

Effect of Adding Garlic (*Allium sativum*), Black Seed (*Nigella Sativa*) and their combination on Some Bio-Physiological Parameters in young common carp (*Cyprinus carpio* L.)

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ABSTRACT:

This study has been executed to elucidate the effect of different levels of garlic (*Allium sativum*) and black seed (*Nigella sativa*) in fish diet of common carp *Cyprinus carpio* L. on some Bio-Physiological Parameters. This study has been executed for a period 8 weeks after an adaptation period of 20 days in the fish laboratory of Animal Science Department, College of Agricultural sciences/ University of Sulaimani, Iraq. It was conducted by using 260 fish (their average weight was 29 ± 1 g) of common carp *C. carpio* L. This experiment was distributed into 9 treatments with three replicates for each treatment as follow; T1 with 0%, T2 10% black seed, T3 1% garlic, T4 10% black seed with 1% garlic, T5 5% black seed, T6 5% garlic, T7 10% black seed with 0.5% garlic, T8 5% black seed with 1% garlic, and T9 5% black seed with 0.5% garlic. T6 with 0.5 % Garlic was higher significantly in Gonadosomatic index as compared to other treatments, in opposite to it the Spleenosomatic index which was lesser significantly than other treatments, in Condition factor the T4 with 10% Black seed + %1 Garlic was higher significantly than other treatments, T5 with 5% Black seeds powder and T6 with 0.5 % Garlic were lesser significantly as compared to other treatment in Hepatosomatic index. No significant differences observed in Hepatosomatic index and Spleenosomatic index, T4 with 10% Black seed + %1 Garlic elevate significantly the Gonadosomatic index, control, T3 with 1 Garlic powder, T6 with 0.5 % Garlic, T7 with 10% Black seed + % 0.5 Garlic were significantly higher in condition factor. no significant differences observed in fish weight without viscera, significant differences found among the treatments in Fish weight without viscera & head. After ten days the a significant differences observed in Fish weight without viscera, the control and T8 with 5 % Black seed + % 1 Garlic were lower significantly in Fish weight without viscera & head.

Keywords: Garlic (*Allium sativum*), Black Seed (*Nigella Sativa*), Bio-Physiological Parameters, common carp

INTRUDUCTION

Aquaculture is the farming of aquatic organisms in Inland and coastal areas, involving intervention in the rearing process to enhance production of the stock being cultivated (FAO, 2012). Special attention has been laid upon feed additives that serve special purposes, such as micronutrient requirements, disease control, activation of immune system and some other nutritional and physiological purposes, use of herbs as drugs in disease management is gaining success, because herbal drugs are cost effective, ecofriendly and have minimal side effects. The inclusion of herbal feed supplements often provides cooperative action to various

physiological functions (Madhuriet *et al.*, 2012). The same authors postulated that traditional herbal medicines seem to have the potential immunostimulating, thus, the use of herbs is an alternative to antibiotics in fish health management. The non-specific immune system of fish is the first line of defense against invading pathogens, Neutrophils and phagocytes, lysozyme and complement are some important indices of non-specific immunity in fishes (Ahilanet *et al.*, 2010).

Black seed (*Nigella sativa*) is cultivated in the Mediterranean region and Asia (Diab, *et al.* 2008). Some researchers used black seeds as enhancer for performance growth and immune system of some fish species, it is promising protein source used in fish feeding (Abd-Elmonem *et al.* 2002; John *et al.*, 2007). On the other hand, Garlic (*Allium sativum*) was also used as a growth promoter in tilapia *Oncorhynchus niloticus* (Diab *et al.*, 2002), in which it increased body gain, feed intake, and feed efficiency. It gave similar results in the study of conducted by Abd-Ellatif and Ebraheem, (1996).

Defense system of fish is very important against microorganisms, therefore, using organic, inorganic, or synthetic matters as immunostimulant has been used as a better alternative to antibiotics to control fish diseases (Findlay and Munday, 2000; Cuesta *et al.*, 2004). Hence, in the last decade there has been increasing interest in the modulation of the non-specific immune system of fish as both treatment and prophylactic measures against diseases (Mirsa *et al.*, 2006). Prepared diets not only provide the essential nutrients that are required for normal physiological functioning, but also may serve as a medium by which fish can receive other components that may affect their health (Gatlin, 2002) Feed additives such as probiotics or medicinal plants enhance not only the growth rate, but also can positively stimulate the non-specific defense mechanisms of fish, *Bacillus spp.* may modulate the mucosal immune systems enhancing their resistance to enteric pathogens and so improved survival and growth of tilapia (Gullian and Thompson, 2004; Eid and Mohamed, 2008).

