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Effects of hybridization between Nile tilapia (*Oreochromis niloticus*) and blue tilapia (*Oreochromis aureus*) on immune response to *Aeromonas hydrophila*

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Abstract

Resistance to *Aeromonas hydrophila* infections (mostly occur during environmental changes, stressors, change in the temperature, in contaminated environments), in three genotypes of tilapias, Nile tilapia (*Oreochromis niloticus*), blue tilapia (*Oreochromis aureus*) (males and females) as well as their crossbred hybrid (♂*O. aureus* x ♀*O. niloticus*) was studied. Based on comparison of non-specific immunity; the crossbred hybrid (♂*O. aureus x* ♀*O. niloticus*) showed the highest percentage of phagocytic activity and phagocytic index in comparison to purebred genotypes. Meanwhile, the purebred ♀*O. aureus* showed the lowest levels for the former non-specific immune parameters at the base line level and post vaccination with the *A. hydrophila* vaccine. Similarly, the crossbred hybrid (♂*O. aureus* x ♀*O. niloticus*) showed the highest antibody titers against *A. hydrophila* pathogen following booster vaccination and challenge infection by the virulent *A. hydrophila*. Meanwhile, the vaccinated mortality percentages and the relative level of protection (RLP %) were the lowest and the highest respectively for the crossbred hybrid (♂*O. aureus* x ♀*O. niloticus*). These results suggest that there is a potential for genetic improvement of disease resistance in aquatic animals since a specific variation exists in immunity against a specific pathogen and consequently, hybrid may be used to create a broad genetic base for future selection programs.

Keywords: Purebred, *Oreochromis niloticus*, *Oreochromis aureus*, inter-specific hybrid tilapia, *immune response, Aeromonas hydrophila*.

1. Introduction

Current methods to control infectious diseases consist of hygiene, vaccination, drug therapy and eradication of infected populations. Improving infectious disease resistance by genetic means is an attractive alternative because of its prospects for prolonged protection. Interspecific hybridization has been used in several fish species to increase genetic and phenotypic variation for both commercial and research purposes suggesting the possibility of such genetic improvement (Bartley *et al.*, 2000). Strain and species differences in disease resistance (e.g. Gleeson *et al.*, 2000*;* Shaw *et al.*, 2001*;* Overturf *et al.*, 2003) and stress response (Noga *et al*., 1999*;* Barton, 2000) were previously demonstrated in fish. In tilapia, differences were found between strains and species for several stress responses (Palti *et al*., 1999; Rezk *et al.*, 2002*;* Cnaani *et al.*, 2004), non-specific immunity (Balfry *et al.*, 1997; Palti *et al.*1999*;* Solis *et al.*, 2007) and disease resistance (Balfry *et al.*, 1997*;* Cai *et al.*, 2004). On the other hand, variable proportions of crossbreds showing heterosis for growth rate, survival, disease resistance and reproductive traits have been obtained in the cultured species for examples; channel catfish; rainbow trout; common carp and the Pacific oyster. Similarly, there are numerous papers in the literature about the performance of cultured tilapias, including diseases; particularly in the over wintering stage and some addressed the heterosis of hybrid tilapia (♂*O. aureus* x ♀*O. niloticus*) *(*Cai *et al.*, 2004*)*. However, the potential heterosis for disease resistance of the hybrid has not been addressed well yet. Therefore, the present study aimed to investigate which of the following three genotypes of tilapias; *O. niloticus* and *O. aureus* and their interspecific hybrid (♂*O. aureus* x ♀*O. niloticus*) has the greatest ability to respond immunologically to *A. hydrophila infection*. Percentage of Phagocytic activity (PA) phagocytic index (PI), antibody titers and relative level of protection were evaluated.

