



### Research Article

## Effect of Different Feed Restrictions on Growth, Biometric, and Hematological Response of Juvenile Red Tilapia (*Oreochromis* spp.)

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### Abstract

The management of red tilapia culture can be improved through restriction of feed. This study aimed to determine the impact of feed restriction time and re-feeding on the growth and hematological performance of juvenile red tilapia. This study used an experimental method with a completely randomized design consisting of five treatments with four replications, namely the fish were given food for 28 days (A), 7 days of feed restriction followed by 21 days of refeeding (B), 14 days of feed restriction followed by 14 days of refeeding (C), 21 days of feed restriction followed by 7 days of refeeding (D), and 28 days of feed restriction (E). The parameters observed were growth, biometry, and hematological values. The results showed that during the feed restriction period there was a significant decrease in growth, condition factors, hepatosomatic index along with viscerosomatic index with the length of time for feed restriction, although fasting for up to 28 days did not cause fish mortality. Hematological parameters such as RBCs, WBCs, hematocrit, and hemoglobin decreased significantly, but after re-feeding, they increased significantly in consecutive times. As for the blood glucose levels decreased during feed restriction and gradually increased after re-feeding. This study stated that feed restriction fish gained weight continuously during re-feeding, but no displaying compensatory growth. The results suggested that the decrease in hematological parameters did not indicate stress levels in fish, but reflected a lack of nutrition condition.

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## 1. Introduction

One type of tilapia that is popular and has high economic value besides black tilapia (*Oreochromis niloticus*) is red tilapia (*Oreochromis spp*). Red tilapia is a very popular fish in Asia and Latin America and has a high selling price due to its color (red color) which resembles several marine fish species. The high market demand is not matched by efforts to improve genetic quality, which in its development, red tilapia cultivation experiences several obstacles, namely slow growth, high mortality, and high color variation (presence of black spots) (Pongthana *et al.*, 2010; Santos *et al.*, 2014). This problem is caused by several factors, including the unavailability of superior seeds and parents, random mating because there is no information on the origin of the fish used, and improper cultivation methods (Hamzah *et al.*, 2017).

In order to improve the management of red tilapia cultivation to improve growth performance and increase productivity, breeding methods have been carried out including selection and hybridization. Another way that can be done through nutritional restriction intake, namely by fasting and re-feeding methods. The nutritional restriction has been widely suggested as a management strategy in aquaculture to maximize growth (Oh *et al.*, 2008; Yengkokpam *et al.*, 2013). Several studies have reported that efforts to improve the meat quality and productivity of tilapia can be done by exploring the effects of fasting as a feeding strategy on growth performance and survival. Fasting techniques in fish can also be used to improve the quality of the final product by reducing muscle fat content (Grigorakis and Alexis 2005; Zhang *et al.*, 2008). In addition, other benefits of the feeding strategy are to reduce water quality problems, reduce mortality due to disease, reduce production costs, and accelerate the growth rate of fish after being fed back after fasting (Fox *et al.*, 2010; Davis and Gaylord, 2011).

Based on literature studies, it has been reported that restriction of feed through fasting leads to compensatory growth, increased feed efficiency, and reduced production time, after re-feeding (Hayward *et al.*, 1997; Nicieza and Metcalfe, 1997). In black tilapia it was reported that fasting for 5-10 days followed by feeding for 42 days resulted in partial compensatory growth indicated by an increase in fish body weight, but still had a lower weight than unsatisfied fish (Nebo *et al.*, 2013). Furthermore, Sakyi *et al.* (2020) on black tilapia monosex which was given a short-term fasting treatment from 3 to 21 days was reported to have a significant effect on growth performance which gave a positive response after being fed again for 21 days.

Physiological changes in fish can occur during fasting. In general, fish will experience stress as a defense response, thus affecting the physiological

activities of animals. Fasting will cause changes in the body's metabolism to release more energy and increase protein production as a response to the body's defense from oxidative damage, this indicates that the fish is experiencing stress (Navarro and Gutiérrez, 1995). One indicator that can be used to assess physiological responses in blood characteristics is based on hematological features (Jenkins *et al.*, 2003; Gupta *et al.*, 2012). Hematological evaluation of fish provides important information regarding the physiological response of fish to external changes in the environment and is indirectly used to determine the health and stress conditions of fish (He *et al.*, 2015; Sharma *et al.*, 2017). The normal range of hematological values in fish is influenced by internal and external factors including fish species, age, sex, sexual maturity cycle, water quality, stress, and nutritional status (Caruso *et al.*, 2011; Fazio, 2019). Monosex black tilapia which was fasted for 15 days and refeeding for 30 days showed a negative effect on hematological and immunological parameters (Moustafa and El-Kader, 2017). Meanwhile, marine fish such as sturgeon (*Acipenser persicus*) fasted for 7-28 days and refeeding for 28 days showed no negative effect on hematological parameters (Yarmohammadi *et al.*, 2015).

