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EFFECT OF *ALLIUM SATIVUM* AS ANTIBIOTIC AND CHLORAMPHENICOL ANTIBIOTIC ON HEMATOLOGICAL AND BIOCHEMICAL CHANGES IN *OREOCHROMIS NILOTICUS*

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Abstract

The present investigations show the effects of garlic and chloramphenicol on physiological changes in *Oreochromis niloticus*. Fish (100gm±1) were classified into seven groups control group and six treated groups, with three replicates each. Treatment groups had a different level of garlic (*Allium sativum)* (4, 8, and 16gm/kg diet) and chloramphenicol (10, 20, and 40mg/kg diet) added to their diets; the control group diet was free from garlic and chloramphenicol. Blood parameters, erythrocyte count (RBC), hematocrit and hemoglobin content in fish treated with 16gm/kg garlic and 20mg/kg chloramphenicol were significantly higher than in control. There were no significant differences in the mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). However, the values obtained for the white blood cells (WBCs) were significantly higher for all groups treated with garlic and chloramphenicol than that control group. There was significant increase in the total protein but decreased in total lipids, cholesterol and triglycerides levels in the groups treated with 16gm/kg garlic and 20mg/kg chloramphenicol than that control group. Plasma glucose, AST, ALT, ALP, uric acid and creatinine were decreased significantly with increasing doses of garlic and chloramphenicol than that control group. The values obtained for the calcium (Ca), sodium (Na) and potassium (K) were significantly higher for all groups treated with garlic and chloramphenicol than that control group. garlic and chloramphenicol administration led to significant hypolipidaemia, accompanied by decreases in liver total lipids and a significant rise in the white muscle total lipids. Garlic and chloramphenicol administration led to the liver and muscle TFAA decreased. Thus, it can be concluded that the treatment of fish with garlic and chloramphenicol can improve fish health by increase the activity of fish due to improvement the changes in blood aspects. Also, the garlic is natural, cheaper and better than chloramphenicol for improvement health of fish.

Keyword: Oreochromis niloticus, garlic, chloramphenicol, blood aspects.

INTRODUCTION

Allium sativum, commonly known as garlic. It is beneficial effects, such as anticancer, antidiabetic, cardioprotective, antioxidant potential as well as its possible harmful effects. The major constituents of the plant, such as sulfur-containing compounds, terpenoids, and phenoids (E)-Ajoene, (Z)-Ajoene, Allicin. Alliin, Allixin, γ-Glutamyl-S-2-propenyl cysteine, Diallyl disulfide, Methyl allyl disulfide, S-allyl-cysteine and 1,2-Vinyldiithin (Kuete, 2017). Garlic (*Allium sativum* L.) is considered one of the twenty most important vegetables, with various uses throughout the world, either as a raw vegetable for culinary purposes, or as an ingredient of traditional and modern medicine. Furthermore, it has also been proposed as one of the richest sources of total phenolic compounds, among the usually consumed vegetables, and has been highly ranked regarding its contribution of phenolic compounds to human diet (Natália and Petropoulosa, 2016). The immune stimulants result in enhancing the activity of the non-specific defense mechanisms and increasing disease resistance (Abass *et al.*, 2018 and Raa, 1996). Garlic increases the welfare of fish, and it can help in the control of pathogens, especially bacteria and fungi (Corzo-Martinez *et al.*, 2007). Disease outbreaks were recently identified as a major constraint to aquaculture production and trade, with a consequent effect on the industry’s economic development (Yunxia *et al.*, 2001). (Latife *et al.*, 2016 and Abraham and Ritu, 2015) demonstrated that bacterial infection can negatively alter blood biochemical profile of C. gariepinus, and dietary supplementation of garlic extract would help improve the resistance of fish to E. tarda infection in culture condition. Over the past 20 years’ various chemotherapeutics have been used to treat bacterial infections in cultured fish (Aoki). The use of disinfectants and antimicrobials has shown limited success in preventing or curing aquatic diseases (Subasinghe, 1997). Probably known as one of the earliest medicinal plants (Farahi *et al.*, 2010), the use of garlic in aquaculture became popular for providing protection against diseases or inducing fish feeds as a growth promoter. Garlic was used as a growth promoter in tilapia (Diab *et al.*, 2002; Shalaby *et al.*, 2006; Metwally, 2009 and

