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Effect of Marjoram *Origanum majorana* L. Extract on Growth Performance and Some Haematological Parameters of Common Carp *Cyprinus carpio* L.

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Abstract: The current study examines the effects of different levels of marjoram *Origanum majorana* extract (0, 0.5, 1.0, and 1.5 %) on growth performance and haematological parameters in diets of *common carp Cyprinus carpio*. Four units of the Recirculating Aquaculture System (RAS) were used. Each unit had three plastic tanks (30×30×60 cm), representing four treatments with three replicates, each tank containing ten fish with an average weight of 16.31 ± 0.02g - 16.48 ± 0.10. Fingerlings were fed at a rate of 3% of the total fish weight. All diets contained an average of 33.89 ± 0.067 % crude protein and about 20.32±0.16 (kJ/g) of gross energy .Fish are fed twice daily, six days a week. The study lasted eight weeks (56 days) during November and December 2021. According to our findings, a group of fish fed 1.5% marjoram extract had the highest rate of final body weight, daily weight increase, relative growth rate, specific growth rate, food conversion ratio, and feed conversion efficiency. The 1.5 % marjoram extract treatment had the highest rate of total protein 5.68± 0.84 g/dl, albumin 4.48± 0.48 g/dl, globulin 1.17± 0.54g/dl and 3.82 ± 0.51% albumin/ globulin. Based on the results, it can be concluded that the use of 1.5 % *O. majorana* extract was best for growth, and blood profile. The results suggest that the inclusion of *O. majorana* can improve the nutrient efficiency, growth performance, and haematological parameter of *C. carpio* fingerlings without negative effects on the fish.

Keywords: Cultured fish, Growth performance, Haematological parameters, *Origanum majorana*.

Introduction

The common carp, *Cyprinus carpio* L., is an important species for freshwater aquaculture in Iraq (Mohammad, 2021). Enhancing the growth performance and disease resistance of farmed fish presents substantial issues for fish farmers (Nicolae *et al*., 2016). In particular, food supplements that improve the fish's physiological and behavioral abilities are frequently required for intensive common carp production. Due to active principles of

substances such as alkaloids, flavonoids pigments, phenolics, terpenoids, steroids, and essential oils, medicinal plants have various activities such as antistress, growth promotion, hunger stimulation, immunostimulant, aphrodisiac, and antibacterial capabilities (Prerna & Neeru, 2014). These compounds are now more common in the diets of finfish and shellfish aquaculture because a large number of their derivatives are used as growth and immunostimulant agents (Tripathy *et al*., 2017).

The use of medicinal plants (phytochemicals) for various reasons, such as sex reversal compounds (Gholipour *et al*., 2014), growth enhancers (Zaki *et al*., 2012), immune stimulant, and antipathogenic (Yilmaz *et al*., 2015), has increased dramatically in recent decades.

One of the relatively new methods for enhancing the health of aquatic farmed species is the use of medicinal plants as immune stimulators or growth promoters (Citarasu, 2010). The study aimed to use marjoram *O. majorana* extract to enhance the growth and immunity of common carp fish.

Materials & Methods

Preparation of aqueous extracts of *O. majorana*

The leaves of *O. majorana* were acquired from a local market in Basrah, Iraq, and properly washed under running tap water to eliminate dust particles. The leaves were sun-dried for five days in a clean tray (0900 am and 1400 pm). 50 g of powdered *O. majorana* leaves were weighed into one-liter Ellen Meyer flasks, 250 ml of deionized water was added, and the mixture was then placed in a vibrating incubator at 35 °C for 24 hours to make the leaf aqueous extract. The solution was filtered through Whatman filter paper (No. 1), the residue was removed, and the filtrate was stored at -20 ºC until it was used.

Diet preparation

Three isonitrogenous and isocaloric experimental rations (diets 2, 3, and 4) were made with varying levels of *O. majorana* (0.5, 1.0, and 1.5 %, respectively). *O. majorana* was not included in the control diet (diet 1). The ingredients for the feed were ground and milled into pellets of 3 mm diameter. Pellets were packed in polyethylene bags, sealed airtight, labeled after drying, and then placed in the freezer until ready to feed (Table 1).

Rearing system

Inside the laboratory, four recycling aquaculture systems (RAS) units were used. Each unit had three plastic tanks (30 × 30 × 60 cm) arranged in two rows on an iron holder, and each unit contained a glass tank (30 × 40 × 90 cm) for water filtration. To increase the oxygen level in each tank, electric air pumps were installed, and heating equipment was used to maintain the desired temperature. The study lasted eight weeks (56 days) during November and December 2021 at the fish laboratories of the Department of Vertebrates, Marine Science Centre and Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah. Common carp weighing an average of 16.395±0.068 g, has been used. Twelve plastic tanks measuring 30 × 40 × 60 cm were placed with 120 fish, three replications of each treatment, and 10 fish per tank. Before the feeding trial, the fish were acclimated for 14 days.

