Abbassa Int. J. Aqua., V0l.14 No.(1) 2021. **1 - 22**

USING OF AQUA FRESH AS FEED ADDITIVES FOR NIL TILAPIA FINGERLINGS TO STUDY GROWTH PERFORMANCE, FEED CONVERSION AND IMMUNO RESPONCE

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Received 10/ 1/ 2021 *Accepted 21/ 2/ 2021*

ABSTRACT

The experiment was conducted to evaluate the effect of AQUA FRESH on growth performance, immunity and disease resistance of Nile tilapia (*Oreochromis niloticus)*. AQUA FRESH is a commercial feed additive was added to the experimental diets at 0.00, 0.5, 1.00, and 1.50 g/kg feed and fed to four fish groups for 12 weeks. Fish groups fed on the experimental diets showed improved growth performance, feed utilization and body composition significantly (P≥0.05) compared to fish fed on the control diet. The result of body composition showed protein and lipid content were increased significantly with increasing levels of AQUA FRESH. Blood serum immune responses of *O. niloticus* showed enhancement of IgM level, lysozyme activity and NBT concentration in all diets than in control diet. Antioxidant activity improved in blood serum was observed in fish fed on experimental diets. The blood serum α-amylase and lipase enzymes activity were significantly increased (P≥0.05) in fish fed on experimental diets than control group. These results revealed that using AQUA FRESH were improved the growth performance, body composition, immunoresponse, digestive enzyme activity and antioxidant activity. The best group was that obtained AQUA FRESH at 1.5 gm./kg feed under the experimental condition.

Key words: AQUA FRESH, Feed additives, Nile tilapia, growth performance, digestive enzymes, immunoresponse, antioxidants activity.

INTRODUCTION

Feed represents around 50 – 80% production cost in aquaculture. Proper nutrition is one of the critical factors in aquaculture. Successful aquaculture

depends on a nutritionally balanced diet and low cost of production. Functional feed additives not only improve the growth performance of the fishes but also improve the health performance of the fishes. Functional feed additives are organic and eco-friendly to fishes and environment. These functional feed additives include prebiotics, probiotics, seaweeds, mushrooms, microalgae, enzymes, organic acids, mycotoxin binders, photogenic or photobiotic compounds and yeasts (Bharathi *et al.*, 2019). The concept of functional feed is paradigm in aquaculture industry and to develop diets with supplemented feed additives for improving the health and disease resistance of cultured fishes (Gatlin and Li, 2004).

Short-chain organic acids (C1–C7) and their salts or mixtures, commonly known as acidifiers, are promising alternatives for antibiotic growth promotants (AGP) and have been receiving growing attention from aquaculture researchers (Luckst 2008a; Ng and Koh, 2011). Organic acids, such as benzoic, formic, lactic and propionic acids have traditionally been used as storage preservatives in food and feed ingredients for preventing product deterioration caused by fungi and microbes (Ricke 2003; van Dam, 2006). Organic acids, their salts or combinations thereof, have been successfully used in livestock feeds as alternatives to antibiotics (Alp *et al.*, 1999; Partanen and Mroz, 1999). Commercial mixtures of organic acids are widely used to control pathogenic bacteria, such as Salmonella species, in feed ingredients and livestock feeds (Van Immerseel *et al.*, 2002).

The decline in capture fisheries was partially offset by the rapid growth of the aquaculture industry. The trend of studies to enhance the resistance of aquatic organisms to disease, as well as feed utilization, and growth performance of cultured organisms is fundamental in various sectors of the aquaculture industry (El-Haroun *et al.*, 2006).

Some reports have shown that antibiotics may improve growth performance by killing intestinal bacteria, leading to enhanced use of amino acids by the host organism (Rawles *et al.*, 1997). The use of antibiotics has a

significant effect on the microorganisms present in the aquaculture water and accumulated in them, and it is transmitted through the food chain to other organisms, including humans (FAO, 2002). Patterson and Burkholder, (2003) replaced the use of antibiotics by using vaccines, dietary supplements of probiotics, prebiotics and immunostimulants may reduce fish disease. Use of antibiotics in the aquaculture has increased the potential harmful effects on human and animal health as well as the aquatic environment (Cabello, 2006).

