Effect of Genetic Selection for Increased Body Weight at Harvest on Disease Resistance and Immune Responses of Nile tilapia *Oreochromis niloticus*

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Abstract: The potential effect of selection for growth and related traits of Nile tilapia *Oreochromis niloticus* on disease resistance and a variety of specific and nonspecific immune parameters was investigated between two different lines of *O. niloticus* (selected bred line SBL derived from the 8th generation selected for increased body weight at harvest and a random bred line RBL), via an experimental challenge through the intraperitoneal route with *Aeromonas hydrophila*. Prior to challenge; SBL showed observed enhancement of survival rate and significant increases ($P < 0.05$) of growth performance compared to RBL group after a rearing period of 180 days in circular earthen ponds. Mortalities due to challenge were higher in RBL group than those recorded in SBL group, SBL group revealed significant increases in the hematological indices than the RBL group, which were consistently higher in females than males before and after the challenge. Plasma total proteins, albumin, α, β and γ globulins were significantly higher in SBL group, which also showed a significant increased levels in plasma glucose in comparison to RBL group, in which plasma cortisol recorded significantly higher levels than SBL group. Results of the immune responses (leukocyte phagocytic activity, respiratory burst activity, lysozyme activity, and Plasma Immunoglobulins) showed significant increases in SBL group compared to RBL group.The results of the present study revealed a positive effect of selection for growth of *O. niloticus* on disease resistance and immune responses, which indicate the possibility of indirect selection for disease resistance in breeding programmes in which growth and size are the selected traits in *O. niloticus*.

Keywords: Genetic selection, disease resistance, *Oreochromis niloticus*

INTRODUCTION

Tilapia is and will continue to be an important fish, particularly for the lesser-developed countries in the tropics (FAO, 2001). A number of biological characteristics make tilapia excellent candidates for culture; acceptance of formulated feeds, efficient food conversion ratios (Jauncey, 2000), tolerance of handling (Little, 2000), tolerance of high stocking densities (Popma and Masser, 1999), tolerance of marginal water quality (Fitzsimmons, 2000), year-around spawning (Beardmore *et al.*, 2001) and market demand (Harvey, 2005). Also because of its numerous desirable traits such as fast growth, hardiness, its suitability for culture in a wide range of farming systems, its relatively short generation time of about six months and its suitability for investigation of the application of genetics in aquaculture, from conservation of genetic resources to breeding programs (Eknath and Velasco, 1993). In addition to the possibility of increasing its productivity through selective breeding and genetic improvement programs, and this is what has been achieved already by the successful application of several programs of genetic selection.

Aquaculture production has been rapidly increased in the recent years, much of which has come from increasing the intensity of existing systems, increases in stocking densities can make fish more susceptible to stress and disease which in turn may cause severe losses of stock. Despite the encouraging trends, several constraints have negative impact on the growth of aquaculture. Diseases are one of these primary limiting factors (FAO, 2004).

Reducing the economic loss due to diseases is considered to be one of the major goals of aquaculture development worldwide. That is why some basic procedures can be used to reduce or control fish disease. These include treatment with drugs, culling of diseased fish and only replacing with new stocks after ponds or tanks are disinfected, vaccination of stock or genetic selection to improve disease resistance. The current breeding objective in aquatic species has focused almost exclusively on the improvement of body weight at harvest or on growth related traits (Nguyen and Ponzoni, 2006). Improving infectious disease resistance by genetic means is an attractive alternative because of its prospects for prolonged protection. The significant genetic variation in disease resistance found in different fish species (reviewed by Chevassus and Dorson, 1990; Fjalestad *et al.*, 1993; Wiegertjes *et al.*, 1996) suggests the possibility of such genetic improvement.

The concept of genetic selection to improve disease resistance is an attractive strategy for disease prevention and receiving great deal of attention, since the other methods described face a variety of setbacks, Antibiotics, for example, are frequently used to control disease, However, use of antibiotics has been seriously criticized for development of antibiotic-resistant bacterial strains (FAO, 2006). In addition, numerous studies have shown that most antibiotics can suppress the natural immunity of fish, increasing the risk of infection from non-bacterial pathogens such as viruses, fungi and parasites (Lunden *et al.*, 1999, 2002). Vaccines are often administered to prevent disease and they are usually delivered via injection instead of dietary supplementation or immersion. Although some have been successfully applied to certain fish diseases they are not yet available for many fish diseases. Moreover, the associated handling stress on the fish

while delivering the vaccine may impair the efficacy of the vaccines to a great extent.

The aim of the present study was to investigate and evaluate the effect of selection for growth and related traits of *O. niloticus* (through eight generations of selection) on disease resistance and immune responses, and to test the possibility of using some immune parameters as indirect selection criteria in selective breeding to enhance disease resistance of *O.niloticus*.

MATERIALS AND METHODS

The fish and the environment:

Experiments were conducted at the Abbassa Research Station of The World Fish Center, Regional Research Center for Africa and West Asia (Abbassa, Abu Hammad, Sharkia, Egypt) as a part of their on-going selection program for increased growth performance of *O. niloticus*.

The Experiment consists of two stages:

1. The first stage of the experiment was extended to six months, at the end of which survival and growth performance were determined.
2. The second stage of the experiment was conducted to estimate the immune responses of the fish after challenge with *A. hydrophilla*.

The experimental fish were derived from an on-going selection program for increased growth performance in Egypt. A total of 2000 individuals of *O. niloticus* with average body weight of 4.03 ± 0.24 g and 3.82 ± 0.48 for the Selected Bred line (SBL) and the Random Bred Line (RBL) respectively was used in this study. Individuals of SBL (1000 individuals) were originated from 30 families derived from the 8th generation selected for increased body weight at harvest. Fish were equally and randomly distributed between 4 circular earthen ponds (100 m^2) at a stocking density of 5 fish.m^{-2} and fed on a basal diet of 25% protein at a ratio of 3% of their total biomass twice daily at (8 am and 2 pm) 7 days a week throughout the rearing period (180 days).