Water quality measurement

Water temperature (°C), dissolved oxygen ($mg.L^{-1}$), pH, and salinity (PSU) of water in the aquaculture system (RAS) were measured using a water quality multimeter (Taiwanese origin). The experimental plastic tanks were filled with de-chlorinated tap water (60L) and the water was replaced 50% every week to keep a healthy environment with enough oxygen.

Table (1): Ingredients and proximate composition (%) of experimental diets containing varying levels of *O.majorana* leave extract.

Ingredients	Control (Diet 1)	Diet 2	Diet 3	Diet 4
Fish meal (60 % protein)	32	32	32	32
Soybean meal (44% protein)	22	22	22	22
Wheat brain	30	29.5	29	28.5
Yellow corn	15	15	15	15
Vitamin premix[1]	0.5	0.5	0.5	0.5
Mineral's premix[2]	0.5	0.5	0.5	0.5
O. majorana leaves extract	0	0.5	1	1.5
Proximate composition %				
Moisture	4.2±0.18^{a}	3.8±0.21^{ab}	3.6±0.24^{b}	3.5±0.26^{b}
Crude Protein	33.98±0.054^{a}	33.92±0.057^{a}	33.86±0.59^{a}	33.80±0.06^{a}
Ether Extract	14.1±2.52^{a}	13.6±2.87^{a}	13.3±3.12^{a}	13.3±3.43^{a}
Ash	1.7±0.12^{a}	1.6±0.15^{a}	1.2±0.18^{a}	1.5±0.16^{a}
NFE[3]	41.02±0.63^{a}	40.91±0.71^{a}	40.88±0.87^{a}	41.10±0.92^{b}
Fibre	5±0.32^{a}	5.17±0.30^{ab}	5.16±0.29^{ab}	5.8±0.24^{b}
GE ($kJ.g^{-1}$) *	20.56±11.15^{a}	20.33±1.26^{a}	20.19±1.39^{a}	20.19±1.52^{a}

Different letters in the same rows indicate significantly different at $P< 0.05$.

*According to Hassan *et al*. (2018): Protein 23.6 kJ. g^{-1}, lipid, 39.5 $kJ.g^{-1}$, and NFE 17.0 kJ. g^{-1}

[1]Vitamin premix (per kg of premix): thiamine, 2.5 g; riboflavin, 2.5 g; pyridoxine, 2.0 g; inositol, 100.0 g; biotin, 0.3 g; pantothenic acid, 100.0 g; folic acid, 0.75 g; para-aminobenzoic acid, 2.5 g; choline, 200.0 g; nicotinic acid, 10.0 g; cyanocobalamine, 0.005 g; atocopherol acetate, 20.1 g; menadione, 2.0 g; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU.

[2]Mineral premix (g/kg of premix): CaHPO4.2H2O, 727.2; MgCO4.7H2O, 127.5; KCl 50.0; NaCl, 60.0; FeC6H5O7. 3H2O, 25.0; ZnCO3, 5.5; MnCl2.4H2O, 2.5; Cu (OAc) 2. H2O, 0.785; CoCl3.6H2O, 0.477; CaIO3. 6H2O, 0.295; CrCl3.6H2O, 0.128; AlCl3.6H2O, 0.54; Na2SeO3, 0.03.

[3]Nitrogen-Free Extract (calculated by difference) = 100 – (protein + lipid + ash + fibre).

Growth parameters

The experiment lasted for 56 days during November and December 2021. At the end of the experimental period, the following growth and feed utilization parameters were calculated (Hepher, 1988): weight gain (WG), relative growth rate (RGR), specific growth rate (SGR), feed conversion ratio (FCR), food conversion efficiency (FCE), and survival rate.

Weight gain

$$WG = W2\ (g)\ -\ W1\ (g)$$

Relative Growth Rate

$$RGR = [(W2\ (g)\ -\ W1\ (g))/W1]\ \times 100$$

Specific growth rate

$$SGR = \ (ln\ W2\ (g)\ -\ ln\ W1\ (g)\ /(t2 - t1\ )\ \times 100$$

Where lnW2 is the natural logarithm of the final weight at the time T2, lnW1 is the natural logarithm of the initial weight at the time T1 and T2-T1 is the period between the two weights.

