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THE EFFECT OF ZYMOFERMENT ON THE GROWTH, HEALTH AND IMMUNITY OF NILE TILAPIA (*O. niloticus*).

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SUMMARY

This study was conducted to evaluate the effect of adding Zimoferment on growth performance and immune status of Nile tilapia (*O. niloticus*). One hundred sixty eight Nile tilapia (*O. niloticus*) with an average body weight of 40+1.02 g were divided in 4 treatments x 3 replecations, 42 fish/group, 14 fish in 12 experimental glass aquaria for 60 days. Four isonitrogenous and isocaloric diets containing 35% crude protein and 2.7 kcal/g of metabolizable energy were formulated in which Zimoferment was added, 0%, 1%, 1.5% and 2%. Feed consumption and body weight were recorded biweekly during the experimental period. At the end of the experiment blood samples were collected from all fish to determine the immune status. Results demonstrated that Zimoferment has good potential as Zimoferment up to 1.5% in the Nile tilapia (*O. niloticus*) diets improve growth performance and immune status of fish. The highest growth performance and the best feed efficiency were observed with fish fed diets containing 1% and 1.5% Zimoferment. The lowest feed efficiency and growth performance were shown in the fish fed on diets containing 0% and 2% Zimoferment.

The phagocytic assay was performed and indicated that the increase of both percentage of phagocytosis and phagocytic index in the group fed on 1.5% Zimoferment compared with the other groups.

The challenge of the experimental groups was performed using a pathogenic strain of *Pseudomonas florescences*, via intra peritoneal route, 10 *O. niloticus* from each group were injected separately by 0.2ml of 24hr broth culture containing 10^6 CFU/ml *Pseudomonas florescences*. Fish clinical abnormalities and mortalities were recorded and reisolation of the Pseudomonas florescenc-

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es was carried out from the clinically ill *O. niloticus*.

It was found that the groups received the Zimoferment with concentration 1% and 1.5% exert the maximum protection against pseudemonas florence in comparison with the groups received 0% and 2% Zimoferment which manifested the typical picture of the septiceamia. It was concluded from the obtained results that Zimoferment may have positive impact on the growth performance and immune status of Nile tilapia (*O. niloticus*) under experimental conditions.

INTRODUCTION

Intensification of fish culture has made the producers think wisely of developing suitable husbandry strategies, based on novel nutritional and management practices that enhance performance and prevent disease without significant increase in the cost of production (lovell, 1989).

Fish producers had incorporated antibiotics as growth promoters in their feeds to enhance growth and feed efficiency. However, public health concern prompted the authority to ban the use of the antibiotics. An alternative strategy was to focus on the use of feed additives of natural origin to avoid the side effect of any xenobiotes and to strengthen the immune system of the fish allowing the fish to defense itself in a natural way without interferences (Fuller, 1992; Klaenhammer and Kullen, 1999).

Nile tilapia, dubbed the aquatic chicken" (Maclean 1984) is one of the most important cultured fish species in many parts of the world including Egypt (Davlin, 1991 and Pullin, 1997). Since tilapias have become a top priority fish in the tropics, a need for a calculated formulation of diets that exert the maximum growth performance, health and immunity was a must.

Zimoferment as a feed additive contains many ingredients that showed promising results in the field of improving the growth and performance in addition to the defense mechanism in poultry and large animal industry (Zouelfakar& Ezzeldeen, 2003).

Baker's yeast, Saccharomyces cervisiae, a single celled eukaryotic fungi used primarily in the production of bread and beer, has been suggested for use in the aquaculture, because it is readily available in large quantities and has the capacity to adapt to high salt concentrations (Adler, 1994 and Nikosketiahen et al., 2001). The cell wall of *S. cervisiae* consists of glucan, manno proteins and chitin. Reports of enhanced survival and evidence of immune stimulation due to purified B-glucan, are more numerous than for yeast both for terrestrial and aquatic organisms (Itami et al., 1993) and Schitz et al., 1999), thus encouraging further research.