AbdelHakim *et al.,* 2010), in Asian seabass (Talpur and Ikhwanuddin, 2012), in sterlet sturgeon (Lee *et al.*, 2014), and in Seabass fry (Saleh *et al.,* 2015). Fish culture is an important industry in which the production of fish worldwide increases every year. Intensification of fish adversely affects fish health and tends to produce a poor environment for fish, increasing their susceptibility to infections (Sakai, 1999). However, the use and abuse of antimicrobials in aquaculture can help to growing, but they result in the selective pressure exerted on the microbes and encourage the emergence of resistant bacteria by transferring resistance-genes to bacteria not exposed to antibiotics. In addition, the antimicrobial besides having a negative impact on the environment, result in drug residues in the treated fish (FAO/WHO/OIE 2006). Antimicrobials can generate cross-resistance against human antimicrobials, which could pose a hazard (Witte *et al.,* 1999). Vaccination may prevent fish disease outbreaks, but the development of vaccines against many intracellular pathogens has not yet been successful. Therefore, the immediate control of all fish diseases using only vaccines is impossible, and commercial vaccines are expensive for fish producers, and may not be available against the encountered and emerging diseases (Raa *et al.*, 1992). Not only application of immune stimulants have more effective approach to controlling disease in aquaculture through the enhancement of natural disease resistance, but also contribute to health management in aquaculture through the enhancement of immune competence and disease resistance in fish (Sakai, 1999). The garlic has several beneficial effects including antioxidant, antihypertensive, and antimicrobial properties (Sivam, 2001).

The present study was aimed at evaluating the effect of garlic (*Allium sativum)* and chloramphenicol an antibiotic and an immune stimulant, on the hematological parameters and plasma activities of protein, lipid cholesterol, triglycerides, ALT, ALP, AST, uric acid, creatinine and electrolytes (Ca, Na and K) of *Oreochromis niloticus*.

MATERIALS AND METHODS

The study was conducted in Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharkia, Egypy. The fish were transferred to the physiology Laboratory. Fish were exposed to different concentrations of (4, 8 and 16 gm/kg of garlic and 10, 20 and 40 mg/kg of chloramphenicol). Control diet fishmeal was free from both garlic (*Allium sativum)* and chloramphenicol. Treated diet were formulated from fishmeal, garlic and chloramphenicol. Chloramphenicol as powder and Garlic without skin were purchased from the local market and the garlic was dried in a Freeze Drier and ground to become powder. chloramphenicol and garlic were transformed into pellet form by Food grinder. After being dried, the pellets were transferred to plastic bags and stored in a freezer at average -3°C until immediately prior to feeding as in table 1. The Nile tilapia (*Oreochromis niloticus*) were divided into four experimental groups each containing 10 animals as follows Control group and three groups of (4, 8 and 16 gm/kg of garlic and 10, 20 and 40 mg/kg of chloramphenicol). The fish treated and fed three times daily for 40 days.

Table 1. Composition of experimental diets (g/100g dry diet).

Items	Experimental diets						
	Control	Garlic 4gm/kg	Garlic 8gm/kg	Garlic 16gm/kg	Chloramphenicol 10mg/kg	Chloramphenicol 20mg/kg	Chloramphenicol 40mg/kg
Fish meal	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Soybean meal	24.00	24.00	24.00	24.00	24.00	24.00	24.00
Starch	47.00	43.00	39.80	29.75	37.00	27.80	7.75
Corn oil	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Natural garlic	-	4.00	8.00	16.00	-	-	-
Chloramphenicol	-	-	-	0.25	10	20	40
Vit mix *	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Min mix *	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cellulose	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00

**Vitamin mix:* Each 2.5kg contained vit A 10 mIU, D 3 mIU , E 10gm , B_1 1g , B_2 4g , B_6 1.5g , B_{12} 10mg , Pantothenic 10g , Nicotinic acid 20g , Folic acid 1000 mg , Biotin 50 mg and Choline Chloride 500 mg.

***Min mix:* Each kg contains 727.78 g $CaHpo_4$. $2H_2o$, 127.5g $MgSo_4$. $2H_2o$, 60g NaCl, 25g $FeSo_4$. H_2o, 5.5g $ZnSo_4$, 0.48g $CoSo_4$. 7 H_2o, 0.3g Ca $(Io_3)_2$ $6H_2o$, 0.13g $CrCl_3$. 6 H_2o and 50g Kcl.

Hematological analysis:

At the end of the experiment, blood samples were collected from the fish caudal vein by a sterile syringe containing heparin as an anticoagulant. Blood samples were placed into microtubes (2.0 mL) containing sodium heparin (50 IU) anticoagulants. All samples were collected in the early morning hours and were processed for hematological analysis. Samples were transported in a refrigerated cooler to the physiology Laboratory. Blood smears were prepared in duplicate and were stained with rapid hematological dye. The total cell count (erythrocytes, leukocytes, and thrombocytes) were performed by the diluent/dye direct method outlined by Soivio & Oikari, 1976 and Natt and Herrick (1952) in a Neubauer chamber at a dilution of 1:100. Following the total cell count of nucleated cells (leukocytes and thrombocytes) in the Neubauer chamber, a differential count of leukocytes and thrombocytes were performed in the stained sample. The packed cell volume was determined by the microhematocrit technique described by Jain (1986). Blood was used for erythrocyte count (Dacie and Lewis 1984), hemoglobin content (Vankampen, 1961) and hematocrit value (Britton, 1963) determination. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated using the formulae mentioned by Dacie and Lewis (1984). Plasma was obtained by centrifugation at 3000rpm for 15min and the non-hemolyzed plasma was stored in a freezer at -20°C until analysis. Plasma protein content was determined by the Biuret method described by Wootton (1964). Glucose concentration was measured according to Trinder (1969), using Boehring Mannheium kits. Total lipids, cholesterol and triglycerides were determined calorimetrically using a kit supplied by El Nasr Pharmaceutical Chemical Co., according to Knight *et al*. (1972). Electrolytes, Creatinine, uric acid, ALP. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically using kits supplied by Diamond Diagnostics, according to Reitman and Frankel (1975).