The fasting method has been applied as a reference for aquaculture management in other fish but based on several studies it is reported that it is still unclear, where there is no optimal protocol information in applying the method of fasting and re-feeding in tilapia aquaculture. Therefore, this study aims to determine the growth performance and index of the biological condition of fish and evaluate the hematological parameters of fish that provide an overview of the nutritional conditions of juvenile red tilapia reared after being exposed to feed restrictions.

## 2. Materials and Methods

### 2.1 Materials

Juvenile red tilapia were obtained from a single spawning batch, in the Research Institute for Fish Breeding. The test fish is the second generation (G2) of red tilapia from the family selection. In this research, the fish were kept in a concrete pond (20 units) size of 2x1x1 m<sup>3</sup> filled with water with a volume of 1500 liters and a continuous flow of fresh water at a rate of 1 L min<sup>-1</sup>. The total number of fish used was 560 fish (average body weight, 35.58 ± 0.29 g) with stocking density for each pond, namely 28 fish. Before being given the treatment, the fish were acclimatized with feed at satiation of the biomass weight with a frequency of 2 times a day (morning and evening) for 1 week.

### 2.2 Methods

#### 2.2.1 Experimental design

The study consisted of five treatments with four replications, namely the fish were given food for 28 days (A), 7 days of feed restriction followed by 21 days of refeeding (B), 14 days of feed restriction followed by 14 days of refeeding (C), 21 days of feed restriction followed by 7 days of refeeding (D), and 28 days of feed restriction (E). During the experimental period, fish were reared under the following conditions: water temperature: 26-29.5°C; pH: 7.6-7.9; and DO: 4.8-6 mg/L. During the maintenance of fish, they are given commercial feed with a protein content of 30-32% with a frequency of 3 times a day (8.00 am, 11.30 am and 04.00 pm) at satiation. In order to maintain water quality, 60% water supply and water replacement are carried out every two days.

## 2.3 Data Collected

### 2.3.1 Growth performance

Growth sampling was carried out at the start of maintenance, the end of the feed restriction period, and during refeeding, including the measurement of length and weight. The parameters observed in this study included absolute growth, daily growth, specific growth rate, and feed conversion ratio. At the end of the study, feed consumption and survival rate were measured. The number of samples observed was 15 fish/replicates.

### 2.3.2 Biometric

Biometric measurement to determine the condition factor of the fish (CF) was carried out at the end of the fasting period by measuring the total length and final body weight of each individual fish. Then the hepatosomatic index (HSI) and viscerosomatic index (VSI) were measured by dissecting the fish and removing the liver and visceral parts of each treatment (3 fish per replication).

### 2.3.3 Hematological

Hematological measurements were carried out at the beginning of maintenance (control) day 0, the end of the feed restriction period (day 7, day 14, day 21) and the end of maintenance (day 28). For each blood sampling, the number of fish samples taken were 3 fish per replication (12 fish from each group), were randomly sampled and anesthetized with phenoxyethanol at 0.3 mL<sup>-1</sup> by immersion. Blood samples were taken using a 0.5 ml syringe intramuscularly on the dorsal part of the fish that had been given 10% ethylene diamine tetra-acetic (EDTA) as an anticoagulant to prevent blood hemolysis. The blood sample is then inserted into the K3EDTA blood tube which is then taken to the laboratory to analyze the total number of red blood cells, the total number of white blood cells, hematocrit value, hemoglobin level, and blood glucose level

## 2.4 Data Analysis

The data were analyzed using the one-way variance test (ANOVA) and then continued with the Duncan test to determine which treatment had the most effect. All statistical analysis were performed using SPSS 22 computer program. The difference was considered significant at  $P < 0.05$  and all data were presented in the form of mean  $\pm$  standard deviation.

### 2.4.1 Calculation of the growth performance

The calculation of growth parameter data is carried out based on the following formula. Absolute weight growth (weight gain) is the difference between the final weight with the initial weight of maintenance, calculated with the formula ( $\Delta W = W_t - W_0$ ) (Zonneveld *et al.*, 1991). The specific growth rate (SGR) is the daily growth rate or the percentage of fish weight added per day, calculated with the formula as follows:

$$(\text{SGR} = \frac{\ln(W_t) - \ln(W_0)}{t} \times 100)$$

Feed conversion ratio is calculated with the formula based on National Research Council (1977) as follows:

$$\left( \frac{F}{(W_t + D) - W_0} \right)$$

Description:

$W_0$  = initial weight

$W_t$  = final body weight

$t$  = the period of days in the feeding period

$F$  = total feed

$D$  = weight of fish death

Survival rate the ratio of the number of fish that live until the end of the maintenance with the number of fish at the beginning of maintenance, calculated using the formula from Effendi (1997).

