THE DEVELOPMENT OF THE TAIL KIDNEY AND TESTIS OF STARVED GENETICALLY MALE (GMT) NILE TILAPIA, *Oreochromis niloticus* L.

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Abstract

Oreochromis niloticus L. (Nile tilapia) was delayed-fed and starved to determine its effect on the tail kidney and testis. After yolk absorption, the fish was starved for 2 (group 2), 4 (group3), 6 (group4), 8 (group 5) days while the fishes in the control (group 1) were fed immediately after yolk absorption, for comparative analysis. Ten fishes on the 2nd, 4th, 8th, and 30th day of the resumption of feeding were processed using the paraffin technique, and the tail kidney and testis were then examined. Based on the results, delaying the onset of feeding caused growth and developmental retardation in both organs. Severe retardation of both organs was mostly observed in group 5, wherein the fishes were starved for the longest duration (8 days). However, some stages elicited growth, which was independent of the groups. There could have been some mechanisms used by the organism at certain life stages, in which optimal allocation of nutrients was done, which reduces the effect of delayed feeding, and thus allowing some considerable growth.

Introduction

The Nile tilapia, *Oreochromis niloticus* L., is one of the most-cultured fish in the Philippines, mainly due to its economic importance. Worldwide harvest of farmed tilapia has now surpassed 800,000 metric tons, and tilapia is second only to carps as the most widely farmed freshwater fish in the world. This fish can easily adapt to virtually any freshwater environment, from ponds to lakes, to even swamps, marshes and brackish waters. It even has the ability to use various food sources and readily takes prepared feeds from fry to adult size. More importantly, *O. niloticus*' ability to reproduce and breed easily makes it very economical to grow. Sometimes, however, this ability becomes more of a liability than an asset. Their ability to spawn repeatedly causes overcrowding in ponds, and eventually causes their growth to become stunted.

O. niloticus takes care of its egg by holding or storing them in the mouth. In mouthbrooding fishes, eggs are incubated and hatched inside the mouth of the mother and the hatched fry are only released when they have absorb the reserved yolk and are able to swim.

However, in some circumstances, such as the danger of predation, the mother would instinctively delay the release of her broods to safeguard them from being eaten. While this practice may be effective in ensuring the survival of fry, it also results in the delay of first feeding or the inability to feed efficiently.

Feeding is one of the most important functions of an organism. The basic functions of an organism – its growth, development, and reproduction – all take place at the expense of energy, which enters the organism in the form of its food. The first stage in the life cycle of a fish, like *O. niloticus*, is completed at the expense of the food reserves, which it receives from the maternal organisms. This makes delayed first feeding a compromising strategy, especially for the young. Feeding delays can likewise reduce fry survival. Death becomes inevitable for those unlucky fry, but for those who survive such ordeal, certain changes are likely to be observed.

However, studies found out that delaying the onset of feeding could be a practical, economical, and cost-efficient strategy for fish farmers. Analyses, based on trials at seven participating commercial farms in the Philippines, showed that delayed feeding led to reductions in feed costs amounting to approximately 37% without a significant loss of crop value at harvest. Though there may seem to be a lot at stake, especially for the fish, fish farmers still practice this strategy mainly because of its ability of increasing profitability (Brown *et al*., 2000; Bolivar and Brown, 2003).

Changes in the organs and organ systems of the fish due to delaying the onset of feeding could cause detrimental effects on the fish's growth and survival. Reproduction and osmoregulation are some life processes, which could be affected by delayed feeding. The ability to reproduce, as well as to osmoregulate, can directly affect the fish's growth and survival, making the study on the testis and kidney important in the reproductive success, as well as maintenance of homeostasis, of the fish.

The objective of the study is to determine if delaying the onset of first feeding will have an effect on the organism's tail kidney and testis. The study aims to determine the occurrence of any significant morphological, histological, and pathological differences due to delayed first feeding.

Materials and methods

Collection and breeding of experimental fishes

Brood eggs of different paternal sources were collected from females (XX) crossed to supermales (YY) and incubated separately until hatched at the Freshwater Aquaculture Center, Central Luzon State University (CLSU) in Munoz, Nueva Ecija.. The hatched fry were carefully monitored to determine when they have absorbed their reserved yolk and are ready to eat exogenous food. The broods, which have at least a thousand fry, were selected to be used for the experiments.

After yolk absorption, the average weight of the fry was determined and each group were stocked separately in plastic container with water and provided with aeration. When a group was scheduled to be fed, the fry were then stocked in a 1-m^3 fine mesh installed in an earthen pond previously fertilized to enhance the growth of natural food. In addition, supplemental food using commercial fry mash was also given following actual hatchery practice. The fry was reared for about 30 days.