Basically, glucan molecules are of two types α-glucan (dextran, starch) and β-glucan (cellulose, zymosan, laminarin, lichenin). β-glucans have the ability to activate the immune response and act as biological response modifiers (BRM) (Miura *et al.*, 1996).

Among different immunostimulants used in aquaculture is β-glucan which forms the major constituents of cell wall of some plants, fungi, bacteria, mushroom, yeast, and seaweeds. They induce and enhance resistance against bacterial and viral infectious diseases by stimulating innate humoral and cellular defense mechanisms (Meana *et al.*, 2013). Development of more efficient administration methods will facilitate the routine and prophylactic use of glucans as natural immunostimulants of fish (Vetvicka, *et al*., 2013). Larger molecular weight glucans activate leukocytes, stimulating their phagocytic, cytotoxic, and antimicrobial activities, and production of reactive oxygen species (ROS) (Akramiene *et al.*, 2007). IgM is regarded as the main molecule bearing antibody activity (Solem and Stenvik, 2006). Whereas IgM antibodies are acting mainly in the serum. In addition to immunoglobulins, the non-specific factors like (hemo) lysins, (hem) agglutinins (lections), lytic enzymes; lysozyme, C-reactive protein, antibacterial peptides, and complement are also present. β- glucan also increased production of cytokines, complement and lysozyme production and antibody formation (Raa *et al.*, 1992).

Lysozyme is a conserved antimicrobial protein that is critical to host defense. All lysozymes share the ability to hydrolyze bacterial cell wall peptidoglycan (PG) (Callewaert and Michiels, 2010).

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). Garlic extract exerts had antioxidant action by scavenging reactive oxygen species (ROS), enhancing the cellular antioxidant enzymes, SOD, CAT and increasing glutathione peroxidase in the cells (Metwally, 2009). Antioxidants enzymes are the first line of defense which able to protect cells against oxidative damage by neutralizing ROS (Nordberg and Arner, 2001).

The main objective of this study is investigated the effect of a commercial feed additive AQUA FRESH on growth, feed use, body composition, immune response, digestive enzyme activity, and antioxidant activity in Nile tilapia *(O. niloticus)*.

MATERIALS AND METHODS

Four experimental diets were formulated to 30 % crude protein and 8 % lipid with 0.00, 0.5, 1.0 and 1.5 g/kg of AQUA FRESH (Cannal Aqua cure comp., Egypt). The main ingredients in the experimental diets are shown in table (1). One hundred and thirty of Nile tilapia, *O. niloticus*, fingerlings (with mean body weight (12.19 ± 0.01 gm.) were obtained from Central Laboratory for Aquaculture Research (CLAR), Agriculture Research Center, Egypt. Fish were acclimation for two weeks and fed commercial diet containing 30 % crude protein. Ten fish were frozen at −20 C° for initial proximate whole body analysis. The fish were distributed equally at rate 10 fish / 200 L aquarium. Each aquarium was aerated by using small air-bumps. Water replaced by dechloronated water from a storage tank. Fish were fed on experimental diets at a rate of 3% of its body weight daily along the experimental period (12 weeks). At the end of the study, growth and feed utilization parameters were calculated as follows:

Weight gain (g) = W2 –W1

Specific growth rate (SGR; (%g / day) = 100 (Ln W2 – Ln W1) / T

Where W1 and W2 are the initial and final weights, respectively, and T is the experimental period (days). Ln = natural logarithm

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

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

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 12 weeks of feeding, Fish were anaesthetized using buffered tricaine methane sulfonate (20 mg /L) and blood samples were collected from the caudal vein of the fish of each treatment using syringe moistens with heparin to evaluate NBT activity. It was determined based on measuring Nitro-Blue Titrazolium activity (NBT) following the method described by (Siwicki, 1989). Another blood samples were collected without anticoagulant. The blood Samples were centrifuged at 3000 ×g for 5 min. The serum were collected in Eppindorf tube and preserved at -20°C for lysozyme activity assay according to (Schaperclaus *et al.*, 1992).

Table 1. Formulation and its chemical analysis of the experimental diets on dry matter basis.

