THE DEVELOPMENT OF INTEGUMENTARY AND SKELETAL SYSTEMS OF STARVED NILE TILAPIA, *Oreochromis niloticus* L.

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Abstract

When young fishes are exposed to stressful conditions, such as starvation, the development of the skeletal and integumentary systems can be greatly affected. In this study, the effects of delayed initial feeding on the development of these systems in *Oreochromis niloticus* were examined using histological techniques. Results show a general decrease in the over-all size of the fishes as the number of days of delayed initial feeding increased. There is also a short delay in the formation of scales in the skin and the layers decreased in thickness. There is a noticeable decrease in mucous cells and the narrowing of the hypodermis suggests that adipose or fat cells are less in more starved fish. However, there is no noticeable effect in the skeletal system because only cartilage was still present. Thus, it can be said that delaying the initial feeding of the fish caused a subsequent delay in the development of the skin layers and the formation of scales, therefore making the fish susceptible to disease and environmental stress.

Introduction

Since the early times, fish has already been cultured and harvested for food. The Nile tilapia was one of the first fish species cultured. Now, worldwide harvest of tilapia has surpassed 800,000 metric tons and tilapia rank second to the carp, as the most widely farmed fish in the world (Pompa and Masser 1999). They are kept in hatcheries and fish farms in order to facilitate their reproduction and nutrition. Although the environmental condition in this setup is safe from predators, delayed first feeding could also occur when nursery containers are not available or prepared. First feeding fry are held in hatching jars until containers are already available. This delay could also occur when the farmer unconsciously failed to check that the fry have already absorbed their yolk and are ready to eat exogenous food. Though their yolk could provide them the energy and nutrition, their first exogenous food is also necessary for the essential nutrients needed by the body in order to grow and develop.

New feeding strategies, such as the use of natural feeds and the delay in first feeding of the fish, are now implemented in fish farms in order to increase profits (Brown *et al.*, 2000; Bolivar and Brown, 2003). However, these strategies may affect the physiological processes and overall health of the fish. These changes may result to the delay of the growth and development of the fish and may also affect its reproductive success. The reproductive success of an organism depends on its health and survival. Reproduction of fishes is very important in fish farms because the more offspring or fish fry, the more profit the farmers would have. However, as consumers, people choose fishes which are high in quality.

The delay in first feeding could also be related to starvation or malnutrition. Malnutrition is the inadequate intake of any of the required nutrients. Nutrients are needed by the fish for the anabolism of important substances, such as proteins and nucleic acids.

The integumentary system is the fish's first line of defense against the harsh environmental conditions. Without the protection of the skin, the fish would not survive the environment and the various diseases it could suffer. The skin of the fish would also be an indication for quality in fish farms. Damaged and unhealthy skin may result to fewer customers and buyers and lesser profits.

The skeletal system of the fish also serves as the main structure for stability and protection of visceral organs. Malformed bones could easily be seen as an indication of low quality fish. Also, the delay in the formation of bones would lessen the reproductive success and survival of the fishes.

The objective of the study is to determine if the delaying of first seeding will have an effect on the integumentary and skeletal systems of fry, reared for one month.

The significance of this study is for tilapia fish farmers to know about the effects of feeding strategies, such as the delay of the first feeding of fish, on the skeletal and integumentary systems of their fishes. Negative effects of these strategies, on the skin and bones of their fishes may result to a decline in the quality of their fish, particularly the fish proteins, and also to the profits of their livelihood. Also, other possible effects such as the increase in the susceptibility of the fish to diseases, could be determined in order to come up with new feeding strategies.

Materials and methods

Collection and rearing of fish

Brood eggs of different paternal sources were collected from females crossed to YY males and incubated separately until hatched. Careful monitoring of the hatch fry was done to determine when they have already absorbed their reserved yolk and are ready to eat exogenous food. The broods that were selected to be used for the experiment were those that had at least a thousand fry.

The different broods were divided into 6 groups consisting of 200 fry. The different groups within the brood were given their first food at different times. Group 1 was the control group and was fed immediately after yolk absorption. Group 2 was fed 2 days after yolk absorption; group 3 was fed 4 days after yolk absorption. Group 4 was fed 6 days after yolk absorption, and finally group 5 was fed 8 days after yolk absorption.

The average weight of the fry was determined after the absorption of the yolk sac and each group was placed or stocked separately in plastic containers with water and provided aeration. Whenever a group was scheduled to be fed, the fry was stock in a $1m^3$ mesh hapa installed in an earthen pond that was previously fertilized to enhance the growth of natural food. Supplemental food using commercial fry mass was given to stimulate actual hatchery practice in addition to the natural food. The fish were reared for one month.

Histology

After fixation, 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 micrometers and stained with hematoxylin and eosin.

