}	0.84±0.005^{bc}	0.82±0.011^{c}

AChE: acetylcholinesterase (10─3); T3: triiodothyronine (ng mL─1); T4: thyroxine (ng mL─1).
Data were represented as means ± SD. Different letters in each column indicate significant differences ($P < 0.05$).

Discussion: The current study demonstrated that the dietary inclusion three levels of sage significantly improved the production performance and feed utilization in common carp reared in cage culture. It is possible that this may be due to promote appetite, stimulates the secretion of pancreatic enzymes, important factors in nutrient digestion and assimilation (13). Various studies reported that the use of herbal plants, due to the extensive presence of bioactive compounds includes (alkaloids, carbohydrate, fatty acids, glycosidic derivatives (e.g., cardiac glycosides, flavonoid glycosides, saponins), phenolic compounds (e.g., coumarins, flavonoids, tannins), poly acetylenes, steroids, terpenes/terpenoids (e.g., monoterpenoids, diterpenoids, triterpenoids, sesquiterpenoids), and waxes), improves growth performance and feed utilization besides enhanced appetite and changed the composition, diversity, and/or activity of the population of beneficial bacteria in the gut microbiota while inhibiting pathogenic bacteria in aquatic species (17). These bioactive compounds may produce bile and stimulate the secretion of digestive enzymes by the pancreas, which in turn causes the digestion of nutrients (7). Also, it has been suggested that herbal bioactive extracts affect gut morphology and its structure which may improve the digestion and absorption of nutrients (34). In line with the present study, (22) reported that, dietary supplementation with 1% sage has a superior effect on growth performance of koi carp fingerlings. Similar studies (26) also reported that fish fed a diet supplemented sage (*Salvia officinalis*) displayed high significance in the growth performance of juvenile gilthead sea bream (Sparus aurata). Most recently, (18) reported that dietary incorporation of sage (2 – 4 g/ kg diet) enhanced growth, feed efficacy.

Body composition is a good indicator of the physiological condition of a fish but it is relatively time consuming to measure (20). The current study demonstrated that the dietary inclusion three levels of sage significantly increase the moisture and did significantly change protein, lipid and ash contents. The findings are consistent with previous studies. (18) did not find any significant difference with dietary addition of sage to European sea bass (Dicentrarchus labrax) diet. On the other hand, significant alterations in fish body composition were reported with medicinal plant supplements, e.g., 1) garlic (*Allium sativum*) in Asian Sea bass (Lates calcarifer) (1) and rainbow trout (*Oncorhynchus mykiss*) (3); curcumin in Nile tilapia (*Oreochromis niloticus*) (12); moringa (*Moringa oleifera* Lam.) in gibel carp (Carassius auratus *gibelio* var. CAS III) (35); turmeric, rosemary and thyme in Nile tilapia (*Oreochromis niloticus*) (15). Variations in the results of the research might be ascribed to fish-related variables (sex, species, size, and physiological condition), habitat or environment (water characteristics), management system, and nutrition (32).

Blood parameters in common carp bass fed on experimental diets showed normal ranges for healthy fish. The findings of current study indicated that the sage is a positive dietary additive to improve hematological parameters.

Furthermore, fish fed on a diet containing sage showed higher RBCs, Hb, MCHC, HCT and MPV. Increasing in RBC, Hct and Hb facilitates tissue oxygenation and elimination of carbon dioxide (31). Increase in MCV and MCHC in the fish fed diet containing 200 mg kg^{-1} marjoram indicates a possible hematopoietic effect of the sage, as new and young RBC are larger in size and contain higher amount of Hb (8). In our study, Htc and Hb levels for common carp fed supplemented diets showed significant differences compared to the control group. This suggests that herbs can improve the performance of the oxygen transport and promote a better tissue perfusion (25).

The current findings is coinciding with (18) who found a significant increase in RBC and Hb with dietary supplementation of sage to European sea bass (*Dicentrarchus* *labrax*). Smilarly, (31) reported that dietary supplementations of sage significantly increase RBC, Hb, Hct, MCHC, MCH and MPV in common carp, *Cyprinus carpio*. Furthermore, (9) found that dietary addition of sage significantly increase RBC, Hb, Hct, MCHC, MCH and MCV in Beluga, Huso huso. In contrast, dietary supplementation of sage to common carp resulted in significant reduction in the level of WBC, GRA and MCV. This is disagreeing with (9, 18 and 31).

