

EFFECTS OF DELAYED FIRST FEEDING ON THE DEVELOPMENT OF THE DIGESTIVE TRACT AND SKELETAL MUSCLES OF NILE TILAPIA, *Oreochromis niloticus* L.

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Abstract

To compare the effects of starvation before initial feeding on the development of the digestive tract and skeletal muscles of the genetically male tilapia (product of a cross between the supermale YY father and ordinary XX female), fish were starved for 2, 4, 6, and 8 days before first feeding. The development of the digestive tract and skeletal muscles from 0-150 days post-hatching (dph) of the Nile tilapia (*Oreochromis niloticus* L.) were investigated by comparing fish that were previously deprived of first feeding for up to 8 days upon hatching, with those that were immediately fed upon hatching.

Starvation caused an approximately 2-day delay in the initial development of the digestive tract and the formation of muscle fibers. At 30-150 dph, histological differences between unstarved and previously starved fish were no longer evident, except for the more abundant goblet cells seen at 90-150 dph in the anterior and posterior intestine of the unstarved fish.

These results suggest that delaying first feeding for up to 8 days in the genetically male Nile tilapia (GMT) may cause initial delay in the developmental histology of the digestive and skeletal muscle tissues. Resumption of normal feeding enables the fish to recover in terms of the histology of the gut and muscles, but not in total body length and weight and gut length where, at 30-150 dph, the unstarved fish consistently had statistically higher measurements compared to the starved fish.

Introduction

Tilapia commonly grown for food, such as *Oreochromis niloticus* (Nile tilapia), are mouth-brooders. As such, they exhibit a high degree of parental care.

After fertilization by the male, the female takes the eggs into her mouth for incubation. The eggs hatch inside the mouth, and the sac-fry remain in the mouth for

protection. When the fry totally absorb their yolk and become free-swimming, the female tilapia normally releases them from their mouth and they are left by themselves to search for exogenous food (Popma and Masser, 1999).

However, in a natural environment where predators abound, the female tilapia may delay the release of her fry for their protection. While this instinctive practice may be effective in ensuring fry survival, it, however, delays the first exogenous feeding of the fry.

Delay in first feeding may also transpire in commercial hatcheries, for instance, when there is insufficiency of nursery containers by the time the fry are released by the mother. It may also happen when farmers fail to notice that the fry have already totally absorbed their yolk and are ready to eat exogenous food.

For mouth-brooding tilapia such as *Oreochromis niloticus*, where the anti-predator tactic of buccal-rearing prevails, partial or total food deprivation of fish larvae or fry during the transition from endogenous to exogenous feeding may seriously impair growth and survival (Hewitt *et al.*, 1985). Such effect may be partly attributed to abnormalities in the growth and development of tissues or histogenesis in the digestive tract and skeletal muscles of the developing fry, which in turn may have great implication on the quality of food fish production.

Baylous and Herrera (1993), in a study on the early development of the gastrointestinal tract in *O. niloticus*, found that there is a correlation in gastroenterohistogenesis and feeding habit in *O. niloticus*. The gastrointestinal tract of the *O. niloticus* fry is undifferentiated while undergoing endogenous feeding. Differentiation starts at the reduced yolk stage to prepare the digestive tract for exogenous feeding, and at 28 days post-hatch, the stomach and intestines are already fully developed.

Normal development of the gastrointestinal or digestive tract, however, may be altered, once first feeding or exogenous feeding is delayed. For initial feeding, fry and fingerling require a diet higher in proteins, lipids, vitamins and minerals and lower in carbohydrates, since they are rapidly developing muscles, internal organs and bones. Sub-adult fish, on the other hand, need more calories from fat and carbohydrates for basal metabolism and a smaller percentage of protein for growth. Adult fish need even less protein. The absolute amount of food the fish eat increases as they grow larger. The amino acids that make up that protein need to be available in certain ratios. Broodfish, however, may require elevated protein and fat levels to increase reproductive efficiency (Santiago *et al.*, 1985; Chang *et al.*, 1988).

The need for these nutrients, as early as the “critical” stage, where the sudden transition from endogenous to exogenous feeding occurs, is so significant that their absence or deprivation may lead to several problems in the fry’s growth and development.

This study therefore, focused on the effects of delayed first feeding on the development of Nile tilapia, *O. niloticus* L. Specifically, it aimed to study the histogenesis of the digestive tract and skeletal muscles in *O. niloticus* when first feeding was delayed.

The negative effects of delayed initial feeding observed from the study may help propel immediate solutions on how to maximize tilapia survival despite first feeding delay, without having to sacrifice the quality of food fish. Likewise, in addition, the study may encourage farmers to come up with remedies to address the possible effects of delayed first feeding. For tilapia researchers, the outcome of this study may provide new ideas for future investigations.