Items	Aqua Fresh and all ingredients (g/kg diet)			
	Group1 Control (0.0)	Group2 (0.5)	Group3 (1.0)	Group4 (1.5)
Fish meal	125	125	125	125
Soya meal	365	365	365	365
Yellow corn	320	320	320	320
Wheat bran	90	90	90	90
Vegetable &fish oil	40	40	40	40
Starch	25	24.50	24.00	23.50
Vit. And Min. mixture	35	35	35	35
AQUA FRESH	0.00	0.50	1.00	1.50
Total	1000	1000	1000	1000-
	Chemical analyses			
Moister	9.45	10.18	10.02	10.10
Crud protein	30.25	30.18	30.23	30.25
Ether extract	8.11	8.20	8.58	8.36
Crude fiber	6.19	6.09	6.10	6.11
Ash	10.46	10.56	10.64	10.67
Nitrogen free extract (NFE)*	44.99	44.97	44.45	44.61
GE** (K cal / 1kg	4324.7	4318.6	4347.7	4336.5

*Nitrogen-Free Extract (calculated by difference) =100 – (protein + lipid + ash + fiber). NRC, (1993).

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

After collecting blood samples from the experimental fish and the blood serum used for determination of 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 were measured according to (Habig *et al.*, 1974). Amylase activity was quantified according to Bernfeld (1995). Lipase activity was determined according to manufacturer instructions of commercial kits obtained from (Bio diagnostic, kits, Egypt).

The results were obtained 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

Growth performance:

The results of present study investigated the effect of AQUA FRESH on growth performance, feed utilization, survival rate, of Nile tilapia showed in Table (2). The effect of AQUA FRESH on growth rate , as final weight, relative growth rate, specific growth rate, and survival rate indicated that there were a high significant increase in all groups which fed on AQUA FRESH feed additive in diets compared to control group1 as illustrated in Table (2).

Table 2. Growth performance of Nile tilapia fed on AQUA FRISH after 12 weeks (Means± S.E.).

Items	Aqua Fresh g/kg diet			
	Group1	Group2	Group3	Group4
Initial weight (g)	12.30 ±0.152 [a]	12.37 ±0.27 [a]	12.10 ±0.13 [a]	12.00±0.204 [a]
Final weight (g)	29.50 ±0.894 [d]	34.99 ±0.73 [c]	41.66 ±0.6 [b]	44.95 ±0.843 [a]
Weight gain (g)	17.20 ±1.02 [d]	22.62 ±1.00 c	29.56 ±0.52 [b]	32.95 ±1.046 [a]
RGR	1.40 ±0.08 [c]	1.81 ±0.10 [b]	2.44 ±0.30 [a]	2.74 ±0.07 [a]
SGR (%/day)	1.040 ±0.488 [c]	1.236 ±0.51 [b]	1.469 ±0.01 [a]	1.569 ±0.04 [a]
Survival rate (%)	93.33 ±3.333 [a]	96.333 ±3.33 [a]	100.0 ±0.00 [a]	100.0 ±0.00 [a]

Means having the same letter in the same row is not significantly different at P≥0.05.

Feed utilization:

Feed utilization of Nile tilapia fed diets containing different levels of AQUA FRESH indicated that, feed intake, protein intake and protein efficiency ratio were significantly increased ($p \leq 0.05$) with increasing AQUA FRESH levels in experimental diets compared to control group. Contrarily, the best feed conversion ratio and protein production value (PPV) decreased significantly ($p \leq 0.05$) in all fish groups fed AQUA FRESH compared to control diet as illustrated in Table (3).

Table 3. Feed utilizations of Nile tilapia fed on AQUA FRISH after 12 weeks (Means± S.E.).

Items	Aqua Fresh g/kg diet			
	Group1	Group2	Group3	Group4
Feed intake g/fish	36.14 ±0.89 [b]	37.97±1.41 [b]	46.73 ±1.10 [a]	45.14±1.28 [a]
Protein intake g	10.72 ±0.263 [b]	11.26±0.42 [b]	13.85 ±0.326 [a]	13.38±0.38 [a]
FCR	2.11 ±0.08 [a]	1.68±0.05 [b]	1.58 ±0.06 [bc]	1.37 ±0.08 [c]
PER	1.61 ±0.06 [c]	2.01±0.067 [b]	2.13 ±0.089 [b]	2.47±0.144 [a]
PPV	23.17 ±0.212 [ab]	23.09±0.62 [a]	22.44 ±0.53 [b]	22.56±0.83 [b]

Means having the same letter in the same row is not significantly different at P≥0.05.