Garlic in fish feed decreases both total cholesterol and low-density lipoprotein (LDL C) in addition to reducing blood pressure (Adler and Holub, 1997). The consumption of its powder may decrease the accumulation of lipids in the liver, increase the excretion of total bile acids in feces, and increase the antioxidant capacity in hamsters (Yaoling *et al.*, 1998). Diab *et al.*, (2002) reported a high growth performance in Nile tilapia *O. niloticus* fed on 2.5% garlic powder / kg diet. Nye and Austin (2009) found that added garlic powder to the fish diet led to an increase in the rate of food intake, stimulating growth and increase feed conversion value. In one of the tests conducted on rainbow trout to observe the effect of different concentrations of garlic powder 5%, 10%, 15%, 20% on growth, the best growth rate was found in the group fed on a diet containing a high concentration of garlic, with better survival and activity compared to control group (Nye and Austin, 2009).

Abou-Zeid (2002) showed that *Allium sativum* supplementation positively affected *O. niloticus* biomass and specific growth rate (SGR). Feed intake increased with the increasing of *A. sativum* and chloramphenicol levels, while feed conversion ratio decreased with the increasing of *A. sativum* levels and increased by increasing chloramphenicol levels (Shalaby *et al.*, 2006). Khattab *et al.*, (2004) found that the dietary of Biogen containing garlic, increased feed intake, Feed conversion ratio (FCR), protein efficiency ratio (PER), and body composition (crude protein, ether extract, ash, and moisture) in fish. Higher growth performance has been recorded for fish fed garlic supplemented diets in the studies of Dias *et al.*, (2002) and Metwally (2009).

The aim of the present work is to study the combined effect of adding black seeds and garlic powder in different levels to fish diet along with starvation trials on some physio-biological

parameters such as Hepatosomato index, Gonadosomatic index (GSI), Splenosomatic index, Condition factor, Fish weight without viscera, and Fish weight without viscera & head.

MATERIALS AND METHODS

Experimental Fish and Tanks: Experimental Fish and Tanks

Young Common carp (*C. carpio*) was obtained from a local fish hatchery located in Daqoq, Middle of Iraq. A total number of 260 fish ranging in weight between (29-30g) were purchased and used for this study. The fish were sorted depending on size then weighed and put in experimental oval plastic tanks with a capacity of (100 L) each. Fish were distributed randomly on 26 plastic tanks at the rate of 10 fish per tank. Three tanks stocked with 30 fish were used as replicates for each treatment. During the accumulation period (20days) fish were fed for satiation with a control diet containing 24-25% protein. The experiment was conducted for 8 weeks in fish laboratory of Animal Science Department, Faculty of Agricultural sciences of Sulaimani University, Sulaimani, Kurdistan region, Iraq.

A total of twenty six plastic tanks containing (100 L) of water were used in this experiment. Every tank was artificially aerated with an air pump and stocked with 10 fish. The numbers of treatments in the trial were eight with three replicates for each treatments. Water quality (temperature, D_O, pH) was monitored weekly to ensure healthy environment. Tanks were daily cleaned by siphon method to remove feces and remained non-consumed feed.

To test the effect of black seed (BS) and garlic powder (G), eight treatments were designed as follows:

Two levels of garlic (0.5 and 1%), two levels of black seed levels (5 and 10 %), four combinations between garlic and black seed (10% BS & 1% G, 5% BS & 0.5% G, 10% BS & 0.5% G and 5% BS & 1% G) as the following.

T1: Control Commercial ration

T2: Diet with 10% Black seeds powder

T3: Diet with 1% Garlic powder

T4: mix Diet 10% Black seed + %1 Garlic powder

T5: Diet with 5% Black seeds powder

T6: Diet with 0.5 % Garlic

T7: mix Diet 10% Black seed +% 0.5Garlic

T8: Mix Diet 5% Black seed +% 1 Garlic

T9: Mix Diet 5% Black seed +% 1 Garlic

Various components including the experimental feed additives (Black seeds and Garlic powder) were ground, weighed and mixed together according to the pre-determined feed formulation and then water added at 30% to make suitable paste. Paste was passed through meat mincer at proper mesh size to make pellets with 3mm size. They were then dried and cut to the desirable size. Compositions of the different rations are shown in Table 1.



Table (1): Formulation of the experimental diets showing the percentage of various components of the supplemented ration

Treatments	Animal concentrate	Soybean cake	Corn	Local Barley	Wheat Bran	Premix	Black seed	Garlic powder
T1: Control	10	25	15	22	25	3	-	-
T2 10 % Black seeds	10	15	15	22	25	3	10	-
T3 1 Garlic powder	10	24	15	22	25	3	0	1
T4 10% Black seed + % 1 Garlic	10	15	14	22	25	3	10	1
T5 5% Black seeds powder	10	15	12	20	25	3	5	0
T6 0.5 % Garlic	10	27	14.5	20	25	3	0	0.5
T7 10% Black seed + % 0.5 Garlic	10	25	11.5	15	25	3	10	0.5
T8 5 % Black seed + % 1 Garlic	10	25	11	20	25	3	5	1
T9 5 % Black seed + % 1 Garlic	10	25	11.5	20	25	3	5	0.5

Physio-Biological parameters:

All the fish samples are scarified and soon the abdominal cavity is opened to remove, all organs to be weighed at once. They were calculated as follow:

Hepatosomato index % = liver weight (g)/body weight (g) x 100 (Lagler, 1956).

