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Effect of adding *E. globulus* (Myrtaceae) on growth, immunological and biochemical parameters in *Cyprinus carpio* L.

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ABSTRACT

The present study was aimed to evaluate the efficiency of *E. globulus* on growth performance on Common carp. For this purpose, 100 fingerlings of *Cyprinus carpio* weight ranged between 65.18 -65.36 g were randomly distributed into five treatment groups. Fish were fed with different *E. globulus* concentrations: 0.25% (T2), 0.5% (T3), 0.65% (T4) and 1% (T5). The first group (T1) was serve as control group without any addition of *E. globulus* . After 28 days of feeding, blood samples were collected for determination of hematological and biochemical parameters. The feed trail results indicate that treatment groups supplemented with *E. globulus* had significantly greater growth rates, hematological and biochemical parameters ($P \leq 0.05$) as compared to the control group. The highest values were observed in the T4 supplement with 1.5 percent *E. globulus*. Our findings indicate that the food supplement of *E. globulus* enhanced the hematological, biochemical, and growth rate parameters of *C. carpio*. This shows that the carp are using this feeding supplement.

1. Introduction

In contrast to chemically synthesized medications, studies have used safer alternative treatments, such as non-synthetic feed additives added to fish food to enhance growth and production, stimulate the immune system, boost resistance to a variety of diseases, and reduce side effects[1]. *E. globulus* is an alternative fragrant medical herb that lowers feed consumption and positively affects body

gain. It is a member of the Myrtaceae family and has drawn more attention for its medicinal and pharmaceutical antioxidant activity because of its extremely high levels of 1,8-cineol, p-cymene, alpha-pinenes, limonene, geraniol, flavonoids, coumarins, tannins, and many phenolic acids are the components of volatile oils[2] . *E. globulus* is a bronchial antispasmodic and an expectorant. Also *E. globulus* used for

antifungal, antibacterial, antiprotozoal, antiviral and antioxidant properties [3]. the aimed of the current study to evaluation the efficiency of diet E. globulus on growth performance, haematologically (WBC and RBC, countPCV value, Hb content), biochemically profile (albumin, total protein and globulin in C. carpio).

2. Material and Methods:

2.1 Experimental Fish

The current study was carried out in the Ichthyology Laboratory, Faculty of Veterinary Medicine/Diyala University, Iraq, from February 10, 2022, to April 22, 2022. 100 carp juveniles in all, weighing between 65.18 and 65.36 g at birth, were gathered from a commercial farm in Diyala, Iraq. Bring the fish into the lab, where they will spend five minutes submerged in a salt bath containing 2% sodium chloride. gets rid of fungal diseases and external parasites. Following a 14-day period of adaption, 100 fish were chosen at random and placed into ten glass aquariums measuring 150 x 40 x 20 cm. Each aquarium held 10 fish and was filled with tap water devoid of chlorine and had air pumps. Two parts). Iterate/Process). Each pool underwent five treated (T2 = 0.5%, T3 = 1.0%, T4 = 1.5%, and T5 = 2% E. globulus). As a control, one group (T1) did not receive any supplements containing eucalyptus globulus. For 56 days, each of the treatment groups received twice-daily

feedings at 1% of body weight. Every two weeks, the fish were all weighed. Every day, clean the aquarium by clearing off fish waste and food residue. Dissolved oxygen, pH, and temperature were measured every day in 6.5 ± 1.5 mg/L25, 5.5 ± 1.3 and ± 2.0 °C, among other chemical and physical properties of the water. Following a 28-day feeding trial, blood samples were taken in order to measure biochemical and hematological markers.

2.2 Calculating the biochemical and hematological parameters

Take blood samples from the tail paw and place them in dipotassium EDTA-containing vials. As stated by Hrubc and Smith4, Hb content, PCV levels, and red and white blood cell counts were ascertained. After centrifugation, serum samples were separated and kept at -20°C until they were needed for biochemical analysis (total protein, albumin, and globulin).

2.3 Analytical Statistics

Mean $\pm$ Standard error is used to express data. One-way and two-way analysis of variance (ANOVA) were used to look for significant differences between the treatment and control groups using SAS software (V.9.1). At the $P < 0.05$ level, significant changes between means were identified using the least significant difference (LSD).

