Alexandria Journal of Veterinary Sciences

www.alexjvs.com

AJVS. Vol. 54 (1): 57-69. Jul. 2017

DOI: 10.5455/ajvs.271187

Insight into The Role of Alternative Starvation-Refeeding Regime in *Oreochromis niloticus* Fingerlings Performance

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Key words:
Aeromonas hydrophila, Fingerlings, *Oreochromis niloticus*, Refeeding, Starvation

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Abstract

Knowledge of how fish respond to starvation periods could provide a basis for improved nutrition, rearing, disease control and thereby help to optimize *Oreochromis niloticus* culture. In the current study, the effect of alternative starvation-refeeding regime was monitored on some growth, hematological and serum biochemical parameters in Nile tilapia fingerlings; followed by checking the role of these feeding schedules in combating the disease challenge. The study was performed in glass aquariums at the aquaculture research unit, Sakha, Kafr El-Sheikh governorate, Egypt using 375 *Oreochromis niloticus* fingerlings weighing 29±0.5 gram. Fingerlings were subjected to different starvation periods (7, 10, 14, 21 days), followed by 30 days of refeeding on a commercial fish ration (25% protein). Fish growth parameters, hematological and serum biochemical parameters were recorded before starvation, before refeeding and after 30 days of refeeding. The experimental challenge was carried out to determine the effect of different starvation intervals followed by refeeding regimes on the immunity of *O. niloticus* fingerlings; against bacterial infection with *Aeromonas hydrophila* through recording mortality rates and the histopathological finding.

The collected results revealed that fingerlings can recover all the hematological and serum biochemical parameter values of all food deprived groups close to the normal level of full-fed control fish; concluding that *O. niloticus* can easily be cultured on suitable economic short-time feeding regime with subsequent refeeding in alternate days without any significant differences in fish size and final production (reducing the food amount and cost required for production cycle). Besides, short-term starvation prior to a bacterial infection followed by subsequent refeeding could promote the defense mechanism of the fish to fight against *Aeromonas hydrophila.*

1. INTRODUCTION

Nile tilapia *(Oreochromis niloticus)* is considered as the most important food fishes in the world due to many characteristics; the ease of its breeding in captivity and its growing at the wide varieties of water conditions (Biswas *et al*., 2005). To cope with the increased demands of Nile Tilapia especially in developing countries, the intensive culture of tilapia under controlled management systems is widely expanding; where tilapia can withstand a wide range of water temperature, dissolved oxygen (DO), salinity, pH and light intensity (El-Sayed and Kawanna, 2004).

One of the most important problems in fish production is to obtain a good balance between fish growth and food consumption. So, it is equally important to know both the growth and the nutritional needs of fish and to know the best feeding strategy for species (Jobling, 1993; Goddard, 1996; Jørgensen *et al.*, 1996; Gokcek *et al.*, 2008). Although there is a positive relationship between growth rate and feeding frequency (Riche *et al.*, 2004; Riche, 2008); Crampton (1991) revealed that it might not be necessary for daily feed to obtain maximum growth rates. On the other side, De Silva and Anderson (1995) investigated that beyond a certain level, excessive feeding has no influence on growth and may result in poor growth.

Naturally, fish may undergo intervals of poor food supply during their life-cycle due to seasonal changes in food availability or migration (Sridee and Boonanuntanasarn, 2012). Fish, unlike mammals, can survive over prolonged periods of starvation. Therefore, fasting is used as a technique to reduce flesh lipid content to improve product quality (Rasmussen *et al.*, 2000). Decreasing the food is a strategy to decrease feed costs, which is a vital step in the intensive fish culture management (Lovell, 1998). It is necessary to determine the fish response to different feeding regimes to detect the optimal interval of feed deprivation (Najafi *et al.*, 2015).

The effect of different starvation periods and subsequent refeeding on growth, hematological and serum biochemical parameters were checked in *Oreochromis niloticus* monosex fries (Moustafa and Abd El-Kader, 2017). The results revealed that prolonged starvation had significant negative effects on growth, hematological and serum biochemical parameters and they concluded that starvation-refeeding regimes in *Oreochromis niloticus* fries is of no value and has no economic profit.

The present study was conducted to: a) provide an economic suitable feeding protocol minimizing fish feed amount and subsequently, the total feeding cost. B) Provide a new insight into the beneficial role of alternative starvation-refeeding regimes preferably short-time starvation prior to bacterial infection to obtain better capability to battle against *Aeromonas hydrophila* infection in cultured *Oreochromis niloticus* fingerlings.

2. MATERIALS & METHODS

The current study was carried out in fish research center, Aquaculture research Unit Sakha, Kafr El-Sheikh governorate, Egypt for a period of about two months.

2.1. Ethical approval

Animal ethics committee, faculty of veterinary medicine, Kafr El-Sheikh University, Egypt, approved the protocol and conducting of the study.

