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Effects of different starvation intervals and refeeding on growth and some hematological parameters in *Oreochromis niloticus* Monosex fries

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Abstract
Nile tilapia (*Oreochromis niloticus*) is one of the most important food fishes allover the world. The current study was aimed to investigate the effect of different starvation periods and subsequent refeeding on growth, hematological and serum biochemical parameters in order to detect to which extent fry can tolerate starvation periods and compensate its growth during the refeeding period. This study was performed in glass aquariums at the fish research center, Sakha, Kafr El-Sheikh governorate, Egypt using 375 *Nile Tilapia* monosex fries weighing 3.5±0.5 gram. Fries were subjected to different starvation periods (4, 7, 10, 15 days), followed by refeeding on a commercial fish ration (30% protein) for 30 days. Fish growth parameters, hematological and serum biochemical parameters were recorded before starvation, before refeeding and after refeeding for 30 days. The obtained results revealed that; fries starved up to 10 days could restore all checked parameters close to the control levels. However, those fries subjected to prolonged starvation (15 days) showed significant negative effects on growth, hematological and serum biochemical parameters. It can be concluded that starvation-refeeding regimes in *Oreochromis niloticus* fries is of no value and has no economic profit.

Keywords: Fry, hematology, *Oreochromis niloticus,* refeeding, starvation

1. Introduction
Tilapia, which is a quick growing species, is considered as everyman's fish and has become a top priority in aquaculture due to their ability of using natural foods efficiently, being herbivorous in feeding nature, resistant to handling and to diseases, ease of reproduction in captivity, tolerant to a wide range of environmental conditions and can easily be bred [1].
In hatchery, delay in stocking and feeding of fry may occur when nursery containers are not available or insufficient. Partial or total food deprivation of fish fry during the transition from endogenous to exogenous feeding may seriously affect growth and/or survival rate [2]. So, the determination of the potential harmful effect would be important to fish farmers to avoid the delay in stocking and feeding of tilapia fry.
The ability of fishes to tolerate starvation differs between species. Some authors [3, 4] revealed that the majority of fishes may only tolerate several days or weeks of starvation, whereas some fishes such as European eel, *Anguilla anguilla*, are reported to survive nearly four years of starvation [5].
Increasing evidence has indicated that starvation is an important cause of poor performance in the feeding, survival, and growth of fish larvae both in nature and in aquaculture [6-9], particularly for first-feeding larvae [10, 11]. At the onset of feeding, even a short period of starvation can cause severe nutritional problems, leading to drastic mortality and morphological deformities [12]. Compensatory growth following different feeding regimes, has been reported in several fish species [13- 20].
The aim of this study is to investigate the effect of different starvation periods and subsequent refeeding on growth, hematological and serum biochemical parameters in order to detect to which extent fry can tolerate starvation periods and compensate its growth during the refeeding period.

2. Materials and Methods
The current study was carried out in fish research center, 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*) monosex Fries weighing 3.5±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 fries were divided into 5 groups, 75 fish/each group (three replicates of 25 fish / each group). Fishes were put in glass aquariums (60 × 60 × 100 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 4, 7, 10, 15 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 30% 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 60 days.
Fish growth parameters, hematological and serum biochemical parameters were recorded in Fries 3 times/ group (before starvation, before refeeding and after refeeding 30 days.).
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] × 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. 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 the described method [21]. Blood samples were divided into two parts; one part for hematological parameters and the other part for serum biochemical parameters.
The erythrocytes and leukocytes were determined according to the method described by [22]. Hemoglobin concentration was determined using the cyanomet-hemoglobin method with Drabkin's solution according to [22] and Packed cell volume determination according to [23].

2.5 Lysozyme concentrations assays
The lysozyme activity of sera of the blood of diseased fish was assayed according to the method described by some authors [24], based on the ability of lysozyme to lyses Gram positive lysozyme sensitive bacterium; *Micrococcus lysodeikticus*.

2.6 Blood serum biochemical analysis
Serum total proteins were determined according to the known method [25] at the wave length 540 nm, Serum albumin was estimated colorimetrically at wave length 550 nm according to previously described method [26]. Globulins content was calculated mathematically. Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically at the wave length 540 nm [27]. Glucose level (mg/100 ml) was determined using glucose enzymatic PAP [28] kits obtained from Bio-Merieux (France).

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 ($P<0.05$).

