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GROWTH PERFORMANCE AND DIGESTIVE ENZYME ACTIVITY OF NILE TILAPIA FISH (*Oreochromis niloticus*) FASTED PERIODICALLY IN STAGNANT EARTH POND

Kinerja Pertumbuhan dan Aktivitas Enzim Pencernaan Benih Ikan Nila (*Oreochromis niloticus*) yang Dipuasakan Periodik di Kolam Tanah Stagnan

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ABSTRACT

Nile Tilapia (*Oreochromis niloticus*) is a freshwater fish species with high economic value and is widely cultivated due to its physiological adaptability and high tolerance to environmental conditions and available feed resources. One problem in aquaculture is suboptimal feed utilization efficiency. A fasting program followed by adequate feeding is a feeding management strategy expected to support growth acceleration and improve feed utilization efficiency. This study aimed to evaluate the effect of one week of fasting on muscle glycogen levels, amylase, protease, and lipase enzyme activities, feed utilization efficiency, feed conversion ratio (FCR), daily growth rate, and survival rate in tilapia (*O. niloticus*) fry. The data observed included muscle glycogen levels, amylase, protease, and lipase enzyme activities, feed consumption (FGR), feed utilization efficiency (FEE), feed conversion ratio (FCR), daily growth rate (DGR), and survival rate (SGR). This research activity was conducted from April to May 2025, in a family-owned fish pond, Palangka Raya, Central Kalimantan. This study used an experimental method with a completely randomized design (CRD) with 4 treatments and 3 replications. The treatments tested were feeding fasting with treatment A (daily feeding), B (1 day fasting in 1 week), C (2 days fasting in 1 week) and D (3 days fasting in 1 week). The test fish used were tilapia (*O. niloticus*) seeds with an average weight of 2.01-2.60 g. Feeding was carried out once a day between 08.00 and 12.00 with the amount of feed given per day of 3.75% of the fish biomass weight. The test fish were kept at a stocking density of 30 fish/container. The maintenance container used a hapa net measuring (1x1x1) m3, with a maintenance period of 28 days. The feed used was commercial pellet feed with a protein content of 39%. The results showed that fasting duration significantly ($P<0.05$) affected muscle glycogen levels and amylase, protease, and lipase enzyme activities, but not significantly ($P>0.05$) on EP, FCR, LPH, and TKH of tilapia fry. Treatment B (1 day of fasting per week) produced the highest muscle glycogen levels of 140.14 mg/100 mL, the highest EP value of 88.50%, the lowest FCR value of 1.14, the highest LPH value of 2.74%/day, the highest harvested biomass value of 146.63 g, and the highest TKH value of 87.78%.

Keywords: Fasting, Digestive Enzyme Activity, Growth, *O. niloticus*

ABSTRAK

Ikan nila (*Oreochromis niloticus*) merupakan salah satu spesies ikan air tawar yang mempunyai nilai ekonomis tinggi dan banyak dibudidayakan karena memiliki kemampuan adaptasi fisiologis dan toleransi tinggi terhadap kondisi lingkungan dan sumber daya pakan yang tersedia di lingkungannya. Salah satu permasalahan pada akuakultur adalah efisiensi pemanfaatan pakan yang belum optimal. Program pemuasaan yang diikuti pemberian pakan yang cukup merupakan salah satu strategi manajemen pemberian pakan yang diharapkan bisa menunjang akselerasi pertumbuhan dan meningkatkan efisiensi pemanfaatan pakan. Penelitian ini bertujuan untuk mengevaluasi pengaruh durasi pemuasaan dalam seminggu terhadap kadar glikogen otot, aktivitas enzim amilase, protease dan lipase, efisiensi pemanfaatan pakan, rasio konversi pakan, laju pertumbuhan harian serta tingkat kelangsungan hidup pada benih ikan nila (*O. niloticus*). Data yang diamati meliputi kadar glikogen otot, aktivitas enzim amilase, protease dan lipase, jumlah konsumsi pakan (JKP), efisiensi pemanfaatan pakan (EP), rasio konversi pakan (FCR), laju pertumbuhan harian (LPH) dan tingkat kelangsungan hidup (TKH). Kegiatan penelitian ini dilaksanakan pada bulan April hingga bulan Mei 2025, di Kolam Ikan milik keluarga, Palangka Raya, Kalimantan Tengah. Penelitian ini menggunakan metode eksperimental dengan rancangan acak lengkap (RAL) 4 perlakuan dan 3 ulangan. Perlakuan yang diujikan adalah pemuasaan pemberian pakan dengan perlakuan A (pemberian pakan setiap hari), B (1 hari dipuasakan dalam 1 minggu), C (2 hari dipuasakan dalam 1 minggu) dan D (3 hari dipuasakan dalam 1 minggu). Ikan uji yang digunakan adalah benih ikan nila (*O. niloticus*) dengan bobot rata-rata 2,01-2,60 g. Pemberian pakan dilakukan 1 kali sehari antara pukul 08.00 dan 12.00 dengan jumlah pemberian pakan per hari sebesar 3,75% dari bobot biomassa ikan. Ikan uji dipelihara dengan padat tebar 30 ekor/wadah. Wadah pemeliharaan menggunakan jaring hapa berukuran (1x1x1) m^3, dengan lama pemeliharaan 28 hari. Pakan yang digunakan adalah pakan pellet komersial dengan kadar protein 39%. Hasil penelitian menunjukkan bahwa durasi pemuasaan berpengaruh nyata ($P<0,05$) terhadap kadar glikogen otot, aktivitas enzim amilase, protease dan lipase, tetapi tidak berpengaruh nyata ($P>0,05$) terhadap EP, FCR, LPH dan TKH benih ikan nila. Hasil perlakuan B (1 hari dipuasakan dalam 1 minggu) memberikan nilai kadar glikogen otot tertinggi sebesar 140,14 mg/100 mL, nilai EP tertinggi sebesar 88,50%, nilai FCR terendah sebesar 1,14, nilai LPH tertinggi sebesar 2,74%/hari, nilai biomassa panen tertinggi sebesar 146,63 g, dan nilai TKH tertinggi sebesar 87,78%.

