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Impacts of Immunostimulant Yeast (*Saccharomyces cerevisiae*) Supplemented Feed on Growth and Blood Profile of Java Barb (*Barbonymus gonionotus*)

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Abstract

The research aims to analyze the impacts of immunostimulant yeast (*Saccharomyces cerevisiae*) supplemented feed on growth rate and blood profile in Java Barb [*Barbonymus gonionotus* (Bleeker, 1850)] fingerlings. The Java Barb of 200 fingerlings had an average weight of 5.64 g ± 0.87 g, and an average length of 6.32 cm ± 0.58 cm. The experiments employed a Completely Randomized Design with five treatments, and each had four repetitions for a period of 49 d (days). Various dosages of *S. Cerevisiae* were added to the feed. The treatments were T1 (0 g kg^{-1} feed), T2 (1 g kg^{-1} feed), T3 (2 g kg^{-1} feed), T4 (3 g kg^{-1} feed), and T5 (4 g kg^{-1} feed). The results showed that *yeast* as supplemented feed could boost the growth and blood profiles of Java Barb. The blood profiles include improvement in total blood cells (TCC μL^{-1}), red blood cells (RBC), white blood cells (WBC), hematocrit (HTC) compared to control. The highest value of efficiency of feed utilization (EFU) was 72.36 % and followed by FCR (feed conversion ratio), PER (protein efficiency ratio), RGR (relative growth rate), and SR (survival rate) with the values of (1.63, 4.21, 4.18) % d^{-1}, and 92.33 % respectively. The optimal dosages of *S. cerevisiae* in the feed for EFU, FCR, PER, and RGR ranged from 2.38 g kg^{-1} feed to 3 g kg^{-1} feed.

Keywords: Digestibility, Enzyme, Fisheries feed, Immunity, Resistance

1. Introduction

Java Barb [*Barbonymus gonionotus* (Bleeker, 1850)] is an Indonesian native fish that is easily cultivated; hence, farmers have practiced intensively in aquaculture (Rachmawati *et al*., 2019b). One of the problems faced by the aquaculture farmers was decreased in water quality due to unconsumed fish feeds and water waste. This condition could inhibit fish growth and rising fish diseases that could decrease fish production. Thus, it made farmers lose in profit. The studies to administrate the fish growth have been done through giving biofloc, probiotic, herb, and immunostimulant. Biofloc was implemented on fish and shrimp (Kuhn *et al.*, 2010). Moreover, de La Banda *et al.* (2010) has applied probiotic on fish and shrimp cultivation. Furthermore, Goda *et al*. (2012) also reported the *S. cerevisiae* supplemented feed could boost fish appetite; therefore, it improved growth and survival rate.

To spur growth and immunity in the fish, fish farmers could utilize immunostimulants that can be obtained from seaweed, bacteria, or yeast. Manoppo and Magdalena (2015) also discovered that the utilization of immunostimulants could raise growth and the immune system in the fish and crustaceae. According to Abu-Elala *et al*. (2013), *S. cerevisiae* is one of the immune-stimulants. In addition, Tewary and Patra (2011) also suggested that *S. cerevisiae* can trigger digestibility due to digestive enzymes to boost fish growth. The *S. cerevisiae*-supplemented feed could boost the digestibility of feed and protein. It could also increase efficiency utilization of the feed and the growth (Razak *et al*., 2017).

The immune response rate of fish could be increased by adding *S. cerevisiae* in the feed, as reported by Abu-Elala *et al*. (2013). Next, the *S. cerevisiae* could be used as an immunostimulant, because it is rich in elements such as β-1-3 glucan (50 % to 60 %). Moreover, it could generate its immunity in the fish and crustacean in which β-glucan in the yeast was able to boost the immunity and disease resistance of the fish (Manoppo and Magdalena, 2015). Moreover, Sheikhzadeh *et al*. (2012) mentioned that *S. cerevisiae* contains immunostimulants such as β-1,3 glucan, nucleate acid, manna oligosaccharide, chitin, non-starch nucleate acid, and polysaccharide. The β-1,3 glucan effectively increased the immune system in some species of fish at the dosage of 1 g kg^{-1} feed (Dhanaraj *et al*., 2010). Jarmolowicz *et al.* (2011) disclosed that the addition of beer yeast at the dosage of 4 % to 6 % could boost non-specific immune system in the fingerlings of pikeperch [*Sander lucioperca* Linnaeus, 1758)].