Materials and methods

Samples of Nile tilapia fry were taken and reared at the Phil-FishGen Project of the Freshwater Aquaculture Center-College of Fisheries of the Central Luzon State University (FAC-CLSU) in Muñoz, Nueva Ecija. Tissues for histological studies were prepared at the Developmental Biology Laboratory of the Institute of Biology (IB) and at the Natural Sciences Research Institute (NSRI) of the University of the Philippines, Diliman, Quezon City. Photomicrography was done at the General Equipment Laboratory of the Institute of Biology. Processing of samples for scanning electron microscopy (SEM) was done at the Electron Microscopy Service Laboratory of the National Institute of Molecular Biology and Biotechnology (BIOTECH), UP Los Baños, Laguna. SEM viewing was conducted at the Electron Microscopy Laboratory of the Department of Mining, Metallurgical and Materials Engineering (DMMME) of the College of Engineering, UP Diliman, Quezon City, using Leica S440. Transmission electron microscopy (TEM) was conducted at St. Luke's Medical Center, Quezon City, using JEOL 1010.

Egg incubation and rearing of larvae

Brood eggs were collected from a female (selected synthetic strain) and were crossed to a YY male. The fertilized eggs were incubated in down-welling hatching jars. Upon hatching, the fry were monitored to determine when they had already absorbed their reserved yolk and were ready for first feeding (exogenous).

Preliminary testing

A pre-trial was performed to determine how long a fry survives without receiving its first exogenous food. Three glass aquaria per batch of fry (one parental source) were filled with tap water. Two batches of fry were used. Two hundred fry with newly consumed yolk sac were stocked in each aquarium. The fry were not fed. The number of dead fry were recorded daily until total mortality was observed. Siphoning of waste and replenishing of water in the set-ups were done daily.

Preparation of experimental set-ups

One thousand (1000) fry taken from one broodfish were used for the experiment. They were divided into 5 groups with each group containing 200 individuals. The number of set-ups was based on the result of the pre-trial experiment which revealed that after 8 days post-hatching (dph), 50% mortality was reached. The different groups were initially fed at different periods starting from day 0 (estimated period when the fry had totally absorbed their yolk and starts taking in exogenous food) and done at an interval of two days.

The first group called T1 (control), which was fed right after yolk absorption, was immediately stocked in a 1m³ fine-meshed “hapa” installed in fertilized earthen pond. Supplementary food using commercially available Tateh fry mash was given to the fry. The four other groups, which, were kept starved, were temporarily held in plastic basins provided with continuous water flow to maintain optimum water quality. Every after two days for eight days, one group was being stocked in a separate “hapa”, where they were first fed. These four groups were designated as T2 (starved for two days), T3 (starved for four days), T4 (starved for six days), and T5 (starved for eight days).

Sampling was done at 0 (before feeding starts), 2, 4, 6, 8, 20, 30, 60, 90, 120 and 150 days after first feeding or days post-feeding (dpf) and 0 (day of hatching), 2, 4, 6, 8, 20, 30, 60, 90, 120 and 150 days post-hatching (dph). For every sampling schedule, five individuals were taken for histological examinations. Fifteen individuals per group were sampled on 150 DPH, five of which were used for light microscopy, another five for scanning electron microscopy (SEM), and the last five for transmission electron microscopy (TEM).

Total body weights and lengths of three samples taken randomly from every group were measured before and after first feeding and during each sampling period. Moreover, the gut length of samples aged thirty to one hundred dph was measured.

Histological procedures

To maintain the original form and shape of the fish samples for histological analysis, the latter were immobilized slowly by placing a few drops of 10% formaldehyde into the sampling vials containing the fish samples. The digestive tract of fish bigger than two cm were dissected before fixation. Also, an approximately 1 cm³ skeletal muscle sample taken from the dorsal part of the fish was cut from the samples longer than two cm.

The fixed fish tissues were dehydrated through graded alcohols (50%, 70%, 80%, 95% and absolute ethyl alcohol), cleared in xylene and subsequently embedded in paraffin. Serial sections (6 µm) were cut from each block and mounted on acid-washed slides. Sections were dewaxed, rehydrated and stained with haematoxylin and eosin (H&E), dehydrated and mounted in entellan.

All prepared slides were observed under a Zeiss (Axioskop) light microscope with Canon EOS ELAN II camera attachment.

Scanning electron microscopy

Anterior intestine and skeletal muscle specimens used for SEM were soaked in 1.0% tannic acid in 0.1M cacodylate buffer (pH 7.4) following the post-aldehyde wash step. They were placed in this solution for two hours, then transferred to 1.0% osmium tetroxide (OsO₄) using the same buffer for two hours at room temperature. Tissues were then washed, dehydrated, critical-point dried through CO₂, mounted on aluminum stubs, and sputter-coated with gold for viewing in a Leica S440 scanning electron microscope.