Kata kunci: Pemuasaan, Aktivitas Enzim Pencernaan, Pertumbuhan, *O. niloticus*

INTRODUCTION

One of the main problems in fish farming, including tilapia (Oreochromis niloticus), is low feed utilization efficiency. Therefore, the solution to control this is to evaluate the determinants of the sustainability of the tilapia aquaculture business by optimizing fish growth and health management. This effort can be achieved through several strategies, including managing the amount and frequency of feeding, which is applied by designing a periodic fish fasting program. This is because tilapia is a fish that has high potential to utilize ecological niches in its cultivation environment, so that feeding management regulations can be controlled more effectively and efficiently. The diversity and biodiversity of phytoplankton, microalgae, and zooplankton in freshwater ecosystems are quite significant in their contribution to the conversion of natural feed into tilapia carcasses. Djauhari *et al.,* (2024) reported that tilapia fry fed a probiotic-containing consortium of probiotic bacteria with a density of ≥ 107 CFU/g, fed once daily and at a feeding rate of 3%, produced the best feed conversion ratio and feed efficiency compared to feeding rates of 4.5% and 6%. Feeding a 50 mL coconut water-based fermented feed to tilapia fry at a daily feeding rate of 3.75% per day and at a feeding rate of once daily improved feed conversion ratio, feed efficiency, specific growth rate, average individual weight, and harvest weight over a 28-day rearing period (Markhamah *et al.,* 2025).

Mustofa *et al.,* (2018) confirmed that different fasting periods in carp significantly affected feed utilization efficiency, FCR, and relative growth rate, but no evaluation of digestive enzyme performance was conducted. The results of the treatment without fasting gave the highest relative growth rate and absolute length growth value, while the treatment of 1 day of fasting followed by 1 day of feeding gave the highest feed utilization efficiency value and the lowest FCR value. Elbialy *et al.,* (2022) said that the decrease in body weight of tilapia during the fasting period is highly dependent on the duration of fasting and the length of refeeding period applied, the longer the fasting period, the greater the decrease in total fish biomass weight. Although after long-term fasting the fish were re-fed as normal conditions, body weight gain was very slow and even tended to experience stunted growth, this means that the compensatory response or capacity to catch up on growth lag is highly dependent on the length of fasting and the length of refeeding period. The results of research by Shuai *et al.,* (2023) revealed that there was a decline in the population of piscivorous fish with carnivorous characteristics in river ecosystems along with a decrease in the availability of natural food such as small fish, aquatic insects and fish eggs which is thought to be due to the invasion of the tilapia population which is more dominant in preying on these natural foods. The dynamic balance between the tilapia population and natural feed contributes to maintaining the continuity of the pond ecosystem's carrying capacity while maintaining good fish health. Furthermore, another positive impact directly felt by tilapia farmers is a reduction in total dependence on expensive commercial feed, resulting in reduced operational costs.

Analysis and evaluation of the impact of reducing the amount and frequency of feeding value through the application of periodic fasting values for tilapia in cultivation ponds, each of which has specific and unique physical, chemical and biological properties on the activity of digestive enzymes and muscle glycogen levels that are positively correlated with the main parameters of fish growth and survival, has not been widely carried out so far. The purpose of this study was to evaluate the effect of periodic fasting of tilapia on the activity of amylase, protease, lipase enzymes, muscle glycogen levels, feed conversion ratio, specific growth rate and survival rate of tilapia.