Gonadosomatic index (GSI) % = Gonads weight (g)/ Body weight (g) x 100

Spleen index % = Spleen weight (g)/body weight (g) x 100

Condition factor= Fish weight / Body length³

Fish wt. without viscera% = Fish weight without viscera (g)/body weight (g) x 100

Fish wt. without viscera& head % = Fish weight without viscera & head (g)/body weight (g) x 100

Statistical Analysis: Analysis of variance is conducted using the general linear models (GLM) procedure of XLSTAT. Pro. 7.5 one way (ANOVA). Fisher’s L.S.D test’s was used to compare between means of the control and experiment treatments.

RESULTS & DISCUSSION

T6 with 0.5 % Garlic was higher significantly in Gonadosomatic index as compared to other treatments, in opposite to it the Spleenosomatic index which was lesser significantly than other treatments, in Condition factor the T4 with 10% Black seed + % 1 Garlic was higher significantly than other treatments, T5 with 5% Black seeds powder and T6 with 0.5 % Garlic were lesser significantly as compared to other treatment in Hepatosomatic index as shown in table (2).



Table (2): Effect of Adding Garlic (*Allium sativum*), Black Seed (*Nigella Sativa*) and their combination on some biological Parameters in young common carp (*C. carpio* L.) in first time.

Treatments	Hepatosomatic index	Gonadosomatic index	Spleenosomatic index	Condition factor
T1: Control	2.109 ab	2.216 bc	0.230 ab	1.223 bc
T2 10 % Black seeds	2.145 ab	1.231 cd	0.238 a	1.053 c
T3 1 Garlic powder	2.841 a	2.610 b	0.244 a	1.164 bc
T4 10% Black seed + %1 Garlic	2.594 ab	0.686 d	0.241 a	1.471 a
T5 5% Black seeds powder	2.051 b	2.353 bc	0.199 ab	1.260 b
T6 0.5 % Garlic	2.050 b	4.893 a	0.127 b	1.159 bc
T7 10% Black seed + % 0.5 Garlic	2.514 ab	0.652 d	0.211 ab	1.076 bc
T8 5 % Black seed + % 1 Garlic	2.158 ab	1.649 bcd	0.290 a	1.150 bc
T9 5 % Black seed + % 1 Garlic	1.887 b	0.568 d	0.191 ab	1.162 bc

Mean values with different mention letter within a column differ significantly ($P \leq 0.05$).

No significant differences observed in Hepatosomatic index and Spleenosomatic index, T4 with 10% Black seed + %1 Garlic elevate significantly the Gonadosomatic index, control, T3 with 1 Garlic powder, T6 with 0.5 % Garlic, T7 with 10% Black seed + % 0.5 Garlic were significantly higher in condition factor as shown in table (3).

Table (3): Effect of Adding Garlic (*Allium sativum*), Black Seed (*Nigella Sativa*) and their combination on some biological Parameters in young common carp (*C. carpio* L.) in second time.

Treatments	Hepatosomatic index	Gonadosomatic index	Spleenosomatic index	Condition factor
T1: Control	2.086 a	1.897 b	0.185 a	1.191 abcd
T2 10 % Black seeds	3.063 a	2.007 b	0.199 a	1.141 bcd
T3 1 Garlic powder	3.050 a	1.034 b	0.242 a	1.187 abcd
T4 10% Black seed + % 1 Garlic	2.360 a	4.594 a	0.277 a	1.090 cd
T5 5% Black seeds powder	2.288 a	1.704 b	0.155 a	1.121 bcd
T6 0.5 % Garlic	2.269 a	1.818 b	0.272 a	1.339 a
T7 10% Black seed + % 0.5 Garlic	2.515 a	2.135 b	0.257 a	1.240 abc
T8 5 % Black seed + % 1 Garlic	3.131 a	1.970 b	0.296 a	1.267 ab
T9 5 % Black seed + % 1 Garlic	3.288 a	1.468 b	0.232 a	1.075 d



Mean values with different mention letter within a column differ significantly ($P \leq 0.05$).

The results of Martins *et al.*, (2002) who verified that addition of *A. sativum* to fish diets was increased erythrocytes number, hemoglobin content, hematocrit value, leucocytes, and thrombocytes. *A. sativum* has some constituents that may play a role in the immune system stimulation and in the function of organs related to blood cell formation such as thymus, spleen, and bone marrow (Jeorg and Lee, 1998) and this may be the cause of the differences in liver and spleen weight that observed in the present results.