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Some researches on the *S. cerevisiae*-supplemented feed to boost growth and immunity were disclosed by Tewary and Patra (2011) in *Labeo rohita*, F. Hamilton, 1822; Tawwab *et al*. (2010) in *Sarotherodon galileaus*, Linnaeus, 1758; Manoppo and Kolopita (2016) in *Cyprinus carpio*, Linnaeus, 1758; Jarmolowicz *et al*. (2011) in *Sander lucioperca* Linnaeus, 1758; Azevedo *et al*. (2016) in *Oreochromis niloticus*, Linnaeus 1758; Rachmawati *et al*. (2019a) in *Pangasius hypothalamus* Sauvage, 1878; and Rachmawati *et al*. (2019b) in *Barbonymus gonionotus* Bleeker, 1850. However, previous research only observed the growth of cultivated fish, and research on the effect of blood profiles has never been carried out. Therefore, it is important to do this research which studies the impact of yeast on the blood profile of Java Barb.

Some benefits of using *S. cerevisiae* as an immuno-stimulant in aquaculture are that it does not leave any residues in fish and the environment, and hazard free for human beings as well. Therefore, the utilization of *S. cerevisiae* as an immunostimulant to increase growth is very important. This study aims to analyze the impacts of *S. cerevisiae* supplemented feed on growth rate and blood profile in Java Barb fingerlings.

2. Materials and Method

The study was conducted at the Laboratory for Fish Health and Environment Assessment, Muntilan, Central Java, Indonesia from April until June 2019. This experiment utilized 500 sample fingerlings of Java barb with an average weight of 5.64 g ± 0.87 g, and an average length of 6.32 cm ± 0.58 cm. (Rachmawati *et al*., 2017). To adjust to feeding and a new environment, the sample fish was first acclimated for 1 wk (week). During acclimatization, fish were fed with commercial feed using ad satiation method. To maintain the water quality, water was being siphoned before feeding. Before the study was conducted, the sample fish fasted for 1 d (day) to clean the metabolism residues hence the initial weight was not affected by the waste weight. The yeast materials for treatments were commercial yeast (*S. cerevisiae*) brand Saf Instant produced by Saf Indonusa - Lessafre Global Group, Indonesia.

Completely Randomized Designed (CRD) was implemented in this research. There were five treatments with four replications. The treatments were a supplementation of yeast (*S. cerevisiae*) into the feed. The feeds used were commercial with brand name Comfeed fish feed, produced by JAPFA Comfeed Indonesia Tbk., composed of 30 % of raw protein, 2 % fat, 3 % raw fiber, 13 % ash, and 12 % water. Treatments were included: T1 (0 g kg^{-1} feed), T2 (1 g kg^{-1} feed), T3 (2 g kg^{-1} feed), T4 (3 g kg^{-1} feed), and T5 (4 g kg^{-1} feed). The dosages of the *S. cerevisiae* in this study were modified from Rachmawati *et al*. (2019a) study. The study recorded that 1 g kg^{-1} feed supplementation of *S. Cerevisiae* was the best dosage to produce the highest feed usage efficiency in fingerling of catfish (*Pangasius hypothalamus* Sauvage, 1878**)**.

After the yeast was added, the protein analysis per treatment was T_0 (30 %), T_1 (30.37 %), T_2 (30.46 %), T_3 (30.39 %), and T_4 (30.53 %). The preparation of the feed was by weighing the *S. cerevisiae* based on the treatments. The weighed yeast was diluted with pure water. The 100 mL pure water was diluted in 1 kg feed (Manurung *et al*., 2013). The diluted yeast was evenly sprayed into the feed based on the Rachmawati *et al*., (2019b) method. Firstly, the feed was put on the plastic tray and then sprayed by suspense yeast. When feed was being sprayed with yeast, the tray was shaken and the feed and the yeast were mixed. Then, the mixture was dried up by letting it at room temperature of 28 °C to 30 °C. After that, the mixture was put in a plastic bag. Then, the bag was labeled by treatment and stored in the refrigerator until it was ready to use. The fix feeding method was used to feed the fingerlings. The method was based on 5 % of the fish weight. The three times daily feeding implemented were in the morning (7 am), afternoon (2 pm), and evening (6 pm). The scale of the fish weight was carried out every week for 49 d. Sampling was carried out once 1 wk; therefore, a 7 wk observation is required to obtain more accurate data.