METHODS

Place and Time

The research was conducted in May 2025 for 28 days, taking place in a fish pond belonging to a local resident in the city of Palangka Raya.

Tools and Materials

The test fish used in this study were tilapia (Oreochromis niloticus) fry with an average weight of 2.18-2.27 g/fish. 360 tilapia fry were obtained from the Mandiangin Freshwater Aquaculture Center (BBAT), South Kalimantan. The rearing container used in this study was a ground pond with a stagnant water system measuring (12x9x2) m3. The test feed used in this study was commercial feed in the form of pellets PF500 with a protein content of 40% (dry weight).

Procedure

In this pond, 12 hapa nets were installed, each measuring (1x1x1) m3. The layout of the hapa nets for each replication was placed randomly. Before the tilapia seeds were spread in the maintenance hapa nets, they were first acclimatized for 7 days in a holding hapa net measuring (3x4x1) m^3. The stocking density of the test fish was 30 fish in one maintenance hapa net. According to Ouattara *et al.,* (2003), the ideal stocking density for tilapia reared in isolated and stagnant public waters ranges from 20-50 fish/m3. Feed was provided according to the fasting schedule for each treatment (Table 1). The amount of feed given per day was 3.75% of the fish biomass weight with a feeding frequency of 1 time a day between 08.00 and 11.00. According to Djauhari *et al.,* (2024) the amount of feed given per day to tilapia fry is between 3-4.5% with a feeding frequency of once a day. The preparation of enzyme extracts from the digestive tract follows the following steps:

1. All enzyme extract preparation procedures are carried out at a temperature of 0 to 4 oC with the aim of keeping the enzyme in an inactive condition.
2. The digestive tract was carefully removed, then washed with distilled water and dried with absorbent paper.
3. The digestive tract was crushed with a mortar until smooth and taken as much as 1 g and homogenized with 10 mL of cold water. Centrifuged at a speed of 15,000 rpm for 20 minutes at a temperature of 4 oC, the supernatant was taken as a crude enzyme extract and used as a sample for enzyme activity testing.

The water quality parameters measured included DO, pH, and temperature. DO was measured using a DO meter, pH using a pH meter, and temperature using a thermometer. DO, pH, and temperature measurements were taken at the beginning, middle, and end of the study. Pond water temperature data during the study ranged from 28.7 to 30.5°C, pH 5.97 to 6.10, and DO 5.8 to 6.2°C.

This research was conducted experimentally using a completely randomized design (CRD) with 4 treatments and 3 replications. The fasting period schedule in one cycle (Refaey *et al.,* 2024; Mustofa *et al.,,* 2018) can be seen in Table 1. Each treatment in this study is as follows:
Treatment A: fish were fed every day without fasting for 28 days of maintenance.
Treatment B: fish were fasted 1 day a week for 28 days of maintenance.
Treatment C: fish were fasted 2 day a week for 28 days of maintenance.
Treatment D: fish were fasted 3 day a week for 28 days of maintenance.

Table 1. Schedule of One Cycle of Fasting Period for Nile Tilapia (*Oreochromis niloticus)*

Day / Treatment	Week	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
A	v	v	v	v	v	v	v
B	v	v	v	-	v	v	v
C	v	v	-	v	-	v	v
D	v	-	v	-	v	-	v

Information: v : Fish are fed
- : Fish are fasted

Data collection

The variables measured included digestive tract enzyme activity, feed intake (FED), feed efficiency (FEE), feed conversion ratio (FCR), daily growth rate (SGR), and survival rate (SR). Water quality data measured included DO, pH, and temperature.

Analysis of digestive tract enzyme activity in tilapia was conducted at the end of the rearing period and conducted in the Fish Nutrition Laboratory, Aquaculture Technology and Management Study Program, Department of Aquaculture, Bogor Agricultural University. The enzyme activities measured were amylase activity based on the method (Bergmeyer & Grassi

1983), lipase activity based on the method of Tietz & Friedrick in Borlongan (1990), and protease activity based on the method (Bergmeyer & Grassi 1983).