To dissolve the hematoxylin in distilled water, it was first heated, then the alum, sodium iodate, citric acid and chloral hydrate were added. In staining, the xylene was deparaffinized then rinsed in water for 2-3 minutes. It was then stained with hematoxylin for 10 minutes and then washed for 3 minutes using running water. Eosin was used for counterstaining which was done for 5-10 minutes. Then, it was dehydrated in 70%, 95%, and 100% alcohol. Xylene was used in clearing and then mounting was performed.

Observation

The skin and skeletal structures of the fishes were observed under 20x and 40x magnification using an ordinary microscope with a 12.5x objective. Pictures of the organs were taken using a digital camera.

Results

Generally, the overall size of the fishes decreased as the number of days in which their initial feeding was delayed increased. The differences of their sizes were more evident when fishes of the same age were compared than the control. Size of the 6-day delayed and 8-day delayed in first feeding fish of the same age was comparably smaller than the control.

Integumentary system

In all groups, the epidermis and dermis were evident (Figs. 1-4). The epidermis was made up of squamous cells, which were flattened at the surface and spherical near the base (Fig. 2). The dermis, on the other hand, was observed as a layer ventral to the epidermis. The stratum laxum or spongiosum was not very evident in the young fish, but was seen in fishes which developed scales. However, the inner stratum compactum was very evident as a wavy and compact collagenous layer. Scales developed from this layer in scale pockets. Meanwhile, the hypodermis (Figs. 1 and 3) was seen as a loose layer between the stratum compactum and the muscles.

The development of the skin in the fish delayed in first feeding for two days was not significantly different from the control fish (Figs. 1 and 3). However, the effects of delayed first feeding was most evident in the fish, which first feeding was delayed for eight days (Figs. 2 and 4). The hypodermis was observed to decrease in thickness as the time of delay increased. Also, mucous cells were fewer in the 6-day delayed and 8-day delayed fish than those in the control.

There was also a delay in the development of scales in the delayed feeding groups (Figs. 2 and 4). In the 2-day delayed group, there was a lag of 2 days in expression of scales. On the other hand, a lag of 8 days could be observed in the fish, which first feeding was delayed for 8 days.

Skeletal system

The development of the notochord and the cartilage tissues were observed in all groups. Generally, cartilage was very evident in all groups. The cartilage of the developing neural spine and pectoral girdles of the fish were most evident. The cartilage found in the fish tissues was mostly hyaline. The cartilage appeared as a group of chondrocytes separated by an intercellular matrix and covered by a perichondrium. The chondrocytes were observed to draw away from the intercellular matrix, forming spaces called lacunae.

In the control fish (T1), which was not delayed in first feeding, the developing neural spine was observed as an oval shaped cartilage. A perichondrium surrounds the group of chondrocytes, which are found in lacunae in the intercellular matrix. The developing pectoral girdle was also observed as an elongated oval-shaped cartilaginous tissue at the lateral sides of the fish.

The neural spine and pectoral girdle cartilage of the delayed feeding groups were not significantly affected by the delay in first feeding. However, there were fewer chondrocytes in the delayed fish groups.

In all groups, the notochord could be seen as a circular gelatinous structure. It was located ventral to the brain or spinal cord and dorsal to the dorsal aorta. Cartilage was also seen in the periphery of the notochord. The notochord was still present in all fish groups suggesting that the fish are still young to degenerate their notochord.

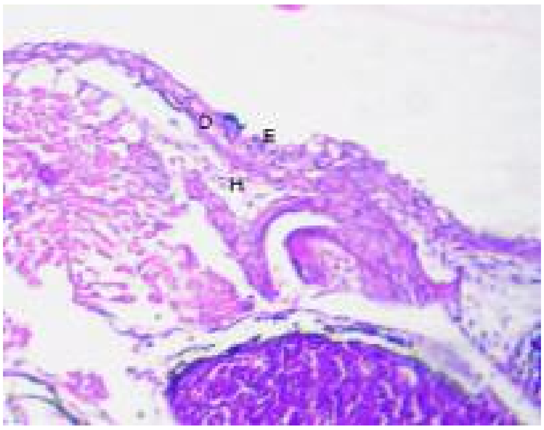

Figure 1. Cross-section of skin of control fish (not delayed in first feeding) after 2 days of feeding. The epidermis (E), which is made of squamous epithelium tissue, and the wavy collagen layer of the dermis (D) could be observed. The hypodermis (H), composed of areolar and adipose tissues, was evident. (H.E. x 250)