The containers used were made from fiberglass by dimension 1.5 m × 1.5 m × 1 m. The fish was reared in 20 containers with a stock density of 25 fish m^{-3}. Freshwater was utilized as cultivation media. Before the water was used, it has been deposited for several days in a reservoir.

The observation on blood profile has been conducted at the beginning (1^{st} d) and the end (49^{th} d) of the study. The observed parameters included total cells (TCC μL^{-1}), red blood cells (RBC), white blood cells (WBC), hematocrit (HCT) (referring to Mohammed *et al*., 2013 on blood profile methodology).

The observed variables included feed efficiency (EF), the ratio of feed conversion (FCR), the protein efficiency ratio (PER), relative growth rate (RGR), and survival rate (SR) which was measured every week. Those variable observations were referring to Rachmawati *et al*. (2018) and Rachmawati *et al*. (2019b), while analysis of blood profile and water quality consisting of temperature, pH, and dissolved oxygen was measured every morning and evening, whereas ammoniac was measured in 1^{st} d, 28^{th} d and 49^{th} d based on the method of APHA (1992). Water quality was maintained through the daily siphon and 30 % of water change every week after sampling. The measure variables are EFU (efficiency of feed utilization) in Equation (1) and RGR (relative growth rate) in Equation (2):

$$EFU = 100\left(\frac{W_2 - W_1}{QF}\right) \quad (1)$$

$$RGR = 100\left(\frac{W_2 - W_1}{T \times W_1}\right) \quad (2)$$

Note: W_1 and W_2 are the initial and final weight, respectively, QF is the feed amount consumed, and T is the days during the feeding period. FCR (feed conversion ratio), PER (protein efficiency ratio), SR (survival rate) are calculated by Equation (3), Equation (4), and Equation (5).

$$FCR = 100\left(\frac{F_1}{WG}\right) \quad (3)$$

Note: FI is feed intake (g) and WG is weight gain (g);

$$PER = 100(\frac{W}{PI}) \quad (4)$$

Note: WG is weight gain (g) and PI is protein intake (g);

$$SR = 100(\frac{C_2}{C_1}) \quad (5)$$

Note: C_1 is an initial count of fish and C_2 final count of fish.

Analysis of variance (ANOVA) and Duncan's Multiple Range Test were used to analyze the observed variables (Rachmawati *et al*., 2019b). The polynomial orthogonal test using SAS9 and Maple12 software was used to calculate the optimal dosage of immunostimulant yeast (*S. cerevisiae*). Water quality parameters were descriptively explained by comparing the rearing conditions to determine the viability.

3. Results and Discussions

Table 1. displayed the observed variables of EFU, FCR, PER, RGR, and SR in treatment T_0, T_1, T_2, T_3, and T_4. The polynomial orthogonal test results of *S. cerevisiae*-supplemented feed on EFU, FCR, PER, and RGR were displayed in Figure 1, Figure 2, Figure 3, and Figure 4. Figure 1 showed that the optimal dosage of *S. cerevisiae* in the feed for EFU was 2.38 g kg^{-1} feed with the EFU value as much as 67.67 %. The best value of FCR (1.58) was obtained from *S. cerevisiae* with 2.43 g kg^{-1} feed, as shown in Figure 2.

Table 1 shows that adding yeast to feed has a significant effect on EFU, FCR, PER, and RGR, but does not show an effect on SR.

Figure 3 displayed the relationship between the dosage of yeast and the values of PER. The optimal dosage of *S. cerevisiae* for PER was 2.62 g kg^{-1} feed giving the value of 4.08. Based on Figure 4, one could calculate the optimal dosage of *S. cerevisiae* for RGR. The optimum dosage was 3 g kg^{-1} feed giving the value of 4.18 % d^{-1}.