1. Amount of Feed Consumption

According to Pereira *et al.,* (2007), the amount of feed consumption (TKP) is calculated using the following formula:

$$JKP = F1 - F2$$

Information:
JKP : Amount of feed consumption
F1 : Initial feed amount (g)
F2 : Amount of remaining feed (g)

2. Feed Efficiency

According to Tacon (1987), the feed efficiency (EP) value is calculated using the following formula:

$$EP = \frac{\Delta B}{JKP} 100$$

Information:
EP : Feed efficiency (%)
ΔB : The difference between the final fish biomass and the initial fish biomass (g)
JKP : Amount of feed consumption (g)

3. Feed Conversion Ratio

According to Tacon (1987), the feed conversion ratio (FCR) is calculated based on the following formula:

$$FCR = \frac{JKP}{\Delta B}$$

Information :
FCR : Feed convertion ratio
JKP : Amount of feed consumption (g)
ΔB : The difference between the final fish biomass and the initial fish biomass (g)

4. Specific Growth Rate

According to Sveier *et al.,* (2000) the specific growth rate (SGR) of fish is calculated using the following formula:

$$SGR = \frac{LnBt - LnBo}{t} 100$$

Information:
SGR : Specific growth rate (% per day)
Bt : Fish biomass at the end of maintenance (g)
Bo : Fish biomass at the start of maintenance (g)
t : Maintenance time (days)

5. Survival Rate (SR)

According to Sveier *et al.,* (2000) the survival rate can be calculated based on the following formula:

$$SR = \frac{Nt}{No} 100$$

Information:

SR : Survival rate (%)
Nt : Number of fish at the end of the study (tail)
No : Number of fish at the beginning of the study (tail)

Data Analysis

Data analysis conducted included the value of feed consumption (JKP), feed efficiency (EP), feed conversion ratio (FCR), specific growth rate (SGR), and survival rate (SR) analyzed using the Microsoft Office Excel 2007 program and statistically tested with Analysis of variance (ANOVA) with the SPSS 17.0 for Windows program. Water quality data were analyzed descriptively.

RESULT

Based on the results of the study, the duration of fasting between treatments and replications in treatments of 1, 2 and 3 days a week did not have a significant effect compared to the control on external growth performance parameters, such as feed utilization efficiency, feed conversion ratio and daily growth rate of tilapia fry maintained for 28 days in stagnant soil ponds as shown in Table 2 and the average value of each parameter is presented in Table 3. It is interesting that the treatment of fasting duration of 1 day a week showed more uniform and consistent values for all growth performance parameters. In contrast to the performance of external growth performance parameters, significant differences were clearly seen in the internal growth performance parameters evaluated in this study, namely muscle glycogen levels, amylase, protease and lipase enzyme activities. Tilapia seeds conditioned with a fasting duration of 3 days a week showed the lowest values compared to other treatments in muscle glycogen levels, amylase, protease and lipase enzyme activities can be seen in Table 4 and the average value of each parameter is presented in Table 5. The effect of fasting duration on external growth performance parameters (FCR, feed efficiency, daily growth rate and survival rate) is shown in Figures 1-4, while the effect of fasting duration on internal growth performance parameters (muscle glycogen levels, amylase enzyme activity, protease enzyme activity and lipase enzyme activity) of tilapia seeds reared in hapa nets in stagnant ponds for 28 days is presented in Figures 5-8.

Table 2. Initial body weight (Wo), final body weight (Wt), initial biomass (Bo), final biomass (Bt), difference between final and initial biomass (ΔB), amount of feed consumption (JKP), feed conversion ratio (FCR), feed efficiency (EP), daily growth rate (LPH), and survival rate (SR) of tilapia fry with fasting periods of 0 days/week (A), 1 day/week (B), 2 days/week (C), and 3 days/week (D) for 28 days of maintenance in stagnant water ponds

Parameter / Treatment	Wo (g)	Wt (g)	Bo (g)	Bt (g)	ΔB (g)	JKP (g)	FCR	EP (%)	LPH (%/hari)	TKH (%)
A1	2,25	6,90	67,35	158,75	91,4	101,74	1,11	89,84	3,07	76,67
A2	2,27	5,49	68,15	131,83	63,68	102,92	1,62	61,87	2,36	80
A3	2,01	4,27	60,19	76,84	16,65	90,84	5,46	18,33	0,86	60
B1	2,14	5,57	64,08	144,72	80,64	83,6	1,04	96,46	2,89	86,67
B2	2,05	4,99	61,61	124,73	63,12	80,38	1,27	78,53	2,54	83,33
B3	2,60	6,09	78,12	170,44	92,32	101,99	1,10	90,52	2,79	93,33
C1	2,01	4,29	60,30	103,07	42,77	65,55	1,53	65,25	1,93	80
C2	2,44	5,77	73,19	121,19	48	79,48	1,66	60,39	1,82	70
C3	2,35	5,46	70,55	136,38	65,83	76,67	1,16	85,86	2,36	83,33
D1	2,08	3,99	62,51	87,84	25,33	55,18	2,18	45,90	1,21	73,33
D2	2,16	4,15	64,77	103,76	38,99	57,2	1,47	68,16	1,68	83,33
D3	2,33	4,92	69,86	132,93	63,07	61,72	0,98	102,19	2,29	90

Table 3. Average values of initial body weight (Wo), final body weight (Wt), initial biomass (Bo), final biomass (Bt), difference between final and initial biomass (ΔB), amount of feed consumption (JKP), feed conversion ratio (FCR), feed efficiency (EP), daily growth rate (LPH), and survival rate (SR) of tilapia fry with fasting periods of 0 days/week (A), 1 day/week (B), 2 days/week (C), and 3 days/week for 28 days of maintenance in stagnant water ponds.