The level of AST, ALT cholesterol, LDL and glucose was significantly decreased with the addition of sage to common carp diets. This is agree with (9) who found significant decrease in AST, ALT with the addition of addition of age (*Salvia officinalis*) Beluga, Huso huso. In contrast, (18) found a significant increase AST, ALT with dietary supplementation of sage to European sea bass (*Dicentrarchus labrax*)

In the present study, the total protein, ALP, albumin, lipase and globulin contents was increased using sage (*Salvia officinalis*) supplemented diets, indicating the improvement of immune system. This is agree with (9) who found the same trend with the addition of age (*Salvia officinalis*) Beluga, Huso huso. Similarly, (18) who found a significant increase in total protein, ALP, albumin, lipase and globulin with dietary supplementation of sage to European sea bass (*Dicentrarchus labrax*)

Cortisol is among the most frequently measured indicators of the fish stress response (5), increased plasma cortisol indicates significant physiological stress, with no signs of acclimation. In the present study, cortisol in serum level was significantly decreased in fish fed diets supplementd by sage compared to the control. These results agree with (21) who reported that dietary supplementation of cineole significantly decreased serum cortisol levels in (*Oncorhynchus mykiss*) under crowding stress. Similarly, (27) pointed out that dietary supplementation of *Moringa oleifera* aqueous extracts to the diets of Nile tilapia (*Oreochromis niloticus*) exposed to hypoxic stress led to a considerable diminish in serum cortisol levels. The present results also showed that dietary supplementation of sage led to a significant decrease in T3, and T4, which have also been previously reported in other studies with different dietary addition and fish species.

Acetylcholinesterase (AChE) is the primary enzyme responsible for hydrolytic metabolism, and a key biomarker of neurotoxicity in fishes (19). Acetylcholine is synthesized in nervous tissue by choline acetylase and degraded further by cholinesterase (28). In the present study, the serum AChE level was significantly increased with the addition of sage to common carp diet. These results agree with (9) who found significant decrease in AChE with the addition of addition of age (*Salvia officinalis*) Beluga, *Huso huso*. Similarly, (28) who reported that the dietary supplementation of synbiotics significantly increased the liver and gill acetylcholinesterase (AChE) level in the Indian major carps Rohu (*Labeo rohita*) juveniles exposed to low pH stress. Conversely, (24) reported that the dietary supplementation of oregano extract (*Origanum vulgare L.*) significantly decreased the level of AChE in the liver of rainbow trout (*Oncorhynchus mykiss*) exposed to the organophosphorus pesticide, diazinon.

Conclusions: In conclusion, the dietary inclusion of sage (*Salvia officinalis)* improved the growth, feed efficacy, non-specific immune responses, blood indices and decrease stress hormones. Therefore, this study suggests that utilized 1.5% herbs can be used as beneficial feed additive to improve growth performance and health status (immune system) of common carp reared in cage system.

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Reference

1. Abdelwahab, A. M., El-Bahr, S. M., and Al-Khamees, S. (2020). Influence of dietary garlic (Allium sativum) and/or ascorbic acid on performance, feed utilization, body composition and hemato-biochemical parameters of juvenile Asian Sea Bass (Lates calcarifer). Animals, 10(12): 2396.
2. Action, S. I. (2020). World Fisheries and Aquaculture. Food and Agriculture Organization, 2020: 1-244.
3. Adineh, H., Harsij, M., Jafaryan, H., and Asadi, M. (2020). The effects of microencapsulated garlic (Allium sativum) extract on growth performance, body composition, immune response and antioxidant status of rainbow trout (Oncorhynchus mykiss) juveniles. Journal of Applied Animal Research, 48(1): 372-378.
4. Association of Official Analytical Chemists, and Association of Official Agricultural Chemists (US). (1925). Official methods of analysis of the Association of Official Analytical Chemists. The Association, 2.
5. Baker, M. R., Gobush, K. S., and Vynne, C. H. (2013). Review of factors influencing stress hormones in fish and wildlife. Journal for Nature Conservation, 21(5): 309-318.
6. Campbell, T. W., and Grant, K. R. (2022). Exotic animal hematology and cytology. John Wiley and Sons.
7. Chelemal Dezfoulnejad, M., and Molayemraftar, T. (2022). Use of dietary rosemary (Rosmarinus officinalis L.) extract as a growth promotor and immunostimulant in common carp. Aquaculture Research, 53(4): 1553-1562.
8. Clauss, T. M., Dove, A. D., and Arnold, J. E. (2008). Hematologic disorders of fish. Veterinary clinics of North America: Exotic animal practice, 11(3): 445-462.
9. Dadras, H., Hayatbakhsh, M. R., and Golpour, A. (2019). Dietary administration of common sage (Salvia officinalis) and coneflower (Echinacea angustifolia) extracts affects growth, blood parameters and immune responses of Beluga, Huso huso. Turkish Journal of Fisheries and Aquatic Sciences, 20(5): 367-374.
10. Dembélé, K., Haubruge, E., and Gaspar, C. (2000). Concentration effects of selected insecticides on brain acetylcholinesterase in the common carp (Cyprinus carpio L.). Ecotoxicology and Environmental Safety, 45(1): 49-54.

