

BIOCHEMICAL AND NON-SPECIFIC IMMUNE PARAMETERS OF HEALTHY NILE TILAPIA (*Oreochromis niloticus*), BLUE TILAPIA (*Oreochromis aureus*) AND THEIR INTERSPECIFIC HYBRID (MALE *O. aureus* × FEMALE *O. niloticus*) MAINTAINED IN SEMI-INTENSIVE CULTURE SYSTEM

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ABSTRACT: *Oreochromis niloticus*, *Oreochromis aureus* and their interspecific hybrid tilapia (♂ *O. aureus* × ♀ *O. niloticus*) maintained under semi-intensive culture system were compared in a preliminary study to explore the variations in blood biochemical and non-specific immunological parameters. Comparisons were performed after one week of acclimation ("base-line" level). Serum cholesterol, albumin, SGPT and SGOT level were significantly higher ($P < 0.05$) in the purebred *O. aureus* than the purebred *O. niloticus* and their crossbred hybrid. The tested genotypes showed insignificant difference ($P > 0.05$) in total protein, globulin and urea. Additionally, the levels of ALP and uric acid were significantly higher ($P < 0.05$) in both ♂ *O. aureus* and the crossbred hybrid (♂ *O. aureus* × ♀ *O. niloticus*). On the other hand, the level of creatinine was significantly higher in the purebred *O. niloticus* followed by the crossbred hybrid and then the purebred *O. aureus* but still without a significant difference ($P > 0.05$) between the latter two genotypes. The phagocytic activity and phagocytic index were significantly higher ($P < 0.05$) in the crossbred hybrid (♂ *O. aureus* × ♀ *O. niloticus*) than the other purebred genotypes. The differences identified suggest that hybrid families from the two species would be used to construct a segregating population for genetic analysis of immunological traits in tilapia. But still, a larger sample size obtained from populations cultured under different managemental practices should be used and challenged to learn if the differences are large enough to produce a segregating population for genetic analysis of immunological traits and disease resistance.

Key words: Purebred, *Oreochromis niloticus*, *Oreochromis aureus*, inter-specific hybrid tilapia normal blood biochemical reference, phagocytic activity, phagocytic index.

INTRODUCTION

Tilapia has become the shining star of aquaculture with farms starting and expanding across the globe while consumption races ahead of even the most ambitious farm building plans. In 2010, the world production of farmed tilapias reached 3.2 million metric tons (Fitzsimmons et al., 2011). In North Africa, Egypt is the second largest producer of tilapia after China (FAO, 2009). The current trend in tilapia farming in Egypt is towards increased intensification of culture systems. Increases in stocking densities can make fish more susceptible to stress and disease which in turn cause severe losses of Tilapia stock. Unfortunately, there are few diagnostic tools available to veterinarians and fish health professionals to evaluate disease in fish. Many of the clinical tools used to evaluate mammalian health are not developed for use in fishes. As the aquaculture industry expands, there is an increasing need for improved diagnostic methods. Hematology and clinical chemistry analysis, although not used regularly in fish medicine, can provide substantial diagnostic information once reference values are established. Although tilapia are the second most frequently cultured fish in the world, there are surprisingly few reports of normal blood values. (Terao and Ogaw, 1984; Palti et al., 1999; Bittencourt et al., 2003; Cnaani et al., 2004; Chen et al., 2003; Mauel et al., 2007). Therefore; established species-specific normal reference values are necessary. Accordingly the

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present study aimed to determine some biochemical parameters and non-specific immune response in three genotypes of tilapia; *O. niloticus* and *O. aureus* and their interspecific hybrid ($\text{♀ } O. niloticus \times \text{♂ } O. aureus$) cultured under semi-intensive pond culture.

MATERIALS AND METHODS

Fish sampling

O. niloticus and *O. aureus* and their hybrid ($\text{♀ } O. niloticus \times \text{♂ } O. aureus$) fry were produced in early September 2008 from a mass spawning of brooders in earthen spawning ponds (Phelps and Popma 2000) in a private fish farm in Behara governorate. They were allowed to grow in deep nursery ponds throughout their nursery and winter period. Thereafter; the fingerlings of each genotype were allowed to grow (two growout ponds for each genotype) through the growing season (April-October 2009). During the course of their growout period sixty apparently healthy fish (170 ± 15 g/fish) were randomly selected from the purebred species *O. niloticus* (n=20) and *O. aureus* (n=20) and 20 fish of their interspecific hybrid ($\text{♀ } O. niloticus \times \text{♂ } O. aureus$). The experimental fish were reared in 100L rectangular glass aquaria supplied with continuous flow of water. Fish were fed once a day at a feeding rate 3% of their body weight till the end of the experiment. Daily water temperature was recorded (23-25°C).