Parameter/ Treatment	Wo (g)	Wt (g)	Bo (g)	Bt (g)	JKP (g)	FCR	EP (%)	LPH (%/hari)	TKH (%)
A	2,18	5,55	65,23	122,47	98,5	2,73	56,68	2,10	72,22
B	2,26	5,55	67,94	146,63	88,66	1,14	88,50	2,74	87,78
C	2,27	5,17	68,01	120,21	73,9	1,45	70,5	2,04	77,78
D	2,19	4,35	65,71	108,18	58,03	1,54	72,08	1,73	82,22

Table 4. Muscle glycogen levels (mg/100 mL), amylase (IU/mL) and lipase (IU/mL) enzyme activities of tilapia fry with fasting periods of 0 days/week (A), 1 day/week (B), 2 days/week (C), and 3 days/week (D) for 28 days of maintenance in stagnant water ponds.

Parameter / Treatment	Muscle glycogen levels (mg/100 mL)	Amylase enzyme activity (IU/mL)	Protease enzyme activity (IU/mL)	Lipase enzyme activity (IU/mL)
A1	132,66	0,594	0,0118	0,1395
A2	106,93	0,659	0,0085	0,1325
A3	130,10	0,594	0,0113	0,1360
B1	129,01	0,626	0,0078	0,1203
B2	140,76	0,659	0,0084	0,1099
B3	150,65	0,646	0,0096	0,1168
C1	141,93	0,691	0,0073	0,1291
C2	127,23	0,672	0,0087	0,1256
C3	144,41	0,620	0,0078	0,1203
D1	78,59	0,497	0,0035	0,0541
D2	69,92	0,464	0,0033	0,0558
D3	78,34	0,464	0,0042	0,0610

Table 5. Average values of muscle glycogen levels (mg/100 mL), amylase enzyme activity (IU/mL) and lipase (IU/mL) of tilapia fry with fasting periods of 0 days/week (A), 1 day/week (B), 2 days/week (C), and 3 days/week (D) for 28 days of maintenance in stagnant water ponds.

Parameter / Treatment	Muscle glycogen levels (mg/100 mL)	Amylase enzyme activity (IU/mL)	Protease enzyme activity (IU/mL)	Lipase enzyme activity (IU/mL)
A	123,23	0,616	0,011	0,1360
B	140,14	0,644	0,0086	0,1157
C	137,86	0,661	0,0079	0,1250
D	75,62	0,475	0,0037	0,0570

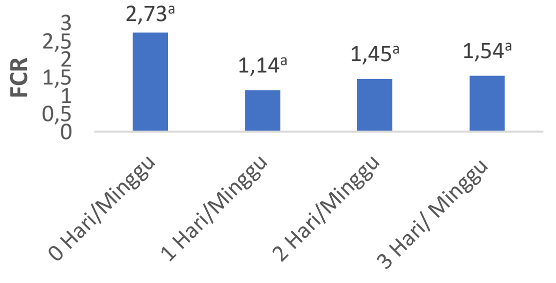

Fig. 1. The effect of fasting duration on the FCR of nile tilapia seeds

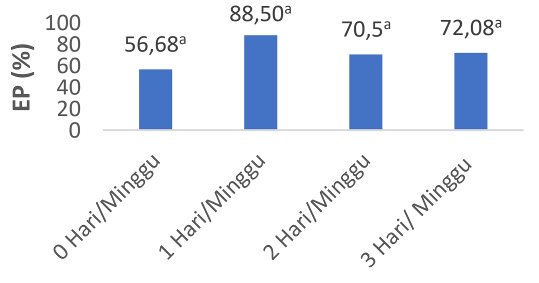

Fig. 2. The effect of fasting duration on the feed efficiency of nile tilapia seeds

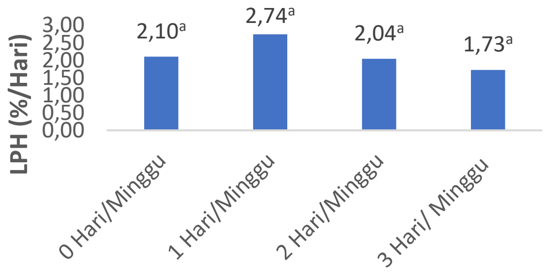

Fig. 3. The effect of fasting duration on…

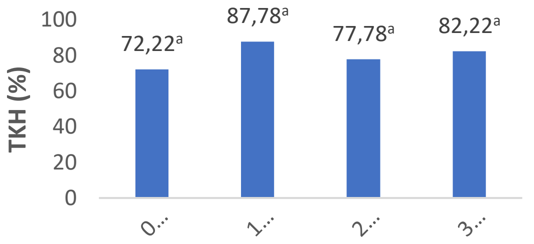

Fig. 4. The effect of fasting duration on the survival rate of nile tilapia seeds