Table 1. The values of the variables

Experiment	Treatments				
Data	T_0	T_1	T_2	T_3	T_4
EFU (%)	48.24 ± 0.18^d	59.75 ± 0.27^c	63.35 ± 0.31^b	72.36 ± 0.19^a	55.63 ± 0.15^c
FCR	2.53 ± 0.32^c	2.18 ± 0.25^b	2.01 ± 0.25^b	1.63 ± 0.18^a	2.12 ± 0.27^b
PER	1.96 ± 0.28^d	2.85 ± 0.24^c	3.27 ± 0.23^b	4.21 ± 0.27^a	2.59 ± 0.25^c
RGR (% d^{-1})	1.67 ± 0.14^d	2.09 ± 0.15^c	3.52 ± 0.22^b	4.18 ± 0.25^a	3.48 ± 0.22^b
SR (%)	75.33 ± 3.52^a	90.33 ± 2.67^a	90.33 ± 2.72^a	92.33 ± 2.78^a	90.33 ± 2.43^a

Note: The mean values with a different superscript in the same column showed significant difference ($P < 0.05$)

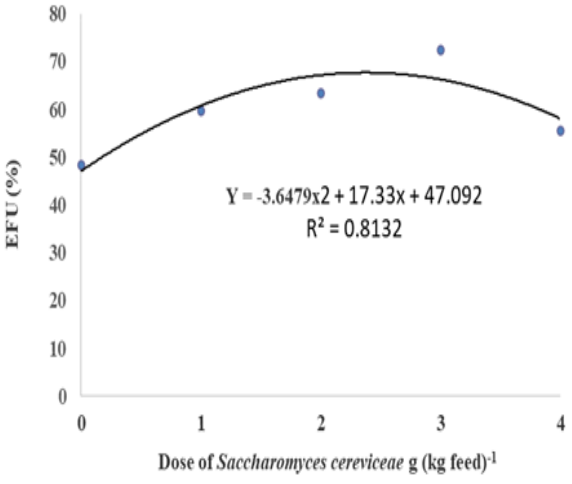

Figure 1. The relation between the immunostimulant yeast (*S. cerevisiae*) and EFU in the Java Barb fingerlings

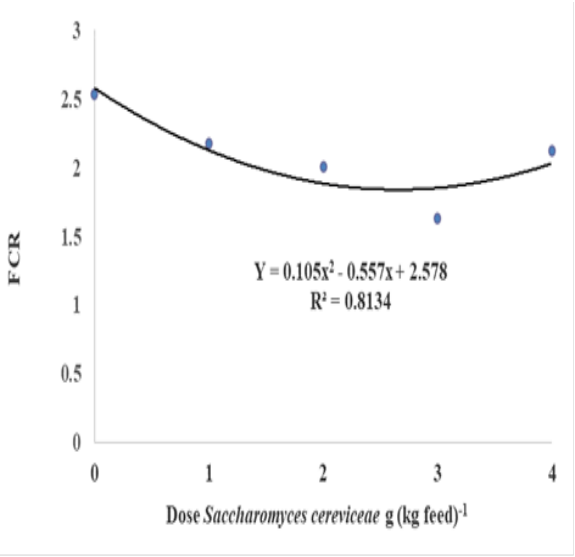

Figure 2. The relation between the immunostimulant yeast (*S. cerevisiae*) and FCR in the Java Barb fingerlings

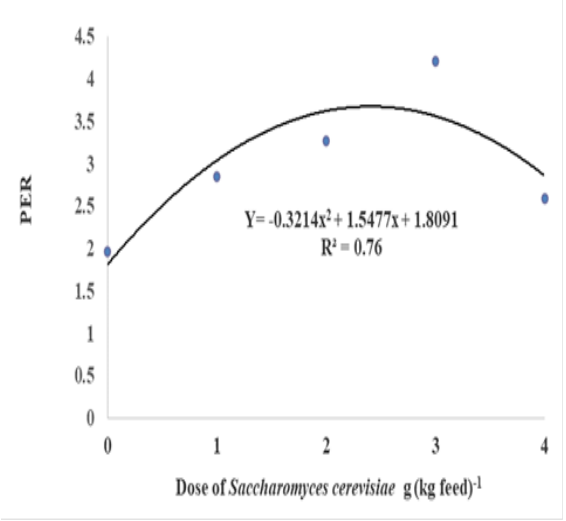

Figure 3. The relation between the immunostimulant yeast (*S. cerevisiae*) and PER in the Java Barb fingerlings

