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EFFECT OF AQUAVIANCE PRODUCT AS DIETARY SUPPLEMENTATION TO IMPROVE GROWTH PERFORMANCE, FEED INTAKE, INNATE IMMUNITY AND ANTIOXIDANT ACTIVITY FOR NILE TILAPIA, (*OREOCHROMIS NILOTICUS*)

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Abstract

An experiment was conducted to evaluate the effect of commercial feed additive (Aquaviance) on growth performance, nutrient utilization, body composition, immune response, digestive enzymes and antioxidants of Nile tilapia (*Oreochromis niloticus)* fingerlings. Aquaviance feed additive was added to the diets at 1, 2 and 3 g/kg feed) and fed to the fish for 12 weeks at a rate of 4% of live body weight for the first 2 weeks, and 3% for the rest of the experimental period. Fish fed on diets with Aquaviance feed additive showed improved growth performance, feed utilization and body composition significantly ($p<0.05$) compared to fish fed on the control diet. The maximum feed consumption was significantly greater with fish fed 2 and 3 g/kg Aquaviance diets. The best growth performance was observed in the group fed with dietary 2 g/kg. Moisture and ash contents did not differ among treatments with Aquaviance feed additive. Protein content increased and lipid content decreased significantly with increasing levels of Aquaviance in diets. In the present study, dietary supplementation of Aquaviance enhance non-specific immune response of *Oreochromis niloticus* as lysozyme activity and respiratory burst activity showed significant increase in group fed 2 g/kg Aquaviance then group fed 3 g/kg followed by 1 g/kg than control group. Improvement in gills and liver Superoxide dismutase activity (SOD), Catalase activity (CAT) and Glutathione peroxidase (GPx) and reduced Malondialdehyde (MDA) activities was observed in the group fed with dietary 2 g/kg which was consistent with the immune response. The highest values of intestinal amylase activity and lipase activities were significantly observed in fish fed 2 g/kg Aquaviance than control group. These results revealed that using

Aquaviance at level 2 g/kg was the best in term of growth performance, feed utilization, body composition, innate immune response, digestive enzyme activity and antioxidant capability. Additionally, Aquaviance additon reduced the feeding cost to produce one kg fish gain; this reduction at 2 g/kg Aquaviance was 23.62%, respectively as compared to control diet.

Key words: Feed additives, Aquaviance, Nile tilapia, growth performance, feed utilization, body composition, innate immune response, digestive enzymes and antioxidants.

INTRODUCTION

Over the years, the total world fishery production decreased slightly and the human consumption for aquatic product increased (FAO, 2010). The reduction in capture fisheries was partly compensated for the fast growth of aquaculture industry. The need for enhanced disease resistance, feed efficiency and growth performance of cultured organisms is substantial for various sectors of the industry (El-Haroun *et al.*, 2006). If growth performance and feed efficiency are increased in commercial aquaculture, the costs of productions are likely to reduce. On the other hands, farmers prefer quality feeds with good feed conversion ratios, enhancing fish growth, survival, yield and successful crops. Though farmers adopt improved technologies to increase production, survival, growth promoters that support optimal performance and animal health is in demand for inclusion in aqua feeds (Dada and Olugbemi, 2013).

One of these commercial feed, Aquaviance which is a blend aromatic plant extracts essential oils and prebiotics (Fructo-oligosaccharides) with target actions to stimulate digestive functions, antioxidant capability, control pathogenic bacteria, and contribute to a good balance of intestinal flora. Due to the success of its essential oil and plant extract based additives for terrestrial animals, Techna is now valorizing its expertise in aquaculture by developing Aquaviance, which is a new feed additive solution designed to optimize performance in aquaculture systems.

Over crowdness in fish farms has led to deterioration of water quality and increase susceptibility of cultured fish to stress and pathogen infections (Sun *et al.*, 2010). Moreover, resulted in oxidative stress, reflected by increased

production of reactive oxygen species (ROS) which are highly reactive molecules and can damage cell structures such as carbohydrates, nucleic acids, lipids, proteins and impairing the cell membrane through lipid peroxidation and alter their functions (Ahmad *et al.,* 2000). Gills and liver is considered a vital metabolic organ exposed to various drugs, chemicals, and toxins. So, it is essential to protect liver by using a successful hepatoprotective agent using natural substances as antioxidant (Sahin *et al.,* 2014) Antioxidants are our first line of defense against unstable molecules known as free radical damage, and are critical for maintaining optimum health and wellbeing.. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) are able to protect cells against oxidative damage by neutralizing ROS (Nordberg and Arner, 2001).