2. Material and methods

2.1. Test fish and experimental design

Three potentially important genotypes of tilapias: *O. niloticus* (both sexes) and *O. aureus* (both sexes) and their interspecific hybrid (♂*O. aureus* x ♀*O. niloticus*) were used in this study. Fish were sampled from a private fish farm in Behara governorate. For each group of fish four replicate aquaria of 5 fish, each used for both vaccine and control randomized in complete randomized design (CRD). The weights of *O. niloticus*, *O. aureus* and their interspecific hybrid (♂*O. aureus* x ♀*O. niloticus*) were 195.26±18.69g, 145.54±13.14g and 252±61.64g respectively. At the initiation of experiment blood was collected from six individual fish (pooled into three samples) for each fish group to provide control serum estimates of PA and PI.

2.2. Experimental condition

The experimental fish were reared in 100L rectangular aerated glass aquaria. Fish were fed with commercially prepared pellet feed every morning (9-10 AM) and afternoon (3-4 PM) at the rate of 1-2% of the body weight. Water qualities in rearing aquaria were maintained by everyday cleaning and water exchange. Daily water temperature was adjusted (23-26°C).

2.3. Vaccination Protocol

A locally isolated and identified pathogenic strain of *Aeromonas hydrophila*, kindly supplied by the Poultry and Fish Diseases Department, Faculty of Veterinary Medicine, Alexandria University, was used for bacterin preparation according to Sakai *et al.* (1984). Fish were acclimatized for one week before vaccination. Fish of each pure genotype (both sexes) and their hybrid were vaccinated via intraperitonea1 (i.p.) injection with 0.2 ml of respective vaccine containing 10^8 cells/ml bacterin (Formalin inactivated and adjuvant bacterial suspension) according to Badran, (1990). Two weeks later all experimental fish were booster injected with *Aeromonas hydrophila*. Blood samples were collected from all vaccinated fish groups (one week after each bacterin injection) for estimation of PA and PI.

2.4. Challenge experiment

All fish groups (vaccinated and non-vaccinated control) were artificially challenged (i.e. two weeks after receiving the booster dose) with i.p injection (0.2 ml of a suspension containing 9×10^7 bacteria/ml of pathogenic *A. hydrophila*). Clinical signs and mortality rate were recorded for one week. Specificity of death was determined by re-isolation of injected bacteria from freshly dead fish during the period of experiment. The relative level of protection (RLP) among the challenged fish was determined *(*Ruangroupan *et al.*, 1986*)* using the following equation:

RLP % =1 – (% of mortality in vaccinated groups/% of mortality in control group) x100

2.5. Blood collection

2.5.1. Detection of non-specific immune response

During the course of the current study, the phagocytic activity and phagocytic index were measured in blood samples taken pre- and one week post- vaccination *(*Kawahara *et al.,* 1991*).* In each occasion, blood samples were collected from 6 fish for each pure genotype (males and females) and 6 fish of their hybrid (♂*O. aureus* x ♀*O. niloticus*). Fish were fasted for 24 h prior to blood sampling; blood was collected with a hypodermic syringe from the caudal vein. The withdrawn blood samples were collected in Eppendorf tubes with anticoagulant (0.1 ml of 4% sodium citrate solution/1 ml blood) used for estimation of phagocytic activity and phagocytic index *(*Kawahara *et al,*. 1991*).*

2.5.2. Preparation of stained antigen and antibody titration

One week post second bacterin and pathogenic *A. hydrophila* injection. Six fish were taken from each treatment group for blood collection (three pooled samples) from the caudal vessel. Serum was then separated in aseptic condition and stored -20°C (Lucky, 1977) for further assay. Stained antigen of *A. hydrophila* was prepared (Collins *et al.,* 1976). Detection of humeral immune response (i.e. antibody titers) to *A. hydrophila* was evaluated by micro-agglutination (MA) test where the agglutination titers were expressed as Log_2 of the highest serum dilution still giving a clear agglutination (Badran, 1990).

2.6. Statistical analysis

Statistical analysis was performed using the one way analysis of variance (ANOVA) to fulfill the requirement of the statistical model

$X_{ijk} = \mu + T_i + R_j + e_{ijk}$

X_{ijk} = Observed value,

μ = Population mean

T_i = Effect of treatment I,

R_j = Effect of replicate j

e_{ijk} = Random error

Statistical analysis System SAS, 2002 was used.