Feed utilization

Feed Conversion Ratio

$$FCR = \ R\ (g)/\ WG\ (g)$$

Feed Conversion Efficiency:

$$FCE = \ WG\ (g)\ /\ R\ (g) \times 100$$

Where R: weight of dry feed intake. WG: wet weight gain (live weight of fish).

Feed intake

$$Feed\ intake\ (FI)\ =\ 100\ \times\ total\ feed\ intake\ /[feeding\ days \times (W1 + W2)/2]$$

Survival rate

$$\text{Survival rate (\%)} = \frac{No.of\ fish\ alive}{Total\ No.of\ fish\ stocked} \times 100$$

Blood collecting

Four fish individual per each treatment were used for blood analysis, and 2.5ml blood samples were collected by cardiac puncture using 5ml disposable syringes into treated Bijou bottles from each treatment. Before analysis, the blood was kept at -20 º C. Total protein ($g.dl^{-1}$), albumin ($g.dl^{-1}$), globulin ($g.dl^{-1}$), and albumin/globulin ratio (A/G ratio) are all measured. The blood analysis was performed using the method described by Velisek *et al*. (2009).

Statistical analysis

The data were analyzed using one-way ANOVA. The Least Significant Differences (LSD) were used to test for mean differences at a 0.5 significant level. The SPSS program version 26 was used for all statistical analyses.

Results

Table (2), shows the physical and chemical properties of the water used in the experimental tanks of various treatments, such as temperature, pH, dissolved oxygen, and salinity. The temperature of water in the rearing system ranged between 24.5-25.09 ºC, the dissolved oxygen concentrations 6.59-7.07 $mg.L^{-1}$, the pH 7.45, and the salinity between 3.03 - 3.06 PSU. Growth performance (final weight, weight gain, relative growth rate, and specific growth rate) improved significantly at ($P<0.05$) with the experimented diets (Table 3). In comparison to the control diet, the diet with 1.5 % *O. majorana* extract produced the highest weight gain (3.67 $\pm$0.07g), Relative growth rate (22.54$\pm$ 1.64%), specific growth rate (0.39$\pm$ 0.02 % day). The results also showed that a group of fish fed with a concentration of 1.5 % marjoram extract had

the best nutritional indicators. Fish fed a 1.5% *O. majorana* extract diet had a better food conversion ratio (1.48 ± 0.41) and higher food conversion efficiency (67.41 ±0.87%), Feed intake ranged between 4.41±0.155g (diet 4), and 4.45±0.145g (diet 1).

Table (2): The physical and chemical properties of the water used in experimental tanks of common carp fish fed different levels of *O. majorana* extract.

Treatments (*O. majorana* %)	Temperature (ºC)	Dissolved Oxygen (mg. l^{-1})	pH	Salinity (PSU)
Diet 1 (control 0 %)	24.5±0.87	7.07±0.06	7.45±0.05	3.03±0.01
Diet 2(0.5%)	25.09±0.46	6.73±0.08	7.46±0.08	3.06±0.01
Diet 3(1%)	24± 0.38	6.59±0.28	7.45±0.07	3.06±0.04
Diet4 (1.5%)	25.45±0.48	6.6±0.15	7.45±0.07	3.04±0.06

Table (3): Growth performance (Mean ± SD) of common carp fed diets containing different levels of *O. majorana* extract.

Item	Diet 1 (0.0%)	Diet 2 (0.5%)	Diet 3 (1.0%)	Diet 4 (1.5%)
Initial weight (g)	16.44 ± 0.13^{a}	16.35 ± 0.11^{a}	16.48 ± 0.10^{a}	16.31 ± 0.02^{a}
Final weight (g)	18.31 ± 0.16^{c}	18.35 ± 0.08^{c}	18.91 ± 0.06^{b}	19.98± 0.29^{a}
Weight Gain (g)	1.86 ± 0.13^{c}	2.0 ± 0.10^{c}	2.43 ± 0.05b	3.67 ± 0.07^{a}
Relative growth rate (%)	11.35 ± 0.84^{c}	12.23 ± 0.69^{c}	14.76 ± 0.41^{b}	22.54± 1.64^{a}
Specific growth rate SGR (% day)	0.21 ± 0.01^{c}	0.22 ± 0.01^{c}	0.27 ± 0.00^{b}	0.39± 0.02^{a}
Feed Intake (g)	4.45±0.145^{a}	4.44±0.095^{a}	4.43± 0.08^{a}	4.41±0.155^{a}
FCR	2.78 +0.91^{b}	2.60 ±0.71^{b}	2.18 +0.29^{ab}	1.48 + 0.41^{a}
FCE %	35.78 ±0.52^{c}	38.42 ± 0.45^{c}	45.77 +0.25^{b}	67.41 ± 0.87^{a}
Survival rates %	100^{a}	100^{a}	100^{a}	100^{a}

