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### EFFECT OF SOME FEED ADDITIVES ON GROWTH PERFORMANCE, BLOOD COMPONENTS OF NILE TILAPIA

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**ABSTRACT:** In a complete randomized design, Two hundred and ten fingerlings (weighing approximately  $0.905 \pm 0.002$  g all fish) were randomly distributed into 21 glass aquaria in 7 treatments (3 replicates per treatment). The first group was fed diet without any treatment (control), the second group was fed on diet supplemented with  $\text{SeO}_2$  (0.3 mg selenium/kg diet) in fish diets, the third group was fed on diet supplemented with organic selenium supplemented; Sel-Plex® (0.3 mg selenium/kg diet) in fish diets, the fourth group was fed the same diet and supplemented with zinc oxide (100 mg zinc/kg diet) in fish diets, the fifth group was fed on diet supplemented with EDTA zinc;  $\text{C}_{10}\text{H}_{12}\text{N}_2\text{O}_8\text{ZnNa}_2$ ; (100 mg zinc/kg diet) in fish diets, the sixth group was fed on diet supplemented with clay 30 g bentonite/kg feed (3%) and the seventh group was fed on diet supplemented with nano-clay 30 g nano bentonite/kg diet. There was no clear effect of dietary feed additives on the water quality in the all experimental groups. All water parameters were stable and within acceptable ranges. Fish groups fed diets supplemented with zinc oxide or selenium oxide recorded higher final live body weight and daily weight gain, then those fed diets supplemented with EDTA zinc, organic selenium and natural clay. Dietary feed additives reduced the mortality rate of fish. Fish group fed diet supplemented with EDTA zinc and nano-clay recorded the lower mortality rate. Fish groups fed diets supplemented with organic selenium and EDTA zinc recorded the best feed conversion ratio. No statistical differences were observed in fish body dry matter, crude protein, ether extract and ash as affected by dietary feed additives. Fish groups fed diets supplemented with EDTA zinc, selenium oxide, zinc oxide or organic selenium recorded the higher final margin and income from body gain. The best final margin was obtained in fish group fed diet supplemented with EDTA zinc.

**Key words:** Nile tilapia, selenium, zinc, clay, growth performance, feed utilization, margin.

### INTRODUCTION

Fisheries and aquaculture remain important sources of food, nutrition, income and livelihoods for hundreds of millions of people around the world. In addition to the increase in production, other factors that have contributed to rising consumption include reductions in wastage, better utilization, improved distribution channels, and growing demand linked to population growth, rising incomes and urbanization. International trade has also played an important role in providing wider choices to consumers (FAO, 2016).

Micro-minerals such as zinc and selenium are essential to fish (Kaushik, 2002 ; Lall, 2002). A low or a high supply of dietary or aqueous trace minerals affects the associated biochemical and physiological responses in fish (Bury *et al.*, 2003). Selenium an essential dietary nutrient plays an important role in normal functioning of the immune system, promoting a normal cellular immune response, and helping the body to resist viral infection (Rayman, 2000). The deficient or excessive dietary selenium levels can cause negative effects on growth, survival rate, peroxidative damage to cells (Lin and Shiau, 2005 ; Liu *et al.*, 2010) and reduced host defense function (Sweetman *et al.*, 2010; Wang *et al.*,

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2013) in fish. Also, Zinc is an essential micro-mineral required for various metabolic pathways, including growth, protein synthesis, energy metabolism, and immunity of animals, including fish (Lin *et al.*, 2013; Houn-Yung *et al.*, 2014). Zinc serves as a functional cofactor for more than 200 metalloenzymes, such as superoxide dismutase, RNA polymerase, alkaline phosphatase, alcohol dehydrogenase, and carbonic anhydrase (Fountoulaki *et al.*, 2010; Luo *et al.*, 2011; Liang *et al.*, 2012; Lin *et al.* 2013). The importance of zinc in the antioxidant protection has been also illustrated in many aquatic organisms (Trevisan *et al.*, 2014 ; Huang *et al.*, 2015). Natural clays are crystalline alumino-silicates characterized by their ability to exchange cations without major changes in structure. Natural clays can adsorb toxic products of digestion and decrease the accumulation of toxic substance in rabbit tissues, thus decreasing the incidence of internal disorders. Addition of natural clays in animal diets improves growth rate, feed conversion and increases return from body gain and final margin (Ayyat and Marai, 1997).

The present study aimed to investigate the effect of some safe feed additive on growth performance and blood parameters of Nile tilapia (*Oreochromis niloticus*).