3. Results

Levels of the innate immunity parameters observed in the current study are presented in Table (1). The PA and PI differed significantly ($P < 0.05$) among the different genotypes; *O. niloticus* (both sexes), *O. aureus* (both sexes), and their interspecific hybrid (♂*O. aureus* x ♀*O. niloticus*) at the base line level and post

vaccination. The hybrid (♂*O. aureus x* ♀*O. niloticus*) showed the highest levels for PA and PI in compare to the other purebred genotypes whereas, the purebred ♀*O. aureus* showed the lowest levels ($P > 0.05$) for PA and PI at the base line level and post vaccination with the *A. hydrophila* bacterin. Similarly, humeral immunological response of different tilapia genotypes vaccinated i.p by *A. hydrophila* bacterin was evaluated (Table 2). The antibody titers were significantly different ($P < 0.05$) among different genotype fish groups, where, the interspecific hybrid (♂*O. aureus* x ♀*O. niloticus*) showed the highest antibody titers against *A. hydrophila* pathogen following second bacterin injection and challenge infection by *A. hydrophila* virulent pathogen. Meanwhile, the vaccinated mortality% and the RLP% were the lowest and the highest respectively for the interspecific hybrid (♂*O. aureus* x ♀*O. niloticus*).

4. Discussion

Generally, (Bakos, 1987; Illyasove *et al.,* 1987) have indicated that hybrids have superior viability and disease resistance. As non-specific immune responses the PA % and the PI of different tilapia genotypes was compared. Phagocytic cells are the most important cellular components of the innate immune system of fish (MacArthur and Fletcher, 1985). Their phagocytic activity is a primordial defense mechanism (Neuman *et al.,* 2001) and an important characteristic of the non-specific immune system (Seeley *et al.,* 1990). In our experiment, the hybrid (♂*O. aureus* x ♀*O. niloticus*) had the highest levels for PA and PI in compare to its tilapia parental species before and after vaccination. A series of studies have been conducted on the resistance to disease caused by *Aeromonas* species based on nonspecific immunity functions, including strain and species variations. Cai *et al.* (2004), reported a significant difference between interspecific hybrid (♂*O. aureus* x ♀*O. niloticus*) and its tilapia parental species to *A. sobria*, based on nonspecific immunity functions. These results are also in agreement with the data put forward by Solis *et al.* (2007), who concluded that *O. niloticus* Rocky Mountain (cross of *O. niloticus* and *O. aureus*) had the best PI and PA than the other tilapia genotypes; *O. mossambicus*, *O. aureus*, *O. niloticus* Egypcia and the hybrid *O. niloticus* Stirling (cross of *O. niloticus* and *O. mossambicus*) studied. On the other hand, the non-specific immune responses of different clones of tilapia (inbred clones and their crosses) were tested using an array of immunological parameters (Sarder *et al.,* 2001). There was some evidence of heterosis in the form of dominance in the inheritance of some of the parameters which were studied. Inheritance appeared to be non-additive (i.e. not intermediate between the two parental clones), the phenotypic values for the crossbred offspring tended to be closer to that of their parental clones with the stronger immune response. However, none of the crossbred tilapia groups exceeded the highest parental value shown by the two parental lines for any of the parameters measured.

On the same manner, results of the humeral immune response strongly support the preceding results of the non-specific immune response parameters measured in the current study. The interspecific hybrid significantly had the highest antibody titers against *A. hydrophila* both after being vaccinated and infected by i.p *A. hydrophila* bacterin and a virulent *A. hydrophila* strain respectively. Meanwhile, the highest RLP% was observed in the challenged interspecific hybrid tilapia. These results supported the results obtained by Sarder *et al.* (2001), who reported that outbred clones, of tilapia, showed significantly higher resistance to *A. hydrophila* compared to their disease susceptible parental clones.