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For the last 50 years, tremendous changes have taken place in the field of aquaculture. These changes came in response to the massive needs for good quality, cheep and palatable animal proteins (Hermino1993).

The intensification and the culture conditions increased the risks of disease occurrence. Bacteria, as etiological agents for the disease are always a threaten factor. Thus, improving the husbandry strategies with novel nutritional and management practices was a must to prevent diseases without significant increase in the cost of production (Lovell, 1989).

A properly functioning immune system is critical in maintaining the fitness and health of an organism, thus, the disease challenge studies are important tools for examining the health status, performance and immunity. This technique provides an opportunity to determine the effect of exposure to xenobiotic (bacteria) on the performance and immunity of the fish species and on their natural habitats (Arkoosh et al., 2005).

Pseudomonas florescence is one of the common bacterial agents that can be awaited in the field of freshwater aquaculture. It is an oxidative, Gram negative opportunistic bacterium normally found in soil and water, the disease occurrence is usually associated with contaminant environmental stress, such as over crowdness, high temperatures and improper nutrition (Roberts, 2001).

The disease itself exerts its clinical picture in affected *O. niloticus* in the form of septicaemia, large cutaneous haemorrhages in the fins and trunk, ulcers, exophthalmia tailrot and high mortalities (Noga, 1996).

The defense mechanisms in fish have not been as extensively studied as those of mammals, but they share a number of some characteristics important in the cell mediated and humeral immunity (Jaso-Friedmann et al., 1990 and Verschuere et al., 2000), cell mediated immunity involves lymphocyte, non-specific cytotoxic cells and phagocytic cells. Macrophages posses a phagocytic activity which is the initial step in the immune response in fish and is the major line of defense for all foreign materials including pathogenic agents (Oliver et al., 1986). Measurement of macrophage activity serves as a bio-indicator of fish health and reflects the impact of environmental stress and chemical contamination (Warinner et al., 1988).

This work was aimed to study the effect of Zimoferment as a feed additive on the growth performance, health and immunity of Nile tilapia.

MATERIAL AND METHODS

1. Experimental fish:

A total number of 168 apparently healthy Nile tilapia (*Oreochromis niloticus*) with an average body weight of 40±1.02 g, obtained from Al Wafa semi-intensive fish farm in Giza governorate were used as experimental fish.

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1.1 Acclimatization:

Oreochromic niloticus were transferred to 12 glass aquaria of 30Ο40Ο80 cm each, supplied by dechlorinated water in the wet laboratory in Dept. of Fish Diseases and Management, Faculty of Veterinary Medicine, Cairo University according to (Tort et al., 2003). Fish were held a period of 15 days for acclimatization. All fish were fed daily on the control diet (containing 0% Zimoferment) at a rate of approximately 3% of their average body weight to be adapted to pelleted feeds. After the acclimatization period, the fish were weighted to determine the average initial body weight.

1.2 Environmental conditions:

The aquaria were supplied with sufficient aeration using electric oxygen pumps to maintain a level of 6.5±0.2 mg/l dissolved oxygen, temperature was adjusted to be 22±2°C, pH value was measured weekly by electric digital pH-meter for a value of 7.1±0.1 and a hardness of 150mg/l as calcium carbonate. The values were measured according to the American Public Health Association (APHA, 1992). The water was changed twice a week to maintain good water quality according to Alabaster and Lioyd, (1980).

2. Experimental design:

One hundred sixty eight Nile tilapia (*O. niloticus*) with an average body weight of 40+1.02 g were divided into 4 treatments x 3 replecations, 42 fish/group (14 fish in 12 experimental glass aquaria) for 60 days. Four isonitrogenous and isocaloric diets containing 35% crude protein and 2.7 kcal/g of metabolizable energy were formulated in which Zimoferment was added 0%, 1%, 1.5% and 2% as shown in table no(1). Feed consumption and body weight were recorded biweekly during the experimental period. By the end of the 6 week, five fish from each group were sacrified and the kidney were collected for conducting the phagocytic assay.