## MATERIALS AND METHODS

The present study was carried out at the Wet Laboratory of the Animal Production Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. The experimental period lasted 84 days from May to August, 2016. In a complete randomized design, Two hundred and ten fingerlings (weighing approximately  $0.905 \pm 0.002$  g) all fish were randomly distributed into 21 glass aquaria ( $35 \times 70 \times 40$  cm -  $0.074$  m<sup>3</sup> of water) in 7 treatments (3 replicates per treatment). The first group was fed diet without any treatment (control), the second group was fed on diet supplemented with inorganic selenium; SeO<sub>2</sub>; supplemented (0.3 mg selenium/kg diet) in fish diets, the third group was fed on diet supplemented with organic selenium supplemented; YSC-100 USA (0.3 mg selenium/kg diet) in fish diets, the fourth group was fed the same diet and supplemented with inorganic zinc; zinc oxide;

(100 mg zinc/kg diet) in fish diets, the fifth group was fed on diet supplemented with EDTA zinc; C<sub>10</sub>H<sub>12</sub> N<sub>2</sub>O<sub>8</sub> ZnNa<sub>2</sub>; supplemented (100 mg zinc/kg diet) in fish diets, the sixth group was fed on diet supplemented with clay 30 g bentonite/kg feed (3%) and the seventh group was fed on diet supplemented with nano-clay 30 g nano bentonite/kg diet (The nano-clay was obtained from Egyptian Organization For Standardization And Quality Control, Egypt).

All fish groups were fed on basal pelleted diet (Table 1). Fish were fed at the rate of 5% of live body weight and it offered two times at 8.00 and 15.00 hours. The fish in each aquarium were weighed biweekly, and the feed weight was adjusted after each fish weighing. About 25% of the water in the aquarium was daily replaced by aerated fresh water. Each aquarium was supplied with air pump to supply fish with oxygen.

The glass aquariums were supplied with well-aerated and dechlorinated tap water from storage tank. Air was compressed to each aquarium *via* air stones by air pumps during the experimental period. The diet remains and fish wastes of each aquarium were removed by siphoning using plastic tube before the second daily feeding to further analysis and minimize leaching. Every second day, each aquarium was partially cleaned including the fish feces and the water partially changed (about 50%).

The proximate chemical on wet weight of whole-body composition including crude protein, crude fat, crude ash and moisture of body composition was determined using the standard procedures of AOAC (2005). At the end of the experimental period (12 weeks), fish were fasted for 12 hours immediately prior to blood sampling and five fish per glass aquaria were randomly chosen and anesthetized with 120 mg/l amino-benzoic acid (Sigma-Aldrich) before the drawing of blood. The blood was extracted from the caudal vein and divided in two sets of tubes. One set was left with no anticoagulant in order to clot at 4°C and centrifuged at 5000 rpm for 5 min at room temperature. All blood parameters [Total protein, Albumin and globulin, Uric acid, Creatinine, Aspartate amino transferase (AST) and Alanine amino transferase (ALT). Red blood cells (RBC) and White blood cell (WBC), Hemoglobin (Hb)] were measured in private laboratory.

**Table 1. Formulation and chemical composition of the basal experimental diet**

<b>Ingredient</b>	<b>Percent in basal experimental diet</b>
<b>Fish meal</b>	17
<b>Soybean meal</b>	33
<b>Corn meal</b>	20
<b>Corn gluten</b>	6
<b>Wheat bran</b>	17
<b>Sunflower oil</b>	3
<b>Dicalcium phosphate</b>	2
<b>Vitamin mix<sup>1</sup></b>	0.5
<b>Mineral<sup>2</sup></b>	0.5
<b>Carboxymethyl cellulose</b>	1
<b>Total</b>	100
<b>Proximate composition (%)</b>	
<b>Crude protein</b>	32.62
<b>Eather extract</b>	5.65
<b>Crude fiber</b>	4.53
<b>Gross energy (Kcal/100g)</b>	4210.0
<b>Price(L.E/Kg)</b>	7.20

1. Each one Kg of vitamin mixture contained: Vitam. A 72000IU, Vitam. B<sub>1</sub> 6 mg, Vitam. B<sub>3</sub> 12000 IU, Vitam. B<sub>6</sub> 9 mg, B<sub>12</sub> 0.06 mg, Vitam. E 60 mg, Vitam. k12 mg, Pantothonic acid 60 mg, Nicotinic acid 120 mg, Folic acid 6 mg, Biotin 0.3 mg and Choline chlorids 3mg.

2. Each one Kg of mineral mixture contained: Zinc 1.3g, manganese 930mg, iron630mg, copper 105 mg, iodin10.5 mg, selenium2.1 mg.

Water quality control was measured biweekly analysis before replacing the water in the aquarium during the experimental period. All the water quality parameters were within the acceptable ranges for fish growth (Boyd, 1984). Water temperature was measured in each aquarium daily using a mercury thermometer of 0 to 100°C range. Dissolved oxygen was measured directly by using oxygen thermometer apparatus (XSI model 58, Yellow Spring Instrument Co., Yellow Springs, Ohio, USA). Ammonia (NH<sub>4</sub>-N mg/l), Nitrate (NO<sub>3</sub> -N mg/l), Nitrite (NO<sub>2</sub> - Nmg/l) and pH were measured by using the Hach kit model HI 83205 (Multiparameter Bench Photometer, Hanna

Instruments, Romania). Continuous aeration was provided by an air blower.