Whole body composition:

The results of present study investigated effect of AQUA FRESH on proximate chemical composition of whole body of Nile tilapia as in table (4). Results indicated after 12 weeks of feeding, there was no significant difference in moisture contents (p≤0.05) among the groups fed on AQUA FRESH comparison with control group. Also, crud protein content and lipid content in flesh fish increased significantly with increasing levels of AQUA FRESH compared to control group. While ash content in nile tilapia were significantly decrease with increasing levels of AQUA FRESH compared to control group as illustrated in Table (4).

Table 4. Effect of AQUA FRESH on chemical composition of whole body of Nile tilapia fingerlings after 12 weeks (on dry matter basis) (Means± S.E.).

Items	Initial	Aqua Fresh g/kg diet			
		Group1	Group2	Group3	Group4
Dry matter %	23.23 ± 0.049 [b]	26.24 ± 0.044 [a]	26.29 ± 0.092 [a]	26.29 ± 0.135 [a]	26.15± 0.07 [a]
Crude protein%	58.63 ± 0.065 [d]	61.35 ± 0.175 [c]	62.61 ± 0.116 [b]	63.56 ± 0.132 [a]	63.54± 0.12 [a]
Total lipids%	18.30 ± 0.070 [d]	19.40 ± 1.73 [c]	21.60 ± 0.56 [b]	21.90 ± 0.067 [a]	21.29± 0.05 [b]
Ash%	22.92 ± 0.327 [a]	19.19 ± 0.32 [b]	15.77 ± 0.11 [c]	14.36 ± 0.19 [d]	14.92 ± 0.09 [d]

Means having the same letter in the same row is not significantly different at P≥0.05.

Immune responses activity:

In the present study, the non-specific immune response of *O. niloticus* showed high significantly increase in concentration of IgM, lysozyme activity NBT and COMP activity in all groups which fed on AQUA FRESH compared to control group. But serum concentration of IgM and NBT in group (2) was showed non-significant difference compared to control group.

Table 5. Immunoresponse of experimental Nile Tilapia fed on AQUA FRISH after 12 weeks (Means± S.E.).

Items	Aqua Fresh g/kg diet			
	Group1 Control (0.0)	Group 2 (0.5)	Group 3 (1.0)	Group 4 (1.5)
IgM	7.07± 0.25 [b]	7.733 ±0.28 [b]	9.65 ±0.42 [a]	10.55 ±0.34[a]
LYSOZYME	1.05±0.06 [c]	1.36±0.04 [b]	1.50±0.04 [ab]	1.63±0.05 [a]
NBT	2.74±0.10 [c]	3.33±0.16B [c]	3.88±0.28A [b]	4.42±0.24[a]
COMP	39.33 ±0.72[d]	41.50±0.56 [c]	45.00±0.68 [b]	50.67±0.84 [a]

Means having the same letter in the same row is not significantly different at P≥0.05.

Antioxidant activity:

The antioxidant activity in present results showed that blood serum, SOD, CAT and GSH activities are highly significant increase (P≤0.05) in all groups of fish fed on AQUA FRESH comparison with group fed on control. While concentration of malonyldialdehyde (MDA) was significantly decreased in all groups of fish fed on AQUA FRESH comparison to control group. These indicated that AQUA FRESH might enhance the antioxidant capability so decreased cell degradation and enhancement of MDA concentration of *O. niloticus* in all groups fed AQUA FRESH than control group as seen in Table (6).

Table 6. Antioxidant activity in blood serum of experimental Nile Tilapia fed on AQUA FRISH after 12 weeks (Means± S.E.).

Items	Aqua Fresh g/kg diet			
	Group1	Group2	Group3	Group4
GSH	0.56±0.01[d]	0.87±0.02 [c]	1.02±0.03 [b]	1.34±0.05 [a]
SOD	3.04± 0.07[d]	4.23±0.17 [c]	5.08±0.12 [b]	5.62±0.20[a]
CAT	34.17±2.15 [d]	43.67±2.65 [c]	54.17±3.08 [b]	63.17±3.68[a]
MDA	17.25±0.55[a]	15.38±0.50 [b]	12.93±0.29 [c]	9.52±0.42 [d]

Means having the same letter in the same row is not significantly different at P≥0.05.

