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Influence of thyme (*Thymus vulgaris)* as feed additives on growth performance and antifungal activity on *Saprolegnia spp*. in *Cyprinus carpio* L.

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Abstract
The present study was aimed to evaluate the efficiency of thyme *(Thymus vulgaris)* on growth performance and antifungal activity on *Saprolegnia spp.* on Common carp. For this purpose, 100 fingerlings of *Cyprinus carpio* weight ranged between 75.18 -75.36 g were randomly distributed into five treatment groups. Fish were fed with different thyme concentrations: 0.5% (T2), 1% (T3), 1.5% (T4) and 2% (T5). The first group (T1) was serve as control group without any addition of thyme. After 56 days of feeding, blood samples were collected for determination of hematological and biochemical parameters. The fingerlings were challenged with *Saprolegnia spp.* post feeding trail (at concentration 1×10^5 zoospore/ml) and their mortalities were recorded up to 15 days. Results of feeding trail in treated groups with thyme supplementation showed significant increase ($P<0.05$) in growth rate, hematological and biochemical parameters compared with the control group, and the highest values were recorded in T4 supplemented with thyme at concentration of 1.5%. On the basis of our data, the dietary supplementation of *T. vulgaris improved* the growth rate, hematological, biochemical parameters and survival rate of *C. carpio* challenged with *Saprolegnia spp.* This indicates the immunostimulant effect and anti-fungal activity of this feed additive in common carp.

Keywords: Common carp, growth performance, *Saprolegnia spp., Thymus vulgaris*

Introduction
Water mold (Saprolegniasis) is one of the main diseases responsible for mass kill and considerable economic losses among freshwater fish, as well as this disease affect fish eggs resulting in heavy mortality during hatching period [1]. Saprolegniasis usually starts as cotton wool like lesion white to dark greyish in colour on the dorsal fin, head, and then extend to all over the body surface to form of focal area [2, 3]. Routine application of disinfectants is a commonly used, the extremely effective fungicide, formalin but it is potentially detrimental to the workers' health and residues in the environment. Other effective antifungal agents is malachite green, however in recent years it has demonstrated that malachite green is mutagenic and carcinogenic for that reason, malachite green has been excluded in some countries[4]. For these reasons researchers trended toward the use of alternative treatments that is required to be not synthetic, safe such as feed additives, which are added to the fish diets to improve the growth and yields and to stimulate immune response and to increase resistance against various diseases as well as lower side effect in comparison with chemically synthesized medicines [5].

One of these alternative medicinal herbs, which affect positively on body gain and which decrease food consumption, include thyme *(Thymus vulgaris*) is an aromatic plant belonged to a family Lamiaceae and has received great attention as both a therapeutic and pharmaceutical agent across the world. Thyme has strong antimicrobial and antioxidant activity due to its very high contents of thymol (40%), carvacrol (15%), cymene, eugenol and 4-allylphenol.Thyme is a bronchial antispasmodic and an expectorant. It has shown antibacterial, antifungal, antiviral, antiprotozoal, and antioxidant properties [6]. This study aimed to evaluate the efficiency of dietary thyme on growth performance, haematological (Hb content, PCV value, RBC and WBC count), biochemical profile (total protein, albumin and globulin) and as antifungal activity against *Saprolegnia spp*. in *C. carpio*.

Materials and Methods
Fish and experimental conditions
This study was carried out at College of Veterinary Medicine/University of Baghdad, Ichthyology laboratory, Baghdad, Iraq, from 14 February to 24 April 2017.