Data analysis:

The obtained data in this study were statistically analyzed for variance ANOVA, LSD (Least significant difference) according to (Snedecor and Cochran, 1982). Differences among treatment means were compared using Duncan's multiple range tests (Duncan, 1995). Data were presented as mean ± SE and significance was declared at ($P < 0.05$).

RESULTS

Results of erythrocytes count (RBC), hemoglobin content, and hematocrit percentage are given in table 2. It shows that diets containing 4, 8, and 16gm/kg diet of garlic and all 10, 20 and 40mg/kg of chloramphenicol increased in all the examined blood parameters, which were significantly different from those of control. Erythrocyte count and hemoglobin content increased significantly in fish fed on diets containing all levels of garlic and chloramphenicol. Similarly, hematocrit values increased significantly in fish fed on 16gm/kg *of* garlic and 40 mg chloramphenicol / kg diet, respectively table 2. The blood indices calculated from the mean values of blood parameters are presented in table 2. It shows that RBC indices include: Mean cell (or corpuscular) volume (MCV), Mean cell hemoglobin (MCH) and Mean cell hemoglobin concentration (MCHC) were significantly increased in Nile tilapias fed on diets containing high levels of chloramphenicol. While, MCV, MCH and MCHC were significantly different among fish fed on diets containing all levels of garlic as shown in table 3. Numbers of WBCs, neutrophils, lymphocytes, eosinophils, basophils, monocytes and thrombocytes of *Oreochromis niloticus* are presented in table 4. Among all groups, the total number of WBCs, lymphocytes, eosinophils, basophils, monocytes and thrombocytes of *Oreochromis niloticus* were significantly higher in the garlic diet than that control group. But there were no significantly different in the WBCs), lymphocytes, eosinophils, basophils, monocytes and thrombocytes of *Oreochromis niloticus* in the chloramphenicol groups than control group. WBCs, lymphocytes, eosinophils, basophils, monocytes and thrombocytes of *Oreochromis niloticus* were evaluated between the garlic was significantly higher than that of the chloramphenicol groups, both of which were significantly increase compared to the control group as shown in table 4.

Table 2. Effect of Garlic (4, 8 and 16gm/kg) and Chloramphenicol (10, 20 and 40mg/kg) on red blood cells (RBCs), hematocrit HCT and hemoglobin (Hb) of *Oreochromis niloticus.*

Doses / parameters	**Control**	**Garlic**			**Chloramphenicol**		
		4gm/kg	**8gm/kg**	**16gm/kg**	**10mg/kg**	**20mg/kg**	**40mg/kg**
RBCs ($x10^6/\mu L$)	1.6±0.3	1.7±0.4	1.8±0.4*	2.1±0.3*	1.65±0.2	1.7±0.2*	2±0.3*
HCT (%)	27.2±1.2	28.1±1.5	29.1±1.4*	30.3±2.3*	27.5±1.5	28.1±2.1 *	31±2.1*
Hb ($g\ dL^{-1}$)	7.1±1.2	7.5±1.5	7.9±1.5*	8.4±1.5*	7.3±1.1	7.9±1.6*	8.2±1.4*

* Significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001 (significant differences between treated groups and control

Table 3. Effect of Garlic and Chloramphenicol o mean corpuscular volume (MCV), Mean cell hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of *Oreochromis niloticus.*

Doses / parameters	Control	Garlic			Chloramphenicol		
		4gm/kg	8gm/kg	16gm/kg	10mg/kg	20mg/kg	40mg/kg
Mean cell (or corpuscular) volume (MCV)fL10^{-15}	15.5 ±1.1	16.0 ± 2.2	17.1 ± 1.2*	18.6 ±3.4**	15.8 ± 2.1	16.1 ± 1.6*	17.1 ±3.4**
Mean cell hemoglobin (MCH) pg 10^{-12}	27.3 ±1.5	28.7 ±2.7	30.5 ±1.5*	31.4 ±2.7*	27.6 ±2.1	29.2 ±1.5*	30.4 ±2.5**
Mean cell hemoglobin concentration (MCHC)g/dL	29.2 ±1.1	28.9 ±2.4	27.5 ±1.5*	26.4 ±2.7**	28.6 ±2.1	27.2 ±1.5*	26.4 ±2.5**

* Significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001 (significant differences between treated groups and control.