Digestive enzymes:

The results of intestinal digestive enzymes (α-amylase activity and lipase activity) were high significantly increase (P≤0.05) in all groups fed on different levels of AQUA FRESH and the highest values of all groups observed in fish fed 1.5 g/kg compared to control group. As can be seen from Table (7).

Table 7. Digestive enzymes activity in blood serum of experimental Nile Tilapia fed on AQUA FRISH after 12 weeks (Means±S.E.).

Items	Aqua Fresh g/kg diet			
	Group1Control	Group2	Group3	Group4
Lipase	23.17 ±0.70[d]	31.50±1.26[c]	36.83 ±0.75[b]	46.50± 1.12[a]
Alpha Amylases	13.57 ±0.67[d]	14.00 ±0.63[c]	14.88 ±1.17[b]	15.80±1.46[a]

Means having the same letter in the same row is not significantly different at P≥0.05.

DISCUSSION

Growth performance and feed utilization:

The results of present study investigated the effect of AQUA FRESH on growth performance, of Nile tilapia (*O. niloticus)* as in Table 2. the final weight gain, relative growth rate(RGR) and specific growth(SGR) rate were highly significant in the groups fed on diets containing AQUA FRESH compared to control group (Table 2).That means the AQUA FRESH product has significantly improved the growth performance in *O. niloticus*. Also acts as a natural growth promoter for Nile tilapia. These improvement are related to AQUA FRESH

product supplementation in fish diets which containing citric acid , lactic acid , acetic acid , formic acid, propionic acid calcium propionate , calcium lactate and prebiotics (β-glucan, mannan and oligosaccharide) for these purpose of stimulating digestive functions and contributing to a good balance of intestinal flora that enhance growth performance. The results are in agreement with action of prebiotics mannan oligosaccharides which effect on growing of microvilli that increase the absorption of nutrients which improving growth performance and fish health (Torrecillas *et al.*, 2007 and Staykov *et al.*, 2007). These results are in agree with (Hung *et al*., 2011) who reported that citric acid, lactic acid, acetic acid, formic acid, propionic acid calcium propionate, calcium lactate and prebiotics (β-glucan, mannan and oligosaccharide) are improve growth performance of Pangasius catfish. The dietary supplemented prebiotics (β-glucan) improved the growth performance of *O. niloticus* **(**El-Mousallamy *et al.*, 2015*)*. In the present Nile tilapia fish fed different AQUA FRESH showed improved survival rate to (100%) than the control group. Similar results showed when lobster juvenile (*Panulirus ornatus*) fed on mannan oligosaccharides supplemented diet obtained by (Sang and Fotedar, 2010).

From the present results, we noticed that when adding AQUA FRESH to the diets in the experiment, was improved the results of the feed intake, protein intake, (FCR) and (FER), (PPV) in all groups fed on AQUA FRESH in comparison to the control group. So AQUA FRESH enhance feed utilization in fish diets due to the effect of prebiotic β - glucan, mannan, oligosaccharides, and the organic acid present in AQUA FRESH composition which had enhanced secretion of digestive enzymes. Agree with oral administration of baker's yeast which improved the enzymatic digestion of complex substances (Tewary and Patra, 2011). Study showing the effect of AQUA FRESH had improved feed intake in many fishes (Hung *et al.*, 2011).

The current results showed that Nile tilapia that were fed on the diets containing AQUA FRESH played a role in enhanced feed intake, nutrients utilization, and digestibility, which was reflected in the chemical composition of the fish body. So we noticed that an increase in the proteins and lipids content in

tilapia groups were fed AQUA FRESH compared to control group. This results are in agreement with the study of commercial live bakers' yeast, *Saccharomyces cerevisiae* as a growth promoter showed high protein and lipid content of Nile tilapia, *Oreochromis niloticus* muscles (Abdel-Tawwab *et al.*, 2008). Also (Genc *et al.*, 2007) reported that increasing rate of dietary prebiotics mannan oligosaccharides in the diet increase protein and lipid contents in muscles of hybrid tilapia (*O. niloticus x O. aureus*).

