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The Role of Herbal Oil Additives in Fresh Water Aquaculture

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Abstract

The current experiments (12 weeks) was carried out to assess the effect of the *Allium sativum* oil (AS) on two stages of *Oreochromis niloticus*. Fish were randomly distributed into four groups each with an initial weights (1.40±0.03 gm & 10.15 ± 0.82 gm) for the fry and fingerlings stage respectively, the first groups as a control, while AS_1, AS_2, and AS_3 were fed the basal diet supplemented with 0.5%, 1.0%, and 1.5 % of AS, respectively, for every stage. Fish were fed (3%) 6 days a week . The results displayed enhancement growth performance, final body weight (FBW), weight gain (WG), daily weight gain (DWG), specific growth rate (SGR),) and protein efficiency ratio (PER), with a significant reduction in feed conversion rate (FCR) in AS_2 (fries) and AS_3 (fingerlings). At the same trend our data were cleared an improvement ($p<0.05$) for the protein productive value (PPV) and fish protein digestibility (APD) through the two test fish groups. Better values of the biometric indices (liver and gut), and condition factor (CF) were recorded for those fed on (2% & 3%) garlic oil for fries and fingerlings respectively, than the control groups (0% AS). Serum levels of cholesterol reduced ($p<0.05$) with increasing the level of AS in diets, while plasma total protein, were improved ascendingly manner with garlic levels. Thus, in the present work, the addition of garlic oil to the Nile tilapia diets proved to enhance growth performance, innate immunity, and physiological status of fish hence, presenting a promising feed additive in aquaculture.

Keywords: *Allium sativum, Oreochromis niloticus, Growth performance, Biochemical and physiological parameters.*

1. Introduction

Fish of importance are farmed, increased intensification of aquaculture has led to many constraints including, poor growth, and poor health. This has made it hard for fish farmers to turn the biological benefits associated with intensive farming systems into economical gain. The wide adoption use of hormones to only increase reproduction appears to be production-oriented, thus unsustainable and unsafe. Hence, improvement of productions in aquaculture should also focus on environmentally friendly and with sustainable methods. Today, huge attention is focused on the use of medicinal herbal extracts to enhance production in aquaculture as replacement to chemical agents. and using this oils in tilapia aquaculture.

A wide range of medicinal oil were reported to enhance growth, appetite, immune response and, antioxidants components for fishes, more studies needed exist the application of herbal oils and their potential influence on fish. More research is therefore its necessary for its use in freshwater fish culture

The main target of using feed additives in fish diets, to enhancement the fish performance, immunity and flesh quality and perhaps for improving the digestibility of the fish [1,2]. Searching for new feed additives is still a very important target [3].

Feed additives have a positive effect on fish performance, so it widely used in aquaculture as growth promoters, immunustimulants, as Garlic, (*Allium sativum*) [1]**.**

Herbs such as garlic (*Allium sativum*) have been described in short notes and they enhancing the fish growth performance. Today we need an excess studies about the immune response for farmed fishes. An active compound of garlic, allicin can induce fish to ingest and increase feed intake.

Garlic (*Allium sativum*) is one of members of family Liliaceae used as a spice and in traditional medicine. It is rich in many minerals and bioactive components which considered has a rich nutritive value. Garlic also contains many valuable compounds such as iodine salts which have positive effects on the circulatory system, silicates which have a positive effect on the circulatory system and sulfur salts with huge effects on cholesterolemia, and control liver diseases [4]**.** *Allium sativium* also contains vitamins such as vitamins A, B and C and linoleic acid [5]. Garlic contains allicin, disulfide, and S-allylcysteine [6]. A wide array of beneficial effects of garlic such as antihypertensive, antihyperlipidemic, antimicrobial,

hypo-glycaemic, hepato-protective and immuno-modulation [7,8]. *Allium sativium* acts as antioxidant and an antihypertensive agent [9], control of pathogens, and may increases the welfare of fish [10].

The potential of herbal products is a astounding as they can be used to augment growth, minimize stress, improve immunity, and prevent various microbial infections in aquatic animals improving their health for the consumption of the end users [11].

Finally herbal oils application in fish culture are still rare, especially its effects on fish immunity, antioxidant activity and stress responses. Hence, this present study was conducted to evaluate the effect of garlic as a feed additive diets, which are considered economical and eco-friendly with fewer side effects, and its impact on growth performance and innate immunity of the Nile tilapia. Also we aims for using garlic oil in the two fish stages which each reared in different conditions like glass aquaria and cement.

2. Materials and methods

2.1. Fish and Experimental design:

This evaluation was performed with Nile tilapia, *Oreochromis niloticus*, two stages (fry and fingerlings) which were held under optimal conditions for a week before the onset of the growth trials. Fries with initial body weight 1.40 ±0.03, were randomly distributed and reared in 70-L glass aquaria (30x40x70 cm), which stocked with 15 fry per each. The aquaria were partially water changed day after day.

Fingerlings with initial body weight 10.15 ± 0.82 g were reared in outdoor concrete rectangular basin (4×10×1.5m), which divided into eight equal parts (2×2.5×1.5m) by fine nets were stocked with density 50 fish for each part. Water were renewed weekly. The two stages of fish were fed twice a day at 9:30 am, and 2:30 pm and weighed biweekly.

2.2. The experimental diets formulation

Fish were randomly dispensed equally into four groups, with three replicates for fries and fingerlings duplicated. Four isonitrogenous (34.90±0.26%) diets and (16.86 MJGE/kg) were prepared. Garlic oil, purchased from the local market produced by El-Captain company (Cap Pharm for extracting oils, herbs, and Cosmotics, El Obour city, Cairo, Egypt. (www.elcaptain-eg.com). The oil was included in diets at 0.5, 1.0, and 1.5 % rates, acted as the AS_1, AS_2, and AS_3 groups, respectively, besides to the control one. Composition and proximate analysis of the experimental diets are presented in Table (1). The test diets were prepared and adjusted to fulfill the recommended nutritional needs of the Nile tilapia according to the National Research Council [12].

The ingredients were mechanically mixed and pelletized using a pellet machine, and the diets were air-dried for 24 h and stored in a refrigerator at 4°C.

2.3. Water quality

Water quality includes ammonia, pH, water temp. and the dissolved oxygen through the 84 days for the glass aquaria or cement ponds detected the normality ranges that adequate for rearing all the fish test groups (fries and fingerlings).