Transmission electron microscopy

Samples (1mm long) of anterior intestine and muscles taken from 150 dph fish were fixed in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer (pH 7.2) containing 3.5% sucrose at 4^oC. Following a total fixation time of approximately three hours, the samples were rinsed in cacodylate buffer and post-fixed in 1% osmium tetroxide in cacodylate buffer, then dehydrated in a graded series of acetones, stained in bloc for one (1) hour in 1% uranyl acetate in 70% acetone, and embedded in Araldite resin. Semi-thin sections (0.5-1 µm) were stained with toluidine blue. Ultra-thin sections were mounted on uncoated copper grids, stained with lead citrate and uranyl acetate and viewed in JEOL 1010 transmission electron microscope operated at 100 kVs.

Analysis of data

Total body weight and length, gut length, the pattern of development of the digestive tract and muscles (i.e. height of the mucosal layer and thickness of the muscularis layer in the stomach, anterior intestine and posterior intestine, the abundance of goblet cells in the anterior and posterior intestine, and the diameter of the hepatic portal vein in the liver), and the density of cells with enzyme activities of fish from each feeding group were compared to determine the effects of delaying first feeding. Data were presented as means ± standard errors. Means were tested statistically using ANOVA (Analysis of Variance) followed by DMRT (Duncan's Multiple Range Test). The 5% confidence level was used throughout. Statistical analyses were performed with Statistical Analysis Software (SAS).

Results

The experimental fish were divided into the following developmental stages: 0 (hatching) - 20 days post-hatching (dph) and 21 - 150 dph. The fish that were immediately fed upon hatching are referred to here as the *unstarved group*, while those that were starved for up to 8 days are referred to as the *starved group*.

Morphological measurements and histology

0 – 20 days post-hatching (dph)

Total body weight and length of the samples used in this study were almost uniform on day of hatching (day 0). This was likewise observable on the 2nd-20th dph, since no definite pattern of increase or decrease in the weight and length of the groups was observed (Figs. 1 and 2).

At tissue level, no consistent pattern in the measurements of mucosal height and thickness of the muscularis in the stomach, anterior intestine and posterior intestine, the

abundance of goblet cells in the anterior and posterior intestine, and the diameter of the hepatic portal vein in the liver was seen at 0-20 dph. This was true between both the unstarved and starved groups.

At the start of exogenous feeding, folds were seen along the length of the esophagus, which became more prominent by ten dph. Mucous-secreting cells distributed among the epithelial cells were present as early as two dph and gradually became more conspicuous at older age. The esophageal wall was surrounded by a circular layer of striated muscle, and by 10 dph, became more distinct from an outer longitudinal muscle layer.

The stomach was observed as an outpocketing as early as two dph in unstarved fish and six to eight dph in starved ones (Figs. 5A-B). At these respective ages, gastric glands appeared, and the early stomach consisted of a submucosa and a thin muscularis of circular muscle fibers.

At two dph, the anterior intestine was lined by a simple columnar epithelium, which was bordered by a layer of microvilli at the apical surface. Goblet cells were present in both anterior and posterior intestines even at an early stage. A distinction between the two could already be pointed out by the appearance and height of the individual mucosal folds present. By day six, the intestines had become much wider, most notably in the anterior segment (Fig. 4A). This was not seen, however, in the starved samples, where, at day six, only the liver mass and an outpocketing identified as the stomach were most evident (Fig. 4B). In general, few morphological changes occurred in the anterior and posterior intestine of fed samples from 0 – 20 dph. However, increases in the size of the organs were evident.

The skeletal muscles at two dph were already well - developed (Figs. 4A-B). The diameter of the muscle fiber bundles increased as the fish grew older. These were surrounded by a clear space occupied mainly by smooth endoplasmic reticulum and mitochondria. The diameter of muscle fibers taken from the older starved samples was the same as those of the unstarved samples.

21-150 days post-hatching (dph)

The differences in weight and length between the unstarved and starved fish from 30-150 dph were statistically significant. The unstarved samples were heavier and longer than those starved for a maximum of eight days (Figs. 1 and 2). Such result was consistent with

Figure 1. Comparison of the effect of delayed first feeding on the weight (grams) of 0-150 dph unstarved and starved *O. niloticus*.

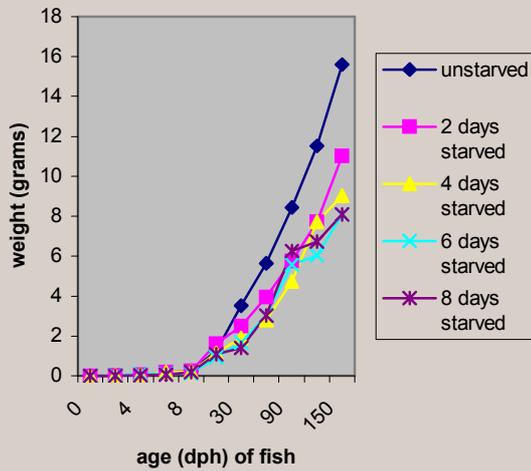


Figure 2. Comparison of the effect of delayed first feeding on the length (centimeters) of 0-150 dph unstarved and starved *O. niloticus* samples.