### 2.4.2 Calculation of fish biometric

The calculation of biometric parameter data is carried out based on the formula according to Jobling (1994). The condition factor (CF) is an indicator of body shape / percentage increase in weight which shows the weight index of the fish against the total length, and is used to determine the physical or biological condition of the fish.

$$(\text{CF}) = \frac{\text{Fish Weight (g)}}{\text{fish length (cm)}^3} \times 100$$

The hepatosomatic index (HSI) is a measure of the relative weight of the liver and the viscerosomatic index (VSI) is a measure of the weight of the fish viscera.

$$(\text{HSI}) = \frac{\text{Liver weight (g)}}{\text{fish weight}} \times 100$$

$$(\text{VSI}) = \frac{\text{Visceral weight (g)}}{\text{fish weight (g)}} \times 100$$

### 2.4.3 Calculation of hematological

Red blood cells (RBCs cellsmm<sup>-3</sup>) were

counted under the light microscope using a Neubauer haemocytometer based on procedures from Nabib and Pasaribu (1989), White blood cells (WBCs, cells mm<sup>3</sup>) were counted using a Neubauer haemocytometer based on procedures from Blaxhall and Daisley (1973). Hemoglobin levels (Hb, g dL<sup>-1</sup>) were determined using the acid hematin method with the Hemometer Sahli-Hellige (g dL<sup>-1</sup>). The scale reading is done by looking at the surface height of the solution being shaken with the stripe scale g% which shows the amount of Hb in grams per 100 ml of blood and expressed as a percentage (% Hb). The determination of the value of the hematocrit (Ht, %) in the blood was carried out using the micro-hematocrit method. The hematocrit value was determined by measuring the percentage of red blood cell volume to the total volume of blood using a microhematocrit reader and expressed as a percentage (% Ht) (Anderson and Siwicki, 1993). Glucose was observed by the colorimetric method using a glucose kit.

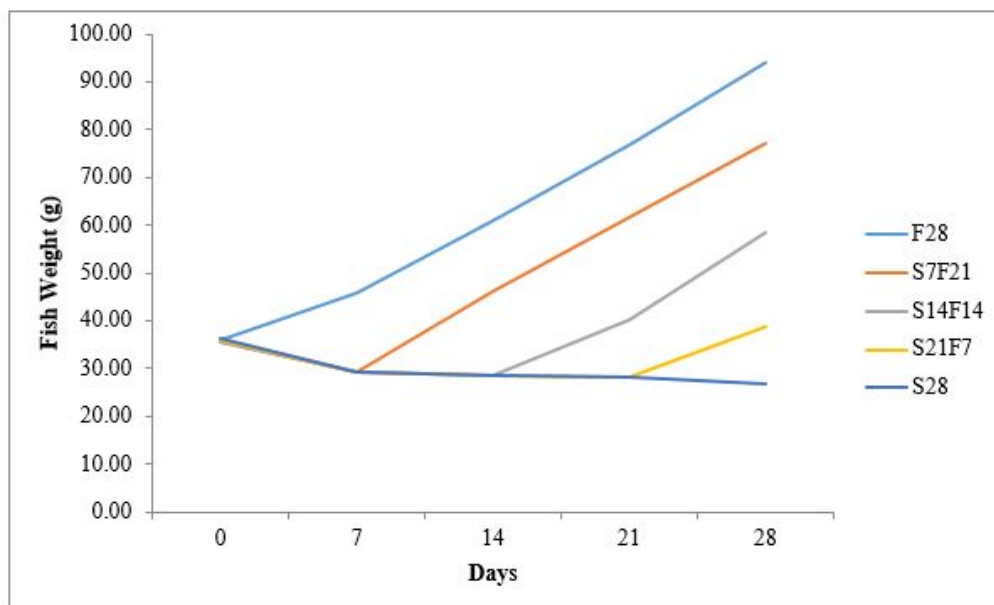
### 3. Results and Discussion

#### 3.1 Growth Performance

During the feed restriction period, body weight decreased gradually in all treatments except control, and a more significant reduction in fish weight was seen at the 28-day feed restriction period (Figure 1). However, at the time of re-feeding, growth began to

increase gradually until the end of growing out. Although after 21 days of re-feeding, the re-feeding group did not achieve the same value of 28 days of feeding. According to Hayward *et al.* (2000), the increase in fish growth is regulated by diet, protein and energy. Fish that experienced starvation can cause energy use to be efficient because their metabolic rate decreases. When re-feeding is done, energy comes from feed protein for growth (Khotimah, 2009). Prakoso and Kurniawan (2017) reported that best tilapia fasted for 7-14 days experienced a decrease in body weight, however, after refeeding 42 days at the end of the experiment, it showed that the fish biomass that was fed restricted for 7 days showed a higher value than control.