A total of 100 fingerlings of *C. carpio* (initial weight 75.18-75.36g) were collected from a commercial farm (Al-Musayyib, Babylon/ Iraq). The fish were transferred to a laboratory and were dipped in a salt bath of NaCl at a concentration of 2.5% for 5 min. to get rid of external parasites and fungal infections. After the acclimation period (14 days), 100 fish were randomly selected and distributed into 10 glass aquaria (dimension 150 × 40 × 20 cm) filled with chlorine-free tap water and supplied with air pump at rate of 10 fish per aquarium (two replicates/ treatment). Five treatments (T2 = 0.5%, T3 = 1.0%, T4 = 1.5% and T5= 2% thyme) were used to each tank. One group (T1) serve as the control did not receive any thyme supplement. All the treatment groups were fed twice a day at rate of 3% of body weight for 56 day. Weight of all fish were measured at every two weeks interval The aquaria were daily cleaned by drain off the food debris and fish feces.Chemo-physical parameters of water including temperature, pH and dissolved oxygen (DO) were recorded daily at 24±2.0 ºC, 6.5±1.3 and 7.5±1.5 mg/l. After 56 days of feeding experiment blood samples were collected for determination of blood and biochemical parameters. All treatment groups were challenged with *Saprolegnia spp*. (At a concentration of 1×105 zoospore/ml). After challenge survival rate were calculated using the following equation:

Survival rate *%*= final number of fish survivor ÷ initial number of fish stocked × 100

Determination of Hematological and biochemical Parameters

Blood samples was drawn from caudal peduncle into Di Potassium EDTA containing tube. Hb content, PCV value, RBC and WBC counts were determined as described by Hrubc and Smith [7]. Serum samples was separated by centrifugation and preserved at-20 ºC until use for biochemical profile (total protein, globulin and albumin).

Statistical Analysis

Data are presented as mean± standard error. One way and two-way analysis of variance (ANOVA) were applied using SAS software (V.9.1) to study the significant differences among treatments and control groups. Significant differences between means were determined using least significant differences (LSD) at level $P<0.05$.

Results

Growth Performance

Data of average body weight of experimental fish (*C. carpio)* are presented in Table 1. The initial weight of all treatment groups at first day of experimental period ranged between 75.18–75.36 g and there was no significant differences among them ($P>0.05$). The effect of thyme supplementation on body weight was observed during the first 15 days. After 15, 30, 45 and 56 days all treatment groups showed significantly difference ($P<0.05$) among them. Moreover, at 56 days of the feeding period, all treatments fed thyme-supplemented diets revealed the existence of a clear increase in the growth rate compared with the control group. Results showed that the body weight increased significantly ($P<0.05$) with advanced age in all groups. The fourth group (T4) exceeded on the other groups. The superiority of this group was detected in the second period at 15 day and continued until the end of experiment. After challenge with *Saprolegnia spp*. the survival rate of carp fish fed different levels of thyme supplemented diets showed 100% and were statistically greater ($P<0.05$) than the control group (20%).

Table 1: Average body weight of C. *carpio* fed different levels of thyme and control diet (mean±SE) during 56 days.

Weight (g) / Treatment	1 Day	15 Day	30 Day	45 Day	56 Day
T1	75.34±0.24^{a}	77.92±0.56^{b}	85.02±0.56^{e}	98.26±1.14^{d}	115.40±0.56 ^{d}
T2	75.18±0.56 ^{a}	79.17±0.27^{b}	88.2±0.15 ^{d}	105.74±0.26^{d}	122.88±0.58 ^{e}
T3	75.24±0.20^{a}	80.97±0.28^{ab}	91.46±0.27^{ab}	114.91±0.42^{b}	138.24±1.15 ^{b}
T4	75.36±0.16^{a}	82.21±0.15^{a}	94.01±0.15^{a}	118.39±0.65^{a}	145.46±1.16 ^{a}
T5	75.26±0.14 ^{a}	79.82±0.14^{b}	89.77±0.14^{bc}	107.51±0.20^{c}	128.56±0.58 ^{c}

Means with different superscript letters in the same column are significantly different ($P<0.05$).