Aquaviance inclusion in aqua feeds targets performance in terms of improved growth and survival, low FCR and improved health status, by control of intestinal flora and stimulation of intestinal enzymes secretion. Aquaviance has been successfully tested in shrimp *(Penaeus vannamei*), Tilapia, Pangasius, Sea bream, Trout, Flounder and is efficient against a wide range of pathogenic strains-*Vibrio anguilarum, Vibrio ordalii, Aermonas salmonicida and Aermonas hydrophila.*

Nowadays, aquaculture represents one of the fastest growing food producing sectors of the world. World aquaculture has grown tremendously during the last fifty years from a production of less than a million tonnes in the early 1950s to more than 65.0 million tonnes in 2007 (food fish and aquatic plants). Particularly Nile tilapia (*Oreochromis niloticus*) accounts for over 80% of the world tilapia production of 3.1 million tons per year (FAO, 2012). The main objective of this study was to investigate the effect of using commercial feed additive (Aquaviance) on growth, feed utilization, body composition, nonspecific immune response, digestive enzyme activity and antioxidant capability of Nile tilapia (*O. niloticus)* fingerlings.

MATERIALS AND METHODS

Four experimental diets were formulated to 31.2% crude protein and 7% lipid with 0, 1, 2 and 3 g/kg of Aquaviance (NOREL Misr Animal Nutrition, Egypt). The chemical proximate chemical composition of the main ingredients in the diets is shown in Table (1). The dry ingredient of each diet was thoroughly mixed, and 100 ml of water was added per kg diet. Afterwards, the mixture (ingredients and water) was blended using a kitchen blender to make a paste of each diet. Pelleting of each diet was carried out by passing the blended mixture through a laboratory pellet machine with a 1-mm-diameter die. The pellets were dried in a drying oven for 24 hours at (65^0C) and stored in plastic bags in a refrigerator at (-20^0C). The caloric value as Gross energy (GE) of each ingredient was estimated on the basis of 5.65 kcal /g protein, 9.45 kcal /g lipid, and 4.11 kcal /g of carbohydrate (NRC, 1993).

All-male Nile tilapia, *O. niloticus*, fingerlings (treated with 17 -methyl testosterone hormone) were obtained from the nursery ponds, Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abu-Hammad, Sharkia, Egypt. Fish were held in a fiberglass tank for two weeks for acclimation during which they were fed a formulated diet containing 31.2% crude protein. Fifteen fish were frozen at −20°C for initial proximate whole body analysis. After that, average fish weights (7.46 g) were distributed randomly at a rate of 10 fish/100-L aquarium. Each aquarium was aerated by using small air-bumps. Settled fish wastes along with a half of the aquarium water was siphoned daily, and replaced by well-aerated and dechloronated tap water from a storage tank. Fish in all treatments were fed the tested diets at a rate of 4% of live body weight for the first 2 weeks, and 3% for the rest of the experimental period. Diets were offered twice daily at 9:00 and 13:00 h for 12 weeks. Fish in each aquarium were sampled biweekly and the amount of feed adjusted accordingly. Dead fish were daily recorded and removed. At the end of the study, fish were individually weighed. Fish growth and feed utilization parameters were calculated as follows:

Weight gain (g) = $W_2 - W_1$

Specific growth rate (SGR; (%g / day) = 100 (Ln W_2 – Ln W_1)/ T

Where W_1 and W_2 are the initial and final weights, respectively, and T is the experimental period (days).

Feed conversion ratio (FCR) = feed intake / weight gain

Protein efficiency ratio (PER) = weight gain / protein intake

Apparent protein utilization (APU; %) = 100 [protein gain in fish (g) / protein intake in diet (g)].

Energy utilization (EU; %) = 100 [Energy gain in fish (g)/energy intake in diet (g)].

Diets and fish were analyzed according to standard methods (AOAC, 1990) for moisture, crude protein, total lipids, and ash. Moisture content was estimated by drying samples in an oven at 85◦C until constant weight was achieved. Nitrogen content was measured using a micro-Kjeldahl apparatus, and crude protein was estimated by multiplying total nitrogen content by 6.25. Total lipid content was determined by ether extraction for 16 h, and ash was determined by combusting samples in a muffle furnace at 550◦C for 6 h. Crude fiber was estimated according to Goering and Van Soest (1970). Gross energy was calculated according to (NRC, 1993).

After 1^{st}, 2^{nd} and 3^{rd} months of feeding, Fish were anaesthetized using buffered tricaine methane sulfonate (20 mg /L) and blood samples were collected from the caudal vein of three fish/treatment using syringe moistens with EDTA to evaluate respiratory burst activity. It was determined based on measuring Nitro-BlueTitrazolium activity (NBT) following the method described by Siwicki (1989). Another blood samples were collected without anticoagulant and allowed to clot for 2 h at room temperature. After that, all Samples were centrifuged at 3000 ×g for 5 min. The separated sera were collected in Eppindorf tube and preserved at -20°C for lysozyme activity assay according to Schaperclaus *et al.* (1992).

Table 1. Ingredients and chemical analysis of the experimental diets (on dry matter basis) containing different Aquaviance.