Table 4. Effect of Garlic (4, 8 and 16gm/kg) and Chloramphenicol (10, 20 and 40mg/kg) on white blood cells (WBCs), neutrophils, lymphocytes, eosinophils, basophils, monocytes and thrombocytes of *Oreochromis niloticus.*

Doses / parameters	control	Garlic			Chloramphenicol		
		4gm/kg	8gm/kg	16gm/kg	10mg/kg	20mg/kg	40mg/kg
Total Leuk. /µL	5212 ±124	5433 ±145*	5355 ±153*	4945 ±111*	5256 ±144*	5365 ±122*	4933 ±151*
Seg. Neutro. /µL	1811 ±112	1991 ±111*	2101 ±222**	2231 ±122**	1999 ±122*	2133 ±211**	2099 ±111*
Lymphocytes/µL	2511 ±131	2722 ±131*	2888 ±111**	2891 ±221**	2599 ±112*	2676 ±143*	2867 ±344**
Eosinophyls/µL	144 ±16	155 ±25*	199 ±11**	204 ±22***	165 ±18*	189 ±33**	199 ±19***
Basophyils/µL	211 ±35	231 ±21*	255 ±21**	266 ±24***	233 ±15*	246 ±41**	263 ±55**
Monocytes/µL	1222 ±211	1452 ±111*	1466 ±131*	1467 ±111*	1311 ±122*	1454 ±133**	1466 ±122**
Thromb/µL	34111 ±2300	34341 ±1511*	34811 ±4333*	34966 ±4455**	33511 ±1131*	34764 ±3452**	34593 ±4422*

* Significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001 (significant differences between treated groups and control.

Total protein content in fish body was significantly higher in the group fed on diet containing 16gm / kg diet of garlic than in all other groups of garlic and chloramphenicol treatments, and control (Table 5). Total plasma protein increased significantly in all groups when compared to control group. The mean value of total plasma lipid level decreased significantly in fish fed on diets containing 8 and 16gm garlic and 20mg chloramphenicol / kg diet, respectively. But, it increased significantly in fish fed on 40mg chloramphenicol / kg diet table 5. Total lipids, cholesterol and triglycerides levels in fish body decreased significantly in fish fed on diet containing 16gm / kg diet of garlic than in all other groups of garlic and chloramphenicol treatments, and control (Table 5). Table 5 shows significant decreases of plasma glucose in fish fed on diets containing 4, 8, and 16gm / kg of garlic diet. But, these values were significantly higher in fish administered with higher levels of chloramphenicol (20 and 40mg / kg diet).

Table 6 show that the level of aspartate aminotransferase (AST) activity in plasma was decreased significantly with increasing levels of *garlic* and chloramphenicol. The highest values were obtained in control group, 4gm/kg garlic and 10mg/kg chloramphenicol group, without significant differences. Plasma ALT activity after treatment with *Allium sativum* and chloramphenicol varied significantly between groups, whereas a significant reduction and was recorded in fish fed on diets containing 4, 8 and 16gm/kg garlic, respectively. The same result occurs in fish fed on 10, 20 and 40mg/kg of chloramphenicol diet table 6. The levels of ALP, uric acid urea and creatinine in plasma were decreased significantly with increasing levels of garlic and chloramphenicol. The highest values were obtained in control group, 4gm/kg garlic and 10mg/kg chloramphenicol group, without significant differences as shown in table 6.

Table 7 show that the levels of Ca, Na and K in plasma were increased significantly with increasing levels of garlic and chloramphenicol. The lowest values were obtained in control group, 4gm/kg garlic and 10mg/kg chloramphenicol group, without significant differences as shown in table 7. But there were higher significant in electrolytes with increasing doses of 16gm/kg garlic and 40mg/kg chloramphenicol group.