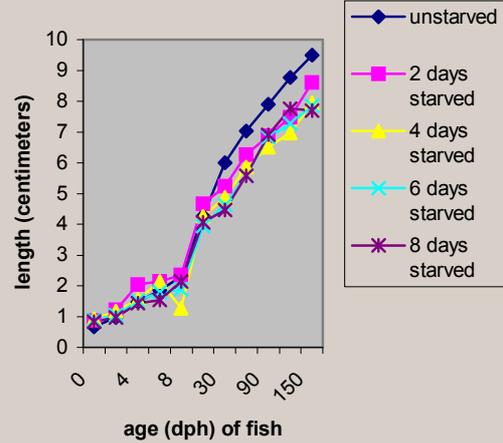


Figure 3. Comparison of the effect of delayed first feeding on the gut length (centimeters) of 30-150 dph unstarved and starved *O. niloticus* samples.

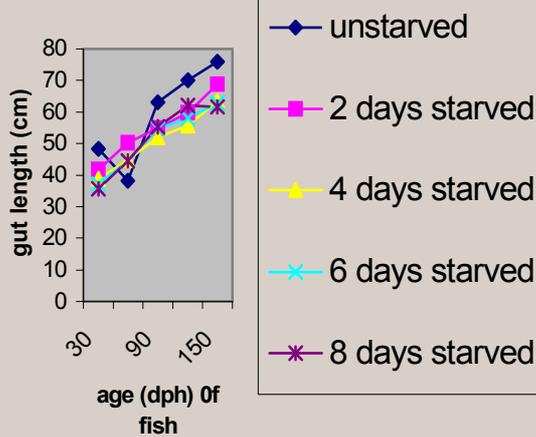




Figure 4. Portions of the cross-section of the anterior abdominal cavity of *O. niloticus* in (A) 6 dph unstarved fish; and (B) 6 dph starved for 6 days fish. Development of the stomach (S) anterior intestine (AI), posterior intestine (PI), liver (L) and pancreas (P) is evident. Only the skeletal muscles (SKM) or muscles (M), the stomach, and liver, are prominent in this section of fry starved for 6 days before initial feeding. H&E, X200.

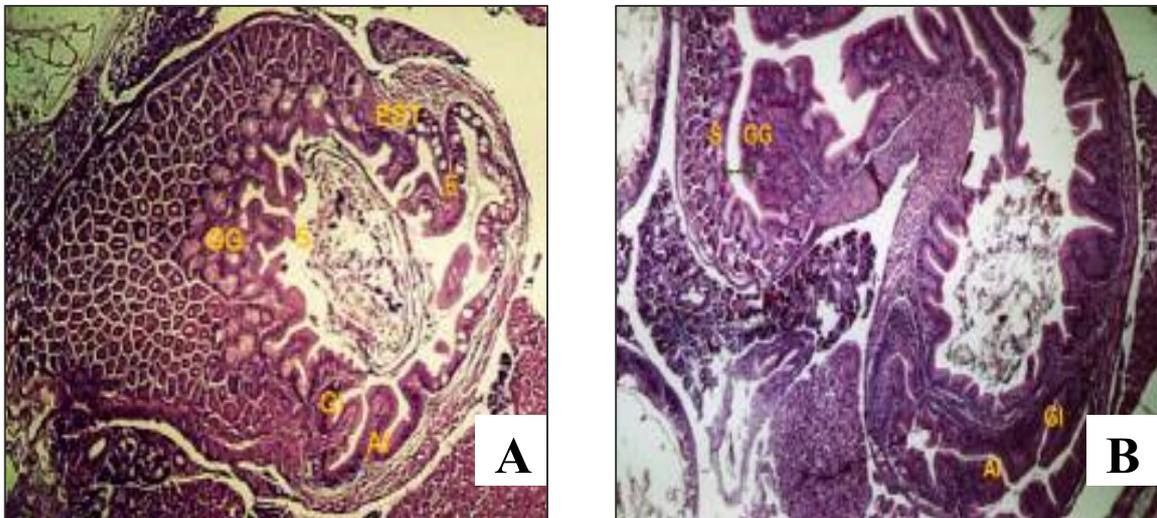


Figure 5. Portions of the cross-section of the anterior abdominal cavity of *O. niloticus* in (A) 6 dph starved for 6 days fish; and (B) 8 dph starved for 8 days fish showing the esophagus-stomach transition (EST) and gastro-intestinal transition (GI). The presence of gastric glands (GG) signifies the onset of chemical digestion. H&E, X100.

the measured gut lengths from thirty to one hundred fifty dph wherein the measurements seen in the unstarved fish were statistically higher than those in the previously starved fish (Fig. 3).

No other new development was seen in the esophagus, where complete differentiation was observed at ten dph. A marked increase in diameter and the separation of the layers of the muscularis were the only noticeable changes at this period.