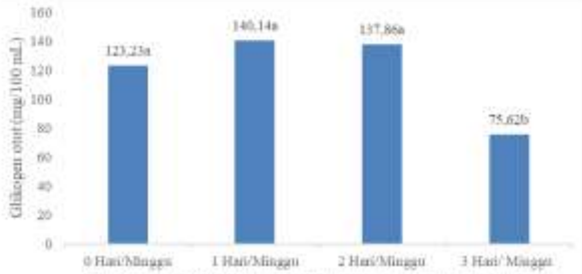

Fig. 5. The effect of fasting duration on muscle glycogen levels of nile tilapia seeds

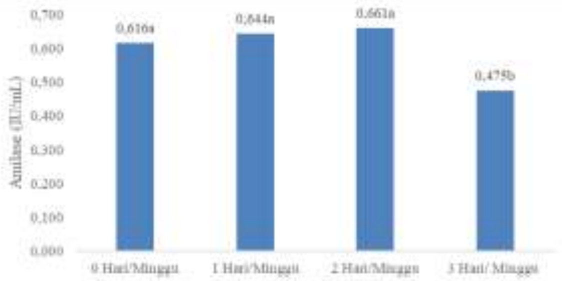

Fig. 6. The effect of fasting duration on amylase enzyme activity of nile tilapia seeds

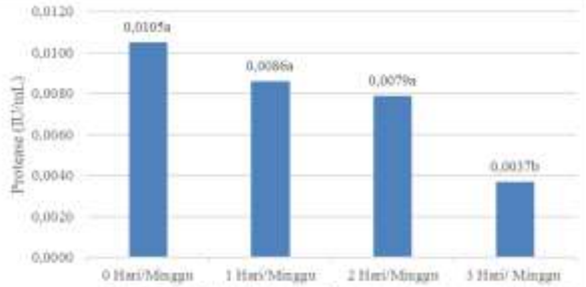

Fig. 8. The effect of fasting duration on protease enzyme activity of nile tilapia seeds

Fig. 7. The effect of fasting duration on lipase enzyme activity of nile…

DISCUSSION

The results of the study showed that in cases of extreme fasting, namely 3 days a week, there was a drastic decrease in muscle glycogen levels in tilapia fish, this is possible because pancreatic β cells greatly reduce insulin production, on the other hand, to maintain the balance of body homeostasis, one effort to obtain energy comes from the catabolism of liver and muscle glycogen into glucose-6-phosphate, as it is known that glucose-6-phosphate is an intracellular form of glucose. Although the glucose-6-phosphatase enzyme that plays a role in dephosphorylating glucose-6-phosphate and releasing glucose into the bloodstream for further utilization by all body tissues is only owned by hepatocytes, but in this study it appears that the role of hepatocytes is thought to be assisted by muscle cells, namely the breakdown of glycogen (glycogenolysis) stored in the muscle tissue of the tilapia body. The supply of energy needs for muscle cells is highly dependent on the level of activity of the fish. In conditions of extreme fasting, glucose bioavailability is very limited, glucose reserves in the form of muscle glycogen are hydrolyzed by the active phase muscles. This is possible because the fish try their best to find and prey on all types of food available in their environment other than pellet feed. Mishra *et al.,* (2025) revealed that juvenile tilapia fish fasted for 5 days and then fed again for 7 days showed a significant reduction in average weight gain, specific growth rate and feed utilization efficiency, but muscle glycogen levels did not show significant changes, either during 1, 3, 5 and 7 days of fasting followed by 7 days of feeding again. During periods of high activity, anaerobic respiration occurs and glucose metabolism produces large amounts of lactic acid which is transported to the liver via the Cori cycle. To meet energy needs, the active phase muscle cells of the fish body not only utilize glucose, but can also use fatty acids (especially resting phase muscle cells) and amino acids. The breakdown of muscle glycogen to pyruvic acid which then enters the Kreb's cycle and in turn produces energy. Pyruvic acid can also be converted to lactic acid through the process of gluconeogenesis which then becomes glucose and the excess is stored as energy reserves in the form of liver glycogen. Glucose is the primary fuel source and an essential substrate for cell metabolism, particularly brain cells and erythrocytes. A logical consequence of this massive energy mobilization is a decrease in growth rate and harvested biomass. Most fish species, including tilapia, possess the ability to adaptively regulate glucose metabolism, which plays a key role in the mechanism of fasting tolerance (Mohapatra *et al.,* 2015). Tilapia experiencing prolonged fasting periods exhibit histopathological changes leading to necrosis of the connective tissue surrounding muscle fibers and the liver, which is crucial for the blood vessel and nerve pathways supplying nutrients to myocytes and hepatocytes. This damage to skeletal muscle and the release of myocyte components into the bloodstream (rhabdomyolysis) and interstitial edema in the endomysium (Elbialy *et al.,* 2022).