2.4. Growth performance and feed utilization.

The fish samples were randomly collected biweekly to investigate the growth parameters that were calculated according to [13] as the following equations

Average weight gain (**AWG**, g /fish) = [final body weight (g) - initial body weight (g)];

daily weight gain, (**DWG**, g /fish /day) = [AWG (g) / Experimental period (days)];

Specific growth rate (**SGR**, %g/day) = 100x (Ln final weight - Ln initial weight) / time intervals (day);

Feed conversion ratio (**FCR**) = feed intake (g) / body weight gain (g);

Protein efficiency ratio (**PER**) = gain in weight (g) / protein intake in feed (g);

Protein productive value (**PPV**, %) =100 [protein gain in fish (g) / protein intake in feed (g)];

Table 1: The experimental diet formulation and chemical composition .

Ingredient (%)	Experimental diet			
	Control	AS_1	AS_2	AS_3
Fish meal	18	18	18	18
Soybean meal	40	40	40	40
Yellow corn	14	14	14	14
Gluten	6	6	6	6
Wheat bran	20	20	20	20
Cr_2O_3	0.5	0.5	0.5	0.5
Vitamins and minerals[1]	1	1	1	1
Sun flower oil	1	1	1	1
Garlic oil	0	0.5	1	1.5
Chemical composition (%)				
Dry matter	89.77	89.82	89.86	89.91
Crude Protein	35.17	34.99	34.82	34.65
Ether extracts	4.12	4.55	4.98	5.41
Ash	7.15	7.15	7.15	7.15
Crude fiber	4.88	4.88	4.88	4.88
NFE[2]	38.45	38.25	38.03	37.82
GE[3] (MJ/ kg)	16.71	16.81	16.90	17.0

1- One kg premix contained:
Vitamins:- 48×10^5 I.U (A), 6×10^2 mg (B_6), 20 mg (Biotin), 8×10^5 I.U. (D_3), 144 mg (E), 400 mg (B_1), 1600 mg (B_2), 4×10^3 mg (Pantothenic acid), 4 mg (B_{12}), 4×10^2 mg (Niacin), 2×10^5 mg (Choline chloride), and 400 mg (folic acid).
Minerals:- 12×10^3 mg Iron, 16×10^3 mg Manganese, 12×10^2 mg Copper, 120 mg Iodine, 80 mg Cobalt, 40 mg Selenium, and 16×10^3 mg Zinc.
2- **NFE,** Nitrogen free extract = (100 –CP - Ash -CF - EE).
3- **GE,** Gross Energy = (CP×5.64+EE×9.65+NFE×4.2)×4.182/100.

Condition factor (CF), which act as an indicator for fish body normality, was measured using the formula of [14]**:** CF =W/ L^3 × 100, where W is wet weight (g) of the tested fish and L is the length in centimeter while Hepato-somatic (HSI) and gastro-somatic (GSI) were calculated as equation by [15]**,** HIS = 100 × liver weight [g] / total body weight [g], and GSI = 100 × gut weight [g] / total body weight [g].

Apparent protein digestibility (APD) was measured by using [16] method. After the end of the trial the final weight was recorded, then the digestion trial was started where the uneaten diet and feces were collected once daily for 15 days by siphoning. Feed or feces was carefully collected before first meal, then 30 min after of feeding, uneaten feed are collected, and after two hours, feces were collected separately then filtered and dried at 60ºC and stored for determined the chemical composition.

2.5. Hematological parameters

2.5.1. Sampling

At the end of the investigation periods, blood samples (0.5-1 ml blood) were randomly taken from 5 fish/ tank (fries) or basin (fingerlings), by puncture of the caudal vein, using the heparinized syringes for some plasma parameters, and syringes without anticoagulant for obtained serum, which was separated by centrifuging at 3000 RPM for 10 min [17].

2.5.2. Biochemical parameters measurement

Plasma were subjected to measuring plasma protein (PTP) according to [18]**.** Blood serum cholesterol was determinated using method of [19]**.** The immunoglobulin's (IgM and IgD) were assessed according to [20]**.**

2.6. Liver biochemical analysis

At the end of the trial, liver samples of 15 fish were taken randomly from each pond, then liver tissues were homogenized by 5 ml distilled and Liver glycogen was measured (g/100 g fresh tissue) using the method by [21].

Hepatic glycogen = (absorbance sample/absorbance standard) ×conc. of standard × (V. of dil. factor/ Wt. of tissues).

Total liver lipids was extracted with a mixture of (2 chloroform:1 methanol), [22]**,** and 0.5 ml H_2So_4 was added to the dried lipid extract, then the liver lipid content was detected as [23].

Hepatic lipid = (Absorbance sample/Absorbance standard) x Conc. of standard × (V. of dil. factor/ Wt. of tissues)**.**

Total liver protein was extracted by homogenization in trichloroacetic acid, then centrifuged (1008 x*g*), and the hepatic protein content was determined as described by [24] and by using the following formula:

Hepatic protein= (absorbance sample/absorbance standard) × Conc. of standard ×(V. of dil. factor/ Wt. of tissues).

2.7. Chemical investigations

Three treated sample were analyzed for adjusting the moisture oven-dried (at 85ºC till constant weight), crude protein using Kjeldahl, total lipids by the method of ether extraction, and the total ash (550ºC / 6 hours) by [25]**.**

2.8. Statistical analysis

The data were subjected to analysis of variance (ANOVA) using general linear models (GLM) procedure, the software used was SPSS (Version 16.0) [26]. Duncan's multiple range tests by [27] was used to compare between means of the control and treated groups, the model of analysis was as follows:

$$\mathbf{Y_{ij} = \mu + T_i + E_{ij}}$$

μ = the overall mean. T_i = the effect of treatment, and, E_{ij} = the random error.

3. Results and discussion

3.1. Water quality

3.1.1. Water parameters in aquaria; temperature (28.3 ± 1.1 °C), dissolved oxygen (6.18 ± 0.4 mg/L), and pH (6.9 ± 0.1), also total ammonia are in normal range (0.035 ± 0.01 mg/L)with a controlled photoperiod (12 h light: 12 h dark) through the experimental period.