Accordingly, the differences found between the different genotypes of tilapia, in the non-specific immune response parameters measured and in the bacterial challenge, are strongly indicative of genetic variation. This particular response could be the result of a combination of the parental traits (Barriga-Sosa *et al.,* 2004), and may be in connection with the fact that it is more resistant to stressful conditions and tolerant to disease (Wang and Xia, 2002). Therefore, the higher disease resistance of hybrids may be a result of heterosis; however the mechanism needs further study.

5. Conclusion

Results of the current study strongly indicate the possibility for genetic improvement of disease resistance in aquatic animals since a specific variation exists in immunity against a specific pathogen and consequently, hybrid may be used to create a broad genetic base for future selection programs. However, the non-specific and specific defense reactions of fish against foreign substance pathogen in particular are influenced to a large extent by the environmental condition (e.g. temperature, nutrition). Therefore, future studies on genetic improvement of disease resistance are needed with respect to the genotype–environment interaction. It is also clear that further investigations will have to focus on humeral nonspecific factors of resistance with a larger sample size to be able to characterize the properties of the available aeromoniasis resistance groups of fish.

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Table 1. Phagocytic activity, phagocytic index, for Nile tilapia (*O. niloticus*), blue tilapia (*O. aureus*) and their hybrid (♂*O. aureus* x ♀*O. niloticus*) (Mean ± SD).

Item	♀ *O. niloticus*	♂ *O. niloticus*	♀ *O. aureus*	♂ *O. aureus*	Hybrid (♂ *O. aureus* x ♀ *O. niloticus)*
[1]*PA before vaccination*	14.33±1.53^{abb}	16.67±1.53^{b}	4.00±1.0^{c}	7.00±2.0^{c}	20.00±3.46^{a}
[2]*PI before vaccination*	7.00±2.0^{bc}	8.67±2.08^{b}	4.67±0.58^{c}	6.33±1.53^{bc}	11.67±1.53^{a}
[1]*PA week After 1st vaccination*	13.33±1.53^{bc}	15.67±1.53^{ab}	11.00±1.0^{c}	13.00±1.0^{bc}	17.34±2.08^{a}
[2]*PI week After 1st vaccination*	5.67±0.58^{b}	6.33±0.58^{b}	4.67±0.58^{b}	6.00±1.0^{b}	12.00±1.73^{a}
[1]*PA week After 2nd vaccination*	15.00±1.0^{c}	18.33±0.58^{b}	13.33±1.53^{d}	15.00±1.0^{c}	20.67±0.58^{a}
[2]*PI week After 2nd vaccination*	7.33±0.58^{c}	10.00±1.0^{b}	6.00±1.0^{c}	6.33±1.15^{c}	12.00±1.0^{a}

Means with different letters at the same row differ significantly at (p<0.05).
[1]Phagocytic activity%
[2]Phagocytic indix

Table 2. Antibody titers (log_2), mortality percentage and RLP% for Nile tilapia (*O. niloticus*), blue tilapia (*O. aureus*) and their hybrid (♂*O. aureus* x ♀*O. niloticus*) (Mean ± SD).

Item	♀ *O. niloticus*	♂ *O. niloticus*	♀ *O. aureus*	♂ *O. aureus*	*Hybrid (♂ O. aureus* x *♀ O. niloticus)*
1 week After 2nd vaccination	4.00±0.0^{bc}	4.33±0.58^{b}	3.00±0.0^{d}	3.33±0.58^{cd}	5.67±0.58^{a}
1 week After bacterial infection	3.67±0.58^{bc}	4.67±0.58^{b}	3.33±0.58^{c}	3.00±0.0^{c}	7.00±1.0^{a}
Control mortality % during 7 days post-challenge	90	80	90	80	70
Vaccinated mortality % during 7 days post-challenge	60	40	70	60	30
RLP%	33.3	50	22.22	25	57.14

Means with different letters at the same row differ significantly at (p<0.05).
NB. The antibody titers for the negative control group (Non-vaccinated and not challenged) is zero because it did not received neither bacterin nor virulent *A. hydrophila*.

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