Experimental groups

Three replicates of the brood were done. Each brood was divided into 5 groups, one group for each group, consisting of 200 fry. After yolk absorption, the groups were given their first food at different times as follows: Group 1: fishes were fed immediately after yolk absorption (control); Group 2: fishes were starved for 2 days before the resumption of feeding; Group 3: fishes were starved for 4 days before the resumption of feeding; Group 4: fishes were starved for 6 days before the resumption of feeding; and Group 5: fishes were starved for 8 days before the resumption of feeding.

Histological and comparative analyses of the different groups

Ten fry on the 2^{nd}, 4^{th}, 8^{th}, and 30^{th} day of the resumption of feeding were processed using the paraffin technique; the fry's tail kidney and testis were then examined. Histological study on the development of both organs was traced. Photomicrographs were taken on the tail kidney and testis. Measurements of organs were also taken and were used for comparative analysis. Appropriate statistical tools were likewise used.

Paraffin technique

The fry were preserved as whole fish. But in bigger fishes, internal organs were dissected out and cut into 0.5 cm thickness before fixation, and were fixed in 10% formalin and Bouin's solution. To neutralize acidity, tissues were soaked in 5% Na_2SO_4 for 4 hours, which was then rinsed in tap water for 5 to 10 minutes.

Dehydration was done in a series of ethyl alcohol concentrations, followed by clearing in xylene and infiltration and embedding in paraffin wax. Blocks were sectioned at 6 μm, stained with hematoxylin and eosin, and mounted on entellan.

Results

Tail kidney

Histology of the tail kidney on the 2^{nd} day of the resumption of feeding

Tail kidneys of the experimental groups were larger than the control. The renal duct was the only structure observed in both control and experimental groups (Figs. 1-2). The average renal duct diameters of the groups with fishes starved for 2 days (Group 2), 6 days (Group 4), and 8 days (Group 5) were smaller than the control. However, the group with fishes starved for 4 days (Group 3) was larger than the control.

Histology of the tail kidney on the 4th day of the resumption of feeding

Tail kidney width of the control was smaller compared to groups 2, 3, & 4. Group 5 showed the smallest kidney width.

The renal duct and glomerulus were observed in both the control and the experimental groups (Figs. 3-4). The control had the largest renal duct and glomerulus diameter, and a larger renal tubule compared to groups 3, 4 & 5.

Starting at this stage, numerous renal tubules were seen in the kidney of both the control and the experimental groups (Figs. 3-4). ANOVA indicated a significant difference between the renal tubule diameter of the control and the experimental groups. Tukey's range test divided the experimental groups into 2 subsets: subset 1 consisting of groups 3, 4, & 5, and subset 2 consisting of the control and groups 2, 3, & 4. Based on the division of the subsets, the significant difference was prevalent between the control and group 5.

Histology of the tail kidney on the 8th day of the resumption of feeding

Tail kidney width of the control was the largest among all the groups. The tail kidney of group 4 had the smallest kidney width.

The renal duct, renal tubule and glomerulus were observed in both the control and the experimental groups. The renal duct diameter of the control was larger compared to groups 2, 3, & 5, with the exception of group 4, which showed the largest renal duct diameter. The glomerulus of group 4 showed the smallest diameter. ANOVA indicated a significant difference with the renal tubule diameter of the control and the experimental groups. The renal tubule diameter of the control was larger compared to both groups 2 and 5, but was smaller compared to both groups 3 and 4. Group 5 had the smallest renal tubule.

Histology of the tail kidney on the 30th day of the resumption of feeding

Tail kidney width of the control was the largest among all the groups. The tail kidney of group 5 had the smallest kidney width.

The renal duct, renal tubule and glomerulus were observed between the control and the experimental groups. However, no growth difference was observed in the diameter of both renal ducts and glomerulus. ANOVA indicated a significant difference with the renal tubule diameter of the control and the experimental groups. Tukey's range test divided the experimental groups into 2 subsets: subset 1 consisting of groups 2, 4, & 5, and subset 2 consisting of the control and groups 1, 2, 3, & 4. Based on the division of the subset, the significant difference was prevalent between the control and group 5. The control has a larger renal tubule compared to that of group 5.

Testis

Histology of the testis on the 2nd day of the resumption of feeding

The testes of the control and groups 2 & 5 were flat and elongated, while the testes of groups 3 & 4 were bean-shaped and rounded.