Ingredients	Control	Aquaviance level (%)		
	0.0	1	2	3
Fish meal (HFM)	11.5	11.5	11.5	11.5
Soybean meal (SBM)	43.3	43.3	43.3	43.3
Ground corn (CNM)	21	21	21	21
Wheat bran (WB)	14	14	14	14
Cod fish oil	2.9	2.9	2.9	2.9
Corn oil	2.3	2.3	2.3	2.3
Vitamins premix[1]	1.5	1.5	1.5	1.5
Minerals Premix[2]	1.5	1.5	1.5	1.5
Starch	2.0	1.9	1.8	1.7
Aquaviance	0.0	0.1	0.2	0.3
Chemical analysis (%)				
Dry matter	92.23	92.53	92.35	92.29
Crude protein	31.2	31.28	31.30	31.32
Crude fat	7.28	7.26	7.24	7.23
Ash	7.18	7.15	7.13	7.11
Fiber	5	4.9	4.9	4.9
NFE[3]	49.34	49.41	49.43	49.44
GE(Kcal/100g)[4]	447.46	448.01	448.02	448.08

1-Vitamin premix (per kg of premix): thiamine, 2.5 g; riboflavin, 2.5 g; pyridoxine, 2.0 g; inositol, 100.0 g; biotin, 0.3 g; pantothenic acid, 100.0 g; folic acid, 0.75 g; para-aminobenzoic acid, 2.5 g; choline, 200.0 g; nicotinic acid, 10.0 g; cyanocobalamine, 0.005 g; a-tocopherol acetate, 20.1 g; menadione, 2.0 g; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU.

2- Mineral premix (g/kg of premix): CaHPO4.2H2O, 727.2; MgCO4.7H2O, 127.5; KCl 50.0; NaCl, 60.0; FeC6H5O7.3H2O, 25.0; ZnCO3, 5.5; MnCl2.4H2O, 2.5; Cu(OAc)2.H2O, 0.785; CoCl3.6H2O, 0.477; CaIO3.6H2O, 0.295; CrCl3.6H2O, 0.128; AlCl3.6H2O, 0.54; Na2SeO3, 0.03.

3- Nitrogen-Free Extract (calculated by difference) = 100 – (protein + lipid + ash + fiber).

4- Gross energy (GE) was calculated from **NRC, (1993**) as 5.65, 9.45, and 4.1 kcal/g for protein, lipid, and carbohydrates, respectivel

Briefly, monthly after blood collection, gills and liver samples were collected from the euthanized fish for antioxidant enzymes assay. The gills and liver tissues were homogenized in 9 volumes of 20 mM phosphate buffer (pH 7.4) containing ethylene diamine tetra acetic acid (EDTA) and 0.1% Triton X-100. The homogenates were centrifuged at 600 ×g for 10 minutes and the supernatants were collected in clean Epindroff tube for antioxidant enzymes assay**.** Superoxide dismutase activity (SOD) was measured according to Kakkar *et al*. (1984), Catalase activity (CAT) was measured according to luck

(1963) and Glutathione peroxidase (GPx) and Malondialdehyde (MDA) activities (as a biomarker of lipid peroxidation) were measured according to Habig *et al*. (1974).

later partial intestine samples were collected and homogenized in 5 volumes v/w of ice-cold distilled water for measuring digestive enzymes(as Amylase and Lipase activity). Extracts utilized for enzyme assays were obtained after homogenization of larvae (35 mg ml−1) in cold 50 mM Tris–HCl buffer, pH 8.0, followed by centrifugation (13.500 ×g; 30 min at 4 °C) and The supernatant was then collected and stored at -80 °C for subsequent analysis. Amylase activity was quantified according to Bernfeld (1995). Lipase activity was determined according to manufacturer instructions of commercial kits obtained from (Biodiagnostic, Egypt).

Finally at the end of experimental period (12 weeks), challenge test was carried out. All untreated and treated fishes were divided into two subgroups; the first group was injected intra peritoneal (IP) with 0.5 ml of pathogenic *Aeromonas hydrophila*. The second group was injected IP with 0.5 ml of saline solution and used as a negative control. Both subgroups were kept under observation for 14 days post challenge during which incidences of daily mortality were recorded. Challenge test were determined according to Brook *et al*. (1988) and Miles and Misra (1983).

The cost of feed required to produce a unit of fish biomass was estimated using economic evaluation. The estimation was based on the local retail sale market price of all the dietary ingredients at the time of the study. These prices (in LE/kg) were as follows: herring fish meal, 17; soybean meal, 5.0; corn meal, 3.50; wheat bran, 3; starch, 4.0; fish oil, 13; corn oil, 11; vitamin premix, 10.0; mineral mixture, 4.0; and Aquaviance, 59.00.