3.1.2. Water parameters outdoor (concrete ponds); mean values (±SD) of dissolved oxygen, water temperature and pH were ranged; 5 - 6 ± 0.4 mg/L 28.6 ± 0.3°C and 7.5 ± 0.2 respectively, where the total ammonia was 0.023 ± 0.01 mg/L; nitrite 0.025 ± 0.013 mg/ L; and nitrate 0.8 ± 0.4 mg/L, for all parameters are within the acceptable range for rearing examined fish .

3.2. Growth performance and biometric indices

As shown in Table (2) and Fig.(1), FBW, WG, DWG, and SGR values were significantly boosted in (AS2 & AS3) for fries, and in (AS3) for fingerlings compared control. The biometric indices (CF) clarified that the higher in AS2 for fry, and AS3 for fingerlings than other groups (Table 2 and Fig. 2). These results illustrated that garlic inclusion in diets of fry (up to 1%) or 1.5% for fingerlings, enhanced ($p<0.05$) growth performance, thus garlic considered as a growth promoter.

Feed additives in fish diets mainly aims to enhance fish performance, immunity and quality of fish flesh. Our findings demonstrated that the best growth performance was observed in fish fed garlic additive, up to 1% garlic for fry and 1.5% for fingerlings, which in an agreement with those recorded by [28,1,29].

The use of *Allium sativum* due to its composition play a beneficial role in animal nutrition [30]. Garlic acts as a growth promoter which improved body weight gain, feed intake and feed efficiency of *Oreochromis niloticus* [27]**.**

The diets supplemented with 1% and 1.5% garlic in current trails were improved survival rate (SR) for the two stages of tilapia. These may due to bioactive compounds that found in garlic oil.

[31] who mentioned that garlic contains allicin, which promotes the performance of the intestinal flora, thereby improving digestion, and enhancing the utilization of energy. Garlic led to improved fish growth and The dietary addition of garlic in any form can promote growth rate, decrease mortality and acts as antioxidant agent in fishes [27, 32, 33, 1, 34, 35].

Table 2. Growth performance for two stages of tilapia.

Fish stage	Diets	Growth performance								
		Initial w. (g)	Initial L. (cm)	Final w. (g)	Final L. (cm)	WG (g)	DWG (g/day)	SGR	CF	SR (%)
Fry reared (indoor)	Cont.	1.41 ±0.02	4.31	12.92^{d} ±1.10	10.85	11.51^{d} ±1.01	0.14^{c} ±0.01	2.64^{c} ±0.21	1.01	91
	AS_1	1.39 ±0.01	3.80	14.73^{c} ±1.09	11.16	13.34^{c} ±1.03	0.16^{b} ±0.01	2.81^{b} ±0.05	1.06	93
	AS_2	1.40 ±0.03	4.08	16.22^{a} ±1.78	10.70	14.82^{a} ±0.99	0.18^{a} ±0.00	2.92^{a} ±0.14	1.32	95
	AS_3	1.41 ±0.02	4.11	16.05^{b} ±0.98	10.91	14.64^{b} ±1.02	0.17^{ab} ±0.01	2.90^{a} ±0.12	1.24	94
Fingerling reared (outdoor)	Cont.	10.51 ±0.82	10.15	36.33^{d} ±1.08	14.42	25.82^{d} ±1.14	0.31^{c} ±0.02	1.48^{c} ±0.01	1.21	90
	AS_1	10.64 ±0.95	10.11	37.74^{c} ±1.21	14.55	27.10^{c} ±1.21	0.32^{bc} ±0.01	1.51^{c} ±0.03	1.22	90
	AS_2	10.55 ±0.74	09.22	38.75^{b} ±1.04	14.47	28.20^{b} ±1.15	0.34^{b} ±0.01	1.55^{b} ±0.02	1.28	93
	AS_3	10.52 ±0.65	09.07	42.01^{a} ±1.17	14.24	31.49^{a} ±1.09	0.37^{a} ±0.02	1.65^{a} ±0.01	1.45	96

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

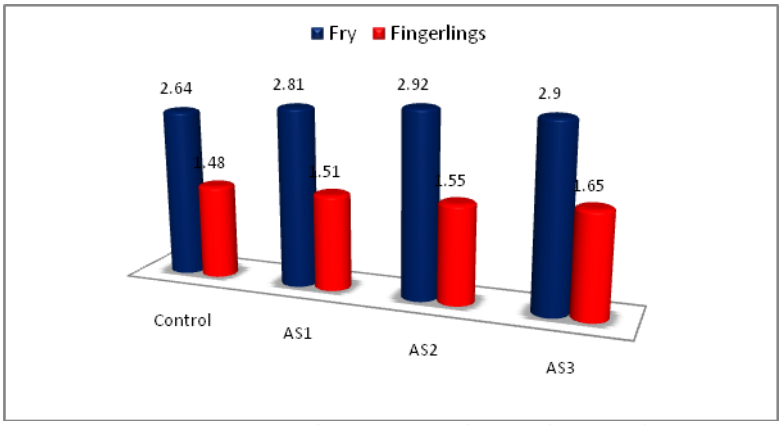


Fig. 1. Comparison between specific growth rates of the two fish stages fed garlic oil.

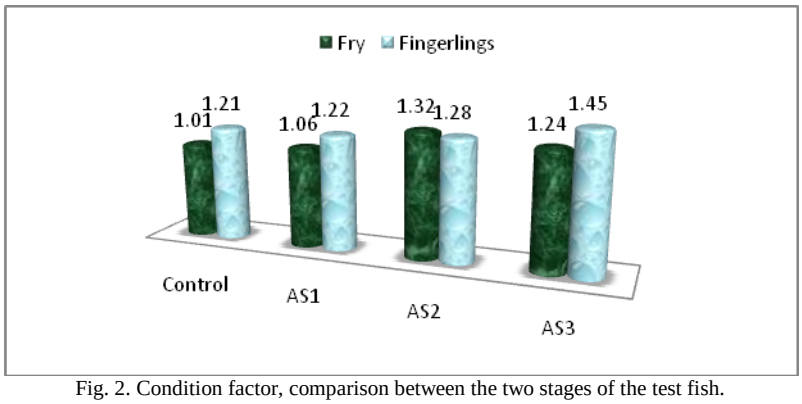


Fig. 2. Condition factor, comparison between the two stages of the test fish.

3.3. Feed utilization measurements.

Feed conversion ratio was the lowest For the fish group fed on diets contained 1 % and 1.5% garlic oil compared to the control for fry stage, while for fingerlings, was the lowest detected in AS_3. Table (3) and Fig. (3 & 4) cleared that the

highest values of PER accompanied with the lowest values FCR were for the second and the third of the additive level of the oil for the two test fish stages (fry and fingerlings) respectively.