Economic evaluation was calculated according to Ayyat (1991) as the following equation: Final margin (Profit) = Income from body gain weight - feed cost. Relative margin = Final margin × Survival rate. Other overhead costs were assumed constant. Price of one kg of diet was 7.20 LE (Egyptian pound = 0.056 US\$) and price of selling of one kg live body weight of fish was 15.0 LE.

The data were statistically analyzed with SAS (2002) according to the following model

$$: Y_{ij} = \mu + T_i + e_{ij},$$

Where,  $\mu$  is the overall mean, T is the fixed effect of treatments ( $i = 1 \dots 7$ ), and  $e_{ij}$  is random error. Significant differences between treatments were tested with Duncan's multiple range test (Duncan, 1955).

## RESULTS AND DISCUSSION

### Water Quality

There was no clear effect of dietary feed additives on water quality in the all experimental groups. All water parameters were stable and within acceptable ranges (Boyd and Tucker, 1998). During the whole experimental period water-quality parameters *i.e.*, averaged water temperature was 28.03°C, dissolved oxygen was 7.52 mg/l, total ammonia was 0.207 mg/l, nitrite was 0.043 mg/l and pH was 7.3. Range of water quality parameters within the acceptable range required for normal growth of tilapia as mentioned by Boyd (1990).

### Growth Performance

The average live body weight of Nile tilapia fingerlings at the beginning of the experiment was  $0.90 \pm 0.002$  grams. The non significant differences between the experimental groups for initial live body weight indicated that the groups at the beginning of the experiment were homogenous (Table 2).

Final live body weight of fish increased by 67.60, 51.53, 68.50, 55.88, 43.14 and 37.92% compared to the control, respectively, at fish group fed diet supplemented with selenium oxide, organic selenium, zinc oxide, EDTA zinc, clay and nano-clay, when compared with those fed diet without any supplementation (Table 2). Fish groups fed diets supplemented with zinc oxide or selenium oxide recorded higher final live body weight, followed by those fed diets supplemented with EDTA zinc, organic selenium and natural clay. Zinc is essential for normal growth of the farmed fish (Prasad, 1979 ; Stahl *et al.*, 1989). Also, Ayyat *et al.* (2004) reported that live body weight of Nile tilapia increased significantly ( $P < 0.001$ ) as affected with zinc supplementation in the diets. Also, Zhoua *et al.* (2009) found that the final weight of carp fish increased significantly when fed selenomethionine compared with the control.

Also, Dlouha *et al.* (2008) reported that Dietary supplementation with selenium enriched alga *Chlorella* increased ( $P < 0.05$ ) body weight. The breast muscle Se concentration was increased ( $P < 0.05$ ) by selenium enriched alga *Chlorella* (0.70 mg/kg DM; 0.36 mg/kg DM in control) supplementation, but not ( $P > 0.05$ ) by SS (0.49 mg/kg DM) supplementation. Also, Zhu *et al.* (2012) found the highest weight gain was obtained in fish fed diets with 1.60 mg selenium/kg diet, which was significant higher than the basal diet with 0.97 mg selenium/kg diet and did not differ significantly with the other treatments. Also, Naiel *et al.* (2012) found that fish growth indicated as live body weight, weight gain and specific growth rate (SGR) increased significantly and the maximum growth was obtained when fish fed diet containing 0.6 g OS/Kg

Daily body weight gain of Nile tilapia fish affected significantly ( $P < 0.001$ ) with feed additives (Table 2). Average daily weight gain increased by 70.04, 53.07, 70.76, 57.76, 44.40 and 38.99% compared to the control, respectively, at fish group fed diet supplemented with selenium oxide, organic selenium, zinc oxide, EDTA zinc, clay and nano-clay, when compared with those fed diet without any supplementation (Table 2). Fish groups fed diets supplemented with zinc oxide or selenium oxide recorded higher average daily gain, then those fed diets supplemented with EDTA zinc, organic selenium and natural clay. The obtained results indicated that dietary zinc or selenium supplementation improved growth rate, these may be attributed to that zinc is involved in numerous aspects of cellular metabolism. It plays a role in immune function (Prasad, 1995 ; Solomons, 1998), protein synthesis (Prasad, 1995), wound healing (Heyneman, 1996), DNA synthesis and cell division (Prasad, 1995). Also, Selenium an essential dietary nutrient plays an important role in normal functioning of the immune system and helping the body to resist viral infection (Rayman, 2000). Eid and Ghonim (1994) reported that fish fed the lowest levels of supplemental zinc (0 and 5 mg zinc/kg diet) had poor growth, while the level over 30 mg/kg diet showed markedly improved growth. Weight gain percent, feed efficiency, serum zinc and bone zinc concentrations indicated that the

**Table 2. Live body weight (g) and survival rate (%) of Nile tilapia as affected by dietary feed additives at different experimental periods**