2.2. Experimental design:

The experiment was performed using 375 Nile Tiapia (*Oreochromis niloticus*) fingerlings weighing 29±0.5 gram; came from a local private commercial fish farm at Kafr El-Sheikh governorate, Egypt. Fish were kept in a fiberglass tank with 1000 L capacity, for 2 weeks for accommodation; during which fish were supplied with maintenance ration.

After the accommodation period, 375 fingerlings were divided into 5 groups, 75 fish/each group (three replicates of 25 fish / each group). Fishes were put in glass aquariums (60 x 60 x100 cm). (25 fish/ aquarium) that were equipped with aeration system.

To investigate the effects of fasting and refeeding on blood hematological and serum biochemical parameters, Groups 1-4 were starved for 7, 10, 14, 21 days respectively, while group 5 was kept as a control (without starvation). After the starvation, fish were refed for 30 days on a commercial fish ration of 25% protein (manufactured by ALEKHWA feed factory; a local Egyptian fish feed factory), with a feeding rate of 3 % total stocking biomass / aquarium, applied twice a day (at 8:00 am & 15:00 pm). Total duration of the experiment was around 2 months.

Fish growth parameters, hematological and serum biochemical parameters were recorded in fingerlings 3 times/ group (before starvation, before refeeding and after 30 days of refeeding).

Mortality rates were recorded in all groups along the experiment period.

2.3. Determination of fish growth parameters:

The fish were totally weighted (25 fish/each replicate/ group) using an electronic balance

Total weight gain (TWG) (g/fish) = final body weight – initial body weight

Specific growth rate (SGR) = [(Ln W2 – Ln W1)/ T] x 100

Where: Ln = the natural log, W2 = final weight at certain period (g),

W1 = initial weight at the same period (g) and T = experimental period (in days).

2.4. Hematological investigation:

Blood samples were taken from the caudal vein along the experiment according the method described by Feldman *et al.*, (2000). 12 fish from each group (4 fish/each replicate) were randomly sampled. Due to the small fish size, blood samples were collected from 3-4 fish and pooled according to (Urbinate & Carneiro, 2006). Blood samples were divided into two parts; one part for hematological parameters and the other part for serum biochemical parameters.

Red blood cells (RBCs) and White Blood cells (WBCs) were counted immediately with a hemocytometer after dilution with Natt & Herrick's solution (Houston, 1990). For hematocrit (Hct) determination capillary tubes were filled with blood and spun in a hematocrit centrifuge at 12,000 g for 5 min and hematocrit values were read as percentage (Karimi *et al.,* 2013). For hemoglobin (Hb) assay Drabkin's solution was added to the blood and then solution was centrifuged (3500 g for 6 min) to remove interferents, afterwards blood hemoglobin concentration (Hb) was determined with a spectrophotometer (Model RA 1000, Technicon Corporation, USA) at 540 NM using the method of Blaxhall and Daisley (1973). The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated (Houston 1990) according to the following formulas:

MCV (fl) = 10 × (PCV per RBC)
MCH (pg) = 10 × (Hb per RBC)
MCHV (%) = 100 × (Hb per PCV)

2.5. Lysozyme concentrations assays:

The serum lysozyme activity was conducted in accordance with the method of Sankaran & Gurnani (1972) which is based on the ability of lysozyme to destroy the peptidoglycan layer of Gram-positive bacteria (*Micrococcus lysodeikticus*). *Micrococcus lysodeikticus* lyophilized cells (Sigma, USA) were suspended in phosphate buffer (pH 7.2) at a concentration of 0.25 mg/ml and used as a substrate solution. 200 µl of serum, diluted with an equal volume of phosphate buffered saline (PBS), were added to 1.3 ml of the substrate solution at 25 ºC and measured immediately at 450 NM. After 30 minutes incubation in a humidified environment at 25 ºC, the optical density was again measured. A lyophilized hen egg-white lysozyme (Sigma, USA) was used to develop the standard curve Demers and Bayne (1997).

2.6. Blood serum biochemical analysis:

Serum total proteins were determined according to Doymas *et al.*, (1981) at the wave length 540 NM, Serum albumin was estimated colorimetrically at wave length 550 NM according to Dumas and Biggs (1972). Globulins content were calculated mathematicaly. Activities of aspartate amninotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically at the wave length 540 NM, according to Reitman and Frankel (1957). Glucose level (mg/100 ml) was determined using glucose enzymatic PAP kits obtained from Bio-Merieux (France) (Trinder, 1969).

2.7. Statistical analysis

Statistical analysis was performed using SPSS software version 16.0, Chicago, IL. Significant difference was determined at a probability level of ($P < 0.05$).

2.8. The experimental Challenge with *Aeromonas hydrophila*

The data about Aeromonas strain identification was obtained by personal communication.