3. Results

3.1 Growth rates

Table 1 displays the average body weight data for the *C. carpio* experimental fish. Each treatment group's beginning body weight on the first day of the experiment ranged from 65.18 to 65.36 g, with no significant difference ($P > 0.05$). Within the first 15 days following supplementation, the effects of *Eucalyptus globulus* on body weight were noted. After 15, 30, 45, and 28 days, there were significant differences ($P < 0.05$) observed in all therapy groups. In addition, all animals fed diets supplemented

with *Eucalyptus globulus* showed a considerably higher growth rate on day 28 of the feeding period as compared to the control group. The weight of each group grew significantly with age ($P < 0.05$), according to the data. T4, the fourth group, outperformed the comparatively speaking, the fourth group (T4) outperformed the other groups. This group's advantage was created on day 15 of the second phase and persisted through the conclusion of the study.

Table 1: The mean $\pm$ SE body weight of *C. carpio* that was fed varying dosages of *E. globulus* and a control diet during a 28-day period.

Weight (g) Treatment	1 Day	15 Days	30 Days	45 Days	56 Days
T1	64.23 $\pm$ 0.13a	76.87 $\pm$ 0.76b	84.13 $\pm$ 0.56e	99.32 $\pm$ 1.24d	114.42 $\pm$ 0.54 d
T2	64.14 $\pm$ 0.55 a	78.13 $\pm$ 0.32	89.21 $\pm$ 0.21 d	111.45 $\pm$ 0.43d	123.87 $\pm$ 0.64 e
T3	66.42 $\pm$ 0.21	81.87 $\pm$ 0.23ab	92.54 $\pm$ 0.32ab	116.76 $\pm$ 0.65b	136.76 $\pm$ 1.65 b
T4	66.65 $\pm$ 0.53a	84.23 $\pm$ 0.42a	95.32 $\pm$ 0.32a	117.54 $\pm$ 0.76a	146.64 $\pm$ 1.76 a
T5	66.32 $\pm$ 0.43 a	78.95 $\pm$ 0.21b	88.66 $\pm$ 0.54bc	109.54 $\pm$ 0.22c	126.55 $\pm$ 0.65 c

In the same column, means with distinct superscript characters indicate a significant difference ($P < 0.05$).

3.2 Parameters related to haematology

Table 2 provides a summary of the haematological parameter data. For every blood parameter, there was no significant change ($P < 0.05$) between the treatment groups. When comparing the RBC count of all E. globulus supplement treated (T2, T3, T4, and T5) to the control group (T1) at 28 days, there was a substantial increase ($P < 0.05$). Additionally, at 28 days, all E. globulus-supplemented treatments (T2, T3, T4, and T5) shown significant increases ($P < 0.05$) in Hb content and PCV% as

compared to T1. Out of all the treatments, T4 had the greatest Hb content and PCV% values. Moreover, comparable outcomes with marginal drops in values relative to pre-challenge values were seen in the Hb concentration and PCV% at 28 days. Conversely, at day 28, the WBC count in T1 of the E. globulus feeding groups (T2, T3, T4, and T5) shown a substantial increase ($P \leq 0.05$). On the 56th day, T4 showed the highest value when compared to the other therapies. Also, all E. globulus supplemented groups showed a substantial ($P \leq 0.05$) increase in WBC count at 28 days.

3.3 Varying leukocyte count

The findings of the differential leukocyte count are shown in Table 4. At 28 and 28 days, lymphocytes at all treatment E. globulus supplement feed groups (T2, T3, T4, and T5) demonstrated a substantial increase ($P < 0.05$) when compared to T1. When comparing the common carp groups (T2, T3, T4, and T5) supplemented with E. globulus to the control feed group T1 on days 28 and 28, there was a significantly higher ($P < 0.05$) at neutrophil levels. On the 56th day, there was a significantly ($P < 0.05$) increase in T4 when compared to the control group and further therapy. Compared to groups (56 days), monocyte-treated groups showed a substantial ($P < 0.05$) increase on day 28. The highest values were seen in T4, which was followed by T3, T5, and T2, in

that order compared to the T1 control group. Compared to the control groups, there was a significantly increase ($P < 0.05$) in eosinophil. There were no discernible variations ($P > 0.05$) in the differential leukocyte counts between the therapy groups.