It is known that the weight loss due to the feed restriction phase ranges from 18.22-23.89% increase according to the length of time for restriction of feed (Table 1). The results based on statistical tests showed significant differences between fish that were fed continuously and those that were given feed restriction ( $P < 0.05$ ). After refeeding (end of experiment), the highest absolute weight gain ( $41.48 \pm 3.34$  g) and SGR ( $3.44 \pm 0.15$  b w day<sup>-1</sup>) were indicated in treatment (B), although the growth in treatment S7F21 is still under control treatment (no compensatory growth) after refeeding period of 21 days. The SGR value in fish farming describes how fast the fish grow every day, where this value can be used to predict the production of fish produced in a certain period.



**Figure 1.** The growth pattern of of red tilapia on different feed restriction and refeeding for 28 day. Description: the fish were given food for 28 days (Control/F28), 7 days of feed restriction followed by 21 days of refeeding (S7F21), 14 days of feed restriction followed by 14 days of refeeding (S14F14), 21 days of feed restriction followed by 7 days of refeeding (S21F7), and 28 days of feed restriction (S28).

Based on the research results, it is known that the growth pattern of fasted red tilapia decreased at the end of the fasting period, but there was an increasing trend of growth after the refeeding period. This shows that during fasting, malnutrition occurs in red tilapia which causes a decrease in metabolic rate, but after the refeeding period, the growth begins to experience an upward trend. Feed restriction through fasting is a widely recommended feed management strategy in aquaculture, where this strategy is believed to have a positive effect on fish growth performance, namely increasing the body weight at harvest, called compensatory growth. This growth is described as the increase in growth resulting from the refeeding process right after a period of restriction or fasting. Fish of many species can grow unusually fast after a period of diet deprivation or restricted feeding, and, in several cases, have caught up in weight with control. According to Secor and Carey (2016), during a longer fasting period, there will be an energy reshuffle from non-carbohydrate compounds, then after the refeeding period, energy recovery occurs marked by increased oxygen consumption and also increased and accelerated growth.

In this study, it was shown that the re-feeding of red tilapia for 7-21 days (end of the experiment) did not produce compensatory growth phenomena, so it took a long time for the refeeding period for the fish to recover energy for growth.

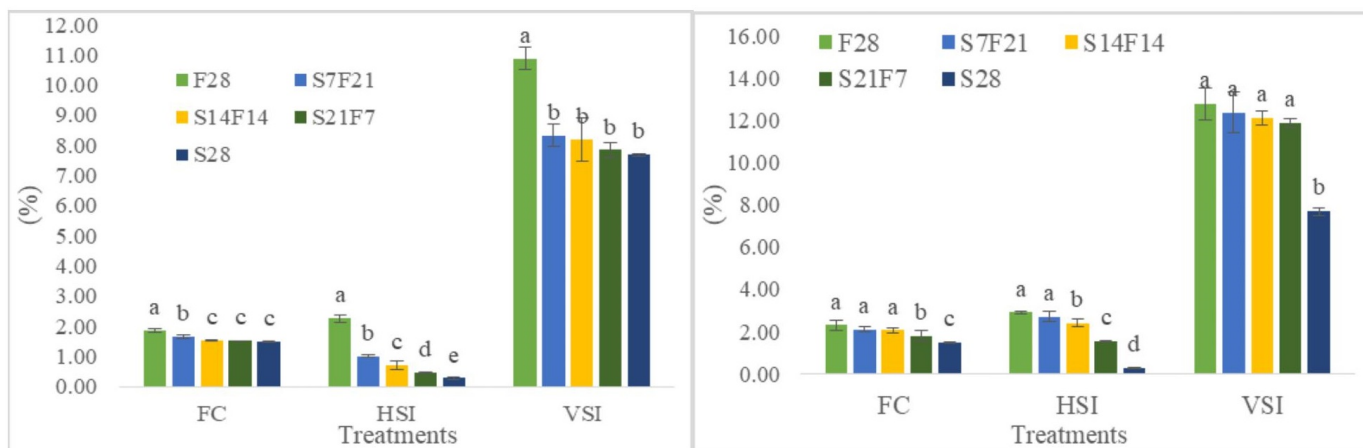
According to Laiz-Carrión *et al.* (2012), compensatory growth is unusually rapid growth after a period of food shortage and starvation, during which fish can reach the same size and weight as fish that have been reared in optimal conditions after receiving a reduction in nutrition. These results are consistent with Moustafa and El-Kader (2017) research that monosex tilapia fasted for 4 to 15 days has lower weight gain (5.81-7.71 g) and SGR (0.89-1.48 %bwday<sup>-1</sup>) compared to controls at the end of the study. Similar reports have been submitted by Gabriel *et al.* (2018) that the compensatory growth response was shown by tilapia *O. mossambicus* which was fasted for 2 days and given 4 days of feed periodically which showed a growth value that was not significantly different from the control and provided optimization in production costs. In others fish, Xu *et al.* (2019) reported on grass carp that grass carp showed total compensatory growth where there was no significant difference in the character of the final body weight between fish fasted for 14 days with 14 days of refeeding compared to fish fed for 28 days. Fu *et al.* (2005) and Zheng *et al.* (2015) stated that in order to maintain the homeostatic process, fish reduce the metabolic capacity of the tissue, resulting in degradation of energy sources (lipids, glycogen, and protein) and cause weight loss due to lack of feed for a long period.