Fish growth, feed utilization, survival rate, and proximate chemical composition data, immunological parameters, antioxidant activity and digestive enzymes assay were subjected to one-way ANOVA. Difference between means

was tested at the 5% probability level using Duncans new multiple range test. All statistical analyses were done using SPSS program V.10 (SPSS, Richmond, USA) as described by Dytham (1999).

RESULTS AND DISCUSSION

The present study investigated the effect of Aquaviance supplementation on growth performance, feed utilization, survival rat, immune response, digestive enzymes and antioxidant enzymes of Nile tilapia (*O. niloticus)*. The obtained results in Table 2 indicated that there were no significant differences in growth performance (final weight gain, relative body weight gain and specific growth rate) between 2 g/kg and 3 g/kg diets containing Aquaviance. The optimum values of these parameters were obtained using diet containing 2 g/kg Aquaviance. Lowest values were observed in fish fed the control diet (Table 3). That means the supplementation of 2 g/kg Aquaviance product has significantly improved the growth performance in Nile tilapia, *O. niloticus*. Supplementation with Aquaviance acts as a natural growth promoter for Nile tilapia. These improvement are related to Aquaviance product supplementation in fish diets which containing aromatic plant extract, essential oils and prebiotics (Fructo-oligosaccharide) for the purpose of stimulating digestive functions and contributing to a good balance of intestinal flora that enhance growth performance.by his action on the prebiotics Aquaviance allows the uniform growing of microvili and increasing by this the absorption surface for nutrients and indirectly fodder use and health witch give a better body growth (Torrecillas *et al.,* 2007 and Staykov *et al.,* 2007). These results agree with Hung *et al.* (2011) reported that the growth performance of 0.2% Aquaviance showed a significantly different with the control. And with Ahmad *et al.* (2014) reported that growth performance increased significantly when fish fed diet containing 2g/kg prebiotics. Also, El-Mousallamy *et al.* (2014) reported that dietary supplementation of prebiotics (β-glucan) improved significantly the growth performance in comparison to the control diet. Ahmad *et al.* (2015) indicated that Nile tilapia fed on diet containing 1.5 and 2g/kg prebiotics showed the higher growth performance parameters. Fish survival rate fed

different Aquaviance product levels (100%) was much higher than the control fish (93.3%). These results indicated that Aquaviance has improved the fish survival rate. Similar results of Aquaviance supplementation on survival rate for Nile tilapia (*O. niloticus*) were obtained by Sang and Fotedar (2010) who found that the survival rate of marron (*Cherax tenuimanus*) fed prebiotics supplemented diets was higher than survival of marron fed the diets without beta glucan supplement.

Table 2. Growth performance of Nile tilapia fingerlings fed different Aquaviance levels for 12 weeks.

Items	Control	Aquaviance levels (g/kg)		
	0.0	1	2	3
Initial weight (g)	7.44±0.01	7.47±0.01	7.45±0.01	7.46±0.01
Final weight (g)	23.99±0.10^{c}	29.39±0.36^{b}	35.53±0.55^{a}	34.32±0.47^{a}
Weight gain (g)	16.55±0.09^{c}	21.92±0.36^{b}	28.08±0.54^{a}	26.85±0.47^{a}
RBWG %	222.25±1.25^{c}	293.44±5.32^{b}	376.91±6.68^{a}	360.05±6.75^{a}
SGR (%g / day)	1.39±0.01^{c}	1.62±0.01^{b}	1.85±0.01^{a}	1.81±0.01^{a}
Survival rate (%)	93.3±3.85	100	100	100

Means having the same letter in the same row are not significantly different at $P < 0.05$

Data in Table 3 shows that feed utilization FI, FCR, FER, PER, APU and EU% of Nile tilapia fingerlings fed diets containing different levels of Aquaviance. Results indicated that feed intake significantly increased ($p<0.05$) with increasing Aquaviance levels in fish diets, and the maximum feed consumption was significantly greater with fish fed 2 g/kg and 3 g/kg Aquaviance diets. Contrarily, FCR decreased significantly ($p<0.05$) at fish fed 2 g/kg and 3g/ kg (1.41 and 1.43, respectively), while the highest FCR was obtained at control diet (1.88). However, diet containing 2 g/kg Aquaviance was the best supplemented level for FI, FCR, FER, PER, APU and EU in comparison to the control and the other treatment. The lowest values were obtained at control diet. These values increased significantly due to increasing levels of Aquavience in fish diet up to 2 g/kg. Elevated FI may be a result of a high demand for nutrients stimulated by the higher growth rate and /or improved appetite related to sensory stimulation by the presence of Aquavience

in the diet. It seems that Aquaviance enhance feed utilization in fish diets due to the effect of essential oils which had enhanced secretion of internal enzymes in digestive tract and the effect of prebiotics which improved the enzymatic digestion of complex polysaccharides including cellulose, organic phosphrous (phytic acid) utilization, and fiber digestion (Tewary and Patra, 2011). There are previous studies showing the effect of essential oils had improved feed intake in many fishes (Hung *et al.,* 2011). These results agree with Ebrahimi *et al.,* 2012; Ahmad *et al.,* 2014; EL-Mousallamy *et al.,* 2014 and Ahmad *et al.,* 2015.