Haematological parameters

The results of haematological parameters are summarized in Table 2. There was no significant difference (P.0.05) between pre challenge and post challenge for all the blood parameters in all treatment groups. RBC count revealed a significant increase ($P<0.05$) at 56 days (pre challenge) and at 70 days (post challenge) in all thyme supplemented treatments (T2, T3, T4 and T5) compared with control group (T1). Also, Hb content and PCV% showed significant increases ($P<0.05$) at 56 days (pre challenge) in all thyme-supplemented treatments (T2, T3, T4 and T5) in comparison to T1. The highest value of Hb content and PCV% were recorded in T4 from all other treatments. Also, similar results were revealed in Hb concentration and PCV% at 70 days (post challenge) with slight decreases in values compared with pre challenge values.

On the other hand**,** WBCs count showed significant increase ($P\leq0.05$) in thyme feeding groups (T2, T3, T4 and T5) in T1 at 56 days (pre challenge). The highest value was observed in T4 on 56th day (in comparison with other treatments. Also, at 70 days WBC count recorded significantly increase ($P\leq0.05$) in all thyme supplemented groups.

Table2: Blood parameter of *C. carpio* with thyme supplement and control diet during 56 days (pre challenge) and at 70 days (post challenge with *Saprolegnia spp*.)

Parameters / Groups	RBC 10^6/mm^3		Hb g/dl		PCV %		WBC 10^3/mm^3	
	56 days	70 days	56 days	70 days	56 days	70 days	56 days	70 days
T1	1.83±0.11^{b}	1.80±0.11^{b}	7.57±1.15^{d}	7.24±1.15 b	22.5±1.15^{b}	22.3±1.15^{b}	18.3±1.15^{b}	19.5±1.70^{b}
T2	1.90±0.11^{b}	1.88±0.11 ^{b}	7.84±1.15^{d}	7.83±1.15 c	25±1.15^{ab}	24.76±1.15^{ab}	20.73±1.15^{ab}	22.35±1.15^{a}
T3	2.15±0.11^{ab}	2.12±0.11 ^{ab}	8.75±1.15^{b}	8.70±1.15 a	27±1.15^{a}	26.25±1.15^{a}	22.27±1.15^{ab}	24.94±1.15^{a}
T4	2.50±0.11^{a}	2.49±0.11^{a}	9.30±1.15^{a}	8.750±1.15a	28±1.15^{a}	27±1.15^{a}	23.01±1.15^{a}	25.56±1.15^{a}
T5	2.05±0.11^{b}	2.04±0.11^{b}	8.33±1.15 ^{c}	8.3±1.15 ^{b}	26.3±1.15^{ab}	26.08±1.15^{a}	21.25±1.15^{ab}	24.83±1.15^{a}

Mean with different superscript letters in the same column are significantly different (P<0.05). WBC, white blood cell; RBC, red blood cell; Hb, haemoglobin; PCV, pocked cell volume

Differential leukocyte count

The result of differential leukocyte count are illustrated in Table 4. Lymphocyte showed a significant increase (P<0.05) in all treated thyme supplemented diet (T2, T3, T4 and T5) in comparison with T1 at 56 and 70 days. Neutrophil showed a significant increase (P<0.05) in common carp groups that supplemented with thyme in diet (T2, T3, T4 and T5) in comparison with the control diet group T1 on 56 and 70 days. A significant increase ($P\leq$0.05) was observed in T4 on 56th days (before challenge) in comparison with further treatment and control group. Monocytes treated groups significantly increased ($P\leq$0.05) after challenged (day 70) than pre challenged groups (56 day). The maximum values were observed in T4 followed by T3, T5 and T2 respectively in comparison with control group T1. Eosinophil showed a significant increase ($P\leq$0.05) in comparison with the control groups. Differential leukocyte count showed no significant differences (P>0.05) between pre challenge and post challenge in all treatment groups.