Measurement of survival and Growth Performance:

Survival Rate was determined according to the following formula:

Survival % = No. of fish counted ÷ No. of stocked fish

The growth parameters of experimented fish were calculated at the end of a grow-out period of 180 days, when all fish were counted and weighed individually (Innes, 1982). Parameters used to evaluate growth performance in this study were weight gain (WG) by a fish, average daily gain (ADG), specific growth rate (SGR) and Condition factor (CF) which are the most commonly used expression of fish growth. ADG, SGR and CF were estimated according to Castell and Tiews (1980), Ricker (1979) and Ricker (1979) respectively.

Bacterial Challenge:

Bacterial Strains:

Aeromonas hydrophila (*A. hydrophila*) Strain was isolated from natural diseased *O. niloticus* and identified in Fish Health Laboratory at World Fish Center, Abbassa, Egypt.

Preparation of A. hydrophila Bacterin:

For preparation of bacterin, the bacterial isolate was inoculated into tryptic soy broth (TSB) and incubated for 24h at 25°C. Formalin (40% w/v) was added to the broth culture at a final concentration of (0.5% v/v) and left 48 hrs at room temperature. The inactivated cells were harvested by centrifugation at 3000 rpm for 10 min., then washed twice in 0.3% formalized phosphate buffered saline (PBS) and resuspended to the density of McFarland standard tube NO.3 (1 x 10^8 cells / ml) for the isolate. After this, the bacterin was tested for its sterility (free from living cells) by streaking it onto tryptic soy agar which showed no growth.

Challenge:

Prior to challenge, fish were maintained under observation for two weeks in glass aquaria (50 × 60 × 70 cm) supplied with aeration, for acclimatization, 320 fish from each line (SBL and RBL) were divided into two subgroups (160 fish each, distributed in 8 aquaria 20 fish in each), Males and Females were kept separately. Two subgroups (one per each line) were vaccinated in a triplicate manner with formalized whole culture of *A. hydrophila* by direct immersion route (McIntosh, 1993); fish were immersed for 30 min in diluted vaccine in a separate vaccine aquarium (1 volume of vaccine to 10 volumes of tank water = 10^8 cells / ml), then they were drained carefully maintaining the vaccine solution in the vaccinating aquarium and returned to their original aquaria after vaccination (40 fish of each group - males and females - were kept untreated as control until the end of the vaccination process). At the end of vaccination process (after 4 weeks); the fish (Vaccinated and Non-Vaccinated) were prepared to conduct the challenge with *A. hydrophila*. All fish were challenged intraperitoneally (IP) with 0.5 ml of 10^8 of 24 h live *A. hydrophila*. Challenged fish were kept under observation for 15 days. The mortalities were recorded and the Relative Level of Protection (**RLP**) among the challenged fish was determined (Ruangroupan *et al.*, 1986) using the following equation:

RLP = 1 - (Percent of Treated Mortality ÷ Percent of Control Mortality) × 100

Blood Sampling and Hematological Parameters:

For collecting blood, fish were anesthetized in water contains Tricaine Methane Sulfonate (MS-222). The Whole blood was collected from the caudal vein of each fish using syringes (1-ml) and 27-gauge needles that were rinsed in heparin (15 unit.ml^{-1}). Samples were divided into two parts; one was used for the estimation of the hematological parameters, and the other was centrifuged at 3000 rpm for 15 minutes and blood plasma samples were separated and kept at –20°C until biochemical and immunological analysis.

(Erythrocytes and Leucocytes) counts were calculated according to Natt and Herrick (1952), Hematocrit (Hct) was determined in microhematocrit tubes; according to (Goldenfarb *et al.*, 1971). Haemoglobin concentration in blood was determined by the cyanomethaemoglobin method, according to Collier (1944) using colorimetric kits (ELITech, France).

Erythrocytes Indices (MCV, MCH, MCHC) were calculated using standard formulas (Seiverd, 1964) and Leucocytes differential count was estimated according to the methodology of (Stoskoph, 1993).

Plasma Biochemical Parameters:

Total protein in the plasma was estimated according to Gomall *et al.* (1949) using colorimetric kits (Bio Systems S.A., Barcelona, Spain). Albumin in the plasma was estimated according to Dumas and Biggs (1972) using colorimetric kits (bioMérieux, France), and to compare sample protein patterns; Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (SDS-PAGE) technique was used. Samples of gel preparation, electrophoresis conditions, staining and destining gels were done according to Laemmli (1970).The absolute values for different protein bands were calculated according to the values of the total proteins (Mahran, 2002). Glucose in the plasma expressed as ($mg.dL^{-1}$) was estimated colorimetrically according to the technique described by Trinder (1959) using colorimetric kits (Spintract, Spain), and Cortisol in the plasma expressed as ($\mu g.mL^{-1}$) was estimated according to Foster and Dunn (1974) using an enzymatic immunoassay (Cobas, Germany).

Immunological Parameter:s

Leukocyte Phagocytic Activity:

Leukocyte Phagocytic Activity was estimated by obtaining four to five drops of blood, from the caudal veins, into heparinized centrifuge tubes, and shaken well. The same volume of a bacterial suspension of *A. hydrophila* was added and the tubes were well shaken again. The tubes were then kept at 28 °C in a water bath for 30 min, and shaken every 10 min. Following incubation they were centrifuged at 3000 rpm for 5 min. The supernatant was discarded, and the upper layer of the precipitate was used to make blood slides (three slides for each fish). Slides were air dried, fixed with methanol for 3 min, and then stained with Swiss staining solution for 5 min. The number of phagocytotic and unphagocytotic leukocytes was counted under the microscope. Phagocytotic ratio was calculated by the formula:

Phagocytotic (%) = Phagocytotic Leukocytes ÷ Observed Total Leukocytes

Respiratory Burst Activity:

The respiratory burst activity of the phagocytes was measured by nitroblue tetrazolium (NBT) assay following the method of Secombes (1990) modified by Stasiack and Baumann (1996).

PLasma Lysozyme Activity:

The lysozyme activity was measured using the turbidity assay. Chicken egg lysozyme (Sigma) was used as a standard and 0.2 mg / ml lyophilised *Micrococcus lysodeikticus* in 0.04 M sodium phosphate buffer (pH 5.75) was used as substrate (Parry *et al.*, 1965).

Measurment of Specific Immune Response:

Plasma total immunoglobulin (IgM) was determined (in the vaccinated subgroups) by the indirect-ELISA according to Loghothetis and Austin (1996) and the interpretation of ELISA readings (mean of OD immersed in the bacterins suspension of equal volumes).