In the stomach, no definite pattern of increase or decrease in the height of mucosal cells and thickness of the muscularis layer relative to age was seen between the adult unstarved and starved fish.

In the anterior intestine, no characteristic pattern of increase or decrease in the height of the mucosal cells and thickness of the muscularis layer was seen between the unstarved and starved groups (Figs. 6A-B). The number of goblet cells, however, became significantly higher in 90-150 dph unstarved group.

In the posterior intestine, an increase in the height of the mucosal cells and thickness in the muscularis layer was remarkable in both unstarved and starved samples. Histological observations had revealed that starved samples had relatively lower mucosal folds and thinner muscularis compared to the unstarved ones. This was very pronounced in the samples starved for eight days. Statistically, however, there was no way of differentiating the feeding groups based on these morphological factors.

In the skeletal muscles, the diameter of each muscle fiber increased progressively but looked similar between the unstarved and starved groups (Figs. 8A-B). This was in fact confirmed in scanning electron micrographs of 150 dph skeletal muscles, where the diameter of the muscle bundles was almost the same for both unstarved and starved groups (Figs. 9A-B).

Electron microscopy

At the cellular level, the goblet cells, which were significantly more abundant in the unstarved samples of 90-150 dph, were also found to be different between the unstarved and starved fish (Figs. 7A-B). Each goblet cell was characterized by an apical surface swollen with mucous granules. More abundant mucous granules were observed in the unstarved fish.

Rough endoplasmic reticulum and prominent Golgi apparatus, which fill the rest of the cytoplasm, were also more abundant in the unstarved fish (Figs. 7A-B).

At ultrastructural level, no differences in the features of the skeletal muscles were observable in both feeding groups. This was similar to the result obtained in light and scanning electron microscopy (Figs. 8A-B, 9A-B). The sarcomeres, considered as the smallest repetitive sub-units of the contractile apparatus, were almost of the same length in both unstarved and starved samples.

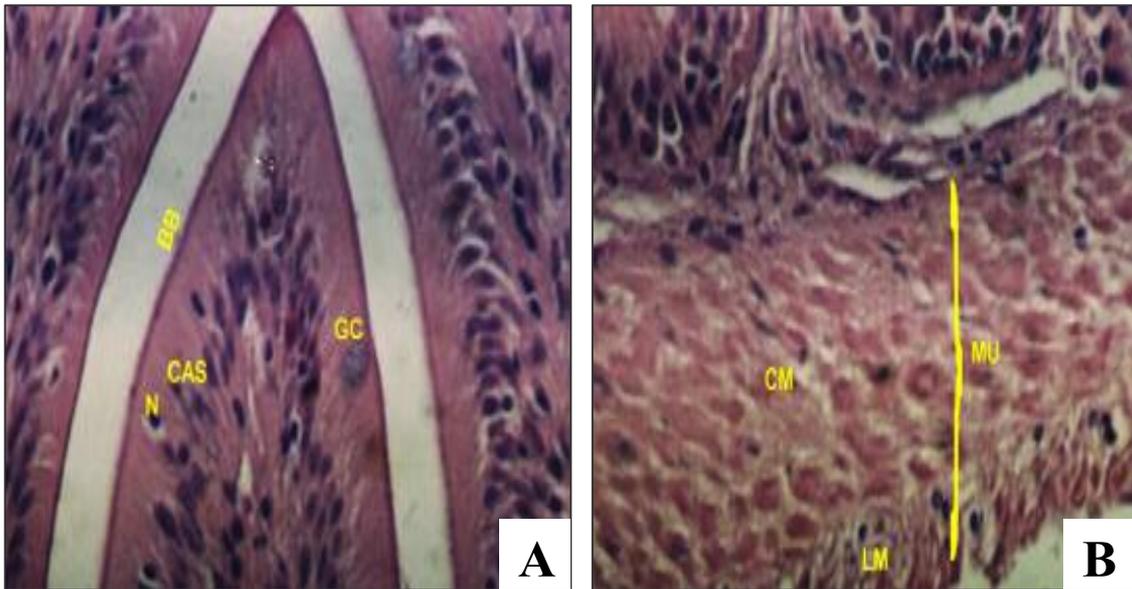


Figure 6. Portions of the cross-section of the anterior intestine of 120 dph unstarved *O. niloticus* showing (A) the mucosal fold; and (B) the muscularis layer (MU). The height of the mucosa (M) and the thickness of the submucosa (SM) and muscularis (MU) are greater in the unstarved fish. Columnar absorptive cells (CAS) with their prominent nuclei (N) are clear. H&E, X1000.