**Table 1.** Growth parameters of red tilapia on different feed restrictions and refeeding for 28 days

Parameters	Treatments				
	F28	S7F21	S14F14	S21F7	S28
Initial weight (g)	35.53±1.42	35.80±0.43	35.96±1.80	35.35±1.50	35.28±1.96
Final weight (g)	94.35±3.01 <sup>a</sup>	77.05±3.04 <sup>b</sup>	58.48±4.4 <sup>3c</sup>	38.87±1.94 <sup>d</sup>	26.82±1.01 <sup>e</sup>
Weight gain( g)	58.35±2.90 <sup>a</sup>	41.48±3.34 <sup>b</sup>	22.64±3.99 <sup>c</sup>	3.52±2.24 <sup>d</sup>	-8.45±1.29 <sup>e</sup>
SGR (gdays <sup>-1</sup> )	3.44±0.15 <sup>a</sup>	2.76±0.17 <sup>b</sup>	0.30±0.26 <sup>c</sup>	-0.30±0.22 <sup>d</sup>	-0.98±0.00 <sup>e</sup>
FCR	1.1.5±0.06 <sup>a</sup>	0.94±0.05 <sup>b</sup>	0.64±0.04 <sup>c</sup>	0.28±0.06 <sup>d</sup>	0.00±0.0 <sup>e</sup>
Feed consumption (g)	2268±46.50 <sup>a</sup>	1544±20.76 <sup>b</sup>	783±40.04 <sup>c</sup>	205±15.77 <sup>d</sup>	0±0.00 <sup>e</sup>
Survival rate (%)	100.00±0.00 <sup>a</sup>	100.00±0.00 <sup>a</sup>	100.00±0.00 <sup>a</sup>	100.00±0.00 <sup>a</sup>	100.00±0.00 <sup>a</sup>

Description: the fish were given food for 28 days (Control/F28), 7 days of feed restriction followed by 21 days of refeeding S7F21), 14 days of feed restriction followed by 14 days of refeeding (S14F14), 21 days of feed restriction followed by 7 days of refeeding (S21F7), and 28 days of feed restriction (S28).

\*The same letter in the same row is not significantly different at  $P > 0.05$  and the different letters(a,b,c,d,e) indicate significant ( $P < 0.05$ ) by Duncan multiple test ( $n=4$ ). The values shown are the average value and standard deviation.



**Figure 2.** Condition factor (CF), hepatosomatic index (HSI) and viscerosomatic index (VSI) of red tilapia of red tilapia at the end of the feed restriction (A) (day 7, day 14, day 21 and day 28) and the end of experimen ( 28 days) (B)\*The same letter is not significantly different at  $P > 0.05$  and the different letters(a,b,c,d,e) indicate significant ( $P < 0.05$ ) by Duncan multiple test ( $n=4$ ). The values shown are the average value and standard deviation.

**Table 2.** Hematological analysis of red tilapia at the end of the feed restriction

Treatments	End of Feed Restrictions				
	Parameter				
	RBCs (cells mm <sup>-1</sup> )	WBCs (cells mm <sup>-3</sup> )	Ht (%)	Hb (gdL <sup>-1</sup> )	Glucose (mgdL <sup>-1</sup> )
F28	1.89±0.09 <sup>a</sup>	10.6±0.46 <sup>a</sup>	27.49±2.29 <sup>a</sup>	9.95±1.14 <sup>a</sup>	67.17±3.31 <sup>a</sup>
F7S21	1.63±0.13 <sup>b</sup>	9.39±0.24 <sup>b</sup>	24.5±0.89 <sup>b</sup>	9.37±0.83 <sup>ab</sup>	54.54±8.37 <sup>b</sup>
S14F14	1.62±0.02 <sup>b</sup>	8.66±0.64 <sup>b</sup>	23.69±1.13 <sup>b</sup>	8.62±0.27 <sup>b</sup>	46.25±8.61 <sup>b</sup>
S21F7	1.60±0.19 <sup>b</sup>	7.28±0.51 <sup>c</sup>	23.09±2.94 <sup>b</sup>	8.23±0.86 <sup>b</sup>	27.00±1.58 <sup>c</sup>
S28	1.64±0.07 <sup>b</sup>	6.28±0.77 <sup>d</sup>	21.77±1.51 <sup>b</sup>	8.61±0.36 <sup>b</sup>	23.96±4.07 <sup>c</sup>

\*The same letter in the same column is not significantly different at  $P > 0.05$  and the different letters (a, b, c, d, e) indicate significant ( $P < 0.05$ ) by Duncan multiple test ( $n=4$ ). The values shown are the average value and standard deviation