Statistical analysis:

Statistical analysis was performed using a two-way ANOVA and Duncan's multiple Range Test (Dauncan, 1955) to determine differences between groups' means (significance level $P \leq 0.05$). Standard errors of groups' means were also estimated. All statistics were carried out using Statistical Analysis Systems (SAS) program (SAS, 2005).

RESULTS AND DISCUSSION

Survival and Growth Performance:

The survival and growth parameters of experimented fish were calculated at the end of a grow-out period of 180 days, when all fish were counted and weighed individually, the SBL group (males and females) showed observed enhancement of survival in comparison with the RBL group (males and females), and within the two groups, there was a significant increase of survival of females in comparison with males. As for the growth performance; SBL group (males and females) showed significant increase of FBW, WG, ADG, and SGR in comparison with the RBL group, males of both groups showed higher values of FBW, WG, ADG, SGR and CF in comparison with the females, while males of the RBL group showed a slight significance in CF compared to the males of the SBL group, and there was no significance observed between females of the two groups, as it is tabulated in Table 1.

Many of the previous studies have found out favorable genetic correlations between disease resistance and growth rate in salmonids (Standal and Gjerde, 1987; Gjedrem *et al.*, 1991; Nilsson, 1992). Gjedrem (2000) stated that by increasing growth rate, a correlated genetic response would be obtained for disease resistance. However, breeding programs for fish that concentrate solely on the enhancement of production traits (i.e., growth, feed conversion efficiency) are likely to have adverse effects on health traits (Henryon *et al.*, 2002). Henryon *et al.* (2002) observed unfavorable correlations between the predicted breeding values for viral haemorrhagic septicaemia virus resistance and the predicted breeding values for the economic traits (growth rates and feed conversion efficiencies) within the rainbow trout population. Rezk, *et al.* (2009) found an evidence of genetic variation in harvest weight and survival rate, after only two generations of selection of this Nile tilapia and the cumulative genetic gain of about 14% in harvest weight, coupled with the favorable genetic correlation of the latter trait with survival rate. Moreover, it is well known that specific growth rate (SGR) and condition factor (CF) are a reflection of the health status in fish.

Table (1): Summary of the two-way analysis of variance (ANOVA) results for Survival and growth performance of *O. niloticus* after a grow-out period of 180 days (M±SE).

	SBL		RBL	
	♂	♀	♂	♀
IBW (g)	$^{A}4.03\pm0.08^{a}$	$^{A}4.03\pm0.08^{a}$	$^{A}3.82\pm0.07^{a}$	$^{A}3.82\pm0.07^{a}$
FBW (g)	$^{A}174.15\pm4.08^{a}$	$^{A}114.61\pm4.97^{b}$	$^{B}145.77\pm2.84^{a}$	$^{B}80.72\pm2.37^{b}$
BWG (g)	$^{A}170.12\pm4.08^{a}$	$^{A}110.58\pm4.97^{b}$	$^{B}141.95\pm2.84^{a}$	$^{B}76.90\pm2.37^{b}$
ADG	$^{A}0.95\pm0.01^{a}$	$^{A}0.61\pm0.01^{b}$	$^{B}0.79\pm0.01^{a}$	$^{B}0.43\pm0.01^{b}$
SGR (%.day^{-1})	$^{A}2.09\pm0.16^{a}$	$^{A}1.86\pm0.23^{b}$	$^{B}2.02\pm0.17^{a}$	$^{B}1.69\pm0.04^{b}$
CF	$^{B}2.02\pm0.03^{a}$	$^{A}1.90\pm0.02^{a}$	$^{A}2.10\pm0.02^{a}$	$^{A}1.87\pm0.02^{a}$

Means within each column preceded by different superscripts (A or B) differ significantly ($P \leq 0.05$) within sex.
Means within each row followed by different superscripts (a or b) differ significantly ($P \leq 0.05$) within line.

Disease Resistance and Immune Responses

Pathological results and Mortalities due to Challenge

After grow-out period, the non vaccinated challenged fish revealed higher mortality in the RBL group than the SBL group, the same trend was observed in the two groups after receiving the vaccine, and within the two lines (SBL and RBL); males recorded higher mortalities than females. Relative level of protection against *A. hydrophila* in the vaccinated group was significantly higher than it was in the non-vaccinated group.

Hematological Parameters:

Hematological parameters are among the important tools for fish disease diagnosis (Ruane *et al.*, 2000; Ranzani-Paiva *et al.*, 2005; Ghiraldelli *et al.*, 2006) and have been considered as important indicators of fish health (Chen *et al.*, 2004). In the present study, mean values of hematological parameters of non-infected groups of *O. niloticus* and those infected with *A. hydrophila* are presented in Table 2. The values of Hb, RBC, WBC, HCT, MCH and MCHC considered in the study were consistently higher in females than males before and after the infection in both SBL and RBL groups. Results indicated that there were significant decreases in Hb, RBC, HCT, MCH and MCHC, contrariwise, WBC and MCV increased significantly in the infected fish compared to the non-infected ones. Collectively, results obtained for SBL group showed significant increases in the studied parameters in comparison with those obtained for RBL group (in both sexes). The decreased values of erythrocytes number, hematocrit and haemoglobin indicate that the infected fish had a severe anemia, and the decrease in the number of erythrocytes together with the increase of its volume (MCV) was a sort of compensation for the haemoglobin oxygen transport. The percent volume of erythrocytes in fish blood gives a clue to the health status of fish and can be helpful in determining any abnormalities arising from the use of probiotics. Hematocrit is an indicator of the health status and can be helpful in detecting any abnormal changes. Anemic fish may have hematocrit as low as 10 % reduced hematocrit may indicate that fish are not eating or are suffering infections (Blaxhall, 1972).

Leucocyte differential counts were characterized by predominance of lymphocytes. Five types of leucocytes, namely Lymphocytes, Neutrophils, Monocytes, Eosinophils and Basophils were identified in the circulating blood of *O. niloticus*. The values of Leucocytes were consistently higher in females than males before and after the infection in both SBL and RBL groups. Results showed significant differences between SBL group and RBL group (in both sexes) where the values in SBL group tends to be higher than those in RBL group.