Antioxidant activity:

The antioxidant activity in present results showed highly significant increase ($P \leq 0.05$) of (SOD), (CAT) and (GSH) activities in serum, of all groups of fish fed on AQUA FRESH in comparison with control group, for keep meut on balance between antioxidants and reactive oxygen species. The antioxidant enzymes are able to clean out the oxygen roots produced by the oxidative reaction that takes place inside cells, and thus these enzymes protect cells and tissues from oxidation (Li *et al.*, 2007). The major antioxidant enzymes, including SOD, CAT and GSH representing the first line of defense against oxidative stress (Farombi *et al.*, 2007). In fact, as the immune response increases, fish cells produce reactive oxygen species (ROS) which are highly in microbial infection (Martinez-Alvarez *et al.*, 2005). In the present study, the groups of fish fed on AQUA FRESH showed significantly reduced in blood serum MDA concentration compared than control group. In addition, AQUA FRESH could inhibit the process of lipid peroxidation in cell of gill and liver tissues by decrease lipid destruction and produce MDA. (Livingstone, 2003) supported the present study by the fact that MDA level is a direct proportion with the oxidative processes caused by free radicals. One probable explanation could be that dietary Probiotic improved the growth performance and immune responses, also it could improve feed utilization which may contribute to dietary antioxidants assimilated diet (Sun *et al.*, 2010).

Immune responses activity:

Many 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, the non-specific immune response of *O. niloticus* showed high significantly increase in blood serum concentration of IgM, lysozyme activity NBT and COMP activity in all groups which fed on AQUA FRESH compared to control group. But blood serum concentration of IgM and NBT in group (2) was showed non-significant difference compared to control group. These results are in agreement with The higher lysozyme activity probable attributed to the high leukocyte production in fish fed on dietary fructo - oligosaccharide and *Bacillus licheniformis* (Zhang *et al.*, 2013). The effect of prebiotic could be ascribed to the growth stimulation of beneficial bacteria such as lactobacilli and befidobacteria, possess lipopolysaccharides which have immunostimulatory properties (Manning and Gibson, 2004). Moreover, acetate, propionate and lactic acid as end products of Fructo- oligosaccharides fermentation play a crucial role in modulating the immune system (Passos and Park, 2003). Furthermore, Fructo- oligosaccharides could interact with toll like receptors (TLR2) expressed on macrophages (Vogt *et al.*, 2013). These 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).

Digestive enzyme activity:

The results of blood serum digestive enzymes (α-amylase activity and lipase activity) were high significantly increase ($P \leq 0.05$) in all groups fed on different levels of AQUA FRESH. Enhanced digestive enzyme activity in fish fed a pre - or probiotics diet was reported by (Suzer *et al*., 2008 and Wang, 2011), also dietary supplemented diet of fructo oligosaccharide (FOS) improves the innate immune response, stress resistance, digestive enzyme activities and growth performance of Caspian roach and turbot fish (Soleimani *et al*., 2012 and Guerreiro *et al*., 2015). Wu *et al*., (2013) who reported that amylase activity of blunt snout bream fingerlings exhibited no significance difference with low

dietary inclusion of FOS, while at high dose level showed significant increase in amylase activity compared with the control group.

Economic evaluation:

The economic analysis of the different experimental fish dites showed that feed cost to produce one kg fish gain was reduced as AQUA FRESH levels increased. Feed cost at 1.5 g/kg AQUA FRESH diet was 8.48 L, while that fed the control diet was 13.06 L. The reduction in feed cost to produce one kg fish gain of treatment containing 1.5 g/kg AQUA FRESH diet was 35.07 % compared with the control diet.

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

Items	Aqua Fresh g/kg diet			
	Group1	Group2	Group3	Group4
Cost / kg feed (L.E)*	6.32	6.5	6.67	6.85
FCR (kg feed / kg gain)	2.11	1.68	1.58	1.37
Feed cost / kg gain (L.E)	13.06	10.04	9.78	8.48
Reduction cost in kg gain (%)	100	20.36	25.11	35.07

*LE = Egyptian pound

CONCLUSION

Commercial AQUA FRESH product contains prebiotics (β glucan, mannan -oligosaccharide), organic acids, when added to the diet of *O. niluticuos* through 12 weeks; improve fish growth performances, the immunoresponse, digestive enzyme activity and antioxidant enzyme activity (SOD,CAT,GSH) with increasing levels of AQUA FRESH and decreased MDA.