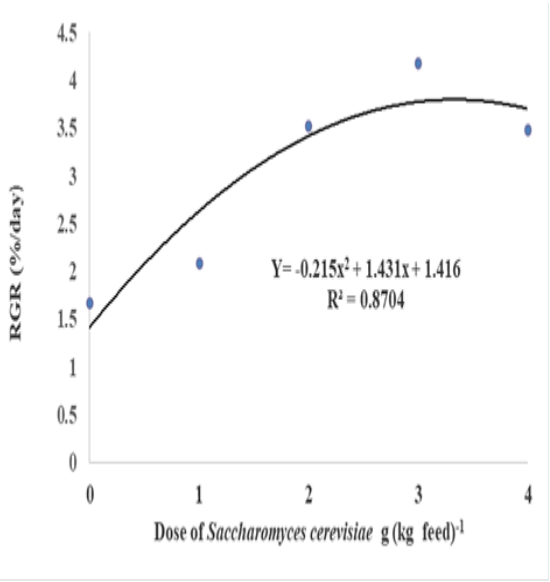

Figure 4. The Relationa between the immunostimulant yeast (*S. cerevisiae*) and RGR in the Java Barb fingerlings

The enrichment of *S. cerevisiae* with the dosages of 1 g kg^{-1} feed to 4 g kg^{-1} feed (T1, T2, T3, T4) in the Java barb fingerlings had higher values of EFU (55.63 % to 72.36 %) than those given the zero-supplementation yeast (48.24 %, as in the treatment T0). It was noted that the *S. cerevisiae* supplemented feed could raise the efficiency of feed utilization. Razak *et al*. (2017) discovered that the *S. cerevisiae* could boost enzymatic activities in the digestive track. Hence, it enhanced the decomposition of complex nutrients into simpler nutrients as proclaimed by Tewary and Patra (2011). The simpler nutrients were easier to absorb, and in turn this enhanced the efficiency of feed utilization. The dosage of 3 g kg^{-1} feed (T3) produced the highest EFU (72.36 %), followed by the treatments T2, T1, T4, and T0 that had values of 63.35 %, 59.75 %, 55.63 % and 48.24 %, respectively. The optimal dosage of yeast in the feed that resulted in the highest EFU may cause the dosage appropriate to enhancing enzyme activities in the digestive track and then improve the efficiency of feed usage. Welker *et al*. (2012) discovered that *S. cerevisiae* as an immunostimulant could enhance the production of the enzyme in the digestive tract to boost digestibility and absorption for nutrients, amino acids, vitamins, and enzymes. Similar results from other studies were reported by Tawwab *et al*. (2010) in *Sarotheredon galileus* Linnaeus 1758, Tewary and Patra (2011) in *Labeo rohita* F. Hamilton 1922, and Rachmawati *et al*. (2019a) in *Pangasius hypothalamus* Sauvage 1978. Results of previous experiments showed that the addition of yeast to feed increases EFU.

The *S. cerevisiae* supplemented feed decreased the FCR of Java barb fingerlings. The treatment T3 with the dosage of 3 g kg^{-1} feed generated the lowest FCR. The dosage was to make maximum feed usage efficiency, so it decreased FCR. Razak *et al*. (2017) supported this discovery. This study found that the *S. cerevisiae*-supplemented feed could raise the efficiency of feed usage and decreased the ratio of feed conversion. In addition, Jarmolowicz *et al*. (2011) discovered that the *S. cerevisiae* supplemented feed caused FCR to increase due to the increase of protein digestibility. Essa *et al*. (2010) also disclosed that the *S. cerevisiae*-supplemented feed raised the FCR and the efficiency of feed utilization.

The protein efficiency ratio (PER) is a number calling the total weight of fish produced per unit of protein in the feed (Tiamiyu *et al*., 2014). Various dosages of *S. cerevisiae* of 1 g kg^{-1} feed to 4 g kg^{-1} feed given to Java barb fingerlings generated higher PER (2.59 to 4.21) compared to the PER (1.96) without the addition of yeast. Goda *et al*. (2012) mentioned that the *S. cerevisiae*-supplemented feed could surge protein digestibility; therefore, it increased the ratio of protein efficiency. Moreover, Rachmawati *et al*. (2019b) stated that the *S. cerevisiae*-supplemented feed could enhance the efficiency of feed utilization and the ratio of protein efficiency. The highest PER (4.21) was achieved in T3 (3 g kg^{-1} feed,) while the lowest PER (1.96) was attained in T0 (0 g kg^{-1} feed). The highest PER was because of the right dosage of the yeast to generate the ratio of protein efficiency, while the lowest PER was due to the absence of yeast.