The remaining fish were challenged by *Pseudomonas florescens* pathogenic bacteria.

Table (1): Groups, number and Zimoferment%

	Group 1 Control	Group 2	Group3	Group4
Number of fish	42	42	42	42
Zimoferment %	0	1	1.5	2
Experimental period	6 weeks			

3. Zimoferment and its composition

Zimoferment (UVL-Italy) contains dry extract of Saccaromycetes, soluble distillers, fermenter yeast, natural phosphorilated yeast, protein concentrate with high content of enzymes and amino-acids. Zimoferment contains 41% crude protein, 1.54% ether extract and 5.3% crude fiber.

4. Experimental diets

All diet ingredients used in this study were obtained from commercial suppliers in Cairo, Egypt. Prior to use, all dry ingredients were passed through a 100-µm mesh sieve, analyzed for their chemical composition according to AOAC (1990). The data obtained was used as a basis for the diet formulations. The dry ingredients were then weighed according to its percentage in the diets, thoroughly mixed with Zimoferment and gelatinized starch (40g in 500 ml water) to facilitate pelletization process and manufactured followed by water until stiff dough was formed. The dough was passed through a special mesh of meat mincer machine. The diet was pelleted into size No.2 (1.2 mm diameter) suitable for fish size (New et al.,1992). Four isonitrogenous and isocaloric diets containing 35% crude protein and 2.7 kcal/g of metabolizable energy were formulated in which Zimoferment was added 0%, 1%, 1.5% and 2% (Table 2).

Table (2): Feed formulation (% of total) and the analyzed composition of the diets (as DM basis).

Ingredients \ Group	Group 1 Control	Group 2	Group 3	Group 4
Yellow corn	32.0	32.0	31.5	31.5
Soybean meal (44%CP)	30.0	29.0	29.0	28.5
Fish meal (72% CP)	25.0	25.0	25.0	25.0
Wheat flour	10.0	10.0	10.0	10.0
Zimoferment	-	1.0	1.5	2
Di-Calcium phosphate	2.3	2.3	2.3	2.3
Gelatin	0.5	0.5	0.5	0.5
Vitamins and Minerals premix*	0.2	0.2	0.2	0.2
Analyzed Composition				
Moisture (%)	9.35	9.45	9.08	9.53
Crude protein (%)	35.13	35.08	35.11	35.14
Crude fat (%)	4.10	4.12	4.13	4.13
Ash (%)	8.15	8.26	7.68	7.95
Crude fibre (%)	3.29	3.29	3.29	3.29
NFE (%)	49.33	49.25	49.79	49.49
Calcium (%)	0.68	0.68	0.68	0.68
Total phosphorus (%)	0.83	0.83	0.83	0.83
Estimated metabolizable energy (kcal/g)	2.67	2.67	2.67	2.67

* Vitamins and minerals premix (per kg): Vit.A 9900 IU, vit.D 2200 IU, vit.E 8.25 IU, ascorbic acid 220 mg, riboflavine 18.2 mg, niacin 10.7 mg, choline chloride 715 mg, pantothenic acid 37 mg, vit.B_{12} 0.014 mg, folic acid 6.1 mg, biotin 0.17 mg, thiamine

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4.1 Analyses of diets and fish

Chemical analysis of the diets and fish was performed according to AOAC (1999). Nitrogen free extract (NFE) was estimated by differences [NFE = 100 - (crude protein% + Crude fat% + Crude fibre% + Total ash%)].

At the termination of the trial, random samples of 3 fish from each group were taken for proximate chemical analysis. The fish samples were kept frozen until the laboratory evaluation began. The whole fish in each sample were passed twice through a feed grinder and analyzed according to AOAC (1990). Diets processing and proximate analyses were performed at Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Cairo University.