Treatment	Initial weight (g)	Weight at 12 weeks (g)	Daily gain at 0-12 weeks	Relative growth at 0-12 weeks (g)
Control	0.90±0.02	24.13±1.31 <sup>d</sup>	0.27±0.01 <sup>d</sup>	185.45±0.73 <sup>c</sup>
Inorganic selenium 0.3 mg/kg	0.90±0.01	40.44±1.69 <sup>a</sup>	0.47±0.00 <sup>a</sup>	191.22±0.37 <sup>a</sup>
Organic selenium 0.3 mg/kg	0.90±0.01	36.56±1.49 <sup>bc</sup>	0.42±0.01 <sup>bc</sup>	190.31±0.37 <sup>ab</sup>
Inorganic zinc 100 mg/kg	0.90±0.02	40.66±1.28 <sup>a</sup>	0.47±0.01 <sup>a</sup>	191.26±0.27 <sup>a</sup>
EDTA zinc 100 mg/kg	0.90±0.01	37.62±0.34 <sup>ab</sup>	0.43±0.00 <sup>ab</sup>	190.61±0.09 <sup>ab</sup>
Bentonite 30 g/kg	0.90±0.01	34.54±1.29 <sup>bc</sup>	0.40±0.015 <sup>bc</sup>	189.76±0.35 <sup>b</sup>
Nano-clay 30 g/kg	0.90±0.01	33.28±0.27 <sup>c</sup>	0.38±0.03 <sup>c</sup>	189.42±0.07 <sup>b</sup>
Significance	NS	***	***	***

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

\*\*\*  $P<0.001$  and NS = Not significant.

minimum zinc requirement of fingerling Nile tilapia is 30 mg Zn/kg diet. Also, Huang *et al.* (2015) found that the percent of weight gain of tilapia increased with increasing dietary zinc. Improved growth rate in fish fed diets supplemented with zinc may be attributed to that zinc is involved in numerous aspects of cellular metabolism. Zinc acts as cofactor to many enzymes and proteins, which are involved in protein synthesis, growth, the nervous system, gastrointestinal tract function, and in reproduction (Classen *et al.*, 2011).

Relative growth rate of Nile tilapia fish affected significantly ( $P<0.001$ ) with feed additives. Relative growth rate increased with supplemented of feed additives in fish diets (Table 2).

### Mortality Rate

Dietary feed additives reduced the mortality rate of fish. Fish group fed diet supplemented with EDTA zinc and nano-clay recorded the lower mortality rate (0.0%), while other fish groups recorded 3.33% mortality rate, but the control group recorded 6.67% mortality rate (Table 3).

Zinc and selenium plays a role in immune function (Prasad, 1995; Solomons, 1998; Lin and Shiau, 2005). Also, Ogino and Yang (1978)

reported that the fish fed a diet low in zinc content (1 ppm) showed an extremely low growth rate and high mortality. A large number of the fish on the zinc-deficient diet were found to suffer from cataracts in the eyes and erosion of the fins and of the skin. On the other hand, Eid and Ghonim (1994) reported that fish fed the lowest levels of supplemental zinc (0 and 5 mg Zn/kg diet) had high mortality, while the levels over 30 mg/kg diet showed reduced mortality rate. The use of selenium as feed additives has important implications for health management in commercial aquaculture facilities.

### Feed Utilization

Daily feed intake increased significantly ( $P<0.001$ ) with feed additives supplementation at the all experimental periods (Table 3). Daily feed intake increased by 53.18, 30.25, 57.32, 26.75, 36.31 and 36.94% compared to the control, respectively in fish group fed diet supplemented with selenium oxide, organic selenium, zinc oxide, EDTA zinc, clay and nano-clay, when compared with those fed diet without any supplementation. Fish groups fed diets supplemented with zinc oxide or selenium oxide recorded higher daily feed intake.

**Table 3. Feed intake (g/day, feed conversion ratio (g food/g gain) and mortality rate (%) of Nile tilapia as affected by dietary feed additives at different experimental periods**

Treatment	Daily feed intake (g)	Feed conversion (g food/g gain)	Mortality rate (%)
Control	0.31± 0.07 <sup>d</sup>	1.13±0.04 <sup>a</sup>	6.67
Inorganic selenium 0.3 mg/kg	0.48±0.04 <sup>a</sup>	1.02±0.03 <sup>bc</sup>	3.33
Organic selenium 0.3 mg/kg	0.40±0.07 <sup>bc</sup>	0.96±0.04 <sup>cd</sup>	3.33
Inorganic zinc 100 mg/kg	0.49±0.09 <sup>a</sup>	1.04±0.02 <sup>abc</sup>	3.33
EDTA zinc 100 mg/kg	0.39±0.06 <sup>c</sup>	0.91±0.01 <sup>d</sup>	0.00
Bentonite 30 g /kg	0.42±0.01 <sup>b</sup>	1.07±0.02 <sup>ab</sup>	3.33
Nano-clay30 g /kg	0.43±0.03 <sup>b</sup>	1.11±0.01 <sup>ab</sup>	0.00
Significance	***	***	---

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

\*\*\* P<0.001.