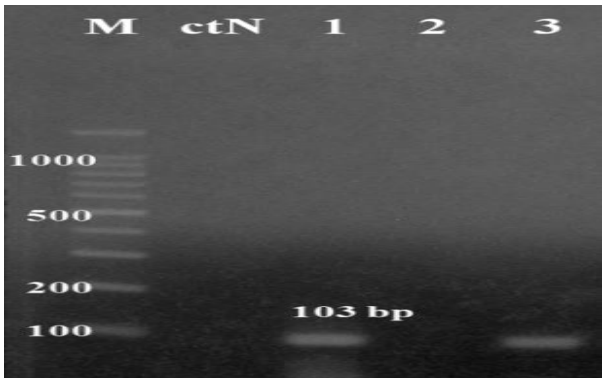
2.8.1. Molecular identification by PCR

DNA Extraction was performed using QIA amp kit (QIAamp: Qiagen inc., USA). Accurately, one ml of the young cell suspension was centrifuged at 10000 g for 10 min at 4oC and the cell pellet was mixed with 600 µl of guanidine hydrochloride buffer (pH 8.0), incubated at room temperature for 30 min and again centrifuged at 10000 x g for 10 min at 4ºC. From that, 500 µl of the supernatant was transferred to another tube and mixed with 100% ice cold ethanol and centrifuged at 13000 x g for 20 min at 4ºC. The supernatant was discarded and the pellet was washed with 95% and 90% ethanol, respectively, followed by centrifugation at 10000 g for 10 min at 4ºC. The pellet was then re-suspended in 50 µl of molecular grade water and then stored at -20ºC until used as PCR template (Haldar *et al.,* 2005).

2.8.2. Primer sequences used for PCR identification system of *A. hydrophila*: Application of PCR for identification of *A. hydrophila* by 16S rRNA was performed essentially by using Primers (Pharmacia Biotech) as shown below.

Primer sequences of A. hydrophila used for PCR identification

Target	Primer Name	Oligonucleotide Sequence (5-3)	Product size(bp)	Reference
Aeromonas hydrophila	16SrRNA-F 16SrRNA-R	5/ GGCCTTGCGCGATTGTATAT /3 5/ GTGGCGGATCATCTTCTCAGA / 3	103 bp	**Trakhna *et al.* (2009)**



Agarose gel electrophoresis of PCR of 16S rDNA(103 bp) for characterization of *A. hydrophila*. Lane M: 100 bp ladder as molecular size DNA marker. ctN: control negative for *A. hydrophila*. Lane1: control positive for *A. hydrophila*. Lane2:negative for *A. hydrophila*. Lane3: positive for *A. hydrophila*

2.9. Histopathological examination

Specimens from liver and kidney of experimentally infected fishes were taken. Specimens were fixed immediately in 10% neutral buffered formalin, dehydrated and embedded in paraffin blocks. Paraffin blocks were sectioned at 4-5 µm thickness and stained with Hematoxilin & Eosin (H&E) and examined under light microscope (Leica) using ×200 and ×400 magnification power according to Bancroft and Gamble (2007).

3. RESULTS & DISCUSSION

In the present study, the effect of starvation and re-feeding was monitored on some growth, hematological and serum biochemical parameters, then monitoring the role of these feeding schedules in combating the disease challenge, Table (1-3). Knowledge of how fish respond to starvation periods in this study could provide a basis for improved nutrition, rearing, disease control and thereby help to optimize *Oreochromis niloticus* culture.

There are no mortalities were recorded among the different groups during the starvation or refeeding periods; indicating that *Oreochromis niloticus* fingerlings can well tolerate all tested intervals of starvation in the experiment

There was a significant decrease in RBCs, Hb and PCV values in all starved groups after different starvation periods (7,10,14& 21 days). However, after 30 days of refeeding, fish restored the normal level of RBCs, Hb and PCV in all starved groups, especially that starved for (7&10 days) which increased than the control group levels at the end of refeeding period. Groups that starved for (14 & 21 days) restored the normal control levels but not exceed it.

The current experiment revealed significant decrease in RBCs count in all starved groups at (7, 10, 14, 21 days). The results are in accordance with that reported in *Mesopotamichthys sharpeyi* after 16-days of food deprivation (Najafi *et al.*, 2015), tiger fish (*Hoplias malabaricus*) after 240 days of fasting (Rios *et al.*, 2005), in *Prochilodus lineatus* after 5 weeks of fasting (Rios *et al.*, 2011), and in the Marble gopy fish (*Oxyeleotris Marmorata*) after 4 weeks of fasting (Sridee and Boonanuntanasarn, 2012). However, the

result disagree with Shoemaker *et al.*, 2003 who revealed that there was no significant difference in RBCs count after 2 and 4 weeks of starvation in Channel catfish (*Ictaluras punctatus*).