3. Results and Discussion
In the current study, the physiological response of *Oreochromis niloticus* monosex fries to different periods of starvation was investigated through fish growth, hematological and serum biochemical parameters as shown in Table (1-3).
Almost, no mortalities were recorded among different groups during the starvation periods. However, the mortality rate was recorded to be 20% and 30% in group 3 (10 days starvation) and in group 4 (15 days starvation), respectively. However, some other authors recorded 50% mortality at 10 days starvation in Nile Tilapia monosex fry [29].
There was a significant decrease in RBCs, Hb, PCV in all starved groups after different starvation periods (4, 7, 10, 15 days). However, after refeeding for 30 days, fries restored normal levels of RBCs, Hb, PCV in Group 1& 2 with 4& 7 days of starvation, respectively, and Group 3 (10 days of starvation) was close to the normal levels but slightly decreased. On the other hand, Group 4 (15 days of starvation) could not restore the normal levels of the control group.
The current experiment revealed reduction in RBCs count in all starved groups at (4, 7, 10, 15 days). The results are in accordance with those reported by in tiger fish [30] after 240 days of fasting, in *Prochilodus lineatus* [31] after 5 weeks of fasting, and in Marble gopy fish [32] after 4 weeks of fasting. However, the result is in contrast to previous authors [33] who reported 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 titre were decreased during starvation in the current experiment. The obtained result is similar to those obtained [34, 35] who reported a decrease in Hb and hematocrit values in starved carp (*Cyprinus carpio*) and Rainbow trout. While, the result is in contrast to some other authors [36-38].
WBCs count was not affected in all groups of experiment; the result which coincided with previous results [20].
ALT & AST were slightly increased during starvation, then restored to normal control levels after refeeding in all groups; the result coincided with previous results [20]. This result may be attributed to that aminotransferase plays a significant role in linking carbohydrate and protein. The increase in AST after starvation appears to indicate their role in protein mobilization as a substrate for gluconeogenesis. On the other hand, the result is in contrast to other researchers [39].
Total protein, albumin, globulin and glucose level were decreased during starvation periods, then restored to control levels after refeeding except in group 4 (15 days of starvation). The decrease in total protein, albumin, globulin

and glucose level during starvation may suggest that during the starvation time, blood protein was used in gluconeogenesis; the result is in agreement with previously obtained results on *C. carpio* [40] and in contrast to other results [41, 42].

In the current study, no change in lysozyme concentration was recorded in all groups during starvation and after refeeding. Similar results ware recorded by others [36]. However, lysozyme titre was increased during starvation only in group 4 (15 days of starvation) then restored to the control levels. The increase in cases of prolonged starvation period was in accordance with some authors [24, 43]. This suggests that fasting duration did not provoke stress responses to cause an increase in lysozyme activity and that the starvation is not a stressor on non-specific immunity system.

Fries body weight could be restored to the control level after refeeding for 30 days while, Group 4 (15 days of starvation) could not restore even to the control level. This might be attributed to remained alive fish became too weak to feed even when food became available.

Nile Tilapia monosex fries can restore all parameters after a short starvation period up to 10 days. More period of starvation has negative significant effect on all parameters and mortality rate increased.

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

Day of sampling	treat	RBCs ($x10^6$ / mm^3)	Hb (g/100ml)	Pcv (%)	MCV (μm^3 /cell)	MCH (pg/cell)	MCHC %	WBCs ($x10^3$ /mm3
zero	G1	2.71±0.01	8.95±0.02ab	26.50±0.11ab	97.60±0.11ab	32.96±0.1	33.78±0.11	35.01±0.09e
	G2	2.72±0.02	9.15±0.01a	26.95±0.21a	98.90±0.12ab	33.58±0.13	33.95±0.09	38.15±0.11a
	G3	2.71±0.01	8.85±0.02b	26.15±0.1b	96.32±0.2b	32.59±0.11	33.84±0.11	37.30±0.09c
	G4	2.72±0.01	9.00±0.01ab	27.00±0.12a	99.26±0.1a	33.09±0.09	33.33±0.09	37.21±0.14d
	G5	2.73±0.01	9.00±0.01ab	27.00±0.15a	98.72±0.03ab	32.91±0.05	33.33±0.04	37.40±0.5b
After starvation	G1	2.20±0.05b	7.10±0.09b	22.00±0.11b	99.77±0.12a	32.20±0.11ab	32.27±0.11c	35.12±0.11d
	G2	2.08±0.03c	6.65±0.14c	20.00±0.13c	96.15±0.14ab	31.97±0.11b	33.25±0.13bc	37.79±0.09a
	G3	1.97±0.01d	6.15±0.05d	18.00±0.11d	91.14±0.09c	31.13±0.09c	34.16±0.1ab	37.05±0.05b
	G4	1.79±0.01e	5.71±0.05e	16.50±0.21e	92.16±0.11bc	31.90±0.09bc	34.62±0.08a	36.82±0.11c
	G5	2.82±0.02a	9.30±0.1a	27.05±0.21a	95.92±0.12ab	32.98±0.09a	34.38±0.09ab	37.01±0.09b
End of experiment	G1	2.93±0.01ab	9.85±0.09b	27.85±0.11a	94.89±0.11b	33.56±0.09a	35.37±0.1ab	35.18±0.1b
	G2	2.91±0.05b	9.85±0.1b	28.00±0.09a	96.22±0.09a	33.84±0.09a	35.18±0.09b	37.67±0.11ab
	G3	2.735±0.01c	8.15±0.08c	26.00±0.14b	95.06±0.09b	29.80±0.1c	31.34±0.05d	37.21±0.09ab
	G4	2.11±0.05d	6.44±0.1d	19.00±0.08c	89.83±0.05c	30.47±0.13b	33.92±0.05c	36.76±0.1ab
	G5	2.94±0.01a	10.00±0.07a	27.90±0.2a	94.73±0.21b	33.95±0.07a	35.84±0.11a	38.47±0.09a