Feed conversion ratio significantly (P<0.001) affected with feed additives supplementation at the all experimental periods (Table 3). Feed conversion improved by 10.10, 15.10, 8.25, 20.11, 5.97 and 2.02% compared to the control, respectively in fish group fed diet supplemented with selenium oxide, organic selenium, zinc oxide, EDTA zinc, clay and nano-clay, when compared with those fed diet without any supplementation. Fish groups fed diets supplemented with organic selenium and EDTA zinc recorded the best feed conversion ratio.

The same trend of the zinc supplementation was observed by Ayyat *et al.* (2004) who found that zinc supplementation in Nile tilapia fish diets improved feed conversion during the whole experimental period. Also, Zhao *et al.* (2011) showed that fish fed with a diet supplemented with 60 mg kg zinc/kg diet from either ZnSO<sub>4</sub> or ZnMet had a significantly greater protein efficiency ratio than those fed with the diets of ≤ 30 mg zinc/kg diet. Li and Huang (2016) reported that fish fed on a diet containing 31 mg kg<sup>-1</sup> endogenous zinc showed the lowest feed utilization when compared with the control group.

Kucukbay *et al.* (2009) reported a linear increase in feed intake and weight gain and improvement in feed conversion ratio were found in sodium selenite or selenomethionine

supplemented fish reared under crowding conditions. On the other hand, Zhu *et al.* (2012) documented that feed conversion ratio, protein efficiency ratio, protein productive value, apparent digestibility coefficients of dry matter and muscle composition were not significantly impacted by dietary selenium supplementation.

Kanyilmaz *et al.* (2014) found that the dietary zeolite treatments had a significant improved on feed conversion ratio of fish.

### Blood Components

Blood hemoglobin content and RBC's counts increased significantly (P<0.001 or 0.05) as feed additives supplemented in fish diets, while WBC's and lymphocytes counts insignificant affected with experimental treatments (Table 4). Blood hemoglobin concentrate increased by 17.04, 12.49, 28.68, 20.56, 18.02 and 7.39% compared to the control, respectively in fish group fed diet supplemented with selenium oxide, organic selenium, zinc oxide, EDTA zinc, clay and nano-clay, when compared with those fed diet without any supplementation (Table 4). Fish groups fed diets supplemented with zinc oxide or EDTA zinc recorded the higher blood hemoglobin concentration. Hemoglobin is an important chemical in red blood cells that carries oxygen, and performs different effect modulation and gas transport duties. Hemoglobin is the main

**Table 4. Hemoglobin, red blood cells, white blood cells and lymphocytes as affected by dietary feed additives on Nile tilapia fingerlings**

Treatment	Hemoglobin (g/dl)	RBCs counts x 10 <sup>6</sup> (cells/ $\mu$ l)	WBCs counts x 10 <sup>3</sup> (cells/ $\mu$ l)	Lymphocytes cells/l
Control	5.51±0.17 <sup>c</sup>	1.39±0.05 <sup>c</sup>	19.96±0.41	60.44±0.59
Inorganic selenium 0.3 mg/kg	6.44±0.09 <sup>ab</sup>	1.69±0.01 <sup>ab</sup>	19.82±0.46	61.82±0.92
Organic selenium 0.3 mg/kg	6.19±0.44 <sup>bc</sup>	1.59± 0.04 <sup>b</sup>	20.08±0.37	59.83±0.34
Inorganic zinc 100 mg/kg	7.09±0.38 <sup>a</sup>	1.66±0.03 <sup>ab</sup>	20.02±0.35	61.57±0.53
EDTA zinc 100 mg/kg	6.64±0.16 <sup>ab</sup>	1.63±0.05 <sup>ab</sup>	19.69±0.24	60.03± 0.71
Bentonite 30 g /kg	6.50±0.24 <sup>ab</sup>	1.62±0.03 <sup>ab</sup>	19.77±0.22	59.85±0.89
Nano-clay30 g /kg	5.91±0.16 <sup>bc</sup>	1.73±0.02 <sup>a</sup>	19.79± 0. 25	60.96±0.70
Significance	*	***	NS	NS

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

\*\*\* P<0.001, \* P<0.05 and NS = Not significant.

transport of oxygen and carbon dioxide in the blood. As with Hematocrit, it is an important determinant of anemia (decreased), dehydration (increased), polycythemia (increased), poor diet/nutrition, or possibly a malabsorption problem. The hemoglobin concentration of fishes is perhaps an index of their physiological activity. We may have in the fishes an illustration of how hemoglobin because of its function in respiration permits organisms to attain habits of definite survival value (Hall and Gray, 1929). Red blood cells count increased by 22.01, 14.60, 19.93, 17.48, 16.69 and 24.46% compared to the control, respectively in fish group fed diet supplemented with selenium oxide, organic selenium, zinc oxide, EDTA zinc, clay and nano-clay, when compared with those fed diet without any supplementation. Fish groups fed diets supplemented with selenium oxide and nano-clay recorded the highest RBC's. Red blood cells main function is to carry oxygen to the tissues and to transfer carbon dioxide to the lungs. This process is possible through the RBC's containing hemoglobin which combines easily with oxygen and carbon dioxide. The application of haematological and serological techniques have proved valuable for fishery biologists in assessing the health of fish and monitoring stress responses either due to fluctuations in environmental condition or due to pollutants. In Nile tilapia, exposure to pollutants for long durations led to marked

anemia and leukopenia (Rizkalla *et al.*, 1997 and Hussein *et al.* 2000).