The way PPV values tends to increase with garlic additive increasing for fingerlings ($p<0.05$), while the best PPV were detected for the fry fed AS_2. [28] stated that garlic extract as a natural phyto-additives supplements in fish diets led to improve utilization indices

The present results suggested that garlic additives were acted like a growth promoter for fry and fingerlings tilapia where these additives enhance nutrient utilization, which was reflected in improvement of weight gain, FCR, PER, PE and SGR for different fishes [36,37,38,4].

Results (Table 3) of apparent digestibility were enhanced with garlic addition in fry and fingerlings diets, this may in turn explain why rich values of growth and feed efficiency of tilapia noticed with experimental diets [36,1].

Present results revealed that the growth promotion effect of diets supplemented with garlic oil can be attributed to the improvement of feed efficiency for the two stages of *Oreochromis niloticus*. The same recorded by [28,32,33] and *O. aureus* [1].

Table 3. Feed utilization for the treated tilapia fed three levels of garlic additive.

Fish stage	*Diets*	*Feed utilization*				
		FI	*FCR*	*PER*	*PPV*	*APD*
Fry	***Control***	$13.40^{d}\pm1.02$	$1.16^{a}\pm0.01$	$2.72^{c}\pm0.10$	$39.80^{d}\pm1.11$	$76.02^{c}\pm1.25$
	AS_1	$15.01^{c}\pm1.21$	$1.13^{b}\pm0.00$	$2.83^{b}\pm0.03$	$43.12^{c}\pm1.23$	$76.81^{b}\pm1.14$
	AS_2	$16.36^{a}\pm1.15$	$1.10^{c}\pm0.02$	$2.89^{a}\pm0.04$	$45.54^{a}\pm1.14$	$79.14^{a}\pm1.32$
	AS_3	$16.22^{b}\pm1.30$	$1.11^{c}\pm0.01$	$2.90^{a}\pm0.02$	$44.85^{b}\pm1.21$	$79.13^{a}\pm1.40$
Fingerling	***Control***	$45.94^{d}\pm1.24$	$1.78^{a}\pm0.03$	$1.78^{d}\pm0.02$	$24.38^{d}\pm1.09$	$77.11^{c}\pm1.26$
	AS_1	$47.37^{c}\pm1.09$	$1.75^{b}\pm0.01$	$1.83^{c}\pm0.01$	$26.12^{c}\pm1.17$	$80.18^{b}\pm1.19$
	AS_2	$48.17^{b}\pm1.11$	$1.71^{c}\pm0.02$	$1.89^{b}\pm0.03$	$27.68^{b}\pm1.25$	$80.93^{a}\pm1.35$
	AS_3	$51.06^{a}\pm1.42$	$1.62^{d}\pm0.01$	$2.01^{a}\pm0.02$	$30.05^{a}\pm1.13$	$81.05^{a}\pm1.48$

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

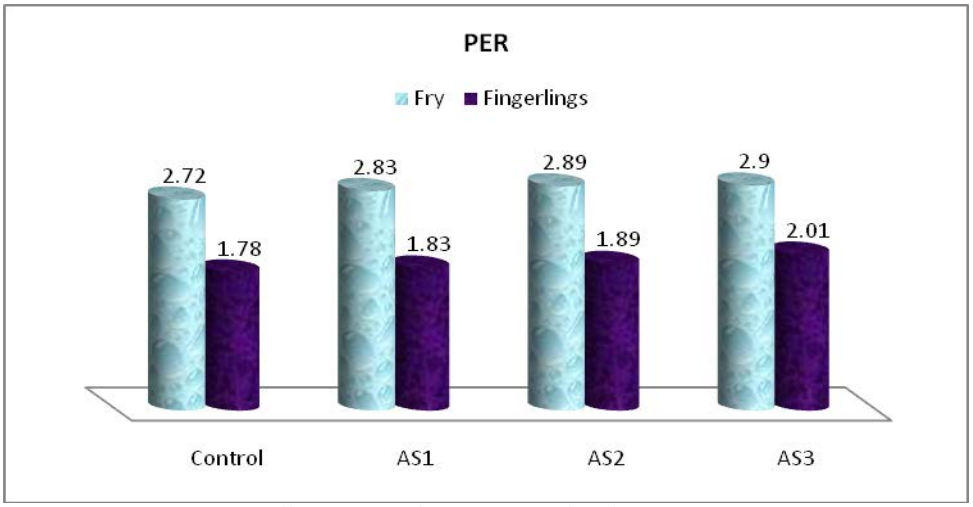


Fig. 3. Protein efficiency ratio of the two stages of the fish treated with garlic oil

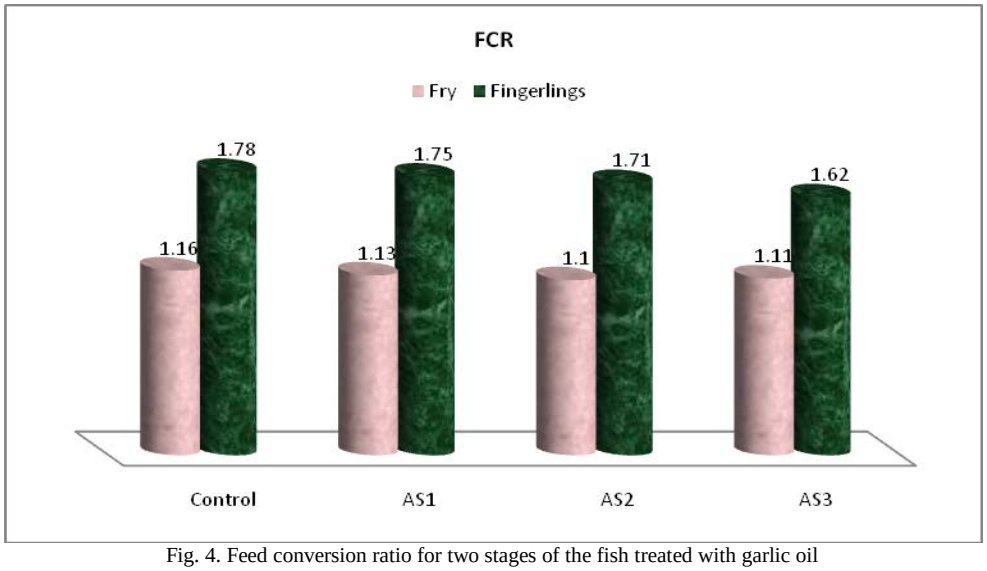


Fig. 4. Feed conversion ratio for two stages of the fish treated with garlic oil

3.4. Biometric indices

In fish nutrition studies, the hepato-somatic index (HSI) is used as an point for the energy reserve status of the fish and is an effective bio-indicator of the detection of dangerous impacts due to the environmental condition [39].