Contrarily, Sarder *et al.* (2001) did not observe significant alteration in the differential counts of white blood cells in tilapia challenged with *A. hydrophila*.

Biochemical Parameters

Profile and Electrophoretic Pattern of Plasma Proteins

The mean values of plasma glucose, cortisol, total proteins and its electrophoretic fractions of non-infected groups of *O. niloticus* and those infected with *A. hydrophila* are presented in Table 3.; infection with *A. hydrophila* showed marked hypoglycemia in infected groups comparing with the non-infected groups. Moreover, SBL group revealed significant increased levels in plasma glucose when compared to the RBL group in both sexes), and within the two lines (SBL and RBL); males recorded higher values of plasma glucose than females. Cortisol levels increased about 2.5 fold after infection with *A. hydrophila*. Results obtained for RBL group showed significant increased levels of plasma cortisol when compared to those obtained for SBL group (in both sexes), where males recorded significantly higher values than females. Groups of infected *O. niloticus* from both lines (SBL and RBL) showed marked drop in the values of total proteins, albumin, α, β and γ globulins comparing to non-infected groups. Moreover, these findings in SBL group revealed significant increases than it is in the RBL group, while A:G ratio was decreased significantly in SBL group than RBL group. Some plasma chemicals may be useful tools to evaluate the health and/or stress condition of the fishes (Sadler *et al.*, 2000, Campbell, 2004, Wagner and Congleton, 2004). Plasma proteins include the humoral elements of the nonspecific immune system (Magnadóttir, 2006). The decrease of total protein concentration detected in the infected group could be attributed to the effect of bacterial toxins on the intestinal villi, digestive enzymes production and loss of food consumption which reflected on the absorbability and digestibility of nutrients. Moreover, the hypoalbuminemia and hypoglobulinemia which were observed in both infected individuals may be due to the histopathological lesions caused by *A.hydrophila* in hepatic tissue which showed evidence of necrosis and

vacuolar degeneration with consideration of the fact that liver is the main site of albumin and some globulins production. The significant increase in the globulins (α, β and γ) of infected group could be attributed to the increase in the level of IgM that mostly found in the β-globulin fraction and released in early infection as a

reported to elevate plasma cortisol (Pottinger *et al.*, 2003, Haukenes *et al.*, 2008) and glucose levels (David *et al.*, 2005), many researchers consider as a "rule of thumb" that fishes undergoing stressful situations exhibit plasmatic increases of cortisol and glucose (Barcellos *et al.*, 1999). Cortisol and glucose are two of the most common stress indicators (Martinez-Porchas *et al.*, 2009). Increase in glucose concentration is a secondary response to stress, and the level of increase is a measurement for stress response (Pottinger and Carrick, 1999).

Regarding the plasma glucose level, comparing to non-infected fish *A.hydrophila* infection showed marked hypoglycemia in infected fish. As it is mentioned by many authors liver plays a key role in glucose metabolism and helps to regulate the blood glucose concentration. According to this, hepatic failure reflected on the blood glucose level, this may explain why the experimentally infected groups showed a significant hypoglycemia compared to non-infected groups, and this may be attributed to the reduced hepatic gluconeogenesis or glycogenolysis (Thrall, 2004). Furthermore, the hepatic tissue of the experimentally infected groups showed evidence of necrosis and vacuolar degeneration. Cortisol has depressive effects on a number of immune responses in fish. In addition, it decreases the activity of antibody producing cells and circulating titres of IgM (Maule *et al.*, 1989; Nagae *et al.*, 1994). The few studies in which selective breeding for stress response was implanted in fish demonstrate the feasibility of using the genetic variance of post-stress levels of cortisol as a selection criterion. These breeding plans were based on the hypothesis that reducing stress responsiveness will result in improved disease resistance. However, it has not yet been proven that there is genetic gain in any economically valued trait, or demonstrated that high stress response is a disadvantage and low stress response an advantage in respect to disease resistance (Pottinger and Carrick, 1999; Fevolden *et al.*, 2002).

Fevolden *et al.* (1993) selected two lines of rainbow trout and Alantic salmon for high stress and for low stress response, challenged them with bacterial pathogens to evaluate their disease resistance; and they found that the low stress response line preformed better against furunculosis and vibriosis.

Immunological Response:

In fish, like other vertebrates, the immune response is a combination of two systems, the innate (non-specific) immune system and the acquired (specific) immune system. Both systems may be divided into two major pathways: cellular and humoral responses. The two systems are not separated; they interact and activate each other for an overall immune response of the organism (Ellis, 1999; Magnadóttir, 2006), and in several vertebrate species, males have a weaker immune system than females due to immunosuppressive androgens (Ahmed *et al.*, 1985; Zuk, 1990; Møller *et al.*, 1998).

Non-Specific Cellular Response

in the immune response of teleost because fishes have only one type of immunoglobulin, IgM-like. Phagocytosis, NBT (nitrobluetetrazolium) or respiratory burst activity and lysozyme activity assays spot the light on the non-specific immune response.

Leukocyte Phagocytic Activity:

Phagocytic cells are the most important cellular components of the innate immune system of fish (MacArthur and Fletcher, 1985). Their phagocytic activity is a primitive defense mechanism (Neumann *et al.*, 2001) and an important characteristic of the non-specific immune system (Seeley *et al.*, 1990). Phagocytosis is a defense reaction of the organisms that may vary according to fish health status (Toranzo *et al.*, 1995). When microorganisms penetrate the body, tissue macrophages, circulating monocytes, and granulocytes enter the infected area to attack the pathogens through phagocytosis, release of oxidative and lytic enzymes, and reactive oxygen species (respiratory burst) as nonspecific defense activities. Phagocytosis by macrophages of antigenic material can start the specific humoral and cellular immune response (Bayne and Gerwick, 2001; Magnadóttir, 2006).

As shown in Table 4, High percentages of phagocytosed bacterium by leucocytes in infected fish groups comparing with the non-infected groups were detected. SBL group showed significant increases in phagocytic leukocytes percentages contrary to those observed in the RBL group (in both sexes). The percentages of phagocytic leukocytes of RBL group were similar for males and females, but in SBL group, they were significantly higher in females than males.