Table 3. Feed utilization of Nile tilapia fingerlings fed different Aquaviance levels for 12 weeks.

Items	Control 0.0	Aquaviance levels (g/kg)		
		1	2	3
Feed intake (g feed /fish)	31.13±0.55^{c}	34.88±0.15^{b}	39.79±0.16^{a}	38.64±0.59^{a}
FCR	1.88±0.03^{a}	1.59±0.02^{b}	1.41±0.03^{c}	1.43±0.01^{c}
FER	53.20±0.86^{c}	62.84±1.18^{b}	70.58±1.62^{a}	69.50±0.85^{a}
PER	1.85±0.02^{c}	2.17±0.04^{b}	2.44±0.05^{a}	2.40±0.02^{a}
APU%	30.97±0.45^{c}	37.91±0.73^{b}	41.94±0.87^{a}	41.40±0.50^{a}
EU%	18.32±0.28^{c}	22.34±0.41^{b}	24.72±0.48^{a}	24.37±0.32^{a}

Means having the same letter in the same row are not significantly different at $P < 0.05$.

The proximate chemical composition of whole body of fingerlings Nile tilapia fed different levels of Aquaviance shown in Table 4. Results indicated that after 12 weeks of feeding, there were no significant difference in moisture and ash ($p>0.05$) contents among diets containing Aquaviance in comparison to control diets. On the other hand, protein content increased and lipid content decreased significantly with increasing levels of Aquaviance in diets. These data suggested that Aquaviance supplementation play a role a role in enhancing feed intake with a subsequent enhancement of fish body composition. Moreover, due to the high feed intake, nutrients utilization, and digestibility, the high changes in protein and lipid content in fish body could be linked with changes in their synthesis and deposition rate in muscles (Abdel-Tawwab *et al.,* 2008). Moreover, due to prebiotics have been reported to enhance amino acid

utilization by killing intestinal infectious micro-flora, thereby increasing amino acid utilization in host. These results agree with those (Genc *et al.,* 2007; Ahmad *et al.,* 2014 and El-Mousallamy *et al.,* 2014) reported that protein contents increased with increasing rates of dietary prebiotics in diets for hybrid tilapia (*O. niloticus x O. aureus*). Similarly, Ahmad *et al.* (2015) who reported that total protein contents of fish increased, while total lipid decreased insignificantly by increasing levels of prebiotics in the experimental diets.

Table 4. Whole body composition of Nile tilapia fingerlings fed different Aquaviance, levels for 12 weeks.

Items	Control	Aquaviance levels (g/kg)		
	0.0	1	2	3
Moisture	75.34±0.02^{a}	74.53±0.05^{b}	74.58±0.04^{b}	74.60±0.06^{b}
Crude protein	62.48±0.22^{b}	63.57±0.04^{a}	63.71±0.03^{a}	63.75±0.02^{a}
Total Lipids	19.32±0.03^{a}	19.26±0.05^{ab}	19.20±0.01^{bc}	19.13±0.01^{c}
Ash	15.85±0.23^{a}	14.60±0.06^{b}	14.66±0.01^{b}	14.64±0.02^{b}

Means having the same letter in the same row are not significantly different at $P < 0.05$

Several parameters such as lysozyme activity, respiratory burst activity, nitric oxide synthase, bactericidal activity, immunoglobulin level, antibody response, etc. are served as a good immunological indicator of fish health status (Chakrabarti *et al.,* 2014). In the present study, dietary supplementation of Aquaviance (FOS, essential oils) showed beneficial Effect on the non-specific immune response of *Oreochromis niloticus* evidenced by the significant increase of serum lysozyme activity and respiratory burst activity in group fed 2 g/kg Aquaviance then group fed 3 g/kg which showed no significance with 1 g/kg than control group. The higher lysozyme activity probable attributed to the high leukocyte production with dietary Aquaviance (FOS) (Zhang *et al.,* 2013) due to the fact that fish lysozyme is mainly produced by neutrophiles and macrophages (Fischer *et al.,* 2006). The immunostimulatory effect of FOS could be ascribed to the growth stimulation of beneficial bacteria such as lactobacilli and befidobacteria, which possess lipopolysaccharides that have immunostimulatory properties (Manning and Gibson, 2004). Moreover, acetate,

propionate and lactic acid as end products of FOS fermentation play a crucial role in modulating the immune system (Passos and Park, 2003). Furthermore, FOS could interact with toll like receptors (TLR2) expressed on macrophages (Vogt *et al.*, 2013) and up- regulated the expression of antimicrobial peptides (Leap) which have important role in innate immune defense and hence disease resistance of fish (Zhang *et al.*, 2014). Parallel to this study in a previous investigations, enhanced lysozyme activity has been recorded in red drum (Zhou *et al.*, 2010), Caspian roach fry (Soleimani *et al.* 2012), turbot (Guerreiro *et al.*, 2013) and blunt snout bream (Zhang *et al.*, 2015). However, dietary FOS showed no significant effect on lysozyme activity in other studies (Grisdale *et al.*, 2008). This contradictory may be attributable to the prebiotic dosage, life stage and/or fish species (Ibrahem *et al.*, 2010).