11. Duncan, D. B. (1955). Multiple range and multiple F tests. biometrics, 11(1): 1-42.
12. Elgendy, M. Y., Shaalan, M., Abdelsalam, M., Eissa, A. E., El-Adawy, M. M., and Seida, A. A. (2022). Antibacterial activity of silver nanoparticles against antibiotic-resistant Aeromonas veronii infections in Nile tilapia, Oreochromis niloticus (L.), in vitro and in vivo assay. Aquaculture Research, 53(3): 901-920.
13. Frankič, T., Voljč, M., Salobir, J., and Rezar, V. (2009). Use of herbs and spices and their extracts in animal nutrition. Acta agriculturae Slovenica, 94(2): 95-102.
14. Gopakumar, G., 2009. History of cage culture, cage culture operations, advantages and disadvantages of cages and current global status of cage farming. In: Course manual: National training on cage culture of seabass. Central Marine Fisheries Research Institute and National Fisheries Development Board, Kochi, 8-12.
15. Hassan, A. A. M., Yacout, M. H., Khalel, M. S., Hafsa, S. H. A., Ibrahim, M. A. R., Mocuta, D. N., ... and Dediu, L. (2018). Effects of some herbal plant supplements on growth performance and the immune response in Nile tilapia (Oreochromis niloticus). Sciendo, 1: 134-141.
16. Hoseinifar, S. H., Sun, Y. Z., Zhou, Z., Van Doan, H., Davies, S. J., and Harikrishnan, R. (2020). Boosting immune function and disease bio-control through environment-friendly and sustainable approaches in finfish aquaculture: herbal therapy scenarios. Reviews in Fisheries Science and Aquaculture, 28(3): 303-321.
17. Hosseini, S. M., Hoseinifar, S. H., Mazandarani, M., Paknejad, H., Van Doan, H., and El-Haroun, E. R. (2020). The potential benefits of orange peels derived pectin on serum and skin mucus immune parameters, antioxidant defence and growth performance in common carp (Cyprinus carpio). Fish & shellfish immunology, 103: 17-22.
18. Hussein, E. E., El Basuini, M. F., Ashry, A. M., Habiba, M. M., Teiba, I. I., El-Rayes, T. K., ... and Dawood, M. A. (2023). Effect of dietary sage (Salvia officinalis L.) on the growth performance, feed efficacy, blood indices, non-specific immunity, and intestinal microbiota of European sea bass (Dicentrarchus labrax). Aquaculture Reports, 28: 101460.
19. Kumar, A., Vasmatkar, P., Baral, P., Agarawal, S., and Mishra, A. (2017). Immunomodulatory and growth promoting effect of dietary fenugreek seeds in fingerlings of common carp (Cyprinuscarpio Lin.). Fishery Technology, 54: 170-175.
20. Mahmoud, R., Aziza, A., Marghani, B., and Eltaysh, R. (2019). Influence of ginger and garlic supplementation on growth performance, whole body composition and oxidative stress in the muscles of Nile tilapia (O. niloticus). Advances in Animal and Veterinary Sciences, 7(5): 397-404.
21. Mirghaed, A. T., Hoseini, S. M., and Ghelichpour, M. (2018). Effects of dietary 1, 8-cineole supplementation on physiological, immunological and antioxidant responses to crowding stress in rainbow trout (Oncorhynchus mykiss). Fish and Shellfish Immunology, 81: 182-188.