Respiratory Burst Activity:

Respiratory burst activity (measured by NBT) is one of the most important bactericidal mechanisms in fish (Secombes and Fletcher, 1992), it was used to determine the activity of the phagocytes, especially neutrophils and monocytes (Jabs *et al.*, 1980). Table 4 clearly showed an increase in neutrophil respiratory burst activity in infected fish groups in comparison with the non-infected groups. SBL group showed significant increases in respiratory burst activity contrary to those observed in the RBL group (in both sexes), the respiratory burst activity of RBL group was similar for males and females, but in SBL group, they were significantly higher in females than males.

Non-Specific Humoral Response:

PLasma Lysozyme Activity:

Lysozyme is an important parameter in the immune defence of both invertebrates and vertebrates. Lysozyme is bactericidal, hydrolysing β-linked glycoside bonds of bacterial cell wall peptidoglycans resulting in lysis. Although primarily associated with defence against

Table (2): Summary of the two-way analysis of variance (ANOVA) results for hematological parameters of *O. niloticus* (M±SE).

	Non infected				Infected			
	SBL		RBL		SBL		RBL	
	♂	♀	♂	♀	♂	♀	♂	♀
Hb (g.dL^{-1})	$^{A}7.64\pm0.22^{b}$	$^{A}8.89\pm0.26^{a}$	$^{B}6.17\pm0.18^{a}$	$^{B}6.73\pm0.19^{a}$	$^{A}7.21\pm0.21^{b}$	$^{A}8.39\pm0.24^{a}$	$^{B}5.82\pm0.17^{a}$	$^{B}6.26\pm0.20^{a}$
HTC (%)	$^{A}29.38\pm0.85^{a}$	$^{A}31.51\pm0.91^{a}$	$^{B}24.63\pm0.71^{b}$	$^{B}27.00\pm0.78^{a}$	$^{A}27.91\pm0.81^{a}$	$^{A}29.93\pm0.86^{a}$	$^{B}23.75\pm0.69^{a}$	$^{B}25.65\pm0.74^{a}$
MCV (fL)	$^{A}104.24\pm3.01a$	$^{A}98.63\pm2.85^{a}$	$^{A}97.08\pm2.80^{a}$	$^{A}100.94\pm2.91^{a}$	$^{A}115.22\pm3.33^{a}$	$^{A}109.03\pm3.15^{a}$	$^{A}105.71\pm3.05^{a}$	$^{A}111.57\pm3.22^{a}$
MCH (pg)	$^{A}28.54\pm0.82^{a}$	$^{A}29.31\pm0.85^{a}$	$^{B}25.22\pm0.73^{a}$	$^{B}26.49\pm0.76^{a}$	$^{A}28.27\pm0.82^{a}$	$^{A}29.03\pm0.84^{a}$	$^{B}24.98\pm0.72^{a}$	$^{B}26.24\pm0.76^{a}$
MCHC (g.dL^{-1})	$^{A}27.38\pm0.79^{a}$	$^{A}29.71\pm0.86^{a}$	$^{A}25.98\pm0.75^{a}$	$^{B}26.24\pm0.76^{a}$	$^{A}24.54\pm0.71^{a}$	$^{A}26.63\pm0.77^{a}$	$^{A}23.63\pm0.68^{a}$	$^{B}23.52\pm0.68^{a}$
RBC (x106.µL^{-1})	$^{A}2.68\pm0.08^{b}$	$^{A}3.03\pm0.09^{a}$	$^{A}2.45\pm0.07^{a}$	$^{B}2.54\pm0.07^{a}$	$^{A}2.55\pm0.07^{b}$	$^{A}2.89\pm0.08^{a}$	$^{A}2.33\pm0.07^{a}$	$^{B}2.42\pm0.07^{a}$
WBC (x103.µL^{-1})	$^{A}38.42\pm1.11^{a}$	$^{A}41.76\pm1.21^{a}$	$^{B}24.63\pm0.71^{b}$	$^{B}35.86\pm1.04^{a}$	$^{A}47.54\pm1.37^{a}$	$^{A}52.75\pm2.38^{a}$	$^{B}40.19\pm1.08^{a}$	$^{B}42.53\pm1.23^{a}$
Lympho. (×103.µL^{-1})	$^{A}29.38\pm0.57^{b}$	$^{A}31.65\pm0.62^{a}$	$^{B}18.64\pm0.36^{b}$	$^{B}27.84\pm0.02^{a}$	$^{A}36.35\pm0.71^{b}$	$^{A}41.77\pm0.81^{a}$	$^{B}30.16\pm0.59^{b}$	$^{B}33.02\pm0.64^{a}$
Neutro.(×103.µL^{-1})	$^{A}6.83\pm0.13^{b}$	$^{A}7.51\pm0.15^{a}$	$^{B}4.50\pm0.09^{b}$	$^{B}6.15\pm0.12^{a}$	$^{A}8.30\pm0.16^{b}$	$^{A}9.78\pm0.19^{a}$	$^{B}7.28\pm0.14^{a}$	$^{B}7.30\pm0.14^{a}$
Mono.(×103.µL^{-1})	$^{A}1.33\pm0.03^{b}$	$^{A}1.53\pm0.03^{a}$	$^{B}0.84\pm0.02^{b}$	$^{B}1.12\pm0.02^{a}$	$^{A}1.79\pm0.03^{b}$	$^{A}2.03\pm0.04^{a}$	$^{B}1.36\pm0.03^{a}$	$^{B}1.33\pm0.03^{a}$
Eosino.(×103.µL^{-1})	$^{A}0.62\pm0.01^{b}$	$^{A}0.78\pm0.02^{a}$	$^{B}0.49\pm0.01^{b}$	$^{B}0.53\pm0.01^{a}$	$^{A}0.77\pm0.02^{a}$	$^{B}0.57\pm0.01^{b}$	$^{A}0.79\pm0.02^{a}$	$^{A}0.63\pm0.01^{b}$
Baso.(×103.µL^{-1})	$^{A}0.26\pm0.01^{b}$	$^{A}0.29\pm0.01^{a}$	$^{B}0.17\pm0.00^{b}$	$^{B}0.21\pm0.00^{a}$	$^{A}0.33\pm0.01^{b}$	$^{A}0.38\pm0.01^{a}$	$^{B}0.27\pm0.01^{a}$	$^{B}0.25\pm0.00^{b}$

Table (3): Summary of the two-way analysis of variance (ANOVA) results for the mean values of some biochemical parameters of *O. Niloticus* (M±SE).