Table2: Blood parameter of *C. carpio* over a 28-day period supplemented with *E. globulus* and fed a control diet.

Parameters Groups	RBC 10 ⁶ /mm ³		Hb g/dl		PCV %		WBC 10 ³ /mm ³	
	28 days	28 days	28 days	28 days	28 days	28 days	28days	56days
T1	1.91±0.12 ^b	1.79±0.32 ^b	7.61±1.17 ^d	7.31±1.22 ^b	22.7±1.22 ^b	22.6±1.21 ^b	18.4±1.42 ^b	20.1±1.63 ^b
T2	1.89±0.12 ^b	1.90±0.22 ^b	7.81±1.21 ^d	7.86±1.42 ^c	24.1±1.22 ^{ab}	24.86±1.31 ^{ab}	21.82±1.22 ^{ab}	23.37±1.23 ^a
T3	2.21±0.21 ^{ab}	2.13±0.32 ^{ab}	8.66±1.21 ^b	8.68±1.21 ^a	28.1±1.21 ^a	27.23±1.32 ^a	22.31±1.22 ^{ab}	24.89±1.21 ^a
T4	2.49±0.21 ^a	2.51±0.21 ^a	9.32±1.21 ^a	8.67±1.43 ^a	28.6±1.21 ^a	26.41±1.21 ^a	23.11±1.22 ^a	25.53±1.22 ^a
T5	2.12±0.21 ^b	2.21±0.21 ^b	8.32±1.21 ^c	8.42±1.21 ^b	26.6±1.32 ^{ab}	27.11±1.21 ^a	21.22±1.42 ^{ab}	24.91±1.14 ^a

In the same column, the means with distinct superscript letters indicate significant differences ($P < 0.05$).

Haemoglobin is represented by the letter Hb; white blood cells, red blood cells, and pocketed cell volume, or PCV.

Table 3: Variations in the leukocyte count across treatment groups 28 days after being challenged with Saprolegnia and 28 days after being fed a different concentration.

Parameters Groups	Monocyte %		Eosinophil %		Lymphocyte %		Neutrophil %	
	28 days	56 days	28 days	56 days	28 days	56 days	28 days	56 days
T1	2.29±0.11 ^e	2.76±0.11 ^e	2.2±0.11 ^e	2.21±0.21 ^e	53.5±0.12 ^e	49.7±0.13 ^d	46.7±0.11 ^e	50.2±0.11 ^e
T2	2.79±0.11 ^d	3.1±0.11 ^d	2.12±0.11 ^d	2.54±0.11 ^d	54.2±0.11 ^d	52±0.11 ^d	49.7±0.11 ^d	55.3±0.11 ^d
T3	4.23±0.32 ^b	4.48±0.21 ^b	2.21±0.11 ^b	2.87±0.12 ^{bc}	58.7±0.12 ^b	54±0.11 ^b	53.5±0.23 ^b	61.7±0.21 ^b
T4	4.32±0.21 ^a	4.87±0.21 ^a	2.13±0.12 ^a	3.21±0.11 ^a	60.2±0.11 ^a	55.1±0.13 ^a	53.5±0.11 ^a	65.2±0.21 ^a
T5	3.53±0.21 ^c	3.78±0.11 ^c	2.21±0.12 ^c	2.87±0.21 ^c	55.7±0.11 ^c	50.6±0.21 ^c	50.6±0.21 ^c	60.4±0.21 ^c

In the same column, the means with distinct superscript letters indicate significant differences (P<0.05)

Table 4: Biochemically characteristics of *C. carpio* fed two different *E. globulus* concentrations at 28 and 56 days.