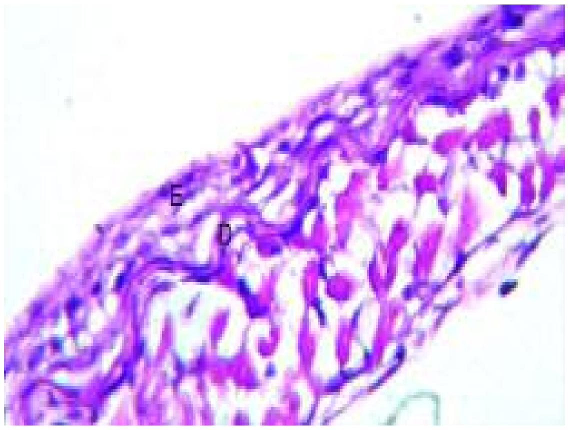

Figure 2. Cross-section of skin of fish, delayed in initial feeding for 8 days, after 2 days of feeding. Only the epidermis (E) and the dermis (D) are evident. (H.E. x 500)

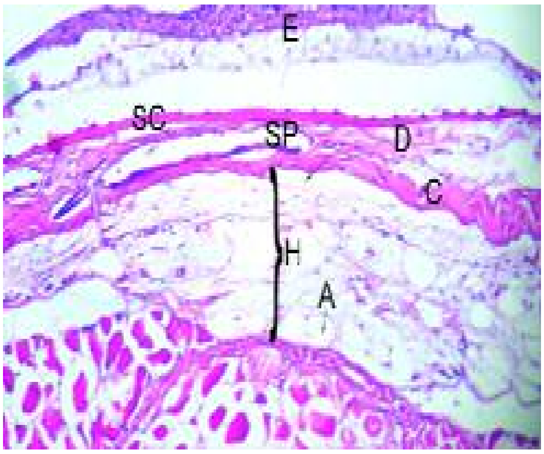

Figure 3. Cross-section of skin of the control fish after 30 days of feeding. Note the epidermis (E) and the dermis (D) with the compact wavy collagen bundles in the stratum compactum (C). The scale (SC) grows from the dermis in scale pockets (SP). The hypodermis (H) has abundant adipose cells (A). (H.E. x 250)

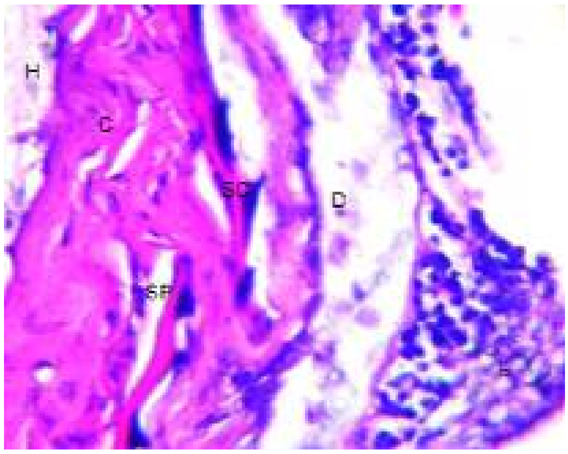

Figure 4. Cross-section of skin of fish, delayed in initial feeding for 8 days, after 30 days of feeding. Note the abnormal epidermis (E) and the dermis (D) with developing scales (SC) in scale pockets. The stratum compactum (C) is also evident. The very thin hypodermis (H) is observed. (H.E. x 250)

Discussion

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 tissues (www.michigan.gov). In the fish, the fats are mainly in the hypodermis, which contains adipose and areolar tissues. In this study, the hypodermis of the fish, which first feeding was delayed, was barely noticeable. As seen in the control fish, the hypodermis is rich in adipose, as well as areolar tissues. The adipose make up the bulk of the hypodermis. In the most starved fishes (T4 and T5), the hypodermis are not evident in the early days and become thinner as they mature. A significant decrease in hypodermis adipose could be observed as the time of delay in first feeding increased, when compared to the control fish.

A study by the Southern Regional Aquacultural Center (2001) in Mississippi, on the effects of nutrition in body composition and subsequent storage quality of catfishes, determined the effects of reduced feeding to fishes. The reduction in the amount of feeds led to a decrease in the overall lipid content of the fish. These would suggest that restricting the diet of the fish, such as delaying their first feeding, would lead to the reliance of the fishes in metabolizing their stored energy, in the form of fats.

Another study by Elie (2000), determined the effects of starvation in humans and animals. One of the most consistent autopsy findings of animals and humans who died of 'pure' starvation, is the virtual absence of depot fat, both subcutaneously and internally. In some studies, fat was found to be replaced by a translucent gelatinous material. Since body fat is the major store reserve of energy which disappears during starvation, it is reasonable to suggest that the initial amount of body fat is a major determinant of the length of survival during starvation.

The subsequent loss of the adipose cells in the hypodermis would then imply that the fish relied mostly in their stored body fat as an energy source. The principal metabolic adaptation during starvation involves a shift away from gluconeogenesis and carbohydrate metabolism to a primary reliance in lipids stored in adipose tissue (Kuzawa 1998).