Plasma total protein and its fractions, creatinine and uric acids significantly (P<0.001 or 0.01) affected with feed additives supplementation in Nile tilapia feed (Table 5). Plasma total protein significantly (P<0.05) increased by 30.60, 23.29, 22.52, 15.24, 20.09 and 19.29% compared to the control, respectively in fish group fed diet supplemented with selenium oxide, organic selenium, zinc oxide, EDTA zinc, clay and nano-clay, when compared with those fed diet without any supplementation (Table 5). Fish groups fed diets supplemented with selenium oxide or organic selenium recorded the higher concentrate of blood total protein. Proteins are the most abundant compound in serum. Decreased levels may be due to poor nutrition or liver damage. Also, plasma albumin significantly (P<0.05) increased by 47.25, 26.42, 41.57, 24.54, 37.52 and 34.56% compared to the control, respectively in fish group fed diet supplemented with selenium oxide, organic selenium, zinc oxide, EDTA zinc, clay and nano-clay, when compared with those fed diet without any supplementation. Fish groups fed diets supplemented with selenium oxide or zinc oxide recorded the higher concentrate of plasma albumin. The same figures for plasma globulin were 13.13, 20.05, 2.54, 5.47, 1.84 and 3.28 compared to the control;

**Table 5. Some plasma constituents as affected by dietary feed additives on Nile tilapia fingerlings**

Treatment	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control	4.68±0.10 <sup>d</sup>	2.39±0.02 <sup>c</sup>	2.28±0.11 <sup>c</sup>	0.27±0.01 <sup>a</sup>	4.91±0.02 <sup>a</sup>
Inorganic selenium 0.3 mg/kg	6.11±0.04 <sup>a</sup>	3.52±0.06 <sup>a</sup>	2.58±0.02 <sup>ab</sup>	0.23±0.01 <sup>b</sup>	4.41±0.13 <sup>b</sup>
Organic selenium 0.3 mg/kg	5.77±0.09 <sup>b</sup>	3.02±0.05 <sup>d</sup>	2.74±0.07 <sup>a</sup>	0.23±0.01 <sup>b</sup>	4.36±0.09 <sup>b</sup>
Inorganic zinc 100 mg/kg	5.73±0.06 <sup>b</sup>	3.39±0.01 <sup>b</sup>	2.34±0.05 <sup>c</sup>	0.23±0.05 <sup>b</sup>	4.30±0.04 <sup>b</sup>
EDTA zinc 100 mg/kg	5.39±0.09 <sup>c</sup>	2.98±0.03 <sup>d</sup>	2.40±0.06 <sup>bc</sup>	0.24±0.05 <sup>b</sup>	4.21±0.06 <sup>b</sup>
Bentonite 30 g/kg	5.62±0.01 <sup>bc</sup>	3.29±0.02 <sup>bc</sup>	2.32±0.02 <sup>c</sup>	0.23±0.03 <sup>b</sup>	4.45±0.06 <sup>b</sup>
Nano-clay 30 g/kg	5.58±0.03 <sup>bc</sup>	3.22±0.01 <sup>c</sup>	2.35±0.02 <sup>c</sup>	0.22±0.05 <sup>b</sup>	4.22±0.03 <sup>b</sup>
Significance	***	***	**	**	***

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

\*\*\*  $P<0.001$  and \*\*  $P<0.01$ .

respectively. Albumin is the major constituent of serum protein. It is manufactured by the liver from the amino acids taken through the diet. It helps in osmotic pressure regulation, nutrient transport and waste removal. Lower levels are seen in poor diets, infection, liver damage or inadequate iron intake. On the other hand, the plasma concentrations of creatinine and uric acids decreased significantly as affected with dietary feed additives. Plasma creatinine significantly ( $P<0.05$ ) decreased by 12.55, 15.30, 14.76, 9.96, 12.18 and 16.97%, compared to the control respectively in fish group fed diet supplemented with selenium oxide, organic selenium, zinc oxide, EDTA zinc, clay and nano-clay, when compared with those fed diet without any supplementation (Table 5). The same trend was observed in uric acid concentration. The decreased values were 10.22, 11.15, 12.38, 14.17, 9.42 and 14.06% compared to the control, respectively. Creatinine is the waste product of muscle metabolism. Its level is a reflection of the bodies muscle mass. Elevated levels are sometimes seen in kidney disease due to the kidneys job of excreting creatinine, muscle degeneration, and some drugs involved in impairment of kidney function.