Table 5. Innate immune response of Nile tilapia fingerlings fed different Aquaviance, levels for 12 weeks.

Fish groups	Lysozyme (µg/ml serum)	Respiratory burst (NBT) activity (µg/ml serum)
Control	0.50 ± 0.01^{c}	0.55 ± 0.05^{c}
1 g/kg	0.76 ± 0.11^{b}	$0.56\pm0.03b^{c}$
2 g/kg	2.51 ± 0.33^{a}	0.79 ± 0.06^{a}
3 g/kg	1.08 ± 0.12^{b}	0.68 ± 0.02^{ab}

Means having the same letter in the same column are not significantly different at $P < 0.05$

The present results in Table 6 showed that gills and liver, SOD, CAT and GPX activities are all improved by the application of Aquaviance (FOS and essential oils) which was consistent with the immune response. This indicated that Aquaviance might enhance the antioxidant capability of *Oreochromis niloticus* in all concentration than control group as seen in Table (6) as the highest increase in 2 g/kg then 3 g/kg and finally 1 g/kg, as was supported by the fact that antioxidant enzymes are capable of scavenging reactive oxygen species and products of lipid per oxidation, thereby protecting cells and tissues from oxidative damage (Li X and Liu, 2007). In fact, as the immune response increases, fish cells produce reactive oxygen species (ROS) which are highly

microbicidal (Martinez-Alvarez *et al.,* 2005). In order to keep an ongoing balance between antioxidants and ROS, the major antioxidant enzymes, including SOD, CAT and GPX representing the first line of defense against oxidative stress were generated (Farombi *et al.,* 2007). In the present study, dietary supplementation of Aquaviance significantly reduced gills and liver MDA content in group feed 2 g/kg more than other groups. In addition, it should be mentioned here that the administration of FOS significantly reduced liver MDA content, indicating again that aquaviance could inhibit the process of lipid peroxide. This was supported by the fact that MDA level is a direct evidence of the toxic processes caused by free radicals (Livingstone, 2003). One probable explanation could be that dietary FOS could improve feed utilization which may contribute to dietary antioxidants assimilated. And, previous studies proved that interval feed immunostimulants such as prebiotics can increase the antioxidant status of fish Consistent with those results, in the present study, fish fed 0.8% FOS two days per week showed higher antioxidant ability compared to that of the control group and that of fish fed 0.8% FOS continuously. This suggested that an optimal feeding mode (D5) of FOS can contribute to the health of blunt snout bream (Sun *et al.,* 2010).

Table 6. Antioxidant activity in gills and liver of Nile tilapia fingerlings fed different Aquaviance, levels for 12 weeks.

Enzyme	SOD (u/l)		CAT (ng/ml)		GPX (ng/ml)		MDA (nmol/ml)	
Groups	Gill	Liver	Gill	Liver	Gill	Liver	Gill	Liver
Control	0.11± 0.02^{d}	0.10± 0.03^{d}	0.09± 0.04^{c}	0.10± 0.03^{c}	0.14± 0.03^{cd}	0.09± 0.04^{d}	0.25± 0.005^{a}	0.20± 0.02^{ab}
1 g/kg	0.13± 0.03^{bc}	0.13± 0.05^{bc}	0.14± 0.04^{abc}	0.13± 0.03^{bc}	0.20± 0.03^{abc}	0.11± 0.02^{cd}	0.19± 0.02^{ab}	0.14± 0.03^{bc}
2 g/kg	0.20± 0.01^{a}	0.19± 0.02^{a}	0.22± 0.02^{a}	0.19± 0.02^{ab}	0.29± 0.05^{a}	0.28± 0.03^{a}	0.09± 0.04^{c}	0.07± 0.04^{c}
3 g/kg	0.22± 0.02^{ab}	0.20± 0.03^{ab}	0.17± 0.04^{abc}	0.16± 0.02^{abc}	0.26± 0.04^{ab}	0.18± 0.01^{bcd}	0.10± 0.03^{c}	0.09± 0.05^{c}

Means having the same letter in the same column are not significantly different at $P < 0.05$