PGCs and somatic cells in the testis were still few in number in this stage. The PGCs are distinguishable from somatic cells by their bigger size, larger nuclei, with round to oval contour, and light cytoplasm.

Histology of the testis on the 4th day of the resumption of feeding

The testes were bean-shaped. The testes increased in size in groups 1 to 4. However, group 5 showed a flat and elongated testis.

PGCs and somatic cells were more numerous and larger in this stage, than in the 2nd day of the resumption of feeding.

Histology of the testis on the 8th day of the resumption of feeding

The testes were bean-shaped. The testis of the control was the largest. All the experimental groups had smaller testes compared to the control, with group 4 having the smallest testis diameter.

PGCs and somatic cells were numerous, especially in the control.

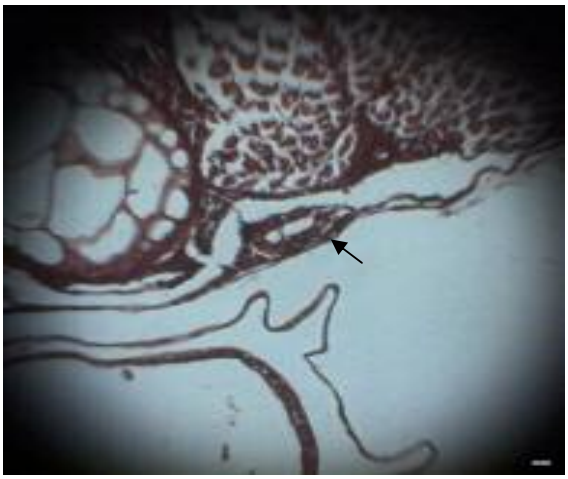

Figure 1. The tail or posterior kidney of the control group on the 2nd day of the resumption of feeding. The renal duct is the only kidney structure visible. **Black Arrow** – Renal Duct. Bar: 10 µm.

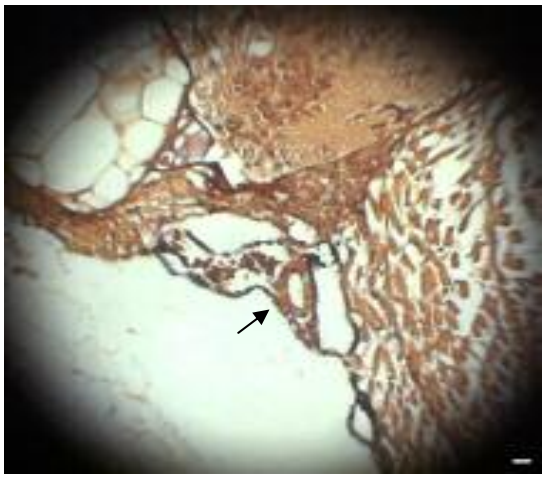

Figure 2. The tail or posterior kidney of the fish starved for 8 days on the 2nd day of the resumption of feeding. The renal duct is the only kidney structure visible. **Black Arrow** – Renal Duct. Bar: 10 µm.

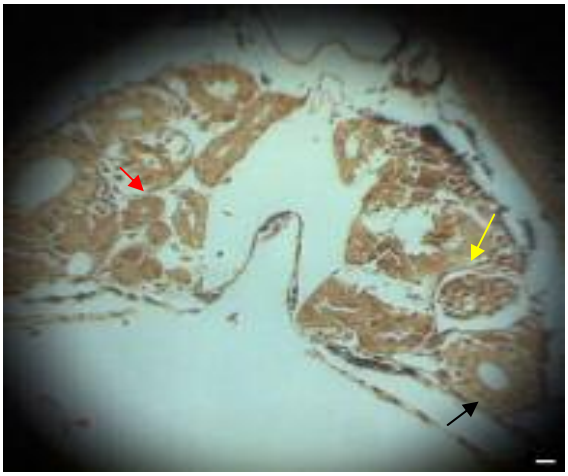

Figure 3. The tail or posterior kidney of the control group on the 4th day of the resumption of feeding. The renal ducts, renal tubules, and glomerulus are now visible. **Black Arrow** – Renal Duct; **Red Arrow** – Renal Tubule; **Yellow Arrow** – Glomerulus. Bar: 10 µm.