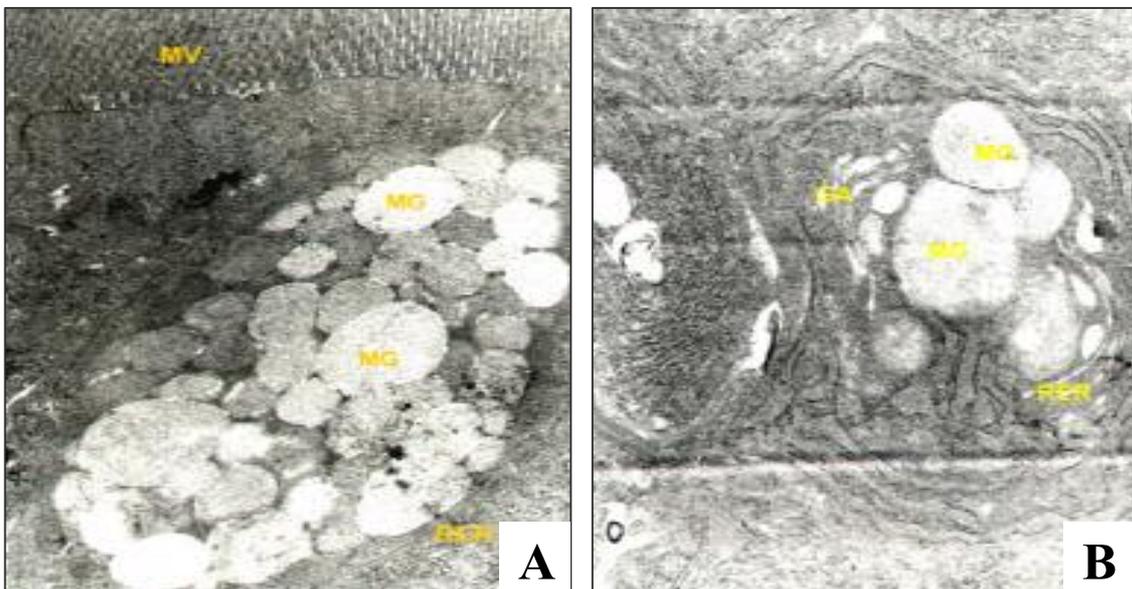


Figure 7. Transmission electron micrographs of the cross-section of the intestinal mucosa of *O. niloticus* showing the goblet cell in (A) 150 dph unstarved fish; and (B) 150 dph starved for 8 days fish. The goblet cell is characterized by an apical surface swollen with mucous granules (MG) which are seen to be more abundant in the unstarved fish than in the fish starved for 8 days. Rough endoplasmic reticulum (RER) and Golgi (GA) fill the rest of the cytoplasm. X21,600.

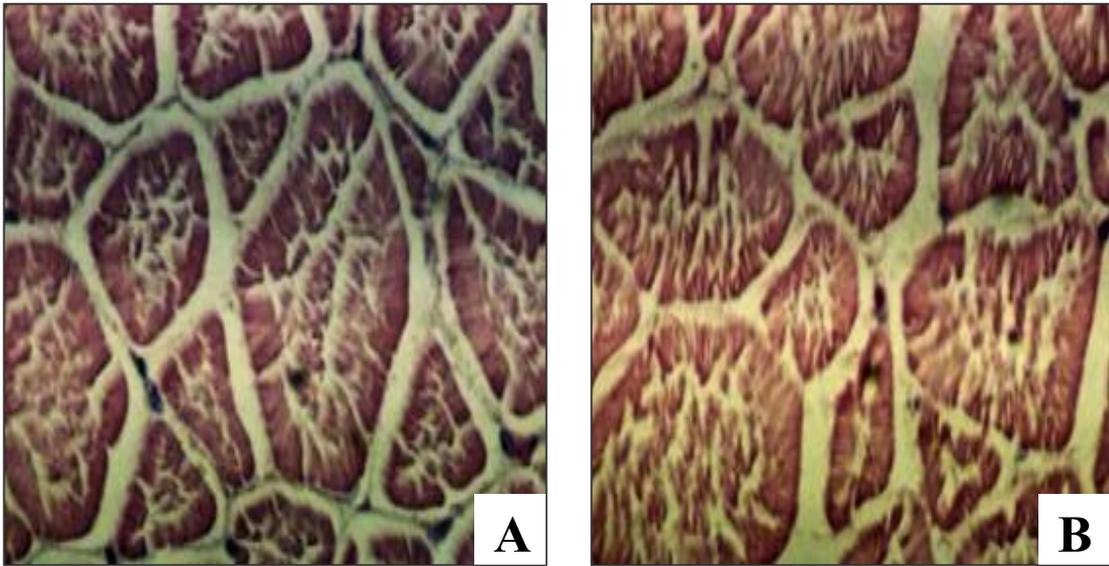


Figure 8. Cross-section of the portions of the dorsal skeletal muscles of 120 dph *O. niloticus* in (A) unstarved fish; and (B) starved for 8 days fish. The diameter of the muscle fiber bundles is the same in both starved and unstarved fish. H&E, X1000.