4.2 Statistical analysis:

All data of the feeding experiments were statistically analyzed using SPSSÆ ver. 11.0 software for personal computer (2005). Means were compared by one-way ANOVA ($P<0.05$) Sendecor and Cochran (1980).

5. Clinical alteration observed in different fish groups

The fish in the feeding trials were inspected for detection of external or internal gross lesions, clinical abnormalities. Examination was carried out according to the method adopted by Noga (1996).

6. The Phagocytic index

At the end of the 6 week, kidney samples were collected from the different fish groups separately to perform the phagocytic assay.

6.1 Preparation of Yeast:

Candida albicans (from Department of Microbiology, Faculty of Vet. Med., Cairo University) was adjusted to 106 C.F.U/ml (colony forming unit in the presence of 1 ml tilapia serum diluted to 1:20 in Hank's balanced salt solution. (HBSS) supplemented by penicillin (100iu/ml) and streptomycin (100mg/ml) according to(Bennani et al., 1995).

6.2 Preparation of phagocytic cells:

Cells were isolated from the pronephros and suspension has been prepared in a number according to (Blazer et al., 1989). The renal phagocytes cells obtained by crushing the pronephros at 4°C in 8ml of HBSS were supplemented with 5% tilapia serum. The cell suspension was loaded on percoll discontinuous density gradient (45 and 31%) and centrifuged for 25min at 400 xg at 4°C. The phagocytes were recovered at the 45-31% percoll interface, washed twice by phosphates buffer saline (PBS) ph.7.2 and resuspeded in 1ml HBSS supplement with 2% tilapia serum. Under these conditions, cell viability was analyzed by trypan blue, exclusion (0.4%) was 95% or greater and the number of viable mon-

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onuclear cells adjusted to be $4x10^6$ cells/ml. The cells were placed in cell culture staining chambers (CCSC) containing sterile round cover slips. After incubation for 1 hour at 5-10% humidified CO_2 incubator and 37°C, the monolayers were rinsed three times with HBSS.. The cells were cover with HBSS containing 2% Tilapia serum and incubated for 24 hr then gently washed three times with HBSS according to (Secombes, 1990).

6.3 Evaluation of Phagocytic activity: (Richardson and Smith, 1981)

C.albicans cell suspensions (106 cells/ml HBSS) were incubated with the prepared monolayers at humidified CO2 incubator at 37°C for 1hr. The cell layers were washed three times with HBSS then stained with Giemsa stain. Under light microscope with oil emersion lens, microscopical fields covered with about 20 phagocytes each were examined. The total number of phagocytic cells, the number of phagocytes with ingested yeast cell and the total number of blastospores within individual phagocyte were determined. The percentage of phagocytes that contained blastospores and the mean number of blastospores per infected phagocyte (Phagocytic index) were calculated according Johnson and Ainsworth (1991) as follow:

$$\text{Percentage of phagocytes} = \frac{\text{The No. of phogocytes with ingested blastospores}}{\text{Total No. of phagocytes}} \times 100$$

$$\text{Phagocytic index} = \frac{\text{The No. of ingest569d blastospors}}{\text{The No. of phogocytes with ingested blastospores}} \times 100$$

7. Challenge assay:

At the end of the 6th week, 10 *O. niloticus* from each group were challenged with 24 hr broth culture containing 10^6 CFU/ml pathogenic *Pseudomonas florescences*, the challenge was performed via I/P route using 0.2ml, according to (Miyazaki, etal., 1984), as shown in table (3), *O. niloticus* were monitored for the alteration in the clinical picture, PM, and mortalities for 7 days post challenge.

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Table (3): The groups, numbers, challenge route and dose & observation time.