Table 3: Differential leukocyte count in treatment groups *C. carpio* fed different concentration for 56 days (pre challenge) and 70 days post challenged with *Saprolegnia.*

Parameters / Groups	Lymphocyte %		Neutrophil %		Monocyte %		Eosinophil %	
	56 days	70 days	56 days	70 days	56 days	70 days	56 days	70 days
T1	54.6±0.05 ^{e}	49.5±0.06^{d}	46.6±0.05 ^{e}	50.3±0.06 ^{e}	2.30±0.03 ^{e}	2.77±0.06 ^{e}	2.0±0.02 ^{e}	2.10±0.02^{e}
T2	54.1±0.06 ^{d}	50±0.06 d^{c}	49.8±0.06^{d}	55.1±0.06 ^{d}	2.80±0.06^{d}	3.0±0.03^{d}	2.04±0.02^{d}	2.60±0.06^{d}
T3	58.6±0.06 ^{b}	53±0.06 ^{b}	52.5±0.06 ^{b}	61.8±0.06 ^{b}	4.20±0.03^{b}	4.50±0.06^{b}	2.15±0.06^{b}	2.83±0.06^{bc}
T4	60±0.06 ^{a}	55±0.06 ^{a}	53.6±0.06 ^{a}	65.1±0.03 ^{a}	4.75±0.03^{a}	4.90±0.06^{a}	2.17±0.03^{a}	3.10±0.06^{a}
T5	55.6±0.06 ^{c}	50.5±0.06 ^{c}	50.5±0.06 ^{c}	60.6±0.06 ^{c}	3.60±0.06 ^{c}	3.80±0.03^{c}	2.05±0.03^{c}	2.73±0.02^{c}

Mean with different superscript letters in the same column are significantly different (P<0.05).

Biochemical profile

The results of biochemical tests are presented in Table 4.Total protein level significantly increased (P>0.05) at 56 days (pre challenge) in all groups compared with control group T1, the high level were recorded in T4.Also, there were significant increases (P<0.05) at 70 days (post challenge) in all groups compared to T1. The highest level was recorded in T4. While, albumin content at 56 and 70 day (post challenge) revealed marginally increase but was non-significant in all treatment groups compared with control group.

Whereas, globulin content at 56 and 70 days showed significantly increase (P<0.05) in treated thyme supplemented diets (T2, T3, T4 and T5) compared with control group T1 at 56 days. Albumin globulin ratio at 56 and 70 days was significantly decrease (P<0.05) in treated groups (T2,T3, T4 and T5) compared with T1.

Table 4: Biochemical parameters of *C. carpio* fed different concentrations of thyme at 56 days (pre challenge) and at 70 days (post challenge)

Parameters / Groups	Total protein g/dl		Albumin mg/dl		Globulin g/dl		A/G %	
	56 days	70 days	56 days	70 days	56 days	70 days	56 days	70 days
T1	3.61±0.06 ^{c}	3.80±1.37 ^{c}	1.20±0.021 ^{a}	1.23±1.34 ^{a}	2.24±0.02 ^{e}	2.50±1.44 ^{e}	0.53±1.44 ^{a}	0.53±0.02 ^{a}
T2	4.44±0.05 ^{cd}	4.52±1.81 ^{cd}	1.22±0.028 ^{a}	1.26±1.75^{a}	2.66±0.19 ^{d}	2.93±0.01 ^{d}	0.45±0.01 ^{b}	0.43±0.19 ^{b}
T3	4.50±0.08 ^{bc}	4.70±1.89 ^{b}	1.24±0.021 ^{a}	1.27±1.83 ^{a}	3.09±0.06 ^{bc}	3.40±2.78 ^{ab}	0.40±6.55 ^{b}	0.40±0.06 ^{b}
T4	4.55±0.02 ^{a}	4.84±2.00 ^{a}	1.25±0.021 ^{a}	1.29±2.02 ^{a}	3.15±0.02 ^{a}	3.45±0.02 ^{a}	0.39±2.78 ^{b}	0.39±0.02 ^{b}
T5	4.45±0.08 ^{c}	4.59±1.98 ^{cd}	1.24±0.028 ^{a}	1.27±1.94 ^{a}	3.06±0.02 ^{c}	3.20±0.02 ^{c}	0.42±0.07 ^{b}	0.41±0.06 ^{b}

Means with different superscript letters in the same column are significantly different (P<0.05).