Moreover, Hb and PCV titer were decreased during starvation in the current experiment. There are conflicting results about effect of fasting on the hemoglobin and hematocrit values. Some studies showed an increase in the hematocrit content in response to fasting periods of 90, 145 and 47 days in the Japanese eel (*Anguilla japonica*), the borbut and European eel, respectively (Sano 1962, Smirnova 1965, Johansson-Sjobeck *et al*., 1975). The increase in the hematocrit was also reported in starved *Beluga sturgeon* by Morshedi *et al* (2011), whereas a decrease in the hematocrit and hemoglobin values were observed in starved carp (*Cyprinus carpio*) and rainbow trout, respectively (Kawatsa, 1966). Reduction in the hematocrit content in Pike (*Esox lucius*), *Hoplias Malabaricus* and *Siberian sturgeon* (Kristoffersson and Broberg, 1971, Rios *et al.,* 2005, Morshedi *et al.*, 2011). On the other hand, Larsson and Lewander (1973) stated that fasting did not affect the hematocrit and hemoglobin content in the starved European eel for 150 days. Furthermore, studies conducted on European seabass (*Dicentrarchus labrax*) and black spot sea bream (*Pagellus bogaraveo*) showed that fasting does not affect the hematocrit and hemoglobin content (Caruso *et al*., 2011).

In the present study, MCV index during periods of fasting and re-feeding remained unchanged. The result is similar to that obtained in starved *Hoplias malabaricus* (Rios *et al.,* 2005), starved *Juvenile Siberian sturgeon* and *Beluga sturgeon* (Morshedi *et al.,* 2011), while Sridee and Boonanuntanasarn (2012) found an increase in the MCV index in starved *Oxyeleotris marmorata*.

Inconsistent with the present results, there was a remarkable increase in MCH and MCHC indices in *Malabaricus Hoplias* after 180 days of starvation which may be attributed to the decrease in RBC volume (Rios *et al.,* 2005). However, Sridee and Boonanuntanasarn, (2012) reported a decrease in MCHC in starved *Oxyeleotris marmorata*. Nevertheless, Morshedi *et al*., (2011) revealed no significant change in the MCH index of starved *Siberian sturgeon* and *Beluga sturgeon*).

WBCs count was not affected in all groups after starvation, but their levels increased after 30 days of refeeding especially groups starved for (7,10 then that starved for 14 days).

From the current obtained results, fasting caused no significant changes in the number of WBCs, while the count of these cells significantly increased after refeeding period. The result is partially similar to that reported in gilthead sea bream (*Sparus aurata*) (Sala-Rabanal *et al.,* 2003). However, WBCs count was reported to be declined in burbot fish (*Lota lota*) (Smirnova, 1965), Rainbow trout (*O. mykiss*) (Kawatsa, 1966), European eel (*Anguilla anguilla*) (Johansson-Sjobeck *et al*., 1975) and in juvenile Siberian sturgeon (*Acipenser baerii*) and Beluga sturgeon (*Huso huso*) (Morshedi *et al*., 2011).

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were slightly increased in all starved groups when compared with the control group levels, then restored to the normal control levels after 30 days of refeeding in all starved groups. The results are in accordance with Yarmohammadi *et al*., (2015). However, Cho *(2009)* and *Cho et al.,* (2006), Cho & Cho (2009) reported no significant differences in ALT and AST levels of olive flounder subjected to 8 weeks fasting between fed and starved groups; but, was increasingly significant in olive flounder after 4 weeks starvation. This may be attributed to that the environmental and physiological conditions can affect AST and ALT levels in response to starvation period.

There was a significant decrease in the Total proteins, Albumin, Globulins and Glucose levels in all starved groups than that of the control group then restored to the control level after 30 days of refeeding for all starved groups except for the long starved group (21 days) which was slightly close to the control group. The total proteins, Albumin, Globulins and Glucose of groups that starved for (7&10 days) recorded to exceed the control level.

The low serum total protein level may be attributed to using of blood protein in gluconeogenesis during the starvation time, and as a fuel source (Friedrich & Stepaniswska, 2001) and Costa *et al.* (2011).

Plasma glucose level was significantly lower in the starved groups than those of control fish, indicating that *O. niloticus* unable to maintain plasma

glucose levels for starvation; the result is in accordance with Power *et al.* (2000) in sea bream (Sparus aurata), De Pedro *et al.* (2003) in tench (Tinca tinca), Perez-Jimenez *et al.* (2007, 2012) in European sea bass (*Dicentrarchus labrax*) and dentex (*Dentex dentex*) and Ceinos *et al.* (2008) in the rainbow trout (*Oncorhynchus mykiss*) have observed a reduction in plasma glucose levels during starvation periods. However, other studies have reported constant plasma glucose levels during the different periods of starvation (Hochachka & Sinclair 1962; Barcellos *et al.* 2010; Caruso *et al.*, 2011; Caruso *et al.*, 2012). The differences in results between studies may be due to differences between species, nutritional status, different tissues for lipid storage, and the duration and severity of starvation (Navarro & Gutiérrez 1995; Pėrez-Jimenez *et al*., 2007; Caruso *et al*., 2010).