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

Table 2: Effect of starvation-refeeding regime on serum biochemical analysis in *Oreochromis niloticus* monosex fries

Day of sampling	Treat	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	7.47±0.04d	88.75±0.21a	5.11±0.01	2.98±0.02a	2.13±0.01	18.36±0.2a	36.05±0.11bc
	G2	7.56±0.04a	88.51±0.19c	5.11±0.02	2.97±0.05ab	2.14±0.05	18.23±0.1c	36.21±0.12a
	G3	7.52±0.05b	87.90±0.09e	5.15±0.02	2.96±0.05b	2.18±0.05	18.12±0.1d	35.97±0.1c
	G4	7.49±0.01c	88.42±0.01d	5.11±0.05	2.97±0.09ab	2.14±0.01	18.31±0.2b	36.04±0.09c
	G5	7.51±0.05b	88.61±0.21b	5.09±0.01	2.96±0.1b	2.13±0.01	18.30±0.1b	36.13±0.09ab
After starvation	G1	7.73±0.1d	89.31±0.17d	4.77±0.02b	2.66±0.04b	2.11±0.05	16.52±0.09b	35.90±0.09b
	G2	7.96±0.12c	90.14±0.09c	4.67±0.05c	2.58±0.01b	2.09±0.05	16.22±0.09c	36.22±0.1b
	G3	8.07±0.09b	100.12±0.07b	4.29±0.01d	2.21±0.05c	2.08±0.04	16.07±0.11d	35.85±0.09b
	G4	8.09±0.09a	109.51±0.11a	4.18±0.01e	2.07±0.05d	2.11±0.03	15.69±0.07e	53.56±0.05a
	G5	7.47±0.09e	88.56±0.13d	5.26±0.01a	3.05±0.02a	2.21±0.01	18.61±0.21a	36.08±0.11b
End of experiment	G1	7.48±0.09b	87.23±0.12c	5.30±0.05b	3.05±0.01a	2.25±0.01b	18.86±0.11b	36.04±0.09c
	G2	7.51±0.11ab	87.11±0.09d	5.29±0.05b	2.98±0.02a	2.30±0.04a	18.70±0.12c	35.79±0.08e
	G3	7.54±0.05ab	86.08±0.21e	5.25±0.05c	2.97±0.01a	2.28±0.06a	18.61±0.09d	36.16±0.11b
	G4	7.58±0.02a	88.21±0.21a	4.71±0.01d	2.57±0.05b	2.14±0.01b	16.97±0.09e	36.98±0.1a
	G5	7.55±0.1ab	87.96±0.11b	5.35±0.02a	3.05±0.01a	2.30±0.01a	18.91±0.09a	35.98±0.1d

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* monosex fries

treatments	Initial Weight (g)	Final Weight (g)	Total Weight Gain(g)	SGR (%/day)	Survival rate %
G1	3.51±0.04c	11.22±0.09b	7.71±0.01b	1.48±0.02b	100
G2	3.41±0.06d	10.98±0.1c	7.56±0.05c	1.37±0.01c	100
G3	3.81±0.03a	10.88±0.12d	7.07±0.01d	1.13±0.01d	80
G4	3.81±0.01a	9.62±0.1e	5.81±0.01e	0.893±0.01e	70
G5	3.71 ±0.01b	12.41±0.09a	8.70±0.01a	1.74±0.02a	100

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

4. Conclusion

From the obtained results in the current study, starvation of Nile Tilapia monosex fries for different periods (4, 7, 10, 15 days) followed by refeeding on a commercial fish diet (30% protein) for 30 days revealed that, fries can recover all growth, hematological and immunological parameters close to

the control level when starved for a period of up to 10 days. However, fries subjected to prolonged starvation (15 days) showed significant negative effects on growth, hematological and immunological parameters. Starvation- refeeding regimes in *Oreochromis niloticus* monosex fries is of no value and has no profit.

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6. Conflict of interests
The authors declare that there is no conflict of interests.

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