In this study showed there was an increase in appetite (Hyperphagia) after a period of refeeding, although the total of feed consumption at the end of the study on day 28 was still not as much as treatment F28 (control) (Table 1). Red tilapia experiencing increased appetite or hyperphagia illustrates that the fish is able to compensate for growth loss during periods of starvation. Also, the FCR and feed consumption value of the feed restriction group was found to be lower than the control and significantly decreased than control ( $P < 0.05$ ). The FCR value shows how efficient the feed is used in producing kg of fish meat (bodyweight). However, in this study, the increase in appetite only occurred for a few days along with a short refeeding period, so there was no compensatory growth. Wang et al. (2000) reported that hyperphagia occurs in hybrid tilapia during refeeding. Hyperphagia occurs when fish can compensate for their loss of growth during fasting.

The hyperphagia process is reported to occur in a matter of days or weeks, this depends on the length of the period of feed restriction and refeeding (Robinson et al., 2006; Gao and Lee, 2012). Periods of hyperphagia are two to three times longer than periods of fasting. Other research results suggested that hyperphagia can restore the structural material reserve ratio (Broekhuizen et al., 1994). The value of feed efficiency has not been seen in fish that are given feed restrictions, because the duration of the feeding phase again is only up to day 21. Caruso et al. (2014) stated that fasting causes a decrease in the overall level of digestive enzymes, then increases after refeeding occurs. Wang et al. (2000) reported, although there was partial compensatory growth in hybrid tilapia reared in seawater, as a result of increased feed intake, even fish tend to have a better ability to absorb nutrients during refeeding. In this study, no mortalities were recorded among different groups during the feed

restriction periods and control. This shows the high resistance of red tilapia to a period of restriction of feed. However, some other authors recorded the mortality rate was recorded to be 20% - 30% in 10 and 15 days starvation, respectively (Moustafa and El Kader, 2017), and 50% mortality at 10 days starvation in Nile Tilapia monosex fry (Fabillo *et al.*, 2004).

### 3.2 Biometric

After feed restriction, CF, HIS, and VSI values decreased significantly ( $P < 0.05$ ) compared to controls with increased feed restriction periods. It can be seen that the lowest CF value is in the 28-day feed restriction treatment of 1.49%, the low CF value in the S28 treatment is accompanied by a low HSI value of 0.27% and VSI value of 7.46%. These results indicate that fasting time greatly affects the nutritional condition of the body of red tilapia (Figure 2).

At the end of the experiment (day 28), the values of CF, HSI, and VSI were seen to have increased as in the control treatment except for red tilapia which was restricted from feed for 28 days (Figure 2). Among the feed restriction treatments, fish that were given a 7-day feed restriction with 21 days refeeding (S7F21) showed that the CF, HSI, and VSI values were almost the same as the control, due to the adequate intake of nutrients from the feed as an energy source. Based on statistical tests, it is known that the values of CF and VSI after the refeeding period, were not significantly different from (F28) ( $P > 0.05$ ) except for S28. As for the HSI value, the S7F21 treatment was not significantly different from the control but significantly different from other treatments. ( $P > 0.05$ ).

The results in this study similar to Xu *et al.* (2019) that on common carp the CF and HSI of experimental fasting for 14 and 28 days showed decreasing trends. Sakyi *et al.* (2020) stated that black tilapia that was fasted for 3-21 days had a condition factor value and a lower HSI than control decreased according to the length of the fasting period which ranged from 1.14-1.54 (CF) and 1.11-2.12 (HSI), but after a refeeding period of 21 days, the value was not significant compared to the control treatment. Likewise, the results of research on the *Chepalus mugil* fish showed that there was a decrease in the value of CF and HSI in fish that were fasted for 10, 20, and 30 days (Akbari and Jahanbakhshi, 2016). According to Robisalmi *et al.* (2021), red tilapia juveniles that were fasted periodically 1-3 days per week for a period of 120 days showed lower values than control ranging from 1.81-2.01 (CF), 1.53-1.91 (IHS), and 9.03-10.07 (IVS). The low value of FK, IHS, and IVS on longer feed restrictions due to the limited amount of feed consumed during rearing resulted in a lower average body weight.

The change of CF, HSI, and VSI are major indicators of physiological conditions and body loss caused by feed deprivation. This is probably the result of the utilization of energy for basal metabolism, where energy requirements were met by liver glycogen reserves, followed by the use of protein contained in fish muscle. CF is also a quantitative parameter of the nutritional adequacy of fish and a simple measure of energy reserves because of its effect on growth. Likewise, HSI and VSI are closely related to the nutritional status of fish.