Plasma transaminase enzymes (AST and ALT) and total lipids concentrations significantly ( $P<0.05$ ) decreased as affected with dietary feed additives, while glucose concentration

significantly ( $P<0.01$ ) increased (Table 6). AST and ALT are reasonably sensitive indicators of liver damage or injury from different types of diseases or conditions, and collectively they are termed liver tests or liver blood tests. However, it must be emphasized that higher-than-normal levels of these liver enzymes should not be automatically equated with liver disease. They may mean liver problems or they may not. Low levels of AST are normally found in the blood. The amount of AST in the blood is directly related to the extent of the tissue damage.

Plasma glucose significantly ( $P<0.05$ ) increased by 9.32, 7.57, 8.04, 7.82, 9.44 and 7.00% compared to the control, respectively in fish group fed diet supplemented with selenium oxide, organic selenium, zinc oxide, EDTA zinc, clay and nano-clay, when compared with those fed diet without any supplementation (Table 6). Fish groups fed diets supplemented with selenium oxide or zinc oxide recorded higher concentrate of plasma albumin. On the other hand, plasma total lipids significantly ( $P<0.05$ ) decreased by 2.96, 3.27, 3.74, 3.23, 2.96 and 4.39% compared to the control, respectively in fish group fed diet supplemented with selenium oxide, organic selenium, zinc oxide, EDTA zinc, clay and nano-clay, when compared with those fed diet without any supplementation.



**Table 6. Some plasma constituents as affected by dietary feed additives on Nile tilapia fingerlings**

Treatment	AST (UI/l)	ALT (IU/l)	Glucose (mg/dl)	Total lipids (mg/dl)
Control	44.38±0.49 <sup>a</sup>	39.02±0.46 <sup>a</sup>	135.20±0.72 <sup>b</sup>	129.12±1.47 <sup>a</sup>
Inorganic selenium 0.3 mg/kg	40.38±0.69 <sup>b</sup>	34.73±0.77 <sup>b</sup>	147.80±2.20 <sup>a</sup>	125.29±0.37 <sup>b</sup>
Organic selenium 0.3 mg/kg	41.05±0.86 <sup>b</sup>	34.29±0.61 <sup>b</sup>	145.43±1.58 <sup>a</sup>	124.90±1.28 <sup>b</sup>
Inorganic zinc 100 mg/kg	41.48±0.72 <sup>b</sup>	34.60±0.90 <sup>b</sup>	146.06±0.75 <sup>a</sup>	124.29±0.21 <sup>b</sup>
EDTA zinc 100 mg/kg	41.22±0.52 <sup>b</sup>	34.30±1.063 <sup>b</sup>	145.76±1.51 <sup>a</sup>	124.95±0.36 <sup>b</sup>
Bentonite 30 g/kg	41.32±0.59 <sup>b</sup>	34.80±1.40 <sup>b</sup>	147.96±2.19 <sup>a</sup>	125.30±1.04 <sup>b</sup>
Nano-clay 30 g/kg	42.29±0.83 <sup>ab</sup>	36.15±0.70 <sup>b</sup>	144.66±2.26 <sup>a</sup>	123.45±0.61 <sup>b</sup>
Significance	*	*	**	*

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

\*\* P<0.01 and \* P<0.05.

Ayyat *et al.* (2004) reported that serum total protein, albumin, creatinine and AST significantly increased with increasing zinc level in fish diet, while ALT significantly decreased. Abdel-Tawwab *et al.* (2007) revealed the increases in total lipid, total protein, albumin and globulin levels, AST and ALT in blood of African catfish as increasing dietary selenium levels. Also, Khalafalla *et al.* (2011) found that increasing dietary selenium level caused significant increases in total protein, albumin and globulin compared to the control diet.

### Body Composition

No statistical differences were observed in fish body dry moisture, crude protein, ether extract and ash as affected by dietary feed additives (Table 7). Ether extract percentages of the flesh insignificantly decreased as affected by dietary feed additives. The results of Zhao *et al.* (2011) showed that the composition of tilapia carcass was also found to be influenced by various levels of dietary zinc. Ayyat *et al.* (2004) reported that fish body composition did not affected significantly with dietary zinc supplementation.

### Profit Analysis

Feed cost, income from body gain and final margin clearly increased with dietary feed additives supplementation in Nile tilapia fish

(Table 8). Feed cost increased by 53.16, 30.00, 57.37, 26.84, 36.26 and 36.84% compared to the control, respectively in fish group fed diet supplemented with selenium oxide, organic selenium, zinc oxide, EDTA zinc, clay and nano-clay, when compared with those fed diet without any supplementation. Also, income from body gain increased by 70.20, 53.01, 70.77, 57.88, 44.41 and 38.97%, respectively in fish group fed diet supplemented with selenium oxide, organic selenium, zinc oxide, EDTA zinc, clay and nano-clay. The same figures for final margin were 90.57, 80.50, 86.79, 94.97, 54.09 and 41.51%, respectively (Table 8). Fish groups fed diets supplemented with EDTA zinc, selenium oxide, zinc oxide or organic selenium recorded higher final margin and income from body gain. The best final margin was obtained in fish group fed diet supplemented with EDTA zinc.