Discussion

Growth performance

In the current study, fish fed diets supplemented with *T. vulgaris* at different concentrations revealed enhanced growth rate. It seems that the thyme improves the nutrient utilization of common carp turning out a better growth of the fish. Most possibly fat was used for energy, and protein was used for growth in thyme diet. Frankic *et al*., [8] have been reported that herbs activate the secretion of pancreatic enzymes, essential factors in food digestion and assimilation. This trend could be related with the higher and significant weight gain obtained with thyme supplementation. Stimulation of growth by *T. vulgaris* has been reported by [9] who found the growth performance of stellate sturgeon, *Acipenser stellatus* fed some

photobiotic (thyme, seabuckthorn) at a concentration of 2% were increase significantly relative to basal diet. Similarly, [10] reported that the thyme supplemented diets significantly improved growth performance of stellatus sturgeons and their optimal growth was obtained at 2% thyme /kg diet.

Also, [11] documented that *Oreochromis niloticus* fed diets supplemented with thyme (1% and 2%) levels had positive effect on growth performance. On the other hand, [12] showed that there was no enhancement in growth performance of stellatus juvenile after feeding 1% of dietary thyme, but thyme supplementation has improved of meat biochemical quality composition, due to a significant decrease in the percentage of water, respectively increasing the percentage of protein. Our results are in agreement with [13] who noticed that in Nile tilapia fed diet supplemented with thyme, rosemary and fenugreek an increase in disease resistance and improved survival rate, which may be attributed to an improvement of immune function.

Haematological Parameters

Blood is a patho-physiological indicator of the whole body and the counts of haematological indices in blood offer an reflection to the health status of fish by detecting any disruption occurring due to the use of immunostimulants [14]

The results of the present study indicated that supplemented thyme in fish diet increased considerably the RBCs count, HB and PCV%. These results are in agreement with [15] who recorded that the RBCs count, HB and PCV% in Nile tilapia fed diet supplemented with 1% of thyme were significantly increase compared to control. Similarly, [16] reported that the PCV, Hb and RBC in rainbow trout, *Oncorhynchus mykiss* fed thymol- carvacrol supplemented diet were slightly higher than the control. Similar results were reported by [17] who found that the RBC count, Hb concentration and PCV in rainbow trout fed dietary carvacrol were significantly higher in comparison to control group. Also, our results are in line in line with the results of [18] who found that the HB, RBC and PCV value in fish fed 1% of thyme supplementation for 45 days had significantly increase compared with control. Also, lymphocyte, neutrophil and monocyte percentages were registered significantly difference in groups fed thyme supplemented diets compared to control. In contrary, [19] reported that the Rosemary administration to *Oreochromis niloticus* were significant reduction in Hb content, compared with control.

Biochemical Profile

Total serum protein represented the most important indicator of the nutritional state of the fish health condition [20]. Some authors reported that the concentrations of total protein, albumin and globulin in plasma represent indicators of liver function and therefore the decrease of serum protein could be attributed to renal excretion or impaired protein synthesis, or due to liver hypo function or disorder [21].

Also, albumin and globulin are two important parts of total protein (TP), and changes in these parameters affect the level of TP. Albumin and globulin concentration are commonly used for evaluating the effect of nutrients on fish immunity. Albumin in fish blood performs the transportation of lipids and helps in the general metabolism of fish [22]. Globulin is considered very important to keeping of good immunity and also have all the immune globulins in blood. Our results are in line with [23] who found that the total protein, albumin and albumin globulin ratio in rainbow trout fed thyme and fennel were higher than control. Similar findings were reported by [24].

Conclusion

Based to our data, it can be concluded that dietary supplementation of *T. vulgaris* improve growth rate of carp fish. The increase in blood parameters (i.e., Hb, RBC and WBC counts), biochemical profile and survival rate of *C. carpio* challenged with *Saprolegnia spp.* following dietary supplementation of thyme in diet indicates the immunostimulant effects and anti-fungal activity of this feed additive. Therefore, we recommend dietary addition of thyme in the diet of carp fish.

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