Parameters Groups	Globulin g/dl		A/G %		Total protein g/dl		Albumin mg/dl	
	28 days	56 days	28 days	56 days	28 days	56 days	28 days	56 days
T1	2.32±0.15 ^e	2.52±1.32 ^e	0.54±0.21 ^a	0.56±0.12 ^a	3.71±0.11 ^c	3.82±1.21 ^c	1.23±0.12 ^a	1.23±1.31 ^a
T2	2.67±0.21 ^d	2.88±0.43 ^d	0.47±0.21 ^b	0.46±0.21 ^b	4.34±0.13 ^{cd}	4.53±1.95 ^{cd}	1.32±0.12 ^a	1.32±0.66 ^a
T3	3.12±0.12 ^{bc}	3.42±2.85 ^{ab}	0.42±0.47 ^b	0.45±0.13 ^b	4.52±0.21 ^{bc}	4.82±1.65 ^b	1.31±0.21 ^a	1.28±0.21 ^a
T4	3.13±0.32 ^a	3.44±0.32 ^a	0.56±0.78 ^b	0.42±0.12 ^b	4.65±0.32 ^a	4.92±1.11 ^a	1.33±0.32 ^a	1.32±0.43 ^a
T5	3.21±0.11 ^c	3.22±0.11 ^c	0.43±0.21 ^b	0.43±0.11 ^b	4.55±0.12 ^c	4.46±1.87 ^{cd}	1.32±0.12 ^a	1.32±0.54 ^a

In the same column, means with distinct superscript characters indicate a significant difference (P<0.05).

3.4 Biochemically profile

Table 4 displays the biochemical test outcomes. After 28 days, there was a substantial increase in total protein ($P > 0.05$). When comparing all groups to the control group T1, the highest amount was observed in T4. Furthermore, at days 56, showed in all groups significantly increases ($P < 0.05$) in comparison to T1. T4 has the greatest level ever noted. When comparing the

4. Discussion

4.1 Growth performance

Fish fed diets supplemented with *E. globulus* at varying doses demonstrated increased growth rate in the current study. It appears that *E. globulus* enhances common carp's ability to utilize nutrients, which leads to improved fish development. In the diet of *E. globulus*, protein was used for growth and most likely fat for energy. Herbs are known to stimulate pancreatic enzyme release, which is crucial for food assimilation and digestion [5]. This pattern may be connected to the larger and more notable weight increase that *E. globulus* supplementation produced. According to research published in [6], stellate sturgeon (*Acipenser stellatus*) fed a 2% concentration of photobiotic (*E. globulus*) showed a substantial boost in growth performance compared to their basal diet. Similarly, [7] found that stellatus sturgeons' growth performance was greatly enhanced by *E. globulus*-supplemented diets, and that 1.5% *E. globulus*/kg food was the ideal growth rate for them. Additionally, it has been

albumin content at 56 and 70 days to the control group, all treatment groups showed a slight but non-significant rise. On the other hand, globulin content in treated *E. globulus* supplemented meals (T2, T3, T4, and T5) at 28 and 56 days shown a significantly increase ($P < 0.05$) when compared to the T1 at 28 days. At 28 and 56 days, there was a substantial ($P < 0.05$) drop in the albumin globulin ratio at treatment groups (T2, T3, T4 and T5) comparative with T1.

shown by [8] that feeding *Oreochromis niloticus* diets enriched with *E. globulus* at levels of 1% and 2% improved growth performance. However, [9] demonstrated that feeding 1% of dietary *E. globulus* did not improve the growth performance of stellatus juveniles. However, *E. globulus* supplementation improved the biochemical quality composition of meat by significantly reducing the water percentage and increase the protein percentage. Our findings are consistent with those of [10] who observed that Nile tilapia feed diet supplement with *E. globulus*, fenugreek and rosemary showed enhanced survival rates and increased illness resistance, this could be connected to an increase in immunological function.

4.2. Haematology parameters

Fish health can be inferred from blood haematological index counts, which identify any disruptions brought on by immunostimulant use. [11] The current study's findings showed that adding *E. globulus* to fish diets significantly raised the percentage of PCV, HB, and RBCs.