Table (4) and Fig. (5&6), HSI was improved for fries fed AS3 (2.18 ±0.01) and fingerlings fed on AS_2&AS_3 (3.07±0.03). The GSI values were fluctuated within the three experimental groups (fries and fingerlings) where increased ($p<0.05$) comparing to control. These findings indicated that garlic oil acts as appetizer.

Table 4. Fish body indices.

Fish stage	*Diets*	*Body indices*			
		Liver weight	*HSI*	*Gut weight*	*GSI*
Fry	*Control*	$0.23^{c}\pm0.01$	$1.78^{d}\pm0.04$	$0.83^{c}\pm0.01$	$6.42^{c}\pm0.21$
	AS_1	$0.29^{b}\pm0.00$	$1.97^{c}\pm0.02$	$1.03^{b}\pm0.01$	$7.01^{a}\pm0.10$
	AS_2	$0.34^{a}\pm0.01$	$2.09^{b}\pm0.03$	$1.07^{a}\pm0.02$	$6.61^{c}\pm0.36$
	AS_3	$0.35^{a}\pm0.02$	$2.18^{a}\pm0.01$	$1.07^{a}\pm0.03$	$6.67^{b}\pm0.14$
Fingerling	*Control*	$1.15^{c}\pm0.01$	$3.16^{c}\pm0.02$	$2.40^{d}\pm0.01$	$6.61^{d}\pm0.23$
	AS_1	$1.10^{d}\pm0.03$	$2.91^{b}\pm0.04$	$2.52^{c}\pm0.04$	$6.68^{c}\pm0.41$
	AS_2	$1.19^{b}\pm0.01$	$3.07^{a}\pm0.03$	$2.67^{b}\pm0.01$	$6.89^{b}\pm0.25$
	AS_3	$1.29^{a}\pm0.02$	$3.07^{a}\pm0.02$	$2.97^{a}\pm0.02$	$7.07^{a}\pm0.12$

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

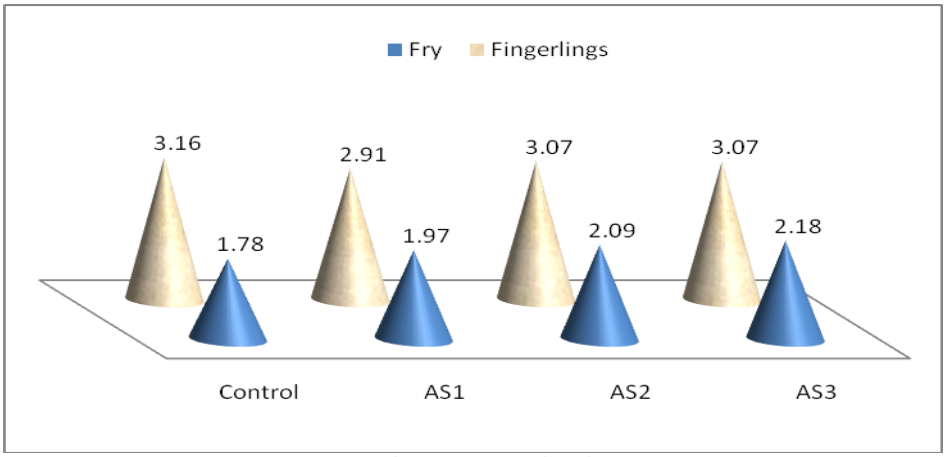


Fig. 5. Hepatosomatic index of the two stages of the fish examined by garlic oil

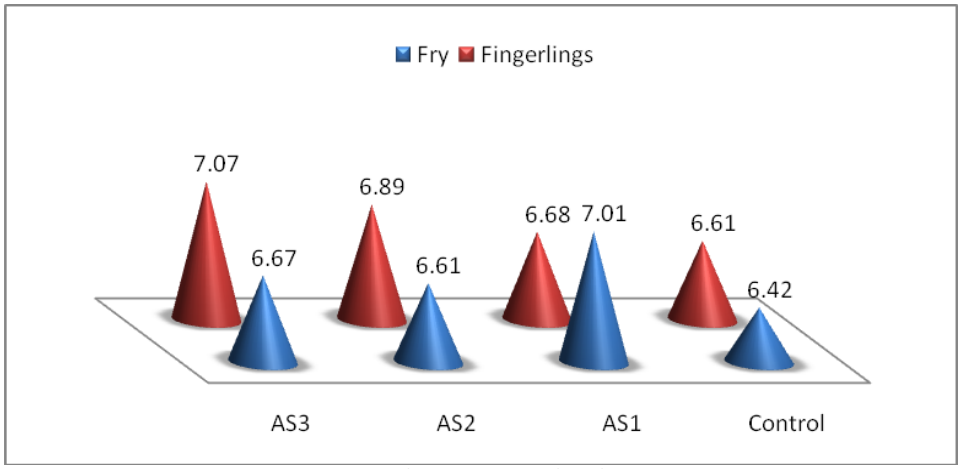


Fig. 6. Gastro-somatic index of the two stages of the fish treated with garlic oil

3.5. Carcass composition of fry and fingerlings.

The highest protein and dry matter content accompanied by lowest lipid content were recorded for the groups that fed AS_3 for the two stages (Table 5; Fig. 7&8).

[34] showed that inclusion of garlic in the diet increased fish protein content and decreased whole body fat in fish. The increase of protein content can be explained by garlic additive in diets which may cause a rise in free amino-acid contents which lead to an enhancement in protein synthesis [29].

Table 5. comparison of carcass composition between the two stages.