There are no significant changes in the lysozyme concentrations except in the prolonged starvation period group (21 days) which showed increased levels; that may be attributed to be a part of immune system response to starvation stress which may be similar to those reported by Demers and Bayne (1997). However, some other factors might have a considerable impact on the test results as the choice of season, photoperiod, temperature and age of the fish (Navarro and Gutierrez, 1995). In contrast to the present result, Caruso *et al*. (2011) found that blood lysozyme was not affected after 31 days fasting in *Dicentrarchus labrax* but in *Pagellus bogaraveo* its value was lower in starved fish. It was also affected in blood and kidney of European eel after 58 days of fasting (Caruso *et al.*, 2011). Eslamloo *et al*. (2017) reported that plasma lysozyme activity significantly increased in fish starved for 1 week, compared with the control.

After 30 days of refeeding for all groups, groups which starved for (7 and 10 days) not only restored the normal control level of the body weight but also exceeded it. The group starved for 14 days restored to the control level, but the long starved group (21 days) couldn't restore even the normal levels of the control group; showed a partial compensatory growth Table (3), which may be due to the fish became too weak to feed even when food became available. The result is in accordance with those reported by Tian and Qin, (2004); where highest restricted fishes can't compensate the growth even after feeding until they were satiated for 5 weeks.

Nikki *et al.* (2004) reported that there is a strong relationship between body weight gain and feed consumption. Full compensatory growth in starved fish during their refeeding periods depend mainly on higher feed consumption, however, no compensation in a restricted feeding regime (Eroldoğan *et al.*, 2008). Partial growth compensation occurs following different starving intervals in gilthead sea breams (*Sparus aurata*) has been reported (Eroldoğan *et al.*, 2006). Compensatory growth was reported in Hybrid sea basses exposed to 2 weeks starvation, Red sea breams exposed to one, two or three weeks of starvation showed full compensatory growth at the end of a 9 week study (Oh *et al.*, 2007) and olive flounders exposed to two weeks starvation and 6 weeks refeeding showed better improvement as compared with a continuous feeding group (Cho and Cho, 2009). On the other hand, no compensatory growth was recorded in gilthead sea breams on 60 day restricted feeding regimes (Bavcevic *et al.*, 2010) and in another study, sea breams exposed to different starvation periods did not show any compensatory growth during a 10 week cycle (Peres *et al.*, 2011). Some similar partial compensation results have been obtained in previous studies carried out on different fish species and feeding models (Jobling, 1993; Hayward *et al.*, 2000; Ali and Jauncey, 2004; Wang *et al.*, 2005, 2009; Eroldoğan *et al.*, 2006, 2008; Mattila *et al.*, 2009; Liu *et al.*, 2011). Additionally, full compensation (Kim and Lovell, 1995; Gaylord and Gatlin, 2001; Zhu *et al.*, 2001, 2005; Tian and Qin, 2003, 2004; Nikki *et al.*, 2004; Oh *et al.*, 2007) and over compensation levels have also been obtained (Hayward *et al.*, 1997; Turano *et al.*, 2007). Yarmohammadi *et al.*, (2015) Findings showed that the juvenile Persian sturgeon starved for 1,2, and 4 weeks showed complete compensatory growth while fish starved for 3 weeks showed partial compensatory growth. This means that the compensatory response depends on the length of feed deprivation.

It has been reported that during starvation, the lipid composition in the fish decreased. In many studies, the growth of fish during compensation growth was found to be faster. In compensatory growth, it has been observed that somatic growth parameters and lipid levels return to their prior levels compared to those during starvation periods (Ali *et al.*, 2003).

Starved fishes have been reported to have higher SGR and feed intake compared to a control group during the refeeding period (Nikki *et al.,* 2004; Turano *et al.,* 2007; Wang *et al.,* 2009). After a long period of restricted feeding (21 days) Tilapia final weight was not significantly different. This result indicates a partial compensatory growth, since the fish deprived of food did not reach the same weight of the continuously fed fish.

Compensatory growth usually precedes a period of food restriction (Dobson and Holmes, 1984; Hayward *et al.,* 1997). Fish subjected to previous nutritional restriction may partially (Miglavs and Jobling, 1989; Jobling, 1993) or completely (Johansen *et al.,* 2001; Maclean and Metcalfe, 2001) regain the weight and can match those who have not been subjected to restriction (Dobson and Holmes, 1984; Kim and Lovell, 1995). By applying this phenomenon in tilapia, the growth rate and feed efficiency may be increased (Wang *et al.,* 2000, 2009; Gao *et al.,* 2015). Reimers *et al.* (1993) found that second sea winter salmon, deprived wholly of their food supplies for two months, were able to recover body weight with faster growth than the unrestricted controls in month after starvation period despite a similar ration was supplied.