Groups	Total number of *O. niloticus*	Challenge bacteria	The challenge route and dose	Observation time
Group 1	10	*Pseudomonas florescences*	0.2ml of 24 hr broth culture containing 10^6 CFU/ml via I/P route	7 days
Group 2	10			
Group 3	10			
Group 4	10			

RESULTS AND DISCUSSION

1. Growth and feed utilization data

Table 4 shows growth and feed utilization data. The addition of Zimofermet at a level of 1% and 1.5% in the diet produced the best growth (final weight and weight gain) with values statistically higher than the other treatments ((P<0.05). The diet supplemented with 2.0% Zimofermint produce final weight and weight gain more than that in control group however it is not significantly different (P>0.05). In general, fish fed with the diet supplemented with 1.5% Zimoferment showed better feed consumption and utilization. These results suggest that addition of Zimoferment at a certain level improve feed utilization and growth performance even under stress condition as growing in experimental glass aquaria. Similar results have been reported by Noh et. al. (1994), who studied the effect of supplementing common carp feeds with different additives and levels. In practical terms, this means the probiot-

Table (4): Growth response and feed utilization efficiency parameters (mean + SD)

	Group 1	Group 2	Group 3	Group 4
Initial Weight (g/fish)	44.14±0.32a	44.63±0.27a	44.16±0.36a	44.35±0.29a
Final weight (g/fish)	85.93±0.75a	79.45±0.62a	105.46±0.83b	89.36±0.78a
Weight gain (g/fish)	41.79±0.75a	34.82±0.62a	61.3±0.83b	45.01±0.78a
FCR	2.23	2.37	2.09	2.34
Diet consumed (g/fish)	93.19±0.87a	82.52±0.94a	128.12±0.79b	105.32±0.59a

Means with the same letter in the same row are not significantly different (P>0.05).

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ics use by a certain level of supplementation can decrease the amount of feed necessary for animal growth (reduce feed intake), improve digestion, absorption and feed utilization which could result in production cost reductions.

2. Analysis of a sample of fish at the end of the experiment

Table 5. demonstrate the proximate analysis of a sample of fish at the end of the experiment. No statistical differences were observed in carcass moisture, protein, fat and total ash content. However, all the Zimoferment-supplemented diets showed high value of the carcass protein than the control group, but the quality of carcass protein was not studied. Carcass protein was clearly related to dietary protein and efficiency of utilization. The obtained results suggest that the addition of Zimofermen improve dietary protein digestibility and utilization, which may in turn explain the better growth and feed conversion. Similar results were obtained by De Schrijver and Ollevier (2000), who reported a positive effect on apparent protein digestion when supplementing turbot feeds with bacterial probiotic.

Table 5: Chemical composition (% on DM basis) of whole body of fish (mean ± SD)

	Group 1	Group 2	Group 3	Group 4
Moisture	76.7±0.90a	77.1±0.84a	75.8±0.97a	76.9±0.95a
Protein	59.9±0.35a	60.3±0.52a	61.7±0.23a	60.7±0.48a
Fat	17.5±0.12a	18.2±0.22a	17.9±0.43a	18±0.32a
Ash	20.2±0.47a	19.8±0.32a	19.9±0.39a	21.7±0.42a

Means with the same letter in the same row are not significantly different (P>0.05).

Table 6: Results of phagocytic assay

	Macrophages			
	Control	1 1%	2 1.5%	3 2%
Total no of phogocytes	180	200	180	160
No.of ingested phagocytes	140	170	165	145
Blastospores within phagocytes	235	310	355	275
% of phagocytosis	78	85	92	91
Phagocytic index	1.6	1.8	2.1	1.8

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3-Evaleuation of phagocytic assay

Macrophages were recognized as key element in host defense which were avidly phagocytic for wide variety of particles, including bacteria and yeast (Roberts ,2001). In the present investigation the in-vitro phagocytosis of *C.albicans* by fish macrophageswas studied. Craig and McLean (2003) and Crumlish et.al. (2000) measured the phagocytosis assay in Nile tilapia when they isolated phagocytic cells from kidney and cultured them in the presence of *C.albicans* .