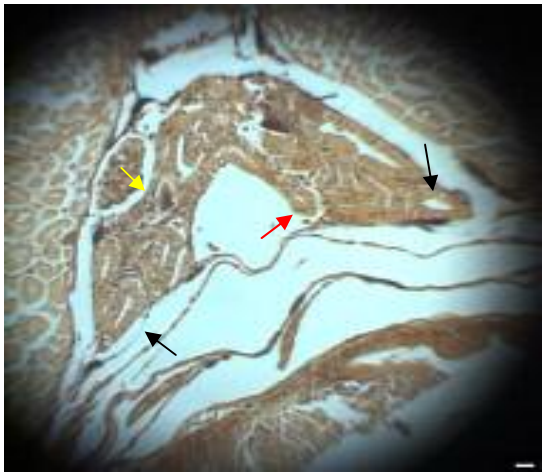

Figure 4. The tail or posterior kidney of the fish starved for 8 days on the 4th day of the resumption of feeding. The renal ducts, renal tubules, and glomerulus are now visible. **Black Arrow** – Renal Duct; **Red Arrow** – Renal Tubule; **Yellow Arrow** – Glomerulus. Bar: 10 µm.

Discussion

The tail kidney and testis of the experimental groups exhibited growth and developmental retardation in most of the stages. It was observed that the control had relatively the same or bigger size of both tail kidney and testis compared to that of the experimental groups. Severe developmental retardation was mostly observed in group 5, wherein the fishes were starved for the longest duration (8 days). The glomerulus and renal ducts in the kidney also showed developmental retardation in some stages. These results are similar with those of Bisbal and Bengston (1995), who found out in their study that growth were strongly dependent on delay of the initial feeding. This was also observed by Amornsakun (2000), who in his study, stated that that larval red-tail catfish should be fed with *Moina* within 3 days after hatching to ensure better survival and growth. Šantiæ *et al*. (1994) found out that sea bass feeding with *Artemia* nauplii may be delayed for 72 hours before producing no harmful consequences in the later development. Also in the study of Ganzonnaret and Fermin (1994) on larval sea bass *Lates calcanter*, growth was adversely affected by delayed feeding that resulted in slower larval growth.

The retardation in growth and development is attributed mainly on delayed feeding and starvation. It is known that delayed feeding and starvation leads to nutrient deprivation. And as nutrient deprivation continues, the animal would reach a point wherein it has exhausted all its reserve stores of nutrients. After all the reserves have been exhausted, the animal will eventually reach a point where the cells of the body are unable to perform the functions necessary for life, such as growth and development. Death results from lack of sufficient blood glucose to provide the energy needs of the brain and hypoglycemic shock occurs (www.michigan.gov).

Before death occurs, several symptoms of starvation are manifested, such a decrease in feeding success, decrease in food-seeking movements, lowered swimming speed, increased of rest stops, and emaciation of the body (Gerking. 1994.). In extreme cases, organisms, especially larval organisms, after suffering from starvation for a very long time, reach a point where they are still alive but are too weak to feed if food becomes available. This was termed as the "point-of-no-return", a term coined by Blaxter and Hempel (1963) in their work with herrings (Bone *et al*., 1995; Blaxter & Hempel, 1963 as cited by Gerking, 1994).

Pathological changes which occur in a starved animal are many and varied. The most striking gross change is a lack of fat in the subcutaneous, visceral, and bone marrow locations, and atrophic changes which occur in the musculature. Serous atrophy, a reddish gelatinous appearance to the fat tissue, is commonly seen in starving animals. The organs of the body also decrease in size and weight. Dibner and Knight (1998), in their study on the growth of three organs (small intestines, liver, and pancreas) of poultry birds, found out that the pancreas was the most negatively affected of the supply organs by delayed feeding. The severity and timing of the restricted diet plays a major role in the future production of starved organisms.

However, in some stages, such as the 2nd day of resumption of feeding for the tail kidney, and 4th day of the resumption of feeding for the testis, growth, rather than retardation, was observed. The size of both organs in the control was smaller compared to that of the experimental groups. The increase in growth may be due to some possible adaptations to delayed feeding, such as increased storage of nutrients in the form of fat. This optimizes the allocation of materials in the body, thus allowing considerable growth in the earlier stages. Thus the growth exhibited during these stages is independent of delayed feeding.

Although it was determined that delayed feeding could lead to growth and developmental retardation of the organs, the mechanism at which growth retardation occurred with respect to the duration of starvation was not very well-understood. The author recommends further study on this area to determine any correlation between the level of growth retardation and duration of starvation. And since growth retardation occurred only in some organs and structures, the author recommends further study on the effect of delayed feeding on other organs and organ systems. In addition, the author also recommends the application of better-refined histopathological techniques that could help improve in the study of the organs, as well as the structures within.

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