**Table 3.** Hematological analysis of red tilapia at the end of experiment (28 days)

Treatments	End of Experiment				
	Parameter				
	RBCs (cells mm <sup>-1</sup> )	WBCs (cells mm <sup>-3</sup> )	Ht (%)	Hb (gdL <sup>-1</sup> )	Glucose (mgdL <sup>-1</sup> )
F28	2.22±0.17 <sup>a</sup>	8.59±0.19 <sup>a</sup>	29.15±1.69 <sup>a</sup>	9.15±0.26 <sup>a</sup>	79.41±2.52 <sup>a</sup>
F7S21	2.03±0.13 <sup>ab</sup>	9.05±0.22 <sup>a</sup>	27.75±1.37 <sup>a</sup>	9.50±0.16 <sup>ab</sup>	77.63±4.29 <sup>a</sup>
S14F14	1.87±0.12 <sup>bc</sup>	7.10±0.01 <sup>b</sup>	23.75±2.40 <sup>b</sup>	8.60±0.38 <sup>bc</sup>	76.31±2.15 <sup>a</sup>
S21F7	1.76±0.1 <sup>4c</sup>	8.80±0.80 <sup>a</sup>	26.25±1.25 <sup>bc</sup>	7.88±0.3 <sup>c</sup>	71.05±3.22 <sup>b</sup>
S28	1.50±0.07 <sup>d</sup>	6.28±0.77 <sup>c</sup>	21.77±1.51 <sup>c</sup>	8.61±0.36 <sup>d</sup>	23.96±4.07 <sup>c</sup>

\*The same letter in the same column is not significantly different at  $P > 0.05$  and the different letters (a, b, c, d, e) indicate significant ( $P < 0.05$ ) by Duncan multiple test ( $n=4$ ). The values shown are the average value and standard deviation.

According to [Goede and Barton \(1990\)](#), the condition factor is a crude measure of the level of energy reserves and fish health, and changes in its values may indicate changes in nutritional status of the fish. Similarly, low HSI values in fish are usually correlated to nutritional problems, because the relative size of the liver is correlated with the nutritional status of the fish. Variations of fish health and its values may be indicated by the changes in the nutritional status of the fish ([Caruso et al., 2012](#)). According to [Maragoudaki et al. \(1999\)](#) that when there is food restriction, the fish will adapt by changing their behavior according to the availability of feed. At the end of the experiment, the condition factor of feed restriction treatment was also similar to control (F28), which indicated that growth occurred in length and body shape of fish, with increased feed consumption.

### 3.3 Hematological

All hematologic parameters at the end of feed restriction were found to decrease significantly ( $P < 0.05$ ) with the length of time for restriction compared to the control ([Table 2](#)), but the Hb value in the control was not significantly different ( $P > 0.05$ ) from S7F21. After refeeding 7-21 days, RBCs, WBCs, Ht, Hb, and glucose values are increasing trend and returned to normal levels such as control fish that were fed every day, although the value was still lower than the control treatment ([Table 3](#)). Based on statistical tests, it is known that the values of RBCs, WBCs, Ht, Hb, and glucose in the control treatment were not significantly different ( $P < 0.05$ ) from S7F21, but significantly different from other feed restriction treatments ( $P > 0.05$ ). At the end of maintenance (day 28), it was known that glucose levels in treatment S7F21 and S14F14 showed a value that was almost close to the control treatment (76.32-79.41 mgdL<sup>-1</sup>).

In this study, it was shown that the feed restriction period (7-28 days) with 7-21 days of refeeding in red tilapia more influenced the nutritional status of the fish which in turn affected hematological values, but did not cause anemia or immune deficiency. The hematological value of an organism can be used to determine the health condition that the organism is experiencing. Physiological deviations in fish cause the components of the blood to also change. But, literature results regarding the effect of feed restriction on hematological value are conflicting. The results of this study are consistent with the report of [Robinson et al. \(2006\)](#), that fasting *O. niloticus* for 1-4 weeks showed a decrease in RBC and Hb values. In the same report, although there were an increase in hematocrit in sturgeon fish that

had been restricted from feed for 6 weeks, the Hb and MCHC values showed a decrease ([Falahatkar, 2012](#)). In this study, feed restriction causes a significant decrease RBCs, WBCs, Ht, and Hb levels which are similar to findings on *Lutjanus guttatus* ([Hernández et al., 2019](#)). However, it is different from the results of [Sakyi et al. \(2020\)](#) that where tilapia that is fasted for 14 days has good immune response with an increase in hematology, while glucose levels decrease but increase with re-feeding. In beluga or great sturgeon fish (*Huso huso*), it is known that there is no change in RBC starvation for 8 days but there is swelling of erythrocytes due to an increase in Ht accompanied by a decrease in MCHC ([Morshedi et al., 2011](#)). In common carp (*Cyprinus carpio*) reported that fasted for 15 weeks showed no significant hematological parameters compared to those fed continuously low protein ([Kondera et al., 2017](#)).