As can be seen from Table (7), intestinal amylase activity and lipase activities were significantly affected showed difference (P>0.05) among all the treatments by dietary Aquaviance levels with the highest values all observed in fish fed 2 g/kg. They were significantly higher than those of the control group and exhibited significant difference (P>0.05) with those of fish fed 1 g/kg and 3 g/kg. Enhanced digestive enzyme activity in fish fed a pre- or probiotics diet was reported in multiple studies (Suzer *et al.,* 2008 and Wang, 2011), these agreed with other studies recorded significant increase of amylase and lipase activity (Soleimani *et al.,* 2012 and Guerreiro *et al.,* 2015). In contrast, Concerning to intestinal amylase and lipase activities, fish fed supplemented diet with FOS showed no significant effect compared to control fed basal diet (Ye *et al.,* 2011; Wu *et al.,* 2013 and Zhang *et al.,* 2014a). This difference may be attributed to fish species, size and dietary dose. This attribution supported by Wu *et al.,* (2013) who reported that amylase activity of blunt snout bream fingerlings exhibited no significance difference with low dietary inclusion of FOS at a level of (0.5-2 g/kg diet) while at a dose level 4 and 8 g/kg diet revealed significant increase in amylase activity compared with the control.

Table 7. Digestive enzymes activity of Nile tilapia fingerlings fed different Aquaviance, levels for 12 weeks.

Enzyme	Control	1 g/kg	2 g/kg	3 g/kg
Amylase (u/l)	84.33 ± 3.47^{d}	84.33 ± 3.47^{b}	96.33 ± 3.68^{a}	65.00 ± 2.48^{c}
Lipase(u/l)	43.33 ± 6.56^{d}	95.66 ± 3.39^{b}	111.32 ± 2.05^{a}	83.00 ± 1.41^{c}

Means having the same letter in the same row are not significantly different at $P < 0.05$

The post challenge mortality is a valuable indicator for monitoring fish health and determining the efficacy of the immunostimulants. In this study after challenge with *A. hydrophila*, the mortality rate of fish fed Aquaviance was significantly lower in 2 g/kg followed by1 g/kg then 3 g/kg all than that of the control group. This suggested that dietary Aquaviance (contains FOS and aromatic essential oils) can enhance the immunity and disease resistance of *Oreochromis niloticus*. According to previous studies, this enhanced disease

resistance may be ascribed to the increase of the antibodies secreted by plasma cells, especially by leukocyte which can represent a prelude of multifaceted inflammatory-like immune response (Bagni *et al*., 2005). Another possible explanation may be that Aquaviance (FOS) stimulated immune system by enhancing IgM production and cytokine modulation as well as improving hostdefenses (Lomax and Calder, 2009), or that FOS serves as a substrate for proliferation of lactic acid bacteria and bifidobacteria, and inhibit the growth of putrefactive or pathogenic bacteria present in the colon through the production of short chain fatty acids (Saulnier *et al.,* 2009).

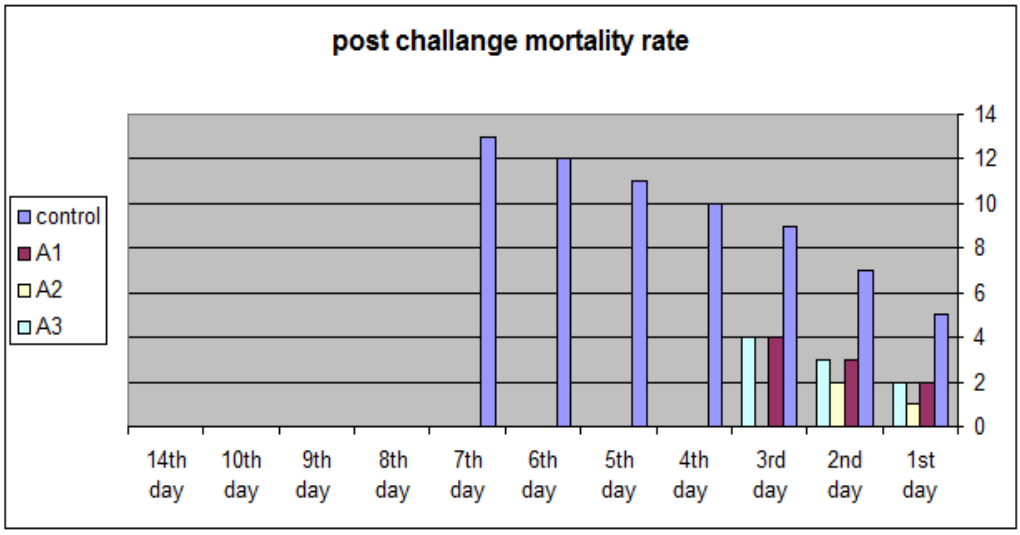

Fig. 1. Post challenge mortality rate % in Nile tilapia fingerlings fed different Aquaviance, levels for 12 weeks.