Different letters in the same rows indicate significantly different at P< 0.05

Haematological parameters are presented in table (4). Total protein, albumin, and globulin levels increased significantly (P<0.05) with increasing *O. majorana* extract levels in the experimental diet, reaching the highest value of 5.68± 0.84 g.dl^{-1}, 4.48 ±0.48 g.dl^{-1}, and 1.17± 0.5g/dl respectively at a group of fish fed 1.5%. The results show significant differences (P<0.05) in the albumin/globulin (A/G) ratio between control and other treatments, while

there were no significant differences at (P>0.05) in the albumin/globulin (A/G) ratio reported between fish fed (0. 5, 1, and 1.5 %) of *O. majorana* extract (Table 4). According to the results, dietary supplementation with 0.5, 1, and 1.5 % *O. majorana* extract significantly increased total protein, albumin, and globulin in diet 2 and diet 4 compared to the control (diet 1). All levels of dietary *O. majorana* extract significantly increased albumin to globulin ratio (A/G ratio), compared to the control treatment.

Table (4): Haematological parameters (protein ($g.dl^{-1}$), albumin ($g.dl^{-1}$), globulin ($g.dl^{-1}$), and A/G ratio) in the blood of common carp fish fed diets containing different levels of *O. majorana* extract.

Treatments	Total Protein $g.dl^{-1}$	Albumin $g.dl^{-1}$	Globulin $g.dl^{-1}$	A/G ratio
Control (Diet 1)	3.57 ± 0.42^{b}	2.64 ± 0.21^{c}	1.09 ± 0.15^{a}	2.42 ± 1.8^{b}
0.5 % (Diet2)	3.59 ± 0.35^{b}	3.59 ± 0.35^{b}	1.11 ± 0.25^{a}	$3.23 + 0.30^{a}$
1.0 % (Diet 3)	4.01 ± 0.13^{b}	3.26 ± 0.11^{b}	1.16 ± 0.48^{a}	2.81 ± 0.29^{a}
1.5 % (Diet 4)	5.68 ± 0.84^{a}	4.48 ± 0.48^{a}	1.17 ± 0.54^{a}	3.82 ± 0.51^{a}

Different letters in the same column indicate significantly different at P< 0.05.

Discussion

The occurrence of fish diseases and the importance of immunostimulants in their protection are currently the subjects of increased public awareness to prevent potential human infection and reduce economic losses brought on by fish diseases (Smith *et al*., 2003). Water quality and diet are complementary, interdependent aspects and any damage can affect a fish's physiological and immune health and increase its susceptibility to disease (Staykov *et al*., 2007; Imran *et al*., 2019). In the present study, the fish fed with an *O. majorana* extract diet at the rate of 1.5 % showed a significant increase ($p < 0.05$) in weight gain (WG), relative growth rate (RGR%) specific growth rate (SGR% day), and a significant decrease in FCR ($P < 0.05$). Medicinal plants or their extracts are used to stimulate growth and improve the physiological and reproductive parameters of fish (Lee *et al*., 2012). The improved WG and FCR in common carp may be due to improved metabolic activities and utilization of nutrients (Li *et al*., 2006) and improvement in the function of the intestinal flora (Weisburger & Chung, 2002). These findings may be due to the activity of marjoram compounds and phytochemicals that promote general health. The results were in agreement with the study of Fath El-Bab *et al*. (2018**)** in which, phytogenic perform their initial activity in feeding as a flavor and thereby influence eating patterns, the secretion of digestive fluids, and total feed intake. All treatments in the current study resulted in a 100 % survival rate. This could indicate that *O. majorana* extract enhances fish growth, feed utilization, and immune function. Improved feed intake and

nutrient digestibility may account for improved fish growth and feeding efficiency when supplemented with *O. majorana*. Furthermore, *Origanum majorana* extract contains a variety of nutrients, including essential oils, vitamins, and minerals, which may aid in the promotion of fish growth. (Citarasu, 2010, Awad & Awaad, 2017; Begnami *et al.,* 2018; Rudiansyah *et al*., 2022). The present results are consistent with those of Abdel-Tawwab *et al.* (2018), who reported that adding cinnamon to the diets of common carp and Nile tilapia fish *Oreochromis niloticus*, respectively, improved nutrient utilization due to the high activity of digestive enzymes that contribute to food metabolism and inhibit pathogenic organisms in the digestive system, increasing the numbers of beneficial microorganism. This enhances feed digestion and nutrient absorption. The content of essential oil and extracts of *Origanum* species containing antimicrobial, antioxidant, and other biological activities may be responsible for the improvement in body weight gain, RGR %, SGR, and survival rate (Milos *et al*., 2000; Aligiannis *et al*., 2001). Essential food components found in medicinal plants include carbohydrates, protein, and fat. These components are essential for the fish body's requirements and are used in a variety of physiological, metabolic, and morphological activities, which influences the increasddse in body content in fish-fed *O.majorana* extract.