Blood analysis and immunological parameters

Each of the biochemical and immunological parameters recorded in this experiment was measured in blood samples taken after one week of acclimation period. Blood samples (n=20) were collected from each pure genotype and their crossbred hybrid. 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 pooled to obtain 10 samples for each genotype and divided in two sets of Eppendorf tubes. The first set (five pooled samples for each pure genotype and their hybrid) 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). The second set were left to clot at 4 °C and centrifuged at 3000 rpm for 15 minutes at room temperature. The collected serum used for determination of total protein (Dumas, 1994), albumin (Reinhold, 1988) using commercial kits produced by Pasteur Lab. Globulin was calculated by subtracting the albumin value from the total protein value of the same sample (Coles, 1998). Albumin/Globulin ratio (A/G) was calculated. Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were estimated according to Anderson and Chalman (1999) using commercial kits produced by Pasteur lab. Serum alkaline phosphatase (ALP) was estimated according to the modified method of Lied et al., (1989) using commercial kits produced by bioMerieux lab. Kidney function was monitored by estimation of some parameters including serum urea and serum creatinine (Crouch, 1997), serum uric acid (Fossatti and Prencipe, 1990). Serum total cholesterol was determined according to Allain (1998) using kits of Quimica Cinica Aplicada S.A. (QCA).

Statistical analysis

One-way analysis of variance (ANOVA) was used (Statistical analysis System SAS (SAS Institute Cary, North Carolina, USA, 2004) 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

RESULTS

Measurements of serum components taken in the current study for purebred *O. niloticus*, *O. aureus* and their hybrid ($\text{♂ } O. aureus \times \text{♀ } O. niloticus$) at the base-line level are presented in Table 1. Serum cholesterol, albumin, SGPT and SGOT level were significantly higher ($P < 0.05$) in the purebred *O. aureus* than the purebred *O. niloticus* and their crossbred hybrid. The tested genotypes showed insignificant difference ($P > 0.05$) in total protein, globulin and urea. Additionally, the levels of ALP and uric acid were significantly higher ($P < 0.05$) in both $\text{♂ } O. aureus$ and the crossbred hybrid ($\text{♂ } O. aureus \times \text{♀ } O. niloticus$). On the other hand, the level of creatinine was significantly higher in the purebred *O. niloticus* followed by the crossbred hybrid and then the purebred *O. aureus* but still without a significant difference ($P > 0.05$) between the latter two genotypes. Levels of the innate immunity parameters at base line level recorded in the current study are presented in Table 2. The phagocytic activity and the phagocytic index were significantly higher ($P < 0.05$) in the crossbred hybrid ($\text{♂ } O. aureus \times \text{♀ } O. niloticus$) than the other purebred genotypes.

DISCUSSION

Blood parameters analyses have proven to be valuable tools to analyze the health status of farmed fish as these indices provide reliable information on metabolic disorders, deficiencies and chronic stress status before clinical symptoms appear (Bahmani et al., 2001). Although tilapia are the second most frequently cultured fish in the world, there are surprisingly few reports of normal blood values. (Terao and Ogaw, 1984; Palti et al., 1999;



Bittencourt et al., 2003; Chen et al., 2003; Mauel et al., 2007). Compared with previously reported blood biochemical values for healthy tilapia, our results were almost similar or varied for most analytes. Yavuzcan Yildiz et al., (1997) reported blood chemistry in 25 small (52 g) tilapia *O. niloticus* and showed higher total protein and albumin (4.60 and 2.96 g/dl) than those measured in our study. Hussein et al., (1996) reported another study on *O. niloticus* (average weight 38.46 g), total protein and albumin (3.40 and 0.67 g/dl), cholesterol (161.3 mg/dl). On the other hand, Chen et al. (2003) identified the blood chemistry in 120 healthy *O. niloticus* (393.2±117g) through a year and showed higher means for Cholesterol (251.9g/dl), globulin (2.67g/dl) and lower albumin (1.32 g/dl). In those studies, the sizes of fish were either smaller or larger than those in our study which in turn could explain the differences in the results.

Table 1 - Means (±SD) measurements of biochemical serum components taken from Nile tilapia (*O. niloticus*), blue tilapia (*O. aureus*) and their hybrid (♀ *O. niloticus* x ♂ *O. aureus*)