As fishes are delayed in first feeding, not enough nutrients are absorbed by the body for anabolic activity. That is when malnutrition and starvation takes place. Malnutrition in fish farms using delayed first feeding as a strategy often happens because the natural food in culture ponds is not adequate to supply the young fish with nutrients. Young fishes are mostly affected because they are still developing structural and physiological structures in their body.

The decrease in the formation of mucus by mucous cells in starved fish, which was found out in this study, may result to a greater tendency for the fishes to catch disease and parasites from their aquatic environment. Mucous cells derive from undifferentiated basal cells as they increase in size and migrate to the surface (aquaticpath.umd.edu). They are also called goblet cells because of their shape. Mucus secretions, composed primarily of glycoproteins, form a slimy protective coat. Functions attributed to this coat include drag reduction, predator evasion, and isolation of superficial epithelial cells from bacteria. Immunoglobulins, also present in mucus, provide additional protection against infection. Also, mucus is secreted in response to stressful external stimuli. Small amounts of nonslimy mucus are secreted by teleost fishes, forming a mucus cuticle (Kent and Carr 2001).

Also, the skin of the fish would be very susceptible to wounding because of the decrease in the width of the stratum compactum or the dermis, which gives strength and support to the skin. The dermis is composed primarily of collagenous connective tissue distinguished as stratum compactum, a dense collagenous matrix providing structural strength and stratum spongiosum or laxum, a loose network of collagen and reticulin fibers. The collagenous structure of the dermis would provide the elastic support to the skin when fish swims (aquaticpath.umd.edu).

Also, a significant delay in scale formation was observed. In the control fish, scales were first observed to develop in 8-day old fish. However, in the T2 group, which is delayed in first feeding for two days, the first development of scales were observed in fishes, which are 10-day old. Though there is a delay of two days, it could not yet be assumed that there is a lag in the formation of scales since there is a gap in the age of the sectioned fish. Nevertheless, the delay in formation was seen in the next group (T3), which is delayed in initial feeding for 4 days. The 12-day old fish first developed scales; however, the younger fish under this group did not form scales even though they are of the same age as that of the control fish, which developed scales (8-day old). The 8-day old and 10-day old fishes of the T4 group (delayed in initial feeding for 6 days) also did not develop scales, while the fish of respective ages in the T1 and T2 group did. The delay in scale formation is more apparent in Table 2. The dermis of *O. niloticus* develops cycloid scales. Cycloid scales are translucent, acellular plates of collagenous tissue, which anchor within dermal scale pockets in the stratum spongiosum but project through the basement membrane into the epidermis. They are covered by a thin squamous epithelium, which remains distinct from epidermal tissue. Scales overlap one-another in an imbricated manner with free ends directed caudad. This scales serves as the armor of the fishes against potential predators and parasites. The delay of the formation of the scales would lead to the susceptibility of the young fish to predator and parasite attack.

The delay of the formation of scales may be attributed to the limited supply of nutrients due to the delay in first feeding. Since the fishes only rely in the minute amounts of plankton in their environment, nutrient intake is also limited. In addition, the delay in formation could also be related to the decrease in the thickness and strength of the epidermis and the dermis, which support the formation and development of scales.

The skeletal system was not greatly affected in the delay of first feeding. However, since hyaline cartilage would be later replaced by osseous bone, the delay in first feeding might have an effect in the onset of ossification. The dorsal cartilage focused on this study would later develop into the neural spine of the fish. It was observed however, that in delayed feeding groups, the cartilage was still present. The cartilage appears initially during the hatching period of the fish (Morrison 2001).

The notochord, which derives from embryonic mesoderm, is composed of soft gelatinous cells surrounded by a membranous sheath – which structure gives it strength and flexibility (Schaffer 2004). The notochord persisted in all groups of fishes. Delay in initial feeding did not cause degeneration of the notochord sheath. The notochord would later degenerate into a pulpy mass in the neural arch.

Further studies should be done to understand better the effects of delayed initial feeding to the development of the integumentary and skeletal system of *O. niloticus.* Also, it is recommended that the development of the two systems be observed until maturity. The effects of delayed first feeding in the skeletal system could be studied better if formation of bones was evident in the age of the fish, focused in the study. Also, more specimens should be processed and observed. The data that would be gathered from future studies may be analyzed statistically to know and firmly establish the significance of the effects of delayed initial feeding to the development of the integumentary and skeletal systems.

It is also recommended that other feeding strategies be implemented in tilapia aquaculture. If delayed feeding would be used, it is advisable not to exceed more than two days in delaying the initial feeding of the fish since delay in first feeding for two days has no significant effects in the integumentary and skeletal system of the fish.

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