Phagocytic cells isolated from anterior kidney (Pronephros) were used to investigate the phagocytic indices because this organ is the main haematopoietic tissue in fish (Braun-Nesje et al., 1982) and the concentration of phagocytic cells is higher in the pronephros than other haemopiotic tissues (Stave et al., 1983).

In the present study, the oral supplementation with zimoferment by concentration 1.5% leading to increasing percentage of phagocytosis and phagocytic index more than concentration 1% and 2% .The results in table (6) indicated a tendency towards declining activity of phagocytes with increasing level of zimoferment than 1.5% and this results agreed with Craig and McLean (2003). The present work concluded that Zimoferment had an impact on the immune system of fish as immuno-modulator.

4-Clinical picture observed in different group of fish

4.1 Clinical picture during feeding experiment

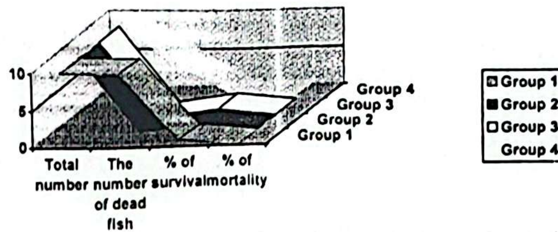
The *O. niloticus* fed in group No. (4) Exerted an adverse effect manifested by increase mortality rates (12 fish out of 42 ie. 28.5%), low growth performance and showed marked gross lesion, darkness of the skin, tail and fin rot. Its worth mentioned that the water quality in the group No. 4 was altered and a clear water turbidity was noticed after feeding times which affect the health condition of the fish in the aquaria, this may be attributed to the high percent of the Zimoferment in the ration rather than the improper manufacturing procedures because this artifact weren't obvious in the other formulas containing less percentage of the Zimoferment.

4.2 Clinical picture post challeng

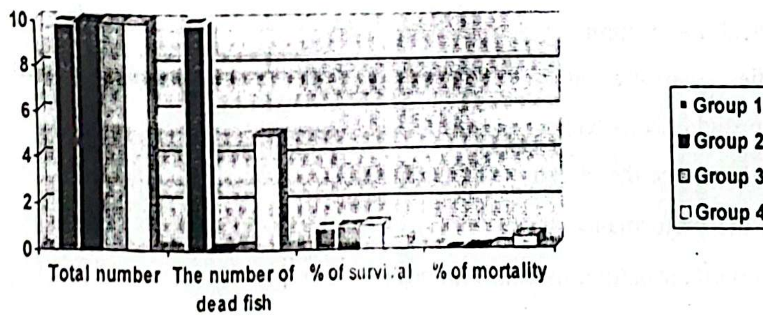
Typical picture of *Pseudomonas florescens* infection recorded in the *O. niloticus* challenged with the pathogenic *Pseudomonas florescens*, the fish were manifested by extensive amount of mucus in the skin with massive streaks of haemorrhages and congested base of the fins together with skin ulceration as shown in photo (1), gill filaments were covered with excessive mucus and the color ranged from pale to severely congested, together with promenant tail rot were as shown in photo (1).

Table (7): Result of the challenge test of the different groups.

Group	Total number	The number of dead fish	% of survival	% of mortality
Group 1	10	10	100%	0%
Group 2	10	0	100%	0%
Group 3	10	0	100%	0%
Group 4	10	5	50%	50%



Line chart (1): Result of the challenge test of the different groups.



Column chart (2): Result of the challenge test of the different groups.