22. Nica, A., Petrea, M. S., Mogodan, A., Simionov, I. A., and Cristea, V. (2021). Effects of dietary vitamin e and sage (Salvia officinalis L.) on growth performance of koi carp (Cyprinus Carpio L., 1758). Scientific Papers. Series D. Animal Science, 64(2): 465-470.
23. National Research Council. (2011). Nutrients requirement of fish and shrimp.
24. Rafieepour, A., Hajirezaee, S., and Rahimi, R. (2019). Dietary oregano extract (Origanum vulgare L.) enhances the antioxidant defence in rainbow trout, Oncorhynchus mykiss against toxicity induced by organophosphorus pesticide, diazinon. Toxin Reviews. 39(4): 397-407.
25. Rummer, J. L., and Brauner, C. J. (2015). Root effect haemoglobins in fish may greatly enhance general oxygen delivery relative to other vertebrates. PloS one, 10(10): 0139477.
26. Salomón, R., Firmino, J. P., Reyes-López, F. E., Andree, K. B., González-Silvera, D., Esteban, M. A., ... and Gisbert, E. (2020). The growth promoting and immunomodulatory effects of a medicinal plant leaf extract obtained from Salvia officinalis and Lippia citriodora in gilthead seabream (Sparus aurata). Aquaculture, 524: 735291.
27. Shourbela, R. M., El-Hawarry, W. N., AM, A. E. L., and Abo-Kora, S. Y. (2020). Potentiality of Moringa oleifera aqueous extract as a growth modulator and antistress in acute hypoxic Nile tilapia Oreochromis niloticus. Iranian Journal of Fisheries Sciences, 19(1): 67-84.
28. Singh, S. K., Tiwari, V. K., Chadha, N. K., Munilkumar, S., Prakash, C., and Pawar, N. A. (2019). Effect of dietary synbiotic supplementation on growth, immune and physiological status of Labeo rohita juveniles exposed to low pH stress. Fish and Shellfish Immunology, 91: 358-368.
29. Sonmez, A., Bilen, S., Albayrak, M., Yılmaz, S., Biswas, G., Hisar, O., and Yanik, T. (2015). Effects of dietary supplementation of herbal oils containing 1, 8-cineole, carvacrol or pulegone on growth performance, survival, fatty acid composition, and liver and kidney histology of rainbow trout (Oncorhynchus mykiss) fingerlings 1, 8 Kinol, karvakrol ya da pulegon içeren bitkisel yağ yem katkılarının alabalık yavrularında (Oncorhynchus Mykiss) büyüme performansı, yaşama, yağ asidi kompozisyonu ile karaciğer ve böbrek histolojisi Üzerine etkileri. Turkish Journal of Fisheries and Aquatic Sciences, 15: 813-819.
30. Tasa, H., Imani, A., Moghanlou, K. S., Nazdar, N., and Moradi-Ozarlou, M. (2020). Aflatoxicosis in fingerling common carp (Cyprinus carpio) and protective effect of rosemary and thyme powder: Growth performance and digestive status. Aquaculture, 527: 735437.
31. Yousefi, M., Ghafarifarsani, H., Hoseinifar, S. H., Rashidian, G., and Van Doan, H. (2021). 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 and Shellfish Immunology, 108: 127-133.
32. Zeilab Sendijani, R., Abedian Kenari, A., Smiley, A. H., and Esmaeili, N. (2020). The effect of extract from dill Anethum graveolens on the growth

performance, body composition, immune system, and antioxidant system of rainbow trout. North American Journal of Aquaculture, 82(2): 119-131.

33. Zervoudakis, G., Salahas, G., Kaspiris, G., and Konstantopoulou, E. (2012). Influence of light intensity on growth and physiological characteristics of common sage (Salvia officinalis L.). Brazilian archives of biology and technology, 55: 89-95.
34. Zeynali, M., Nafisi Bahabadi, M., Morshedi, V., Ghasemi, A., and Torfi Mozanzadeh, M. (2020). Replacement of dietary fishmeal with Sargassum ilicifolium meal on growth, innate immunity and immune gene mRNA transcript abundance in Lates calcarifer juveniles. Aquaculture nutrition, 26(5): 1657-1668.
35. Zhang, P., Yang, F., Hu, J., Han, D., Liu, H., Jin, J., ... and Xie, S. (2020). Optimal form of yeast cell wall promotes growth, immunity and disease resistance in gibel carp (Carassius auratus gibelio). Aquaculture Reports, 18: 100465.