Table 5. Effect of Garlic (4, 8 and 16gm/kg) and Chloramphenicol (10, 20 and 40mg/kg) on total protein, lipids profile, glucose of *Oreochromis niloticus.*

Doses / parameters	Control	Garlic			Chloramphenicol		
		4gm/kg	8gm/kg	16gm/kg	10mg/kg	20mg/kg	40mg/kg
Total protein (g/dl)	6.5±0.2	6. 7 ± 0.3*	6.9 ±0.4*	7 ±0.5*	6.8 ±0.5*	6.7 ±0.2*	6.9 ±0.4*
Total lipid	4.66±0.1	4.55±0.1	4.41±0.2	4±0.1	4.56±0.2	4.3±0.2	4.1±0.2
Cholesterol (mg/dl)	191 ±12	190±11	184±12**	163±11***	190±22	188 ±11*	180±22**
Triglycerides (mg/dl)	3.8 ±0.5	3.5 ±0.1	3.2±0.2*	3±0.1*	3.6 ±0.1	3.4 ±0.1*	3.1 ±0.8*
Glucose (mg/dl)	103 ±1.34	102 ±1.5	100 ±1.6*	97±1.9***	101 ±1.6	97±1.2***	94±1.6***

* Significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001 (significant differences between treated groups and control)

Table 6. Effect of Garlic (4, 8 and 16gm/kg) and Chloramphenicol (10, 20 and 40mg/kg) on liver functions and kidney functions test of *Oreochromis niloticus.*

Doses / parameters	Control	Garlic			Chloramphenicol		
		4gm/kg	8gm/kg	16gm/kg	10mg/kg	20mg/kg	40mg/kg
AST (u/l)	48 ± 0.22	47 ± 0.43	44 ± 0.27*	40 ± 0.56***	46 ± 0.55*	42 ±).65**	41± .38***
ALT(u/l)	23± 0.3	22± 0.5	20 ± 0.11*	18 ± 0.22**	22.3± 0.33	21± 0.4*	19± 0.4**
ALP (u/l)	28± 0.6	27± 0.5	26 ± 0.22*	24 ± 0.3**	27± 0.6	25± 0.4*	23± 0.6**
Uric acid (mg/dl)	14 ± 0.2	14 ± 0.4	13± 0.1	11± 0. 3**	14± 0.3	11± 0.2**	10 ± 0.9**
Creatinine (mg/dl)	0.27 ± 0.2	0.25 ± 0.01*	0.22 ± 0.02**	0.2± 0.02***	0.24 ± 0.03*	).23 ± 0.02*	).21 ±).05**
Urea (mg/L)	53.8± 4.7	52.5± 3*	46.6± 5**	33.6± 2***	49.6± 4*	44.6± 3***	40± 3***

* Significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001 (significant differences between treated groups and control)

Table 7. Effect of Garlic (4, 8 and 16gm/kg) and Chloramphenicol (10, 20 and 40mg/kg) on electrolytes of *Oreochromis niloticus.*

Doses / parameters	Control	Garlic			Chloramphenicol		
		4gm/kg	8gm/kg	16gm/kg	10mg/kg	20mg/kg	40mg/kg
Ca mmol/L	12.5±1	12.7±2*	12.9±1*	12.9±2*	12.6±1*	12.7±2*	12.8±1*
Na mmol/L	155±3	156±4	158±2*	159±4*	155±3	157±5*	158±2*
K mmol/L	5.56±0.2	5.6±0.3	5.8±0.3*	5.9±0.5*	5.55±0.2	5.62±0.4*	5.7±0.1*

* Significant at $p<0.05$, ** highly significant at $p< 0.01$, *** very highly significant at $p < 0.001$ (significant differences between treated groups and control)

Tables 8 & 9 indicate that garlic and chloramphenicol administration led to severe hypoglycaemia after all periods of the experiment accompanied by liver glycogen depletion and elevation of white muscle glycogen at the end of the experiment. The tables also show that garlic and chloramphenicol administration led to significant hypolipidaemia, accompanied by decreases in liver total lipids and a significant rise in the white muscle total lipids. Garlic and chloramphenicol administration led to the liver and musle TFAA in response. This result was accompanied with a transitory decreased in the liver cholesterol and insignificant changes in its levels in white muscle. There were significant decreases in both serum triglyceride and cholesterol levels in response to garlic and chloramphenicol administration, while garlic and chloramphenicol administration led to increase the white-muscle triglyceride content. Garlic and chloramphenicol administration led to decreased the serum levels of both uric acid, creatinine and alkaline phosphatase.

Table 8. Effect of Garlic (4, 8 and 16gm/kg) and Chloramphenicol (10, 20 and 40mg/kg) on some liver constituents in the fish, of *Oreochromis niloticus.*

Doses /	Control	Garlic			Chloramphenicol		
parameters		4gm/kg	8gm/kg	16gm/kg	10mg/kg	20mg/kg	40mg/kg
Glycogen (mg/g dry wt)	119 ±8.2	123 ±8.2*	90 ±6.4**	135 ±11**	101 ±6.7**	124 ±12*	109 ±7**
Total lipids (mg/g dry wt)	74 ±6.2	73.6 ±2	71.0 ±2*	68.6 ±4**	70.9±6*	62.2±4***	46±3***
Triglycerides	28 ±2.4	29.6 ±2	22 ±1.6**	28.2 ±1	19.8 ±1.9***	21.7 ±2***	21 ±2.6***
Total lipid/triglyceride ratio (%)	41 ±5.0	40 ±5	31.1 ±1.9***	42 ±3.3	30.0 ±2***	34.3 ±3***	46.6 ±3**
Cholesterol (mg/g dry wt)	4.13 ±0.24	3.53 ±0.3*	3.5 ±0.2*	3.68 ±0.1*	3.93 ±0.3*	3.38 ±0.3*	3.38 ±0.35*
TFAA (μg/g dry wt)	490 ±41	436 ±50***	430 ±52***	390 ±41***	431 ±51***	430 ±53***	395 ±22***

* Significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001 (significant differences between treated groups and control).