	Non infected				Infected			
	SBL		RBL		SBL		RBL	
	♂	♀	♂	♀	♂	♀	♂	♀
Glucose (mg.dL^{-1})	$^{A}106.81\pm2.07^{a}$	$^{A}100.75\pm1.95^{b}$	$^{B}93.93\pm1.82^{a}$	$^{B}78.89\pm1.53^{b}$	$^{A}133.45\pm2.59^{a}$	$^{A}125.89\pm2.44^{b}$	$^{B}117.36\pm2.27^{a}$	$^{B}98.58\pm1.91^{b}$
Cortisol (µg.dL^{-1})	$^{B}3.86\pm0.08^{a}$	$^{B}2.93\pm0.06^{b}$	$^{A}4.61\pm0.09^{a}$	$^{A}3.51\pm0.07^{b}$	$^{B}9.65\pm0.19^{a}$	$^{B}7.33\pm0.14^{b}$	$^{A}11.52\pm0.22^{a}$	$^{A}8.78\pm0.17^{b}$
Total protein (g.dL^{-1})	$^{A}3.17\pm0.06^{b}$	$^{A}4.16\pm0.08^{a}$	$^{B}1.44\pm0.03^{b}$	$^{B}1.65\pm0.03^{a}$	$^{A}2.90\pm0.06^{b}$	$^{A}4.09\pm0.08^{a}$	$^{B}1.42\pm0.03^{b}$	$^{B}1.62\pm0.03^{a}$
Albumin(g.dL^{-1})	$^{A}0.50\pm0.01b$	$^{A}0.97\pm0.02^{a}$	$^{B}0.38\pm0.01^{b}$	$^{B}0.43\pm0.01^{a}$	$^{A}0.35\pm0.01^{b}$	$^{A}0.68\pm0.01^{a}$	$^{B}0.27\pm0.01^{b}$	$^{B}0.29\pm0.01^{a}$
α - globulin (g.dL^{-1})	$^{A}0.24\pm0.02^{b}$	$^{A}0.32\pm0.02^{a}$	$^{B}0.11\pm0.01^{a}$	$^{B}0.13\pm0.01^{a}$	$^{A}0.14\pm0.00^{b}$	$^{A}0.19\pm0.00^{a}$	$^{B}0.11\pm0.01^{a}$	$^{B}0.11\pm0.01^{a}$
β - globulin (g.dL^{-1})	$^{A}1.15\pm0.06^{b}$	$^{A}1.52\pm0.09^{a}$	$^{B}0.51\pm0.04^{a}$	$^{B}0.59\pm0.04^{a}$	$^{A}1.35\pm0.03^{b}$	$^{A}1.75\pm0.03^{a}$	$^{B}0.97\pm0.12^{a}$	$^{B}1.02\pm0.10^{a}$
γ - globulin (g.dL^{-1})	$^{A}0.92\pm0.07^{b}$	$^{A}1.19\pm0.08^{a}$	$^{B}0.43\pm0.04^{a}$	$^{B}0.48\pm0.03^{a}$	$^{A}1.06\pm0.02^{b}$	$^{A}1.47\pm0.03^{a}$	$^{A}0.78\pm0.09^{a}$	$^{B}0.81\pm0.08^{a}$
A:G	$^{B}0.22\pm0.00^{b}$	$^{B}0.32\pm0.01^{a}$	$^{A}0.36\pm0.01^{a}$	$^{A}0.36\pm0.01^{a}$	$^{B}0.14\pm0.00^{b}$	$^{B}0.20\pm0.00^{a}$	$^{A}0.23\pm0.00^{a}$	$^{A}0.22\pm0.00^{b}$

Table (4): Summary of the two-way analysis of variance (ANOVA) results for the mean values of some non-specific immune response of *O. niloticus* (M±SE).

	Non infected				Infected			
	SBL		RBL		SBL		RBL	
	♂	♀	♂	♀	♂	♀	♂	♀
Phagocytosis (%)	$^{A}52.34\pm1.02^{b}$	$^{A}55.30\pm1.08^{a}$	$^{B}47.78\pm0.93^{a}$	$^{B}48.65\pm0.95^{a}$	$^{A}61.02\pm1.19^{b}$	$^{A}64.41\pm1.25^{a}$	$^{A}55.57\pm1.09^{a}$	$^{B}55.90\pm1.09^{a}$
NBT (mg.mL^{-1})	$^{A}0.69\pm0.03^{b}$	$^{A}0.80\pm0.03^{a}$	$^{B}0.49\pm0.02^{b}$	$^{B}0.56\pm0.02^{a}$	$^{A}1.24\pm0.04^{b}$	$^{A}1.44\pm0.05^{a}$	$^{B}0.89\pm0.04^{b}$	$^{B}1.00\pm0.04^{a}$
lysozyme (µg.mL^{-1})	$^{A}10.87\pm0.10^{a}$	$^{A}11.43\pm0.19^{a}$	$^{B}9.60\pm0.07^{b}$	$^{B}10.28\pm0.11^{a}$	$^{A}13.05\pm0.11^{a}$	$^{A}13.72\pm0.22^{a}$	$^{B}11.52\pm0.09^{b}$	$^{B}12.34\pm0.14^{a}$

Means within each column preceded by different superscripts (A or B) differ significantly ($P \leq 0.05$) within sex.

Means within each row followed by different superscripts (a or b) differ significantly ($P \leq 0.05$) within line.

Gram positive bacteria, Gram negative bacteria can also be lysed by this enzyme. Lysozyme is also known to be an opsonin and activate the complement system and phagocytes (Jolles and Jolles, 1984). It presents in mucus, lymphoid tissue, plasma and other body fluids of most fish species (Grinde, 1989). An increase in the lysozyme concentration in fish blood can be caused by infections or invasion by foreign material (Fletcher and White, 1976; Siwicki and Studnicka, 1987).