Along with the integration of metabolism, namely the fate of glucose metabolism of tilapia during normal, moderate to extreme fasting, the results of the study also showed that the activity of digestive enzymes, including amylase, protease and lipase also decreased. The results of this study are in line with Mishra *et al.,* (2025) that the activity of amylase and lipase enzymes decreased significantly in the 1 and 3 day fasting methods, but returned to normal like the control group (without fasting, fed continuously) after being fed again for 7 days. In contrast, the activity of the protease enzyme initially increased during the 1 and 3 day fasting periods, but returned to normal like the control in the 7 day fasting treatment group after being fed again for 7 days. In extreme fasting conditions, because no pellet feed is consumed and digested, although it is suspected that the fish can still utilize all types of natural food available in the pond, the organs and digestive glands of the fish's body will respond by gradually reducing the production and activity of digestive enzymes, both endogenous enzymes and exogenous enzymes, so that in general the digestive metabolic activity also decreases. Enzymes that experienced significant reductions in activity were amylase, protease, and lipase. α-amylase is an enzyme produced by the pancreas and works in the intestine to hydrolyze amylose (starch) into maltotriose and maltose. Carbohydrates serve as the primary energy source in cells and are central to all metabolic processes. Protease enzymes play a role in hydrolyzing proteins into amino acids. Amino acids are used for the synthesis of enzymes, transporters, and other physiologically important proteins. Amino acids are precursors of several peptide hormones such as insulin and glucagon, as well as amine hormones such as catecholamines. Lipase enzymes hydrolyze fats into fatty acids, which are then oxidized to acetyl CoA to produce energy in the form of NADH. Dai *et al.,* (2024) suggested that the activities of enzymes and hormones that play key roles in the glycolysis and gluconeogenesis pathways coordinate with each other to maintain glucose homeostasis during the transition period from fasting to refeeding in black carp (Mylopharyngodon piceus).

Under normal natural conditions, fish also undergo fasting, particularly when climate change causes significant fluctuations in the physical, chemical, and biological parameters of the cultured water (Yang *et al.,* 2019), including temperature, pH, dissolved oxygen, and phytoplankton and zooplankton density, all of which significantly influence physiological responses. The duration of fasting for tilapia in this study was 48 hours, with one intermittent feed per week (treatment B), two intermittent feeds per week (treatment C), and three intermittent feeds per week (treatment D), while treatment A was continuous (without fasting).

Generally, after a certain fasting period, the fish are re-fed, resulting in highly efficient digestive metabolic responses and nutrient absorption. Intestinal tissue contains two primary and most dominant cells involved in digestive metabolism and nutrient absorption: mucocytes and enterocytes, each of which produces digestive enzymes and plays a key role in the digestion and nutrient absorption processes.

The results of the study showed that all treatments showed significant differences in terms of variation in the size of tilapia fry, this is suspected to be due to the high genetic variability or diversity among the individual tilapia fry used as test animals in this study. The high variability or diversity among the individual tilapia fry in question is primarily thought to be closely related to the aggressive response to the given feed, the efficiency of digestive metabolism and absorption of feed nutrients and growth potential. Fish that have a high aggressiveness character towards feed and are very efficient in digestive metabolism and absorption of feed nutrients can normally consume more feed and are more efficient in utilizing feed nutrients for basal metabolic needs and growth, compared to fish that have a moderate or low aggressiveness character and efficiency of digestive metabolism and absorption of feed nutrients. Fish that are less aggressive in pursuing and consuming feed and inefficient digestive metabolism and absorption of feed nutrients gradually become weaker and lead to high mortality rates due to the minimal energy supply to maintain homeostasis and basal metabolic balance, resistance to environmental stressors, such as daily temperature fluctuations and so on, oxidative stress, let alone energy allocation for growth. A positive correlation is suspected between the performance of mucocyte and enterocyte cells and the aggressiveness of individual tilapia fry. Fish with low feed aggressiveness tend to have low mucocyte and enterocyte cell performance, resulting in fewer feed nutrients being digested and absorbed by all body tissues (low feed utilization efficiency and high feed conversion ratio), the subsequent impact of which is a decrease in growth rate. This extreme condition is suspected to have occurred in the third and first replications of each treatment of fish without fasting and fasting 3 days a week, respectively. Another possible factor is the suspicion that the tilapia fry used in this study may be predominantly female. Benhaim *et al.,* (2017) proved that the high aggressiveness character towards feed tends to be inherent in male tilapia compared to female tilapia, that female tilapia have a rather shy behavioral response and prefer to stay under shelter. The value of individual fish characteristics is influenced not only by the individual's genotype, but also by the conditions of the cultivation environment and social interactions with individuals of the same or other species (Hill *et al.,* 2007). The genotype of one individual can influence the trait value of another individual (Bijma, 2012).