### Conclusion

The results from the present study indicated that growth performance, feed utilization, feed cost, income from body gain and final margin showed significant improvement compared to the control group with dietary feed additives supplementation to feed of Nile tilapia fish. The present study recommends using safe feed additives in Nile tilapia fish production.

**Table 7. Proximate chemical analysis on wet weight of whole-body of Nile tilapia as affected by dietary feed additives on Nile tilapia fingerlings**

Treatment	Moisture (%)	Crude protein (%)	Ether extract (%)	Ash (%)
Control	74.15±0.14	17.91± 0.20	6.91±0.52	3.92±0.33
Inorganic selenium 0.3 mg/kg	73.03±0.25	17.24±0.01	6.74± 1.20	3.24±1.02
Organic selenium 0.3 mg/kg	74.24±0.25	17.28±0.20	4.99± 1.24	3.70±0.22
Inorganic zinc 100 mg /kg	72.79±0.27	16.86±0.30	5.50±0.50	3.82±1.13
EDTA zinc 100 mg /kg	73.44±0.23	17.87±0.24	5.98±1.03	3.62±1.23
Bentonite 30 g /kg	70.12±0.20	17.07±0.32	6.66±1.01	3.73±1.13
Nano-clay 30 g /kg	74.94±0.24	17.46±0.01	5.70±1.01	3.83±1.23
Significance	NS	NS	NS	NS

NS= Not significant.

**Table 8. Economic visibility of Nile tilapia fish as affected by dietary feed additives**

Treatment	Total feed intake (g)	Feed cost (LE)/fish	Total gain (g)	Income from gain (LE)/fish	Final margin (LE)/fish
Control	26.37	0.19	23.26	0.34	0.15
Inorganic selenium 0.3 mg/kg	40.40	0.29	39.56	0.59	0.30
Organic selenium 0.3 mg/kg	34.35	0.24	35.61	0.53	0.28
Inorganic zinc 100 mg/kg	41.49	0.29	39.73	0.59	0.29
EDTA zinc 100 mg/kg	33.43	0.24	36.70	0.55	0.31
Bentonite 30 g /kg	35.95	0.25	33.60	0.50	0.24
Nano-clay 30 g /kg	36.12	0.26	32.34	0.48	0.22

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## تأثير بعض إضافات الأعلاف على أداء النمو، ومكونات الدم في أسماك البلطي النيلي

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في تصميم عشوائي تام، تم توزيع ٢١٠ إصباغية من الأسماك عشوائيا في ٢١ حوض زجاجي في ٧ معاملات (٣ مكررات لكل معاملة)، المجموعة الأولى تغذت على عليقة كنترول دون أي معاملة، وتمت تغذية المجموعة الثانية على عليقة مضاف لها أكسيد سيلينيوم ٠.٣ ملجم سيلينيوم/كجم عليقة، تم تغذية المجموعة الثالثة على عليقة مضاف لها السيلينيوم العضوي (٠.٣ ملجم سيلينيوم/كجم عليقة)، وغذيت المجموعة الرابعة بإضافة أكسيد الزنك للعليقة (١٠٠ ملجم من الزنك/كجم عليقة)، وتغذت المجموعة الخامسة بإضافة إيتا زنك للعليقة، ١٠٠ ملجم زنك/كجم عليقة، تم تغذية المجموعة السادسة على عليقة مضاف لها معادن الطين ٣٠ جم بنتونيت/كجم علف (٣%)، لم يكن هناك تأثير واضح لإضافات الأعلاف الغذائية على جودة المياه في جميع المجموعات التجريبية، وكانت جميع قياسات المياه مستقرة وضمن نطاقات مقبولة، وسجلت المجموعات السمكية التي غذيت علائق مضاف لها أكسيد الزنك أو أكسيد السيلينيوم أعلى وزن نهائي للوزن الحي وزيادة في الوزن اليومي، ثم المجموعات التي أضيف لها الإيتا زنك والسيلينيوم العضوي والطين الطبيعي. وقد خفضت الإضافات الغذائية العلفية معدل وفيات الأسماك، وكانت مجموعة الأسماك المضاف لعلائقها الإيتا زنك والنانو كلاي قد سجلت انخفاض معدل الوفيات، وسجلت مجموعات الأسماك التي غذيت على علائق مضاف لها السيلينيوم العضوي و الإيتا زنك أفضل نسبة لتحويل الغذاء، ولم يلاحظ وجود فروق ذات دلالة إحصائية في نسبة الرطوبة والبروتين الخام ومستخلص الأيثر والرماد المتأثر بالإضافات الغذائية، وسجلت المجموعات السمكية التي تغذت على علائق مضاف لها الإيتا زنك وأكسيد السيلينيوم وأكسيد الزنك والسيلينيوم العضوي أعلى هامش ربح ومكاسب في وزن الجسم، تم الحصول على أفضل هامش ربح نهائي في مجموعة الأسماك المضاف لعلائقها الإيتا زنك.

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