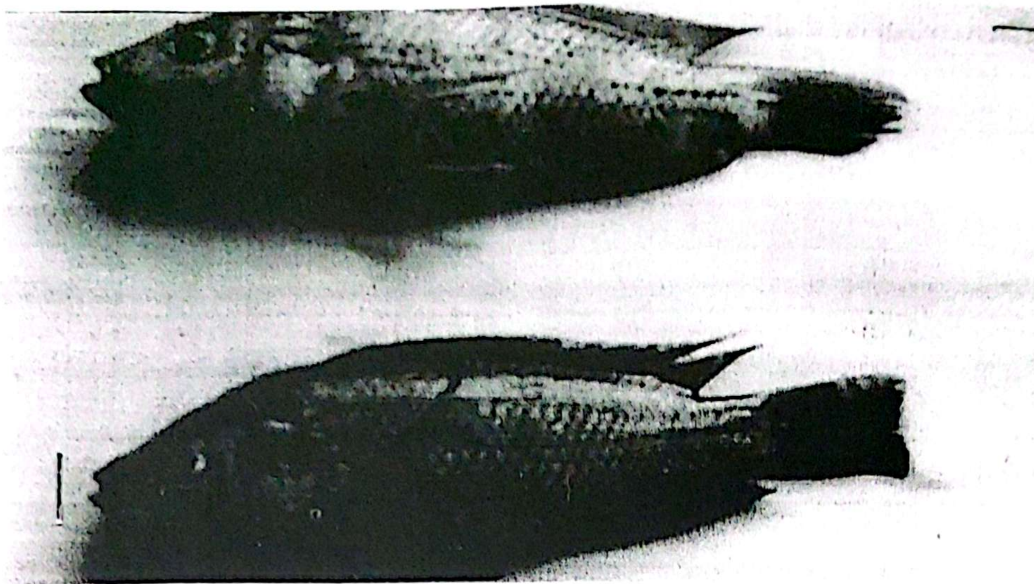


Photo (1): O. niloticus showing extensive skin haemorrhage, tail and fin rot and ulcerative

Mortalities were observed in group (4) and the control group (1) are shown in table No. 7, line chart (1)and column chart(2).

An important tool for examining the status of the performance ,health and immunity is The disease challenge studies, also referred to as host resistance challenge studies, this technique provide an opportunity to determine the effect of exposure to xenobiotic on the performance and immunity of the fish species on their natural habitats (Arkoosh et al, 2005).

In the present study *Pseudomonas florescences* was chosen because its well known for its action as freshwater fish pathogen, it is normally present in soil and water, the disease occurrence is usually associated with environmental stress, such as, over crowdness, high temperatures, improper transportation and feeding (Roberts,2001).

The signs of *pseudomonas florescens* infection recorded manifested by extensive amount of mucus in the skin with massive streaks of haemorrhages and congested base of the fins together with skin ulceration as shown in photo (1), gill filaments were covered with excessive mucus and the color ranged from pale to severely congested, also *O.niloticus* showed clear inflammation on the fin bases together with tail rot.

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The results were in agreement with Ahmed, 1991, who demonstrated the same clinical picture in *O.niloticus* experimentally infected with *P. florescens*, Noga, 1996 cleared that the disease it self exert its clinical picture in affected fish in the form of septicaemia, large cutaneous haemorrhages in the fins and trunk, ulcers, exophthalmia and high mortalities.

The results also cleared that the group received 1%, 1.5% of the zimofermrnt pioneered with the highest immune response as observed by the best challenge results. Mortalities were observed in group (4) and the control group (1) shown in table No. (7), line chart (1) and column chart (2). This can be explained by the fact that un-rational use of the feed additive can be costly and even toxic to fish, this results coincides with the results of Craig and McLean (2003), who mentioned that overdosing of some feed additives (Bio-Mos®) or long term exposure or both can badly affect the performance characteristics of Nile tilapia, they found that there is tendency toward declining the phagocytotic and the survival with increasing levels of (Bio-Mos®). Thus affects the survival and the immune-competence of the Nile tilapia to the challenging environments, this also explain the previous result of the declining activity of phagocytes with increasing level of zimoferment than 1.5%.

It was concluded from the obtained results that Zimoferment may have positive impact on the growth performance and immune status of Nile tilapia (*O. niloticus*) in a calculated doses under experimental conditions.

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