Kafrelsheikh University

Faculty of Veterinary Medicine

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Effect of Seasonal Variation on Expression of Immune Response Related Genes in Fish Hatcheries

A thesis submitted by

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The experiments of this study were performed in fish diseases unit in Animal Health Research Institute (kafrelsheikh) and the Genetic analysis was performed in the department of Animal Wealth Development, Faculty of Veterinary Medicine, Kafrelsheikh University and in Genetics Laboratory of Animal Husbandry and Animal Wealth Development Department, Faculty of Veterinary Medicine, Alexandria University and in the Biotechnology Laboratory in Faculty of Agriculture, Kafrelsheikh University. While the morphometric parameters were performed in the Pathology Department, Faculty of Veterinary medicine, Kafrelsheikh University. This study was conducted from March 2018 till May 2019.

All the larvae samples used in this study were collected from one of the private Nile Tilapia fish Hatcheries in kafrelsheikh governorate in three times in March, August and October covering the beginning, middle and the end of the hatching season in 2018.

The objectives of this study were:

1. Evaluate the immune status of Nile tilapia larval stage at the beginning, middle and at the end of hatching season, This will achieved by:
   A. Morphometrically assessment of larvae parameters including yolk sac diameter, body length and width in different groups representing the beginning, the middle and the last period of the hatching season (March, August and October) respectively.
   B. Detecting the variation in the expression levels of some immune response related genes including *RAG1, SACS, VTg, TLR7, IL-1B, IL8, , HSP27* and *HSP70* in the previously mentioned groups.

2. Study the effect of immune stimulants as a feed additives particularly B. glucan, Vitamin C, and methionine /lysine amino acids on the expression of the previously mentioned immune response genes at two different temperatures (23±1°C and 30±1°C).

To achieve these objectives we used the following methodology:

1. Nile tilapia (*Oreochromis niloticus*) one day old yolk sac larvae, with an average weight of 0.01 g, were collected from a commercial fish hatchery, Kafrelsheikh, Egypt. About 2200 fries were collected in each time for three times in March (Water Temp. 23±1°C), August (Water Temp. 30± 1°C) and finally in late October (Water Temp. 20 ±1°C).
2. From each group of the three collected groups (Group 1, Group 2 and Group 3) of Nile tilapia yolk sac fries, 100 larvae were distributed equally in 10 eppendorf tubes and maintained in liquid nitrogen and transferred to -80 ºc for genetic analysis.
3. Another 100 yolk sac larvae were put in a 10 percentage neutral buffered formalin for further histomorphological examination by using special program (ImageJ analysis foftware).
4. The first experiment: One day old 2000 Larvae that collected in March 2018 were stocked in four glass tanks (500 larvae in each tank) and the temperature maintained at 23±1°C. The larvae were left five days for acclimatization and yolk sac absorption. The first group Fish received basal commercial ration 40% protein. The second group received ration containing B. glucan 0.1%, the third group received ration containing Vitamin C 100% and the fourth group received diet containing amino acid mixture (methionine and lysine). The experiment continued for 21 days then the samples from each group were taken for genetic analysis.
5. The second experiment: One day old 2000 Larvae that collected in august 2018 were stocked in four glass tanks (500 larvae in each tank) and the temperature maintained at 30±1°C. The experiment continue by using the previously mentioned feed additives for 21 days then the samples from each group were collected for genetic analysis.

6. Three individual Larvae /tank were used for RNA isolated then the isolated RNA, cDNA was synthesized and quantitative real time PCR were done to detect the expression levels of the target genes.

The summary of the obtained results

1. Combined analysis of one-day yolk sac larvae measurement and its histomorphology at March, August and October revealed that yolk sac size diameter significantly larger in March than its size during August and October, it is almost double their volume. Also it has opaque contents compared with the October yolk sac which contain large vacuole.
2. Larvae length is also significantly larger at March, The least Larvae height was significantly recorded at October compared with March and August.
3. Expression analysis of one day yolk sac larvae revealed that:
   - ❖ The expression level of *RAG* gene is not affected by thermal variation along the three hatching season intervals, only a mild increase in their expression to almost equal levels at the three studied intervals. Also, mild increase of *Vtg* was observed in October only, and a significant up-regulation of *SACs* gene was observed in October compared with its level in March and August.
   - ❖ The expression pattern of *TLR7*, *HSP27*, *HSP70* and the inflammatory related genes (*IL1B* and *IL8*) were greatly influenced by thermal variation along the studied intervals. They are significantly increased higher in October than in March and their expression nears the control level (1 fold) at August except *HSP27* and *IL8.*
   - ❖ A significant up-regulation in the expression pattern of *HSPs*, immune and inflammatory related genes and the inflammatory related were significantly increased higher in October than in March and their expression nears the control level in August for the *TLR7*, *HSP70* and *IL8*.

4. Effect of different immunostimulants on Nile tilapia larvae at 23°C and 30°C

   - ❖ Mortality percentage: the total mortality is significantly affected by the temperature variants, different treatment and their interaction. The group received the β-glucan has the highest mortality at both temperature variants, The group received the amino acid mix has the least mortality at the two studied group, followed by the Vitamin C treated group, then the control group.
   - ❖ Gene expression of *RAG1, SACS* and *Vtg* did not show any significant difference in their gene expression by any feed additive, at two temperature variants or by their interaction
   - ❖ Similar pattern were observed for the expression of *HSP70* and inflammatory related genes (*IL-1b and IL8*), at different temperatures and treatments with significant up-regulatory effect of amino acid and the least effect for the β-glucan. *HSP70* at different temperatures, treatments and their interaction.
   - ❖ No significant differences in the expression of *HSP27* gene by the different treatments, however significant differences were observed by variant temperatures. For *TLR7*, only a significant effect for treatments was observed with the highest level of expression by amino acid compared with other treatments.

Conclusion:

The immune status of Nile tilapia is affected by thermal fluctuation throughout the hatching season reflected by altered yolk sac size, length, and expression of the immune and stress related genes of the larvae and with the best performances at the beginning of the hatching season (in March). High temperature impair cellular and immune response and increased mortality in fish larvae suggesting narrow thermal tolerance range for the larvae compared with the adult fish. Amino acid mix is recommend as immunostimulant for Nile tilapia larvae, it reduce the mortality

% and improve cellular response, also the use of β-glucan should be prohibited during this developmental stage of larvae.