The results displayed in table (4) showed that the survival rate for the groups subjected to bacterial infection with *Aeromonas hydrophila* was 70%, 60%, 40% and 15% in groups, previously starved for 7, 10, 14 and 21 days, respectively; however, the positive control group showed a survival rate of 30%. The high survival rate in short time starved groups may confirm the role of alternating starvation and refeeding schedule, preferably short-term starvation prior to an infection, to obtain better capability to battle against bacterial infection in fish. The result is similar to those reported by Mohapatra *et al.* (2017) in red sea bream infected with *Edwardsiella tarda*.

Histopathological examination of starved fishes exposed to bacterial infection with *Aeromonas hydrophila* revealed the marked congestion and vacuolation of renal tubular epithelium of the kidney (Fig. 1) and liver showed marked vacuolation of the hepatocytes and perivascular mononuclear cells and heterophils infiltrations in the prolonged starved group starved for 21 days, (Fig. 2) as well as loss of hepatic architecture together with massive heterophils infiltrations in the positive control group (Fig. 3) and atrophy and vacuolar degeneration of the hepatocytes together with area of necrosis surrounded with mononuclear cells and heterophils infiltrations (Fig. 4). But the short term fasted groups starved for 7,10 and 14 days showed mild histopathological changes; Spleen showed mild depletion of the lymphocytes as well as depletion and small size melanomacrophage centers (Fig. 5).

4. CONCLUSION

From the collected results in the current study, starvation of Nile Tilapia fingerlings for different periods (7, 10, 14, 21 days) followed by refeeding on a commercial fish diet (25% protein) for 30 days revealed that, fingerlings can recover all the hematological and serum biochemical parameter values of all food deprived groups close to the normal level of full-fed control fish. Therefore, it could be concluded that *Oreochromis niloticus* can easily be cultured on suitable economic short-time feeding regime with subsequent refeeding in alternate days without any significant differences in fish size and final production. This will reduce tilapia production cost to a great extent. Moreover, it has a great profit in fish culture, both for the rural small scale and commercial tilapia farmers.

Besides, short-term starvation prior to a bacterial infection followed by subsequent refeeding promoted better physiological and immunological functioning in Nile Tilapia fingerlings; and may be a trigger for the defense mechanism in the fish to fight against *Aeromonas hydrophila*. Thus, these alternating starvation and refeeding regime, at the appearance of an infection, can be adopted at the farm level as a counter measure to protect the fish from bacterial invasion

5. Conflict of interests

The authors declare that there is no conflict of interests

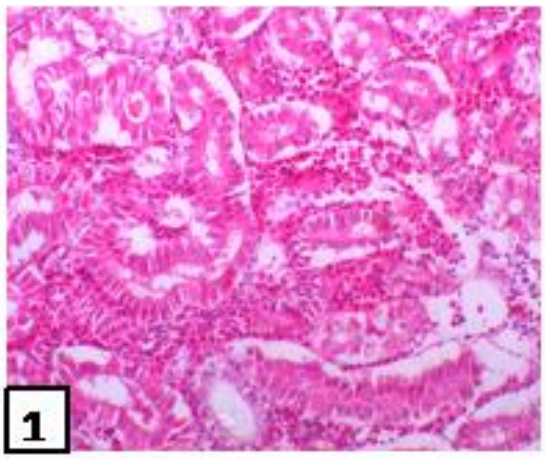


Figure 1: Kidney showing marked congestion and vacuolation of renal tubular epithelium. H&E. X 200.

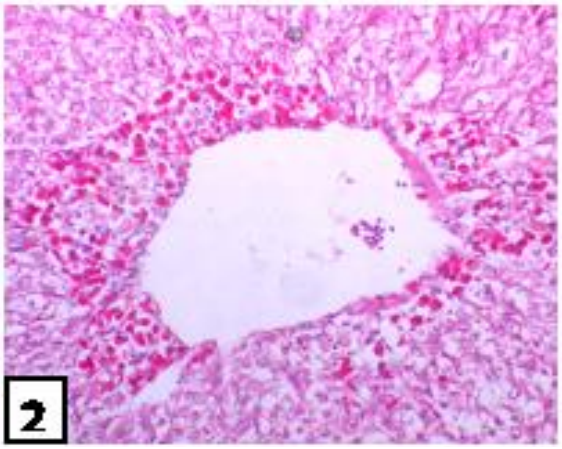


Figure 2: Liver showing marked vacuolation of the hepatocytes and perivascular mononuclear cells and heterophils infiltrations. H&E. X 200.