Fish stage	*Diets*	*Chemical composition*					
		Moisture	*Dry matter*	*Protein*	*Lipid*	*Ash*	*NFE*
Fry	*Control*	73.89^{a} ±1.012	26.11^{d} ±1.16	56.05^{c} ±2.05	15.27^{a} ±1.02	18.00^{d} ±1.06	10.68^{a} ±0.91
	AS_1	72.99^{c} ±1.24	27.01^{b} ±1.11	56.45^{b} ±2.10	15.07^{b} ±1.01	19.21^{a} ±1.03	09.27^{b} ±0.057
	AS_2	72.59^{d} ±2.01	27.41^{a} ±1.13	57.40^{a} ±1.97	14.62^{d} ±1.10	18.85^{c} ±1.11	09.13^{c} ±0.36
	AS_3	73.03^{b} ±1.91	26.97^{c} ±1.08	57.41^{a} ±2.13	14.70^{c} ±1.05	19.05^{b} ±1.06	08.84^{d} ±0.68
Fingerling	*Control*	74.83^{a} ±1.57	25.17^{d} ±1.15	54.42^{d} ±2.08	17.31^{a} ±1.03	18.29^{ab} ±1.02	09.98^{a} ±0.95
	AS_1	74.64^{b} ±1.63	25.36^{c} ±1.24	56.59^{c} ±1.78	16.20^{b} ±1.11	17.78^{c} ±1.12	09.43^{b} ±0.87
	AS_2	74.20^{c} ±1.84	25.80^{b} ±1.09	57.33^{b} ±1.65	15.92^{c} ±1.09	18.32^{a} ±1.08	08.43^{c} ±0.62
	AS_3	73.62^{b} ±1.53	26.18^{a} ±1.18	57.99^{a} ±2.03	15.61^{d} ±1.10	18.26^{b}±1.21	08.14^{d} ±0.84

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

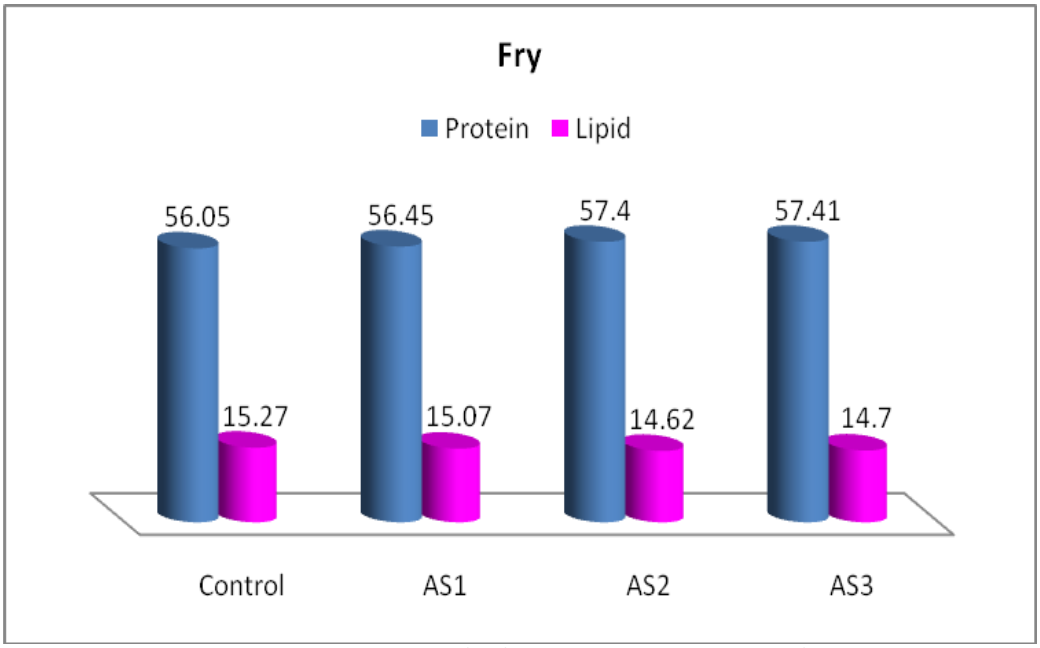


Fig. 7. Lipid and protein percentage in fish flesh treated with the three levels of the additive

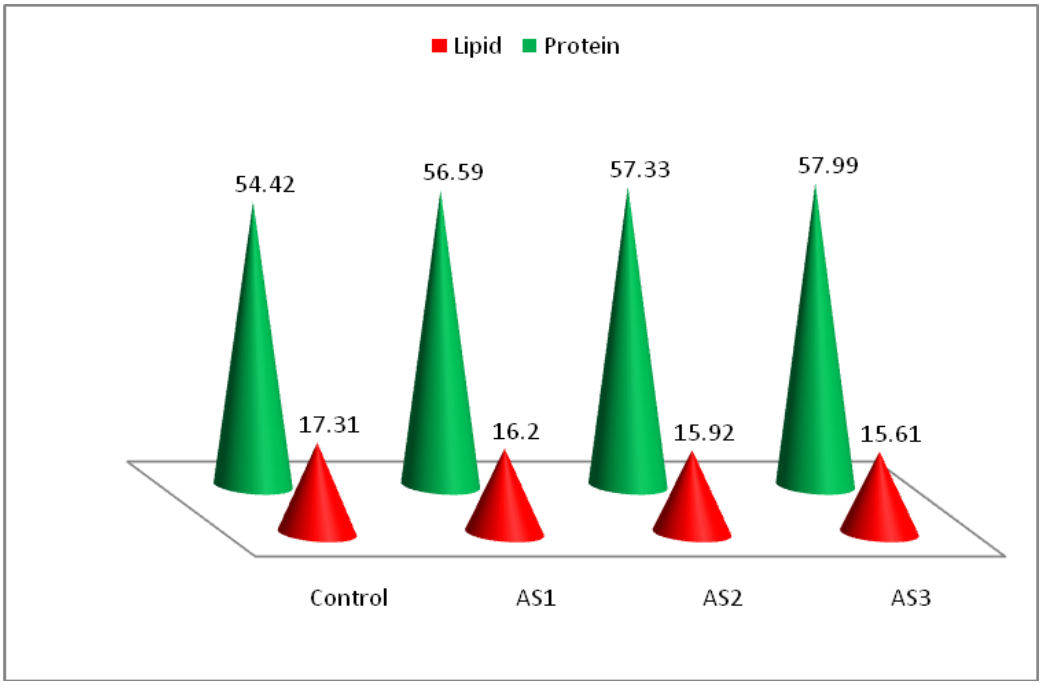


Fig. 8. Lipid and protein percentage in the fingerlings fed the three levels of the additive

3.6. Liver metabolites

The present results in Table (6); Fig. (9) cleared that the highest liver protein content were recorded for AS_2 (fries) and AS_3 (fingerlings), at the same time, values of hepatic lipid were decreased ($p<0.05$). Glycogen values were increased through all test groups ($p<0.05$) compared control. Garlic oil may cause a decrease in liver lipid [29]. [2] demonstrated an increasing trend for liver protein and glycogen, while hepatic lipids showed an opposite trend.