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استخدام الأكوافريش كاضافات علفية لأصبعيات البلطي النيلي لدراسة تاثيره على أداء النمو، التحويل الغذائي والاستجابة المناعية

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الملخـــــص العربـــــي

في هذه الدارسة تم تجميع اصبعيات البلطي النيلي بمتوسط وزن ابتدائي (2.19 ± 0.01) جرام تم تقسيمها الى 4 مجموعات لكل مجموعه ثلاث مكررات، تم تغذية الاسماك على علائق متماثلة في البروتين 30% والدهن 7% وتم اضافة تركيزات مختلفة من المنتج التجاري أكوا فريش (صفر. 0.50، 1.00، 1.50 جم أكوافريش/ كجم من العليقة) استمرت التجربة لمدة 12 اسبوع. تم تغذية الاسماك خلالها بمعدل 3% من وزنها وفي بداية التجربة تم أخذ بعض عينات من العليقة والاسماك المستخدمة لعمل بعض التحليلات الكيميائية وفي نهاية التجربة تم وزن الاسماك لمعرفة معدل النمو كما اخذت عينات الدم من أسماك كل معامله على حدة للحصول على مصل الدم لإجراء الاختبارات المقررة بالتجربة مثل الاختبارات المناعية (IgM, Lysozyme, COMP, NBT) وبعض مضادات الأكسدة مثل انزيم الجلوتاثيون ريدكتيز، الكتاليز ، السوبر أوكسيد ديسميوتيز (SOD, CAT ,GSH) وكما تم قياس تركيز مادة المالونيل داى الدهيد (MDA) وكذلك تم قياس بعض الانزيمات الهاضمة مثل انزيم الفا أميليز (α-amylase) وأنزيم الليبيز(Lipase). كما تم تحليل مكونات جسم الاسماك لكل معاملة. وفى النهاية تم الحصول على تلك النتائج :

- ارتفاع معنوي في معدل النمو وكذلك معدل التحويل الغذائي في الأسماك التي تم تغذيتها على العلائق المحتوية علي منتج الأكوا فريش مقارنة بالعليقة الضابطة.
- لم تتأثر نسبة الرماد والرطوبة بينما ارتفعت نسبة الدهن والبروتين في انسجة عضلات الأسماك التي تم تغذيتها على العلائق المحتوية علي منتج الأكوا فريش مقارنة بالعليقة الضابطة.
- لاحظنا زياده معنوية في نشاط الأنزيمات المضادة للأكسدة مثل مثل انزيم الجلوتاثيون ريدكتيز، الكتاليز، السوبر أوكسيد ديسميوتيز (SOD, CAT ,GSH) في مصل دم الأسماك التي تم تغذيتها على العلائق المحتوية علي منتج الأكوا فريش مقارنة بالعليقة الضابطة.

- انخفاض معنوي في تركيز مادة المالونيل داى الدهيد (MDA) الناتجة من أكسدة الدهون في مصل دم الأسماك التي تم تغذيتها على العلائق المحتوية علي منتج الأكوا فريش مقارنة بالعليقة الضابطة.
- زياده معنوية في الاستجابة المناعية من خلال ارتفاع نشاط (IgM, Lysozyme , COMP, NBT) في مصل دم الأسماك التي تم تغذيتها على العلائق المحتوية علي منتج الأكوا فريش مقارنة بالعليقة الضابطة.
- زياده معنوية في نشاط بعض أنزيمات الهضم مثل انزيم الفا أميليز (α-amylase) وأنزيم الليبيز(Lipase) في مصل دم الأسماك التي تم تغذيتها على العلائق المحتوية علي منتج الأكوا فريش مقارنة بالعليقة الضابطة.

تخلص الدراسة الى ان اضافة المنتج التجاري الاكوافريش لعلائق اصبعيات البلطى النيلى ادى الى تحسين معدل النمو وكفائة التحويل الغذائى ورفع الاستجابة المناعية وكانت افضل نسبة اضافة بمعدل 1.5 جرام/ كيلو جرام عليقة وذلك تحت الظروف التجريبية لهذا البحث.