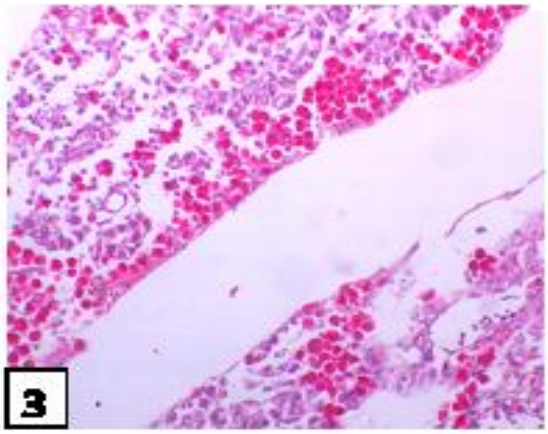


Figure 3: Liver showing massive heterophils infiltrations and loss of hepatic architecture. H&E. X200

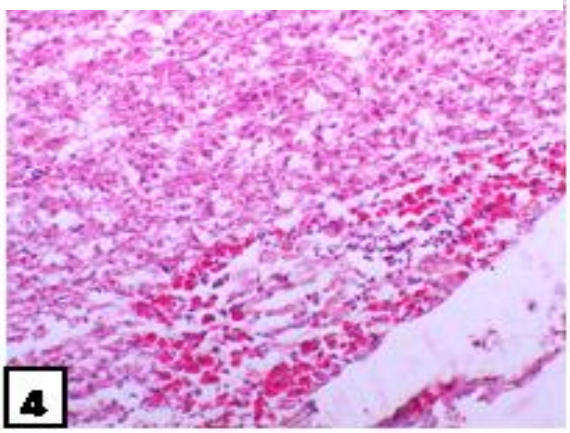


Figure 4: Liver showing atrophy and vacuolar degeneration of the hepatocytes together with area of necrosis surrounded with mononuclear cells and heterophils infiltrations. H&E. X 200.

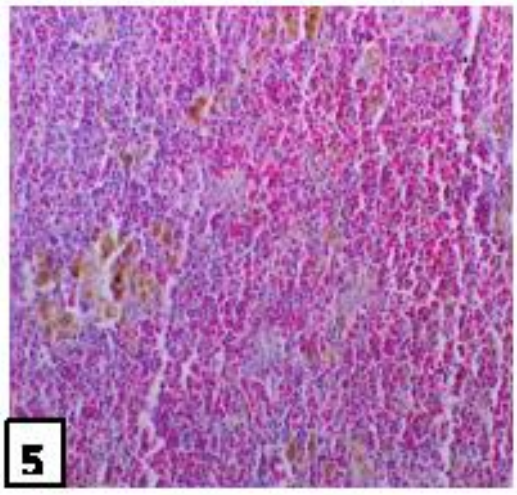


Figure 5: Spleen showing mild depletion of the lymphocytes as well as depletion and small size melanomacrophage centers. H&E. X 200

Table 1: Effect of starvation-refeeding regime on haematological parameters in *Oreochromis niloticus* fingerlings

Day of sampling	Treated groups	RBCs ($x10^{6}$ / mm^{3})	Hb (g/100ml)	PCV (%)	MCV (μm^{3} /cell)	MCH (pg/cell)	MCHC %	WBCs ($x10^{3}$ /mm^{3}
Zero day	G1	2.16±0.01a	7.25±0.05a	21.15±0.16ab	97.69±0.11	33.48±0.09	34.28±0.1	43.11±0.11b
	G2	2.15±0.01a	7.05±0.05b	21.15±0.12ab	98.14±0.1	32.71±0.1	33.33±0.1	42.89±0.2e
	G3	2.13±0.05b	7.15±0.01ab	21.05±0.09b	98.59±0.09	33.48±0.12	33.97±0.14	43.00±0.1d
	G4	2.16±0.02a	7.25±0.03a	21.25±0.11a	98.15±0.14	33.48±0.05	34.11±0.09	43.25±0.05a
	G5	2.13±0.01b	7.15±0.07ab	21.00±0.18b	98.36±0.2	33.49±0.05	34.05±0.09	43.05±0.21c
After starvation	G1	1.95±0.03b	6.46±0.05b	18.05±0.09b	92.33±0.21b	33.07±0.11b	35.81±0.1a	43.37±0.11e
	G2	1.81±0.05c	6.07±0.05c	17.87±0.21c	98.32±0.05a	34.03±0.12a	34.62±0.09b	44.18±0.09a
	G3	1.81±0.01c	6.09±0.05c	17.87±0.11c	98.48±0.09a	33.58±0.1ab	34.10±0.1c	43.50±0.1c
	G4	1.78±0.01d	5.97±0.03d	17.66±0.1d	98.96±0.1a	33.47±0.08ab	33.82±0.13c	43.39±0.1d
	G5	2.31±0.01a	7.75±0.04a	21.65±0.11a	93.52±0.1b	33.48±0.09ab	35.79±0.09a	43.62±0.05b
End of experiment	G1	2.68±0.01a	8.55±0.02a	25.05±0.1a	93.47±0.09b	31.92±0.21a	34.15±0.05a	53.11±0.09a
	G2	2.56±0.05b	8.31±0.01b	24.00±0.1b	93.57±0.11a	32.41±0.09c	34.65±0.09b	51.27±0.1b
	G3	2.43±0.02d	8.11±0.05c	24.05±0.11b	98.77±0.05b	33.33±0.05b	33.74±0.12c	50.18±0.2c
	G4	2.41±0.04e	8.00±0.01d	23.00±0.2d	98.76±0.05c	33.74±0.12e	34.17±0.21d	45.18±0.09e
	G5	2.46±0.05c	8.15±0.01c	23.85±0.09c	93.31±0.12a	32.45±0.14d	34.78±0.05b	45.79±0.05d