In this study, Lysozyme activity of infected fish groups was increased significantly in comparison with the non-infected groups, SBL group (males and females) showed significant increases of lysozyme activity in comparison with the RBL group. Moreover, lysozyme activity of females of the both lines showed significant increases in comparing with males, as it is tabulated in Table 4. However, the use of lysozyme as a selection criterion to improve disease resistance is complicated by the fact that the association between lysozyme activity and disease resistance seems to be highly influenced by the immune status of fish at the time of sampling (Røed *et al.,* 2002). Significant genetic variation in lysozyme activity has also been suggested in Atlantic salmon, although the heritability was rather low (Røed *et al.,* 1993). The lysozyme activity has been shown to be negatively correlated with survival against diseases in Atlantic salmon (Lund *et al.,* 1995) and Nile tilapia (Chiayvareesajja *et al.,* 1999). There have been reported moderate to relatively high heritabilities in serum hemolytic activities in rainbow trout (Røed *et al.,* 1990) and low to moderate heritabilities in spontaneous hemolytic activity were marked in Atlantic salmon (Røed *et al.,* 1992, 1993), which indicated importance of this parameter to use as indirect selection marker to improve disease resistance. The hemolytic activity in Atlantic salmon showed significant genetic variation for this parameter, when measured in serum sampled before and after a single immunization. However, no significant genetic variation was found with samples taken after a second immunization (Røed *et al.,* 1993). This may indicate that the genetic variation in hemolytic activity depends on the immunological status of the fish, which has also been observed in case of rohu. This finding was in agreement with the observation of Lund *et al.,* (1995) and Chiayvareesajja *et al.* (1999), who also reported no significant correlation between hemolytic activity and survival rate in Atlantic salmon and Nile tilapia, respectively, either through challenge against bacterial pathogens or via natural survival record.

Specific Immune Response: Plasma Total Immunoglobulin (IgM):

The humoral immune response of O. niloticus was investigated following injection administration of the formalin killed vaccine A. hydrophila, and as shown in Fig. 1, antibody titre of intraperitoneally vaccinated fish reached its maximum level 4 weeks after vaccination, then it decreased gradually in fifth and sixth week post vaccination. SBL showed significant increase in plasma total immunoglobulin levels compared to RBL. For both lines (SBL and RBL); females tend to show higher levels of plasma total immunoglobulin than males. In the present study, females tend to show higher levels of plasma total immunoglobulin than males. However, Kortet et al. (2003) found that plasma immunoglobulin M (IgM) concentrations were marginally higher among males of cyprinid and roach.

In this study; the SBL produced an increase in IgM values than the RBL. It has been described that the antigen-specific Ig in fish reaches its highest level 3 - 4 weeks after initial immunization (Hung *et al.,* 1997). However, Cuesta *et al.* (2004) demonstrated that the production of IgM begins earlier (at 2 weeks) and lasts longer (up to 6 weeks). It is also worth noting that the observed changes in the total IgM levels were not concomitant with the effects produced by stimulants or stressors on other humoral immune responses, such as complement and lysozyme activities. Thus, this parameter might be considered one of the best candidates for determining the fish immune system status in certain fish-farm situations due to the non-invasive sampling protocol and because of the simplicity and reproducibility of the assay.

The present study was a preliminary attempt to find out the influence of genetic selection for growth of *O.niloticus* on its immune parameters and survival against *A. hydrophila* challenge. The relatively large phenotypic variation that existed indicated the prospects for increasing resistance to aeromoniasis appeared promising.

Generally, there are two important points that need further consideration to include disease resistance as one of the trait in the selective breeding programs of fish. First, it is not possible to develop stocks to protect from all forms of diseases with varied aetiologies as fish require different mechanisms to handle different types of infections. Second, during the selection program the pathogen may likely evolve to survive in the fish. From this angle, development of marker traits may be of help (Sahoo *et. al.*, 2004).

The observed differences in disease susceptibility between selected and non selected lines of *O.niloticus* are unquestionable. The results of the present study indicate the possibility of indirect selection for disease resistance in breeding programmes in which growth and size are the selected traits in *O.niloticus*. Tthis goes along with similar findings that have been reported for salmonids. (Standal and Gjerde, 1987), Atlantic chinook salmon (Beacham and Evelyn, 1992) and halibut (Imsland *et al.,* 2002).

The present study aimed to investigate and evaluate the effect of selection for growth and related traits of *O. niloticus* on disease resistance and immune responses, and to test the possibility of using some immune parameters as indirect selection criteria in selective breeding to enhance disease resistance of *O.niloticus*.

The results of the present study indicate the possibility of indirect selection for disease resistance in breeding programmes in which growth and size are the selected traits in *O.niloticus*, and the use of lysozyme activity as a selection criterion appears to be a promising candidate trait for indirect selection to improve survival rate of *O.niloticus*. According to the association between plasma lysozyme activity and survival rate, broodstock should be selected among

individuals and families with high plasma lysozyme activity.

Further work is required; with a larger sample size will have to focus on humoral nonspecific factors of resistance to be able to characterize the properties of the available resistant groups of fish.

It is also worth to mention that studies aimed at decreasing disease susceptibility in response to the stressful conditions of aquaculture practices will benefit from collaborative efforts of different fields of research such endocrinology (to characterize the hormonal control of stress response), immunology (to study the immune response), pathology and veterinary medicine (to analyze disease infections), physiology (to characterize stressors and their effect on the homeostasis of fish), and genetics (to explore genetic variance and the potential for applying selective breeding for these traits).