This study established the efficiency of *O.majorana* extract at 1.5 % of feed which support growth, survival rate, and haematological parameters. It is essential to recognize how to balance the ratio of nutrients to anti-nutrients to have a favorable effect on the body without affecting the bioavailability of nutrients as *O. majorana* extract contains both high amounts of nutrients and anti-nutrients. It appears that a 1.5 % of *O. majorana* extract added to the diet of common carp will improve the fish's ability to utilize feed as well as their development and hematological parameters.

Conclusion

This study established the efficiency of *O.majorana* extract at 1.5 % of feed which support growth, survival rate, and haematological indices. *O.majorana* extract includes both high levels of nutrients and anti-nutrients, therefore it's critical to understand how to balance the ratio of nutrients to anti-nutrients to have a positive impact on the body without compromising the bioavailability of nutrients. It implies that adding *O. majorana* extract to the diet of common carp at a concentration of 1.5 % will enhance the fish's ability to utilize feed, as well as their development and haematological parameters.

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Contributions of authors

A.H.K: Methodology, collection the data, edit the manuscript.

A.T.Y: Constructed the idea and hypothesis for research; planned the methodology, analysed the data, wrote the manuscript.

K.S.A.: Constructed the idea and hypothesis for research; planned the methodology, analysed the data, review the manuscript.

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Conflict of interest

The authors declared that they have no conflict of interest.

Ethical approval

All applicable national and international guidelines for the care and use of animals were followed.

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تأثير مستخلص نبات البردقوش *Origanum majorana* على معايير النمو وبعض مقاييس الدم لأسماك الكارب الشائع *Cyprinus carpio* L.

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المستخلص: تهدف الدراسة الحالية لمعرفة تأثير اضافة مستخلص نبات البردقوش *Origanum majorana* بتراكيز مختلفة (0.0، 0.5 ، 1.0 ، 1.5 %) في غذاء أسماك الكارب *Cyprinus carpio* على أداء النمو وبعض مقاييس الدم. أستخدم 12 حوض بلاستيكي (60× 30×30 سم). تمثل الاحواض أربع معاملات (كل معاملة بثلاث مكررات)، وكل حوض يحوي 10 اسماك (30 سمكة لكل معاملة) بمعدل وزن يتراوح بين 16.31–16.48 غم في بداية التجربة. غذيت الأصبعيات بنسبة 3% من الكتلة الكلية للأسماك حتى نهاية فترة التجربة. احتوت جميع العلائق على بروتين خام بمعدل تراوح بين 33.80–33.98% وحوالي 16.344± 0.0213 الى 16.695± 0.0201 (ملغم/ كغم) طاقة مهضومة. غذيت الأسماك بمعدل مرتين باليوم لمدة ستة أيام في الأسبوع. كانت مدة التجربة 56 يوماً. أظهرت نتائج التجربة ما يلي: أعلى معدل لوزن الجسم النهائي(FBW) ، الزيادة الوزنية اليومية(DWG) ، معدل النمو النسبي(RGR) ، معدل النمو النوعي(SGR) ، معدل التحويل الغذائي.(FCR) أفضل كفاءة تحويل غذائي(FCE). سجلت للمعاملة الرابعة (1.5٪). وفيما يتعلق بمعايير الدم فإن المعاملة الرابعة سجلت اعلى معدل من البروتين الكلي 5.68±0.84 g/dl، كلوبيولين 1.17± 0.54 g/dl و3.82 ± 0.51 ألبومين/كلوبيولين. اعتماداً على النتائج التي تم الحصول عليها من الدراسة الحالية يمكن الاستنتاج بأن مستخلص البردقوش بتركيز 1.5% كان الافضل للنمو ولصفات الدم. تشير النتائج الى ان اضافة البردقوش يمكن ان يحسن من كفاءة التغذية واداء النمو وصفات الدم لإصبعيات الكارب دون اثار سلبية على الاسماك.

الكلمات المفتاحية: اسماك مستزرعة، أداء النمو، ، مقاييس الدم، *Origanum majorana*.