These findings concur with those of [12] who observed a substantial rise in the RBC count, PCV% and HB at Nile tilapia fed a diet supplement with 1% E. globulus as compared to the control group. In a similar vein, [13] found that rainbow trout (*Oncorhynchus mykiss*) fed a diet supplemented with thymol-carvacrol had somewhat higher PCV, Hb, and RBC than the control. Same reported by [14] , who discovered that rainbow trout fed dietary carvacrol had significantly greater RBC count, Hb concentration, and PCV compared to the control group. Furthermore, our

findings are consistent with those of [15] , who discovered that fish given 1% E. globulus supplementation for 45 days had much higher HB, RBC, and PCV values than fish fed a control group. Additionally, groups given meals supplemented with E. globulus showed significantly different percentages of neutrophils, monocytes, and lymphocytes when compared to the control group. On the other hand, according to [16] reports, *Oreochromis niloticus* treated with rosemary had a significantly lower Hb concentration than the control group.

4.3 Biochemical Profile

The maximum significant index of the feed status of the fish health condition [17] was total serum protein. According to several writers, the levels of globulin, albumin, and in total protein plasma serve as markers of liver function. As a result, a drop in serum protein may be caused by renal excretion, reduced protein synthesis, hepatic hypofunction, or disorders [18]. Additionally, two significant components of total protein (TP) are albumin and globulin, and variations at these parameters impact the quantity of TP. Concentrations of globulin and albumin are frequently use

to assess how nutrients affect fish immune. Fish blood contains albumin, which aids in the overall metabolism of fish and transports lipids [19]. Maintaining optimal immunity and having all of the immunological globulins in blood is thought to be highly dependent on globulin. Our findings are consistent with those of [20] who observed that rainbow trout fed E. globulus and fennel had higher levels of total protein, albumin, and albumin globulin ratio than the control group. [21] published findings that were similar.

5.Conclusion

Carp fish grow more quickly when E. globulus is added to their diet. The immunostimulant effects are indicated by the rise in blood parameters (WBC, RBC counts and Hb), biochemically

profile and survivals rate of *C. carpio* challenged with *Saprolegnia* Spp. after E. globulus was added to the diet. As a result, we advise including E.globulus in the diet of carp fish.

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تأثير إضافة أوراق الكالبتوز على النمو والمؤشرات المناعية والكيميائية الحيوية لأسماك الكارب الاعتيادي

قصي صالح جمعة

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الملخص

هدفت الدراسة الحالية إلى تقييم كفاءة الكالبتوز في نمو أسماك الكارب الشائع، إذ تم استخدام 100 إصبعية تراوحت أوزانها بين 65.18 - 65.36 غرام موزعة عشوائياً على خمسة مجاميع. عدت المجموعة الأولى (T1) كمجموعة سيطرة إذ أنها لم تعامل بأية إضافة، بينما عوملت المجموعات الأخرى على النحو الآتي: المجموعة الثانية بتركيز 25 % من الكالبتوز، المجموعة الثانية بتركيز 5 % من الكالبتوز، المجموعة الثالثة بتركيز 75 % من الكالبتوز في حين عوملت المجموعة الرابعة بتركيز 1 % من الكالبتوز تم جمع عينات الدم على فترتين 28 يوم و 56 يوماً من التغذية لقياس المعايير الدموية والكيموحيوية. إذ اشارت نتائج التجربة في المجاميع المعاملة بالكالبتوز الى وجود ارتفاعاً معنوياً ($P \leq 0.05$) في معدلات النمو بزيادة نسبة المعاملة بالكالبتوز وارتفاعاً معنوياً في المعايير الدموية بشكل طردي مع نسبة المعاملة بالكالبتوز مقارنة فيما بين المجاميع المعاملة وعند المقارنة مع مجموعة السيطرة إذ لوحظت أعلى قيمة في المجموعة الرابعة المعاملة بنسبة 1 % من الكالبتوز. نستنتج من هذه الدراسة الى ان المكملات الغذائية المستخدمة في تغذية الاسماك (الكالبتوز انموذجاً) قد ساهمت في تحسين صورة الدم وبعض المعايير الكيموحيوية في اسماك الكارب العادي.

الكلمات المفتاحية : المكملات الغذائية، الكارب العادي، أوراق الكالبتوز