Ndong and Fall, (2007) who reported that WBC counts increased significantly in juvenile hybrid tilapia fish fed 1% and 0.5% garlic supplemented diet. Also, the astronomic increase of total WBC counts in the 0.5% garlic in diet than in the other treatments suggests that 0.5g/kg is the concentration that stimulates and improves the immunologic functions of the blood. This concentration is appropriate for the overall increase in resistance of fish to environmental stressors.

Martins *et al.*, (2002) who verified that addition of *A. sativum* to fish diets was increased erythrocytes number, hemoglobin content, hematocrit value, leucocytes, and thrombocytes. *A. sativum* has some constituents that may play a role in the immune system stimulation and in the function of organs related to blood cell formation such as thymus, spleen, and bone marrow (Jeorg and Lee, 1998).

From table (4) no significant differences observed in fish weight without viscera, significant differences found among the treatments in Fish weight without viscera & head. After ten days the a significant differences observed in Fish weight without viscera, the control and T8 with 5 % Black seed + % 1 Garlic were lower significantly in Fish weight without viscera & head.

Table (4): Effect of Adding Garlic (*Allium sativum*), Black Seed (*Nigella Sativa*) and their combination on Fish weight without viscera and weight without viscera & head in young common carp (*C. carpio L.*) in two different times.

Treatments	Fish weight without viscera		Fish weight without viscera & head	
	First time	Second time	First time	Second time
T1: Control	85.875 a	83.692 ab	57.467 abc	42.474 b
T2 10 % Black seeds	85.378 a	87.080 a	55.847 abcd	56.768 a
T3 1 Garlic powder	88.989 a	83.855 ab	54.072 cd	55.426 a
T4 10% Black seed + % 1 Garlic	83.002 a	82.298 ab	52.863 d	52.265 a
T5 5% Black seeds powder	85.284 a	83.536 ab	54.778 bcd	54.666 a
T6 0.5 % Garlic	85.522 a	82.033 ab	58.566 a	54.536 a
T7 10% Black seed + % 0.5 Garlic	84.726 a	81.760 b	56.556 abc	53.929 a
T8 5 % Black seed + % 1 Garlic	86.012 a	82.175 ab	57.830 ab	50.990 ab
T9 5 % Black seed + % 1 Garlic	87.883 a	86.289 a	55.287 abcd	56.587 a

Mean values with different mention letter within a column differ significantly ($P \leq 0.05$).

The contents of crude protein, crude lipids, amino acids, water loss rate, and folding endurance of muscle may reflect the flesh quality to some extent. Long-term garlic supplemented feeding may lower the lipid and cholesterol content of fish. Moreover, allicin could activate intestinal proteases, which help to convert feed protein into fish protein, increasing the content of palatable amino acids. Shakya and Labh, (2014) reported that the post-harvest flesh quality and shelf-life of fish fed a garlic-supplemented diet were improved. Metwally found that the protein content in whole fish increased significantly in the group fed a garlic-containing diet, whereas the total lipid and ash contents decreased significantly in the same group. It has been proved that garlic has immunomodulatory properties and is well capable of enhancing protection against pathogens. Hence, it can be concluded that garlic supplemented diets in fish enhance growth rate and improves the immune response in aquaculture. Garlic can also be used as an alternative to antibiotics or chemotherapeutic agents; however, further research is needed under practical conditions (Shakya and Labh, 2014).

Despite many studies on investigated the effects of garlic in aquaculture, little is known about the effect of garlic on flesh quality, currently, there is no perfect system for evaluating the flesh quality of cultured fish, nonetheless, the contents of crude protein, crude lipids, amino acids, water loss rate, and folding endurance of muscle may reflect the flesh quality to some extent. Long-term feeding may lower the lipid and cholesterol content of fish. Moreover, allicin could activate intestinal proteases, which convert feed protein into fish protein, increasing the content of palatable amino acids. Xiang and Liu, (2002) reported that the addition of 25–100 mg/kg garlic to the diet had increased the crude protein content and reduced the crude lipid content of *C. barchypomum*.

Luo *et al.*, (2008) found that a compound from *Eucommia ulmoides* and garlic could improve the flesh quality of grass carp, *C. idellus*. Aly *et al.*, (2008) reported that the post-harvest flesh quality and shelf life of fish fed a garlic-supplemented diet were improved. Metwally, (2009) found that the protein content in whole fish increased significantly in the group fed a garlic containing diet, whereas the total lipid and ash contents decreased significantly in the same group. These results are in agreement with those obtained by (Abdelhamid *et al.*, 2005; Xiang and Liu, 2002; Khattab *et al.*, 2004; Shalaby *et al.*, 2006 and El-Dakar *et al.*, 2008).

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