Table 6. Liver composition.

Fish stage	*Diets*	*Liver metabolites*		
		Protein	*Lipid*	*Glycogen*
Fry	*Control*	$19.63^{d}\pm0.97$	$10.56^{a}\pm0.91$	$2.03^{c}\pm0.03$
	AS_1	$20.21^{c}\pm1.08$	$09.86^{b}\pm0.78$	$2.51^{b}\pm0.02$
	AS_2	$21.04^{a}\pm1.05$	$09.12^{d}\pm0.94$	$2.77^{a}\pm0.04$
	AS_3	$20.68^{b}\pm0.89$	$09.54^{c}\pm0.57$	$2.54^{b}\pm0.01$
Fingerling	*Control*	$18.65^{d}\pm1.04$	$11.27^{a}\pm0.63$	$1.78^{d}\pm0.03$
	AS_1	$20.41^{c}\pm0.96$	$10.05^{b}\pm0.82$	$2.15^{c}\pm0.02$
	AS_2	$20.95^{b}\pm1.05$	$09.82^{c}\pm0.90$	$2.37^{b}\pm0.05$
	AS_3	$21.19^{a}\pm1.09$	$09.75^{d}\pm0.87$	$2.26^{a}\pm0.01$

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

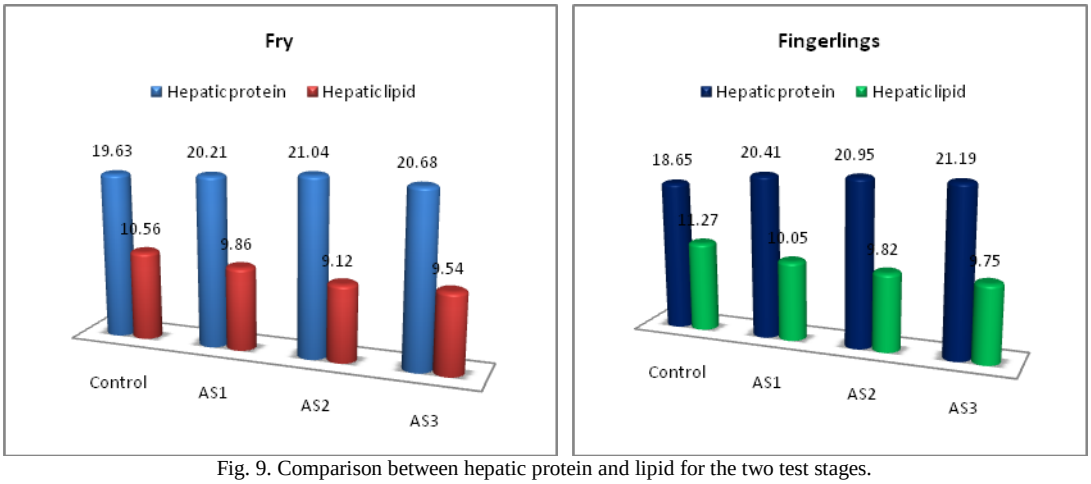


Fig. 9. Comparison between hepatic protein and lipid for the two test stages.

3.7. Biochemical parameters

The results for fish fry stage cleared that the best PTP value was for the AS_2 group, while AS_3 group recorded best PTP in fingerlings (Table 7; Fig. 10 a,b,c). Cholesterol levels (Fig. 11) were reduced through all fish groups at fry and fingerlings stage, comparing with control ($p<0.05$).

Plasma total protein is an important to evaluate the nutritional state of the fish health condition [40,41]**.** [42] stated that total protein and albumin directly responsive to protein intake and its quality. Garlic contains sulfur salts which optimizing cholesterol levels and control liver diseases [3]**.**

[29] recorded that dietary 1.0 % garlic extract improved growth, hematological parameters and immune function of monosex Nile Tilapia fingerlings.

[43] showed that garlic allicin may adjusting and optimizing the activity of lipogenic and cholestrogenic enzymes in liver and [44] added that allicin content of garlic causes inhibition of accumulation of fat in body.

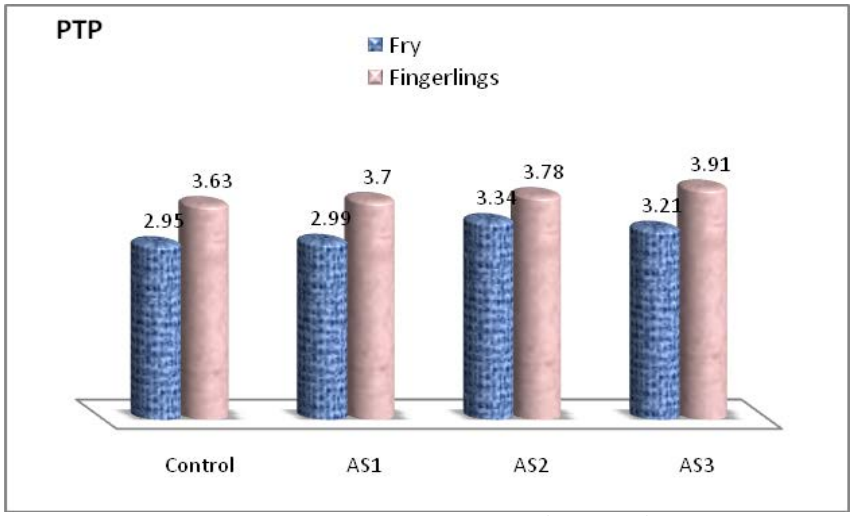
Best values IgD resulted for the fry fed on AS_2, while IgD displayed increasing trend, with the high level of additive in fingerlings stage. Opposite trend was recorded for IgM values, where decreased when garlic oil level increased (Table 7). These results indicated that garlic additive (1% for fry and 1.5% for fingerlings) enhanced fish immunity.