The results on the RGR of the supplementation of *S. cerevisiae* ranged from 1 g kg^{-1} feed to 4 g kg^{-1} feed and between 2.09 % d^{-1} and 4.18 % d^{-1}. These values were higher than without yeast supplementation by 1.67 % d^{-1}. The *S. cerevisiae* supplemented feed could raise RGR, which was due to the increases in protein digestibility and efficiency of feed utilization (Manoppo and Magdalena, 2015). Moreover, Rajagukguk *et al*., (2017) disclosed that the existence of yeast (*S. cerevisiae*) in the fish digestive system could boost enzymatic activities. It can hike protein digestibility and efficiency of feed usage, and in turn, it raised RGR. The treatment T3 (3 g kg^{-1} feed) generating the highest RGR was suggested due to the effective dosage of the yeast (*S. cerevisiae*).

The *S. cerevisiae* supplemented feed was not significant ($P > 0.05$) on the SR of Java barb fingerlings. It was suggested that the feed was not a factor for SR. The factor that affected SR was abiotic one, such as the ability to adapt to the environment, handling, density, competitors, diseases, ages, and the existence of the predators (Abu-Elala *et al*. (2013). The SR of Java barb fingerlings that were fed by supplementation of *S. cerevisiae* ranged from 90.33 % to 92.33 %, while the survival rate for those that were not fed with the

supplementation of *S. cerevisiae* was 73.33 % (Table 1). It was suggested that the yeast (*S. cerevisiae*) contained β-glucan as an agent of immunostimulant to increase the immune system; therefore, the SR was high. This phenomenon supported by Manoppo and Magdalena (2015) stated that the enrichment of n β-glucan was able to be an immunostimulant to improve the immune system in the fish. The results of the blood profile were higher after the fingerlings were fed with the enrichment of *S. cerevisiae* as in Table 2.

The results of the blood profile measurement consisted of total cell count (TCC μL^{-1}), red blood cells (RBC), white blood cells (WBC), and hematocrit (HCT). Those were displayed in Table 2.

Table 2. Total cell count (TCC/μL^{-1}), red blood cells (RBC), and white blood cells (WBC), hematocrit (HCT) in the Java Barb fingerlings

Treatments ($g\,kg^{-1}$ feed)	TCC/µL ($\times 10^6$)	RBC ($\times 10^6$)	WBC ($\times 10^5$)	HCT (%)
T_1 (0)	1.39^b	1.92^b	1.38^b	24.49^b
T_2 (1)	3.23^a	3.25^a	2.63^a	35.13^a
T_3 (2)	3.45^a	3.37^a	2.37^a	36.74^a
T_4 (3)	3.65^a	3.39^a	2.65^a	37.98^a
T_5 (4)	3.56^a	3.29^a	2.83^a	35.83^a
±SD	0.127	0.132	0.263	0.221

Note: The mean values with a different superscript in the same column showed significant difference ($P < 0.05$)

Table 2 showed that the fingerlings that were fed with the *S. cerevisiae* supplemented feed had a significant increase in the total cell count (TCC/μL^{-1}), the red blood cells (RBC), the white blood cells (WBC), and the hematocrit (HCT). It was revealed that the *S. cerevisiae*-supplemented feed increased non-specific immune response in the Java barb fingerlings. The results show that *S. Cerevisiae* is rich in β-1-3 glucan (50 % to 60 %) that increased fish immune system; therefore, fish resisted the pathogen. It was proven by the value of SR that was higher than without *S. Cerevisiae* supplementation. Manoppo and Magdalena (2015) reported that the *S. cerevisiae*-supplemented feed increased non-specific immune response. Abu-Elala *et al*. (2013) also reported that the Java barb fed with *S. cerevisiae* supplemented feed increased erythrocyte, hemoglobin, and leukocyte. Moreover, Welker *et al*. (2012) discovered that the catfish fed with *S. cerevisiae*-supplemented feed for 1 wk had higher red and white blood cells than those with no *S. cerevisiae* supplemented feed.

The observation of water quality during the experimental period was in an infeasible condition for Java Barb cultivation as indicated by the standard quality in the literature. The suitable condition of the water quality affected by the water quality was controlled according to the requirement of Java Barb need.

4. Conclusion

The supplementation of immunostimulant yeast (*S. cerevisiae*) in the feed could raise the growth and blood profiles such as total cell count, red blood cells, and white blood cells in the Java barb fingerlings. The optimal dosages of *S. cerevisiae* in the feed for EFU, FCR, PER, and RGR ranged from 2.38 $g\,kg^{-1}$ feed to 3 $g\,kg^{-1}$ feed.

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