The discontinuous long-term fasting period in treatment D showed that the fish tended to maintain drastic body weight loss. This suggests that the tilapia fry were still able to utilize all available natural food sources in the pond as an energy source. In other words, compensatory growth was still demonstrated by the fish even though the fasting period was the longest per week. This aligns with the opinion of Elbialy *et al.,* (2022) who stated that longer periods of feed restriction must be balanced with periods of refeeding to achieve catch-up or complete growth compensation in tilapia (Oreochromis niloticus).

In general, the decrease in growth rate due to fasting begins with a reduction in muscle and liver glycogen deposits. Excess energy in the form of glucose from carbohydrate breakdown is stored as energy reserves in the muscle and liver. Limited nutrient availability leads to the breakdown of glycogen (glycogenolysis) in the muscle and liver, which is then converted back to glucose, resulting in a decrease in fish body weight. Fish muscle tissue represents 40–60% of the total body mass of fish (Weatherley and Gill, 1985) and primarily represents white muscle, the edible portion of the fish carcass (Zhang *et al.,* 1996; Sänger & Stoiber, 2001). Davis & Gaylord, (2011) revealed that short-term fasting periods are very useful for controlling water quality problems and reducing the phenomenon of water mass reversal due to wide daily temperature fluctuations, thereby reducing the impact of mortality and also as a preventive measure for possible opportunistic pathogen attacks, a balance between the efficiency of feeding and feed utilization due to efforts to optimize the use of ecological niches by fish during the fasting period. Fasting activities and refeeding after a certain fasting period affect muscle volume as one of the most important tissues for aquaculture. Navarro and Gutierrez, (1995) revealed that the short period and high frequency of fasting cause fish to move very actively in search of food and require quite high energy mobilization due to muscle activity and have implications for reducing muscle glycogen deposits. The results of this study indicate that glucose reserves in the form of muscle glycogen are mobilized as an important energy source, in line with Dai *et al.,,* (2024) who found that during short-term fasting in black carp, there was a gradual decrease in muscle glycogen levels between days 5 and 10, but after refeeding, muscle glycogen levels gradually returned to normal levels as before the fish were fasted. Similar research results were confirmed in rainbow trout (Bermejo-Poza *et al.,* 2019) and ureotelic tilapia (*Alcolapia grahami*) in Lake Magadi (De Boeck *et al.,* 2019). Carbohydrate metabolism plays an important role in providing the main energy source in the form of glucose for fish during very limited food availability, especially in the form of pellets (Davis & Gaylord, 2011). Changes in gene expression caused by starvation and refeeding affect muscle metabolism and growth rate and can sometimes interfere with muscle tissue growth (Hornick *et al.,* 2000; Hagen *et al.,* 2009). In bony fish, the normal and continuous growth and development of skeletal muscle depends primarily on the availability of nutrients, both in quantity and quality, modulating the main endocrine system that regulates linear growth in fish, namely GH/IGF-I (Reinecke *et al.,* 2005; Liu *et al.,* 2020) which regulates the metabolism of various nutrients, muscle protein synthesis, and the growth of other body tissues (Butler & Le Roith, 2001).

CONCLUSION

Of all the treatments observed and evaluated for growth performance parameters, only the one-day-a-week fasting treatment showed the lowest average feed conversion ratio, highest feed utilization efficiency, highest daily growth rate, highest harvest biomass, and highest survival rate. This was also confirmed by the lowest digestive enzyme performance parameters and muscle glycogen levels shown by the three-day-a-week fasting treatment. This indicates that tilapia fry reared in stagnant ponds are only able to provide a tolerance response of one day of fasting a week to catch up on growth lag (compensatory growth) which is emphasized by the consistency of growth performance parameter values in all replications. Another assumption is that the individual tilapia fry in the one-day-a-week fasting treatment group may coincidentally have a relatively uniform response related to a high aggressive response to feed and very efficient in digestive metabolism and absorption of feed nutrients as well as a high average growth potential. The next possibility that can be explained is that the tilapia seeds used in this study are suspected to be mixed offspring from the spawning of several pairs of tilapia parents, this is suspected to be one of the causes of high genetic variability or genetic diversity between individuals in dealing with aquaculture environmental factors in general, especially in responding to the fasting treatment.

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