+The fish fed on (1 & 1.5%) garlic oil were recorded the optimum results of immune activity, to illustrate our data, it makes sense to initiate the increase in the specific immune response (IgM) only when the infection becomes harmful, and then return to optimal levels when the infection is reduced [45, 46]**.**

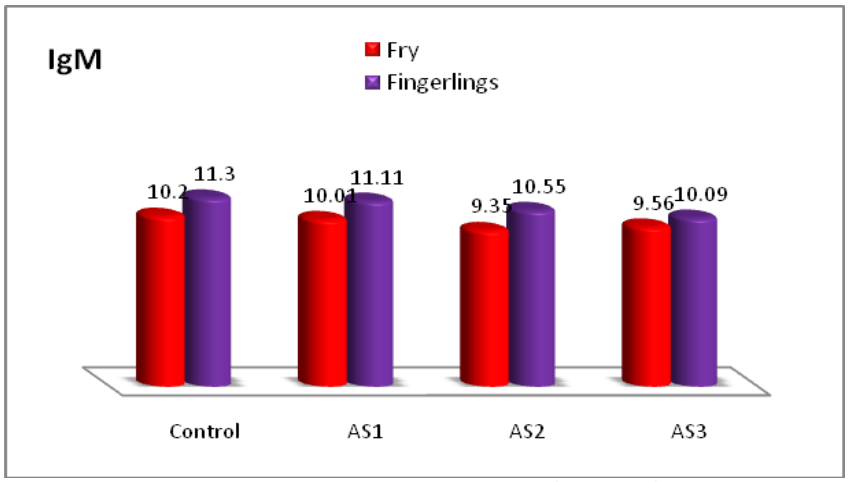
Table 7. Impact of garlic oil on immune response of tilapia

Fish stage	*Diets*	*Blood parameters*		
		PTP	*IgM*	*IgD*
Fry	*Control*	$2.95^{c}\pm0.04$	$10.20^{a}\pm1.01$	$122.40^{c}\pm2.12$
	AS_1	$2.99^{c}\pm0.02$	$10.01^{b}\pm0.94$	$122.50^{c}\pm1.87$
	AS_2	$3.34^{a}\pm0.04$	$09.35^{d}\pm0.86$	$124.25^{a}\pm2.06$
	AS_3	$3.21^{b}\pm0.03$	$09.56^{c}\pm0.67$	$123.50^{b}\pm2.35$
Fingerling	*Control*	$3.63^{d}\pm0.05$	$11.30^{a}\pm0.92$	$132.40^{c}\pm1.97$
	AS_1	$3.70^{c}\pm0.01$	$11.11^{b}\pm0.58$	$135.50^{b}\pm2.41$
	AS_2	$3.78^{b}\pm0.03$	$10.55^{c}\pm0.73$	$135.25^{b}\pm2.35$
	AS_3	$3.91^{a}\pm0.02$	$10.09^{d}\pm0.68$	$137.50^{a}\pm1.54$

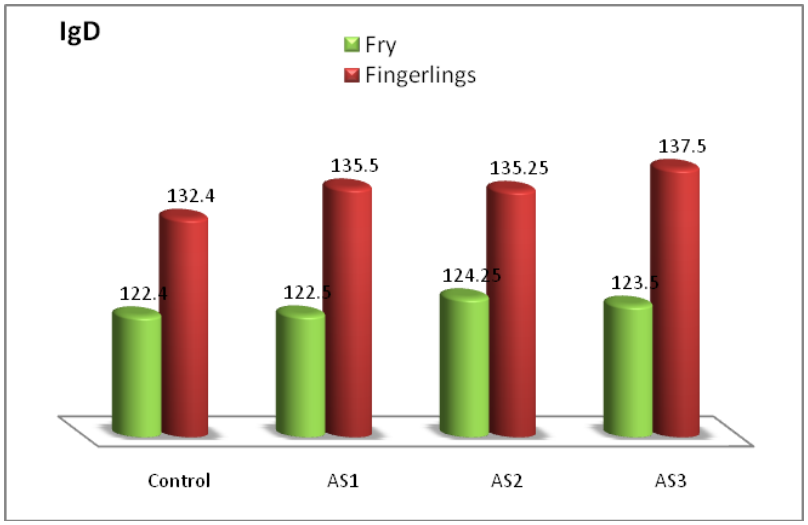
Mean values in the same column with different letters, are significantly different ($P < 0.05$).



a. Comparative evaluate the plasma total protein for the two fish stages.



b. Comparative evaluate the immunoglobulin (IgM) for the two fish stages.



c. Comparative evaluate the immunoglobulin (IgD) for the two fish stages.
Fig. 10. Comparative evaluate the immunity indicators for the two fish stages.

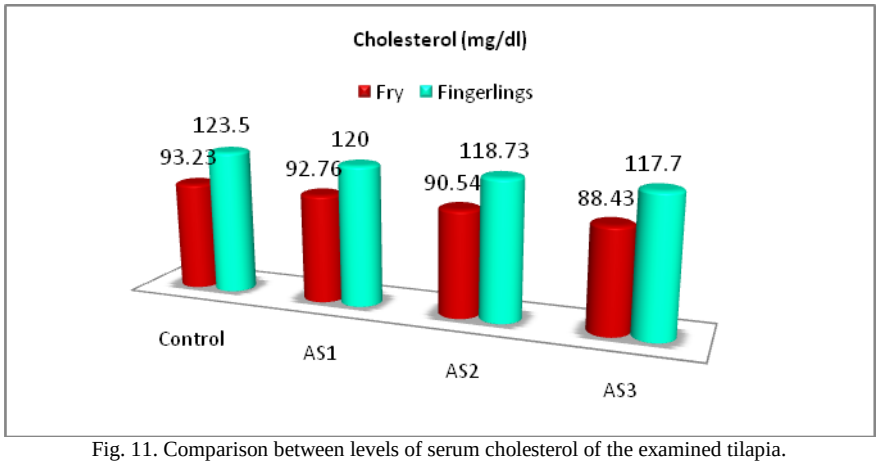


Fig. 11. Comparison between levels of serum cholesterol of the examined tilapia.

4. Conclusion

We would like to summarizing the results of the present studies raveled a positive impact of the additive which used (garlic oil) on the growth performance, feed utilization, biochemical and physiological conditions of the two stages which reared in different conditions, glass aquaria indoor (fry) or cement outdoor (fingerlings).

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