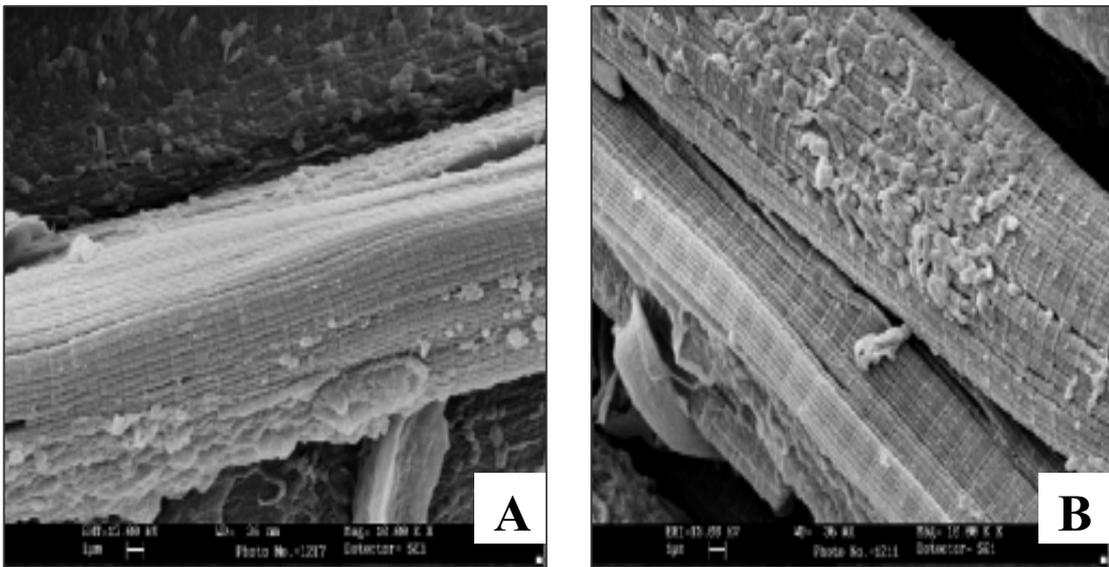


Figure 9. Scanning electron micrographs of the longitudinal section of the dorsal skeletal muscles of *O. niloticus* in (A) 150 dph unstarved fish; and (B) 150 dph starved for 8 days fish. X10,000.

Discussion

Starvation

For the Nile tilapia fry, total yolk absorption signifies the drastic shift from the familiar endogenous feeding to exogenous feeding; that is, the fry, acting in survival instinct, are now forced to look for exogenous food. To deprive them of exogenous food, notwithstanding their readiness, is therefore a form of starvation, which could delay their growth and development for lack of basic nutrients. This deprivation is a type of stress that organisms often encounter in nature (www.biol.unt.edu).

In this study, it was shown that Nile tilapia could withstand food deprivation for a maximum of eight days. Starvation for up to ten days caused more than 50% mortality in the fry population. Several researches have been conducted to understand starvation mortality in both marine and freshwater fry. In their study on feeding resumption, morphological changes and mortality during starvation in Japanese flounder larvae, Dou *et al.* (2002) cited Blaxter and Hempel (1963) to have introduced the concept of point-of-no-return (PNR), which denoted a threshold point during progressive starvation from first feeding when 50% of the larvae were still alive but too weak to feed even when food became available. For Nile tilapia fry, the point-of-no-return starts on the 10th dph. This result was obtained during the pre-trial experiments, the reason why for the actual experimentation, fry were starved for up to eight days only.

Several studies show that different species of fish differ in their ability to withstand delay in first feeding or food deprivation (Dou *et al.*, 2002). Many physiological changes occur as the animal attempts to satisfy its energy requirements. At the cellular level, catabolism continues to supply the substances required for anabolism and continue its vital functions. In the Nile tilapia fry, nutrient reserves derived from their previous yolk may have been utilized to compensate for the lack of nutritional intake.

Pathological changes, which occur in a starved animal, are many and varied. The most striking of which are the absence of fat in the subcutaneous, visceral, and bone marrow locations, and atrophy in the musculature. The organs of the body decrease in size and weight, and the digestive tract of most species is empty (www.michigan.gov). Some of these symptoms were used to differentiate unstarved from the previously starved Nile tilapia.

Morphological measurements

The growth and development of Nile tilapia fry is affected up to maturity, when initial feeding delays reach a maximum of eight days. The effect was seen first at 30 dph (Figs. 1 and 2). This therefore suggests that at an earlier age, between zero to eight dph, starved fish utilize their stored energy reserves to survive, and even catch up with the weight and length of unstarved fish. Though starved fish were able to cope with starvation effects, some other effects of initial feeding delay were magnified at a later age and thus, caused the differences in morphological measurements among unstarved and starved samples. This result finds consistency with Rana (1990) who suggested that a 90% survival rate of *O. niloticus* fry during their early development was practically attainable in commercial hatcheries, if feeding of fry commences no later than five to six dph.