According to [Johnny et al. \(2003\)](#), a decrease in hemoglobin and hematocrit levels can be used as an indication of the low content of feed protein and vitamin deficiency. The lower the hemoglobin level, the smaller the ability will be to carry oxygen into the body. The decrease in blood parameters has been overcome by depression in erythropoiesis, especially the decrease in red blood cells as a result of the decreased erythrocyte decline ([Rios et al., 2005](#); [McCue, 2010](#)). [Kondera et al. \(2017\)](#) stated that fasting causes a decrease in the erythropoietic rate by inhibiting the differentiation of blood cells in the hematopoietic tissue, but does not cause anemia. In this study, the number of RBCs tended to decrease with the length of the fasting time, this indicated that red tilapia did not experience phagocytosis and environmental stressors that caused an increase in the number of white blood cells. A low RBCs value indicates that the fish are in good health or have a good immune response and red tilapia can tolerate dietary restrictions, without an increase in the white blood cell count. According to [Mahajan and Dheer \(1983\)](#) WBC is very sensitive to hunger, so white blood cells have a major function in immunity and cell defense, but there is no significant increase depending on the species.

The value of red tilapia blood glucose levels in this study decreased along with the length of the feed restriction period (7-28 days). These results indicate that blood glucose is used as an additional source of energy for metabolic processes during fish fasting. In this study glucose level of red tilapia was similar to ([Sakyi et al., 2020](#)) that glucose of juvenile tilapia, which was fasted for 3-14 days tended to decrease and in control fish increased. [Hernandez et al. \(2019\)](#) stated that on rose snapper (*Lutjanus guttatus*) indicated



that during short-term starvation (14 day), this fish species exhibited reduced plasma glucose levels. This data suggests that under feed restriction decrease in the glucose level of the plasma indicates a depletion of available glycogen reserves. According to [Li et al. \(2018\)](#), the decrease in glycogen caused by the fasting period is caused by a decrease in glucose levels because it is used as fuel in metabolic processes. In numerous fish species, glycemic maintenance during food deprivation has a direct relationship with the capacity to mobilize glycogen from the liver, noticeable at the beginning of starvation, with a decrease in the weight of the liver ([Pérez-Jiménez et al., 2007](#)). [Mommmsen \(1991\)](#) suggested that during fasting there is an increase in glycogenolysis (the process of breaking down glycogen molecules into glucose) and gluconeogenesis (making glucose from non-carbohydrate compounds, usually occurs in the liver), which aims to maintain glucose levels in the body. Glucose is obtained by breaking down glycogen into glucose which is then used to produce energy. [Martínez-Porchas et al. \(2009\)](#) stated that the fluctuation of glucose levels in fish blood indicates that the fish is hungry or full. If the fish is hungry, and there is no feed intake, the glucose level in the blood will decrease instead.

#### 4. Conclusions

In this study, feed restriction affects the growth performance on red tilapia proportional to the length of the starvation period indicated by decreasing body weight, biometric and hematological values, but does not cause death. The results suggest that the decrease in hematological parameters did not indicate stress levels in fish, but reflected a malnutrition condition.

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#### Author's Contribution

All authors have contributed to the final manuscript. The contribution of each author as follows, Adam Robisalmi and Bambang Gunadi; conceived of the presented idea, developed the theory and performed

the computations. Kariawati Alipin; verified the analytical methods. Bambang Gunadi; encouraged Adam Robisalmi to investigate theory and supervised the findings of this research. All authors; discussed the results and contributed to the final manuscript. Adam Robisalmi and Bambang Gunadi; carried out the experiment. Adam Robisalmi; wrote the manuscript with support from Bambang Gunadi and Kariawati Alipin. Kariawati Alipin; developed the theoretical formalism, performed the analytic calculations and performed the numerical simulations. Both Adam Robisalmi and Bambang Gunadi contributed to the final version of the manuscript. Kariawati Alipin; supervised the project. Adam Robisalmi, Kariawati Alipin and Bambang Gunadi; conceived and planned the experiments. Adam Robisalmi; carried out the experiments. Adam Robisalmi and Kariawati Alipin planned; and carried out the simulations. Adam robisalmi and Bambang Gunadi; contributed to sample preparation. Adam Robisalmi dan Kariawati Alipin; contributed to the interpretation of the results. Adam Robisalmi; took the lead in writing the manuscript. All authors; provided critical feedback and helped shape the research, analysis, and manuscript. Adam Robisalmi and Bambang Gunadi; designed the model and the computational framework and analysed the data, carried out the implementation. Adam Robisalmi; performed the calculations, wrote the manuscript with input from all authors. Kariawati Alipin; conceived the study and were in charge of overall direction and planning. All authors; discussed the results and commented on the manuscript, contributed to the design and implementation of the research, the analysis of the results, and to the writing of the manuscript.

#### Conflict of interest

The authors have no conflicts of interest to declare. All co-authors have seen and agreed with the contents of the manuscript and there is no financial interest to report. We certify that the submission is original work and is not under review at any other publication.

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