Table 9. Effect of Garlic (4, 8 and 16gm/kg) and Chloramphenicol (10, 20 and 40mg/kg) on some white muscle constituents in the fish, of *Oreochromis niloticus.*

Doses /	Control	Garlic			Chloramphenicol		
parameters		4gm/kg	8gm/kg	16gm/kg	10mg/kg	20mg/kg	40mg/kg
Glycogen (mg/g dry wt)	11 ±1.3	14.5 ±1*	10.2 ±0.7**	15.0 ±1**	9.7 ±1.1*	13.2 ±0.8*	13 ±0.8*
Total lipids (mg/g dry wt)	104 ±4	111 ±3*	101 ±10.3*	100 ±10.7*	99.8 ±6*	99 ±9.6*	100 ±9.6*
Triglyceride (mg/g dry wt)	38.8 ±2	40 ±4	37.9 ±3.7	37 ±3.5*	34 ±2.6**	35 ±4.5*	36 ±4.5*
Total lipid/ triglyceride ratio (%)	37.8 ±5.7	37 ±3	38 ±2	33 ±2.8**	33 ±4**	43 ±2**	43 ±1**
Cholesterol (mg/g dry wt)	4.13 ±0.24	3.53 ±0.3*	3 ±0.2*	3.3 ±0.26*	3.4 ±0.38*	3.8 ±0.3*	3.3 ±0.5*
TFAA μg /g dry wt	447 ±22	444 ±40	431 ±47*	388 ±43***	411 ±37**	413 ±22**	388 ±41***

* Significant at p<0.05, ** highly significant at p< 0.01, *** very highly significant at p < 0.001 (significant differences between treated groups and control).