The economic analysis of the different experimental fish diets showed that feed cost to produce one kg fish gain was reduced as Aquaviance levels increased. Feed cost at 2g/ kg Aquaviance diet was 8.89 L, while that fed the control diet was 11.64 L. The reduction in feed cost to produce one kg fish gain of treatment containing 2g/ kg Aquaviance diet was 23.62% compared with the control diet was 14.69%. (Table 8).

Table 8. Economic evaluation of Nile tilapia fingerlings fed different of Aquaviance levels for 12 weeks.

Items	Control (0.0)	Aquaviance levels (g/kg)		
		1	2	3
Cost/ kg feed (LE)	6.195	6.25	6.305	6.36
FCR (kg feed/ kg gain)	1.88	1.59	1.41	1.43
Feed cost/ kg gain (LE)	11.64	9.93	8.89	9.09
Reduction cost in kg gain (%)	100	14.69	23.62	21.90

CONCLUSION

Aquaviance product contains aromatic plant extract, essential oils and prebiotics (fructo-oligosaccharide). The trial using the product in feeding Nile tilapia during 12 weeks indicated that fish growth performances and feed efficiency, the nonspecific immune response, digestive enzyme activity and antioxidant capability has improved with increasing levels of Aquaviance the opposite was true for MDA as well as improve disease resistance. The best combination of FOS and APE was 2 g/kg level significantly improved the growth rates and feed efficiency, the nonspecific immunity, digestive enzyme activity and antioxidant capability when compared to the control and the 1 g/kg and 3 g/kg levels of Aquaviance.

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تاثير المنتج اكوافينس كمكملات غذائيه لتحسين اداء النمو واستهلاك العلف ورفع المناعه والمحتوى الانزيمى لاسماك البلطى النيلى

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الملخص العربي

اجريت هذه الدراسه بقسم بحوث تغذية الأسماك بالمعمل المركزي لبحوث الثروة السمكيه بالعباسه– ابو حماد– شرقيه– مصر.

فى هذه الدراسه قسمت اصبعيات اسماك البلطى النيلى بمتوسط وزن ابتدائى (7.64 جم وزن ابتدائى) الى 4 مجموعات لكل مجموعه ثلاث احواض زجاجيه سعه الواحد منها 100 لتر وبه 10 سمكه . تغذت الاسماك على علائق متماثلة في البروتين (31%) والدهن (7%) تحتوى علي تركيزات مختلفه من اكوافينس (صفر،.1 ،2، 3 جم/ كجم) لمده 12 اسبوع. قدم العلف الى الاسماك بمعدل 4% من الوزن الحى خلال اول اسبوعين ثم 3% خلال باقى التجربه. و في بداية التجربه تم عمل بعض التحليلات الكيميائيه للعلف المستخدم وعينه من اصبعيات اسماك البلطي النيلي المستخدم في التجربه وذلك لتقدير نسبة الرطوبه– الرماد– الدهن– البروتين– الألياف. في نهاية التجربة تم وزن الاسماك لمعرفة معدل النمو كما اخذت 6 سمكات من كل معامله لقياس بعض المكونات الكيميائيه فى جسم الاسماك مثل الرطوبه والرماد والدهن والبروتين والالياف واخذ عينات من السيريم لقياس بعض الاختبارات المناعيه مثل انزيم الليزوزيم و NBT واخذ عينات من الخياشيم وكبد الاسماك لقياس بعض الانزيمات المضاده للاكسده مثل انزيم GPX، SOD،CAT والانزيم خاص باكسده الدهون الغير مشبعه وتم اخذ عينات من الامعاء لقياس بعض الانزيمات الهاضمه مثل انزيمالاميليز والليبيز كما اخذت 10 سمكات من كل معامله وتم حقن فى البطن بجرعه تحت مميته من بكتريا الايروموناس هيدروفيلا الممرضه وتم ملاحظه حاله الاسماك خلال 14 يوم من الحقن. اظهرت التجربه النتائج الاتيه:

ظهور تحسن واضح فى نمو الاسماك وزاد معدل استهلاك الغذاء و تحسنت نسبة معامل التحويل الغذائى بوجود اكوافينس وتحقق افضل أداء نمو للاسماك عندما غذيت علي عليقة تحتوي على0.2 جم لكل كجم علف%. لم تتاثر نسبة الرماد والرطوبة بينما انخفضت نسبة الدهن وزادت نسبة البروتين في في جسم الاسماك المغذاه على العليقة المحتويه علي اكوافينس ولوحظ زياده فى انزيم الليزوزيم و NBT وزياده فى الانزيمات المضاده للاكسده ونقصان فى انزيم MAD وايضا زياده فى الانزيمات الهاضمه ومقاومه الاسماك للبكتريا الممرضه المغذاه على الاكوافينس 2جم/ كجم

نستنتج من هذه الدراسه ان استخدام 2جم/كجم اكوافينس فى علائق اسماك البلطى النيلى يحسن من اداء النمو ويرفع من مناعتها ومقاومتها للامراض ويخفض من تكلفه العليقه.