Item	<i>O. niloticus</i>	<i>O. aureus</i>	Hybrid (♂ <i>O. aureus</i> x ♀ <i>O. niloticus</i>)
Cholesterol (mg/dL)	122.5±26.45 ^b	151.4±45.70 ^a	110.0±10.93 ^b
Total protein (g/dL)	3.35±0.47 ^a	3.63±0.54 ^a	4.02±0.64 ^a
Albumin (g/dL)	1.54±0.30 ^b	1.85±0.14 ^a	1.77±0.27 ^a
Globulin (g/dL)	1.82±0.33 ^a	1.78±0.58 ^a	1.81±0.41 ^a
Albumin/Globulin ratio	0.87±0.21 ^a	1.21±0.59 ^a	1.02±0.21 ^a
¹ SGPT (U/L)	5.60±2.07 ^b	13.2±5.79 ^a	2.75±0.78 ^b
² SGOT (U/L)	64.1±18.9 ^b	175.00±66.5 ^a	73.25±14.55 ^b
Alkaline Phosphatase (U/L)	5.59±1.53 ^b	6.45±0.77 ^{ab}	7.10±0.76 ^a
Urea (mg/dL)	6.10±1.50 ^a	6.80±1.23 ^a	6.20±1.23 ^a
Uric acid(mg/dL)	2.96±0.33 ^b	4.27±1.42 ^a	3.39±0.16 ^b
Creatinine (mg/dL)	0.32±0.22 ^a	0.20±0.07 ^b	0.27±0.02 ^{ab}

Means with different letters at the same row differ significantly at (p<0.05); ¹Glutamic pyruvic transaminase; ²Glutamic oxaloacetic transaminase

Table 2 - Means (±SD) measurements of phagocytic activity% and phagocytic index taken from Nile tilapia (*O. niloticus*), blue tilapia (*O. aureus*) and their hybrid (♀ *O. niloticus* x ♂ *O. aureus*)

Genotype	Phagocytic activity%	Phagocytic Index
<i>O. niloticus</i>	15.5±1.87 ^b	7.83±2.04 ^b
<i>O. aureus</i>	5.5±2.1 ^c	5.5±1.38 ^c
Hybrid (♂ <i>O. aureus</i> x ♀ <i>O. niloticus</i>)	20.0±1.79 ^a	12.0±0.89 ^a

Means with different superscripts at the same column differ significantly at (p<0.05)

On the same manner Palti et al. (1999) reported higher means for Cholesterol (267/dl), total protein (4.5g/dl), albumin (2.2g/dl), globulin (2.2g/dl) and ALP (35U/l) in *O. aureus* than those observed in the current study. Similarly, Hrubec et al. (2000) and Mauel et al. (2007) reported higher values of blood chemistry for hybrid tilapia (*Oreochromis niloticus* x *O. mossambicus* x *O. aureus* hybrids) and (*Oreochromis niloticus* x *O. aureus* hybrids), raised in a high-density aquaculture setting. These deviations could reflect the fact that some parameters could be affected significantly by culture conditions. Ammonia, nitrite, culture density and the culture systems could influence the values obtained (Hrubec et al., 1996, 1997).

Total cholesterol level, which differed significantly among different genotype in the current study, was found to be associated with disease resistance in fish (Maita et al., 1998). Meanwhile, high levels of serum protein, albumin and globulin are thought to be associated with strong innate response in fish (Wiegertjes et al., 1996). Palti et al. (1999) identified significant differences between *O. aureus* and *O. mossambicus* in serum total protein, albumin and globulin. Similarly, the same pattern was observed, with higher values in *O. aureus* than in *O. mossambicus*, *O. niloticus* (wild strain) and *O. niloticus* (red strain) Cnaani et al. (2004). However, the current study identified a significant increase only in the serum albumen content of *O. aureus*. Biochemical differences were also identified in levels of SGPT, SGOT, ALP, uric acid and creatinine. The immunological significance of those differences is currently unknown.

On the other hand, the phagocytic activity and the phagocytic index were significantly higher in the crossbred hybrid (♂*O. aureus* x ♀*O. niloticus*) in compare to its tilapia parental species. These results are 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 phagocytic activity and the phagocytic index than the other tilapia genotypes; *O. mossambicus*, *O. aureus*, *O. niloticus* EGYPTIA and the hybrid *O. niloticus* Stirling (cross of *O. niloticus* and *O. mossambicus*) studied. Phagocytosis is a fundamental and generally efficient mechanism within the innate immune response that provides the host with a continuous surveillance against foreign invaders and is ultimately responsible for the destruction of the phagocytized pathogens (Silva et al., 2002).



CONCLUSION

It is known that the normal values of blood components have genetic and physiological variations. The genetic variation may be due to interspecific factors between species and intraspecific within species. The physiological variations may be caused by age, sex and nutritional aspects. In this study, three tilapia genotypes were of the same age and were sampled from the same culture environment. Therefore, the observed variations of serum biochemical components may reflect the genetic variations in nature (Sifa et al., 2000). In this study we identified significant differences in two parameters of non-specific immunity between two tilapia species and their hybrid. The differences identified between *O. niloticus*, *O. aureus* and their hybrid and those identified in previous studies (Cnaani et al., 2004; Mauel et al., 2007) suggest that hybrid families from the two species may be used to construct a segregating population for genetic analysis of immunological traits and stress response. Further research is needed to determine if the immunological differences are associated with variation in disease resistance.

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