DISCUSSION

Garlic is an important vegetable extensively cultivated in many countries. It is used as food for humans as well as some animals and as remedy for several diseases, chemical complexity and broad-spectrum action, not promoting acquisition of antibiotic resistance. In addition, direct intergalactic effects are feasible because *Allium sativum* antimicrobials are not affected by acid environments (LAWSON, 1996 and Abass *et al.*, 2018). Chloramphenicol supplementation as a feed additive antibiotic has a greater effect than diets without antibiotics in improving live body weight and feed conversion efficiency in broiler chicks. Antibiotics are largely used for prophylaxis and treatment to eliminate or reduce bacterial contamination to a degree that enhances host defense mechanism (SMITH, 1992). The present study aimed to show the effects of garlic and chloramphenicol on the health of Nile tilapia. garlic was used at the levels 4, 8 and 16gm/kg diet and chloramphenicol at 10, 20, and 40mg/kg diet. These doses partially agree with those mentioned by Diab *et al*. (2002) and Abass *et al.*, 2018, who obtained the highest growth performance in *Oreochromis niloticus* with 2.5% garlic / kg diet. Total lipid content decreased significantly with the same levels of *Allium sativum* and chloramphenicol. These results agreement with those obtained by Shalaby *et al.*, 2006; Metwally, 2009; Abdelhamid *et al*. (2002) and Diab *et al*. (2002) who reported that there were no significant changes in fish body composition caused by different garlic levels. The employment of hematological techniques, including evaluation of erythrocytes count, hemoglobin concentration, hematocrit and leucocytes count, has provided valuable knowledge for fishery biologists in the assessment of fish health (Blaxhall, 1972) and in monitoring stress responses (Soivio and Oikari, 1976). These results reflect the health status of fish cultured with all treatments. The present study revealed that administration of garlic or antibiotic induced significant increases in all blood parameters (erythrocyte count, hemoglobin content, and hematocrit value) in treated fish, these results agreement with the results of Abass, *et al.*, 2018 and Martins *et al*. (2002), who verified that addition of *Allium sativum* to fish diets increased erythrocytes number, hemoglobin content, hematocrit value, leucocytes, and thrombocytes. *Allium 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). Also, Faisal (2003) reported significantly increased values of erythrocyte count, hemoglobin content, and hematocrit in catfish *Clarias garepinus* at the 1st and 3rddays after administration of ciprofloxacin, amoxycillin and ampicillin. Blood indices (MCV, MCH and MCHC) are particularly important for the diagnosis of anemia in most animals (Coles, 1986). This study showed a significant increase of MCH and MCHC in fish fed on the highest level of chloramphenicol. So, it is assumed that the decrease or increase of blood indices may be attributed to a defense reaction against chloramphenicol, which occurs by stimulation of erythropoiesis. Changes in the physiological state often reflect alteration of hematologic and blood biochemical values. Clinical chemical analysis is a fundamental tool used to diagnose and predict the outcome of diseases and to monitor the effects of therapeutic, nutritional and environmental management in human and veterinary medicine. In the present study, plasma glucose concentration reduced significantly in fish fed on diets containing the highest levels of garlic diet. These results agreement with those of Kumar and Reddy (1999); Abass, *et al.*, 2018 and Thomson (2003), who found that feeding mice with 45mg garlic / kg body weight for 28 days induced significant decrease of serum glucose levels. Lower levels of plasma glucose in fish have also been reported in the assessment of physiological effects of *Allium sativum* (Sheela and Augusti, 1992). On the other hand, the significant elevated ($p<0.001$) plasma glucose level in fish administered with all doses of chloramphenicol, compared with control, indicated that this antibiotic affects glucose dynamics in *O. niloticus* in order to obtain more energy to withstand and overcome the existing stress condition. Plasma or serum glucose level is often used as an indicator of non-specific stress (Hunn and Greer, 1991). Our results agreement with those of Tarter (1986) and Faisal (2003), who verified that plasma glucose concentration in catfish (*Clarias garepinus*) increased significantly after oral administration of ciprofloxacin, amoxycillin and ampicillin. decreased blood glucose levels might have been due to a glucose splits. Blood serum protein is a fairly labile biochemical system, precisely reflecting the condition of the organism and the changes happening to it under influence of internal and external factors. Significant hyperproteinemia was observed in all fish groups administered with garlic diet and the two highest levels of chloramphenicol. Total plasma protein was significantly high, which agrees with the results of (Abraham and Ritu, 2015; Latife *et al.*, 2016 and Lee *et al.*, 2014 and Hussein *et al*. 2001), who showed that serum total protein content was elevated in Male Albino rats after administration of garlic oil. Increase in the serum total protein level in hyperlipidemic rats treated with *Allium sativum* oil could be attributed to the increase in the immunoglobulin level and total globulin concentration (Hussein, 1996). Also, Faisal (2003) found that serum total protein increased significantly in *Clarias garepinus* after administration of both ciprofloxacin and amoxycillin antibiotics. High serum protein levels have been reported to be indicative of osmoregulatory dysfunction, hemodilution, or tissue damage surrounding blood vessels. Reduction of total lipid in plasma of *O. niloticus* fed on diets containing high doses of *Allium sativum* is in agreement with the study by Adler and Holub (1997), who verified that serum total lipid and total cholesterol decreased significantly in men treated with garlic and fish oil alone or combined. Also, Hussein *et al*. (2001) found that the serum total lipid decreased significantly in albino rats after administration of garlic. Also, the present study showed decreased in plasma lipid in Nile tilapia after administration of antibiotic diet. These results agree with those of Abdelhamid *et al*. (2002), who reported that fat content in muscles of Nile tilapia increased significantly after administration of 10g flavomycin / kg diet. It is also considered to be important in assessing the state of the liver and some other organs (Verma *et al.*, 1981). Therefore, attention has been focused on the changes in AST, ALT and alkaline phosphatase (ALP) activities, which promote gluconeogenesis from amino acid, as well as on the changes in aminotransferase activities in the liver (Rashatuar and Ilyas, 1983).