At 30 dph, it was already possible to dissect out the entire gut of the samples and obtain measurements of their lengths. Gut lengths obtained from the unstarved and starved fish were proportionate with their total lengths. In fact, at thirty to one hundred dph, the gut lengths of unstarved fish were also significantly greater than those which were previously starved for eight days (Fig. 3). This may account for the significantly greater total weight of unstarved fish compared to the starved ones.

In this study, delaying initial feeding to up to eight days did not automatically result in the immediate death of the fish, but neither did the commencement of feeding cause a so-called *compensatory growth* in the samples used. Compensatory growth of warmwater fishes was reported in channel catfish, *Ictalurus punctatus*, hybrid sunfish, *Lepomis cyanellus* and gibel carp, *Carassius auratus gibelio* (Qian *et al.*, 2000). The present study does not confirm the existence of compensatory growth in *O. niloticus*, as far as length and weight measurements are concerned, *vis-a-vis* a longer duration of starvation before initial feeding.

The length and weight of fish are determined largely by the amount of nutrients they absorb and assimilate. The absorption and assimilation processes are greatly observed in the digestive tract and are revealed further in the skeletal muscles. This therefore provides the reason why it was equally important to also investigate the histology of the digestive tract and skeletal muscles.

Histology

The histogenesis or development of the digestive tract and skeletal muscles is partly affected at an early age after first feeding is delayed, because delaying first feeding means depriving the newly-hatched fry from availing a diet high in protein, lipids, vitamins and minerals and low in carbohydrates for developing muscles, internal organs and bones. Studying digestion and energy utilization after first feeding is delayed is therefore helpful in measuring the quality of food fish.

From the moment of hatching, teleost fry must develop efficient structures and mechanisms for capturing, absorbing and digesting food. As in other freshwater and marine species, *O. niloticus* fry at the endotrophic phase possess limited endogenous energetic and nutritive reserves (Calzada *et al.*, 1998). Major morphological and functional changes must occur for the fry to survive one of the most critical stages of their life span: that is, when their yolk reserves are almost totally absorbed.

At the start of exogenous feeding, the tilapia gut appeared as an undifferentiated tube. At two dph, the esophagus was already fully developed including the presence of mucus-secreting cells. Also, gastric glands were already visible in the stomach indicating the genesis of chemical digestion. At six dph, pyloric caeca appeared as slight evaginations of the anterior intestinal epithelium. This represented the last major morphological change of the intestine (Hamlin *et al.*, 2000). Stroband and Dabrowski (1981) as cited by Hamlin *et al.* (2000) referred to the appearance of gastric glands and pyloric caeca, as well as a functional stomach, as indications of transformation from fry to juvenile stages.

The differentiation of structures in the fry which resulted to a digestive system similar to the adult began at two to ten dph in Nile tilapia. For fish starved for eight days, this development was seen to be delayed for two days. This delay in development at a young age may have affected digestion efficiency. At the adult stage, however, such effects were no longer felt. Thus, it may be reasonably concluded that in the adult Nile tilapia, digestion and assimilation were uniform for both unstarved and starved groups.

There were some obvious factors which highlighted the effects of delayed initial feeding. These factors included the abundance of goblet cells in the anterior and posterior intestine of 90-150 dph unstarved fish.

In the unstarved samples, more goblet cells were found. The mucous layer coating the anterior and posterior intestine is secreted primarily by these cells, which were interspersed among the enterocytes covering the mucosal folds. Some of the functions of goblet cell mucus common to all the regions of the digestive tract include trapping sloughed cells and undigested particles and facilitating their clearance, and holding IgA that binds to and inactivates microorganisms. The abundant mucous granules seen in the unstarved fish may not be due to a higher activity in terms of functions just mentioned; instead, it may be attributed to the abundance of raw materials present for its formation.

The basic structure and path of synthesis and secretion of all mucins are similar. Mucins are large glycoproteins that contain greater than 50% by weight carbohydrate arranged as chains of oligosaccharides linked to peptide backbones. Mucin polymers are continually packed into secretory granules on the trans surface of the Golgi and transported to the apical region of the cell. Within seconds after exocytosis, the released mucins are hydrated, resulting in a 600-fold expansion in volume. This addition of water, along with the association of polymers with each other, creates an unstirred layer of mucous gel covering the cell surface (Cross and Mercer, 1993). This efficient process was seen in unstarved fish where the continuous ingestion of food was not delayed or disrupted at any time of development.

Continuing the investigation of the effects of delayed first feeding on Nile tilapia from the fry to the adult stage may provide some explanations for the significant differences in length and weight of unstarved and starved fish, despite similarities in the histology of the digestive tract and skeletal muscles. Further investigation using several biochemical indices like for instance, the amount of plasma glucose, lactate and acetoacetate, brain glycogen and acetoacetate, and liver acetoacetate, glycogen and lactate which, had been investigated in other fish species could be useful for this purpose. It may also be useful to study several hormones in relation to delay in first feeding. Finally, attention must also be focused on some growth and thyroid hormones, and alterations in circulating hormone concentration related to biochemical indices.

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