For each day of sampling: Treatments means within the same column of different litters are significantly different at ($P<0.05$)

Table 2: Effect of starvation-refeeding regime on serum biochemical parameters in *Oreochromis niloticus* fingerlinges

Day of sampling	Treated groups	ALT (U/l)	AST (U/I)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Glucose (mg/dl)	lysozyme (u/ml)
Zero day	G1	6.69±0.04d	78.61±0.21d	5.34±0.05b	3.00±0.01b	2.34±0.01a	24.11±0.2b	30.00±0.11a
	G2	6.72±0.05c	79.64±0.24c	5.36±0.05a	3.10±0.02ab	2.26±0.01ab	23.19±0.11c	29.66±0.09d
	G3	6.76±0.05a	79.80±0.11b	5.33±0.01b	2.99±0.01b	2.34±0.02a	24.17±0.12a	29.18±0.09e
	G4	6.700±0.05d	77.81±0.12e	5.36±0.01a	3.15±0.02a	2.21±0.01b	23.15±0.09d	29.75±0.05b
	G5	6.74±0.05b	79.81±0.12a	5.35±0.01a	3.05±0.01ab	2.30±0.01ab	23.15±0.23d	29.72±0.12c
After starvation	G1	7.13±0.01d	80.11±0.11d	4.51±0.01b	2.45±0.05b	2.05±0.01b	21.29±0.11b	29.97±0.1e
	G2	7.41±0.2c	83.29±0.12c	4.35±0.01c	2.31±0.01c	2.04±0.02b	20.17±0.12c	30.25±0.09c
	G3	7.82±0.08b	87.09±0.18b	4.13±0.05d	2.21±0.01d	1.91±0.01c	19.77±0.09d	30.75±0.21b
	G4	7.97±0.08a	89.18±0.15a	4.02±0.05e	2.11±0.02e	1.91±0.01c	18.44±0.11e	45.13±0.14a
	G5	6.62±0.01e	79.62±0.2e	5.41±0.05a	3.20±0.01a	2.21±0.01a	29.12±0.09a	30.15±0.11d
End of experiment	G1	6.70±0.1c	78.11±0.12e	5.72±0.01b	3.30±0.01b	2.42±0.02a	29.95±0.19a	30.87±0.14c
	G2	6.54±0.05e	79.04±0.17c	5.75±0.01a	3.40±0.02a	2.34±0.01c	29.77±0.11b	30.90±0.09b
	G3	6.74±0.08b	79.60±0.09b	5.69±0.05d	3.30±0.01b	2.39±0.01b	29.53±0.14c	30.82±0.09d
	G4	6.75±0.05a	80.13±0.11a	5.42±0.02e	3.20±0.01c	2.21±0.02e	27.87±0.09d	35.66±0.1a
	G5	6.69±0.01d	78.98±0.12d	5.71±0.05c	3.40±0.01a	2.30±0.01d	29.76±0.21b	30.29±0.13e

For each day of sampling: Treatments means within the same column of different litters are significantly different at ($P<0.05$)

Table 3: Effect of starvation-refeeding regime on fish growth parameters in *Oreochromis niloticus* fingerlinges

Treatments	Initial Weight (g)	Final Weight (g)	Total Weight Gain(g)	SGR (%/day)
G1	29.80±0.1b	49.15±0.21a	19.34±0.09a	0.587±0.01a
G2	30.05±0.09a	48.78±0.2b	18.73±0.1b	0.526±0.01c
G3	28.85±0.12d	42.50±0.09d	13.65±0.11c	0.382±0.01d
G4	29.65±0.13c	39.98±0.12e	10.33±0.05d	0.254±0.02e
G5	29.60±0.1c	43.12±0.24c	13.52±0.09c	0.544±0.01b

Treatments means within the same column of different litters are significantly different at ($P< 0.05$).

Table 4: Effect of starvation-refeeding regime on the mortality rate of *Oreochromis niloticus* fingerlinges challenged with *A.hydrophila*

Mortality	G1	G2	G3	G4	G5 (+ve control)	G6 (-ve Control)
After week	3	3	5	7	6	0
After 2 week	7(3+4)	8(3+5)	12(5+7)	17(7+10)	14(6+8)	0
%	30	40	60	85	70	0

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