Furthermore, AST and ALT activities might be altered by a variety of chemical, biological, and physiological factors or by a disturbance in the Kreb's cycle. Decreased activity of the Kreb's cycle cause a decrease in its intermediates, thereby, ALT and AST compensate by providing –ketoglutarate (Salah and Rogers, 1993). Results of this study showed that serum AST and ALT activities decreased significantly in the fish group fed on all levels of *Allium sativum* and chloramphenicol. These data agreements with those reported by El-Shater *et al*. (1997) and Augusti *et al*. (2001), who found that the lipid parameters and enzyme activities (AST, ALT and ALP) in serum of rats decreased significantly when they were fed on a diet containing 5% *Allium sativum*. Also, Faisal (2003) mentioned reduced AST in serum of catfish after ampicillin administration. These results can be attributed to *Allium sativum*, which may cause stabilized cell membrane and protect the liver against deleterious agents and free radical-mediated toxic damages to the liver cells. This is reflected in the reduction of liver enzymes. *Allium sativum* helps the liver to maintain its normal function by accelerating the regenerative capacity of its cells.The present study was aimed at evaluating the effect of garlic (*Allium sativum)* and chloramphenicol an immune stimulant, on the hematological parameters and plasma activities of protein, lipid cholesterol, triglycerides, ALT, ALP, AST, uric acid, creatinine and electrolytes (Ca, Na and K) of *Oreochromis niloticus*. Data of the present study showed a significant drop in serum alkaline phosphatase (ALP) in response to effect of garlic (*Allium sativum)* and chloramphenicol. Similarly, garlic oil decreased serum ALP in streptozotocin-diabetic rats (Ohaeri, 2001). The present work showed diminished level of serum total free amino acids TFAA accompanied with significant drop in liver TFAA and insignificant change in white muscle TFAA in response to effect of garlic (*Allium sativum)* and chloramphenicol. This result may result from the elevation in insulin release in response to effect of garlic (*Allium sativum)* and chloramphenicol treatment in Nile tilapia. Our data showed that effect of garlic (*Allium sativum)* and chloramphenicol affect the serum total protein and decreased serum urea. This suggests that degeneration of some of the cells in the renal tubules was not great enough to leak serum protein *via* the kidney.In conclusion, the hypoglycaemia, hypolipidaemia, hypochosterolaemia, hypotriglyceridaemia and the decrease in the serum TFAA may be correlated to the elevation of insulin release in response to effect of garlic (*Allium sativum)* and chloramphenicol treatment. In addition effect of garlic (*Allium sativum)* and chloramphenicol enhanced glycogenesis and lipogenesis in white muscle. Effect of garlic (*Allium sativum)* and chloramphenicol diet may improve carbohydrate metabolism in fish, probably leading to lower dietary protein costs.

CONCLUSION

It could be recommended that garlic (*Allium sativum*) may be used as prevention of diseases and for enhancing fish tolerance to environmental stress; therefore garlic powder should be added to the diets of freshwater fish. In conclusion, the present study suggested that the effect of garlic (*Allium sativum)* and chloramphenicol an antibiotic and an immune stimulant, on the hematological parameters and plasma activities of protein, lipid cholesterol, triglycerides, TFAA ALT, ALP, AST, uric acid, creatinine, urea and electrolytes (Ca, Na and K) of *Oreochromis niloticus*.

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تأثير الثوم كمضاد حيوي والكلورامفينيكول المضاد الحيوي على التغيرات الدموية والكيميائية الحيوية في البلطي النيلي

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الملخـص العربــي

تُظهر هذه الدراسة تاثير الثوم والكلورامفينيكول على التغيرات الفسيولوجية في البلطي النيلي. تم تقسيم الأسماك (100 جم ± 1) إلى سبع مجموعات المجموعة الضابطة وست مجموعات معالجة، مع كل ثلاث مكررات. كانت مجموعات المعالجة تحتوي على جرعات مختلفة من الثوم ٤ ، ٨ ، و ١٦جم/كجم والكلورامفينيكول (١٠ ، ٢٠ ، و ٤٠ مجم/كجم المضافة إلى وجباتهم الغذائية ؛ كان النظام الغذائي لمجموعة الضابطة خالية من الثوم والكلورامفينيكول. كانت هناك زيادة معنوية في البروتين الكلي ولكن انخفضت في مستويات الدهون الكلية والكولسترول وثلاثي الجليسريد في المجموعات المعالجة بـ ١٦ جرام / كجم من الثوم و٢٠ مجم / كجم من الكلورامفينيكول من تلك المجموعة الضابطة. اظهرت المعاملات ان الدم وعدد كريات الدم الحمرا ومحتوى الهيماتوكريت والهيموجلوبين في الأسماك المعالجة بـ ١٦ جرام / كجم من الثوم و٢٠ مجم / كجم كلورامفينيكول أعلى بشكل كبير من مجموعة الضابطة. لم تكن هناك فروق ذات دلالة إحصائية في حجم الجسيمات MCV يعني متوسط تركيز الهيموجلوبين MCHC انخفضت البلازما الجلوكوز، AST، ALT، ALP، حمض اليوريك والكرياتينين بشكل ملحوظ مع زيادة جرعات الثوم والكلورامفينيكول من تلك المجموعة الضابطة. ومع ذلك، كانت القيم التي تم الحصول عليها لخلايا الدم البيضاء WBCs أعلى بشكل ملحوظ لجميع المجموعات المعالجة بالثوم والكلورامفينيكول من تلك المجموعة الضابطة. وبالتالي، يمكن الاستنتاج أن علاج الأسماك بالثوم والكلورامفينيكول يمكن أن يحسن صحة الأسماك عن طريق زيادة نشاط الأسماك بسبب تحسن التغيرات في جوانب الدم وأفضل جرعات للتحسين كانت ١٦ جم / كجم من الثوم و٢٠ مجم / كجم كلورامفينيكول. أيضا دلت النتائج ان الثوم منتج طبيعي ور خيص الثمن والأفضل من الكلورامفينيكول لتحسين صحة الأسماك.