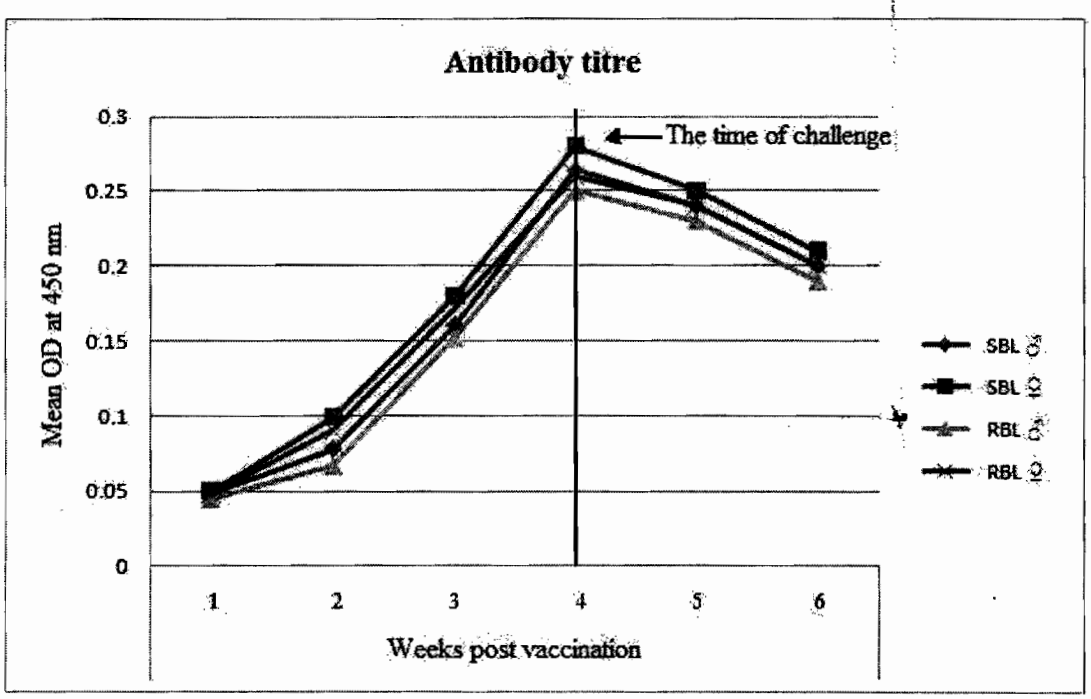

Fig. (1): Antibody titre of intraperitoneally vaccinated *O. niloticus* during the vaccination period and after challenge with *A. hydrophila* measured by ELISA.

In conclusion, the results of the present study reported the positive effect of selection for growth and related traits of *O. niloticus* (through eight generations of selection) on disease resistance and immune responses, which indicate the possibility of indirect selection for disease resistance in breeding programmes in which growth and size are the selected traits in *O.niloticus*, and the use of lysozyme activity as a selection criterion appears to be a promising candidate trait for indirect selection to improve survival rate of *O.niloticus*. According to the association between plasma lysozyme activity and survival rate, broodstock should be selected among individuals and families with high plasma lysozyme activity.

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تأثير الإنتخاب الوراثى للزيادة فى وزن الجسم عند الحصاد على مقاومة الأمراض والإستجابات المناعية لأسماك البلطى النيلى (أوريوكرومس نيلوتيكس)

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تم التحقق من التأثير المحتمل للإنتخاب الوراثى (للنمو والصفات المرتبطه به) فى أسماك البلطى النيلى (أوريوكرومس نيلوتيكس) على الإستجابات المناعية و مقاومة الأمراض بين سلالتين مختلفتين من الأسماك (سلالة منتخبة وراثياً ؛ متحصل عليها بعد ثمانية أجيال من الإنتخاب لصفة النمو والزيادة فى وزن الجسم، وأخرى غير منتخبة وراثياً)، بإجراء إختبار تحدى بإحداث العدوى من خلال الحقن فى الغشاء البريتيونى بميكروب (الإيروموناس هيدروفيلا).

قبل إختبار العدوى ؛ أظهرت مجموعة السلالة المنتخبة تحسناً ملحوظاً فى مدى الإعاشة وكذلك أبدت زيادات معنويه ($P< 0.05$) فى كل من وزن الجسم النهائى والزيادة الوزنية المكتسبة ومتوسط الزيادة الوزنية اليومية ومعدل النمو النوعى مقارنة بمجموعة السلالة غير المنتخبة، وبعد إختبار العدوى ؛ سجلت مجموعة السلالة الغير منتخبة إرتفاع معدل الوفيات مع إنخفاض مستوى الحماية النسبى ضد بكتيريا الإيروموناس هيدروفيلا مقارنة بمجموعة السلالة المنتخبة التى أظهرت بدورها زيادة معنوية ($P< 0.05$) فى مختلف مقاييس الدم المقدرة (العدد الكلى لخلايا الدم الحمراء والبيضاء (بجميع أنواعها)، محتوى الهيموجلوبين، نسبة مكداس الدم، تركيز الهيموجلوبين فى خلايا الدم الحمراء وكذلك متوسط تركيز الهيموجلوبين فى الخلايا) مقارنة بأفراد السلالة غير المنتخبة من أسماك البلطى، كما و أن الإناث قد أظهرت تفوقاً واضحاً على الذكور بزيادة معنوية ($P< 0.05$) فى مختلف المقاييس قبل وبعد العدوى (فى كلتا السلالتين)، وكذلك لوحظ إنخفاض معنوى ($P< 0.05$)فى تركيز البروتين الكلى، الألبيومين والجلوبيولين (بأنواعه)، نسبة الألبيومين / الجلوبيولين، ومستوى الجلوكوز فى بلازما الدم لمجموعة السلالة غير المنتخبة بالمقارنة مع مجموعة السلالة المنتخبة، بينما سجلت أفراد هذه المجموعة (غير المنتخبة) زيادة معنوية ($P< 0.05$) فى مستوى الكورتيزول فى بلازما الدم بالمقارنة مع المجموعة المنتخبة، وأظهرت مقاييس الإستجابة المناعية المختلفة (النشاط البلعمى لخلايا الدم البيضاء، إختبار النشاط التنفسى للخلايا (إختبار النيتروبلو تترزوليم)، بالإضافة إلى نشاط إنزيم الليسوزيم فى بلازما الدم) زيادة معنوية ($P< 0.05$) فى مجموعة السلالة المنتخبة بالمقارنة مع مجموعة السلالة غير المنتخبة من أسماك البلطى النيلى بعد إحداث العدوى بميكروب (الإيروموناس هيدروفيلا).

وقد كشفت النتائج المتحصل عليها عن وجود تأثير إيجابى للإنتخاب الوراثى (للنمو والصفات المرتبطه به) فى أسماك البلطى النيلى (أوريوكرومس نيلوتيكس) على الإستجابات المناعية و مقاومة الأمراض، مما يشير إلى إمكانية الإنتخاب الغير مباشر لصفة مقاومة الأمراض فى برامج التربية بالإنتخاب على أساس النمو والصفات المرتبطة به فى أسماك البلطى النيلى.