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Stimulation of Deprivation Cycles with *Spirulina platensis* Feed Supplementation on *Osphronemus gouramy* Physiological Responses

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Abstract

Spirulina platensis is a phytoplankton, the cell wall composed of complex sugars so easily digested by fish. The present study was carried out to investigate stimulation cycle of feed deprivation with feed supplemented *S. platensis* the best to increase growth, hematological and body composition of gurami (*Osphronemus gouramy*). Groups of 24 fish, each in triplicate, were exposed to four different treatment for a period of 56 days. Sample measurements of growth done every 14 days, hematological and body composition measurements carried out at the end of the experiment. Growth was significantly different between stimulation cycle of feed deprivation and the control ($P<0.05$). Conclusions result showed that stimulation cycles of feed deprivation could not improve growth and hematological, but could improve body composition. Feed deprivation is done to reduce the cost of production, high production costs due to high feed prices. During research on feed deprivation is done by giving commercial feed, this study is to provide feed supplementation *S. platensis*. Thus, the results of this study can be useful for science as *S. platensis* information can be used as a food supplement and and for the people cultivating gurami should be fed daily supplementation of *S. platensis*.

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INTRODUCTION

Spirulina platensis is a blue-green algae planktonic, filamentous, can live in lakes, brackish water and sea water. The cell wall is very soft because it is coated by complex sugars and protein, making it easy to digest (Kozlenko & Henson, 2010). Nutritional composition of *S. platensis* is very complete, consisting of 62% protein, vitamins, antioxidants β-carotene, phytopigment xanthophyl (Kumari, 2011). Henrikson (2000) reported that *S. platensis* contains Gamma Linolenic Acid (GLA), and iron, contain chlorophyll, glycogen, minerals (Allen, 2000) and phycocyanin and porphirin (Belay, 2002).

Supplementation of *S. platensis* in the diet can modulate the immune system (Rabadiya & Patel, 2010), can to a large-scale fish farming (El-Sheekh et al., 2014), and can be used as an essential component for animal feed (Kim et al., 2013). Simanjuntak et al., (2011 a, b), reported that methanol extract of *S. platensis* can enhance the humoral immune response; Immunoglobulin-G and Immunoglobulin-M content in mice that infected with tachyzoite *Toxoplasma gondii*. Furthermore, it was reported that supplementation of *S. platensis* in fish feed can promote the growth and the survival (Ibrahem et al., 2013; Jana et al., 2014; Zeinab et al., 2015; Simanjuntak et al., 2015), and can be used as a raw material for feed (Simanjuntak et al., 2014).

Fish, including gurami, is the main source of protein for human consumption and is important in improving malnutrition. Gurami is a freshwater fish that have economic value because the price is quite expensive and relatively stable. National gurami production increased by 19.86% per year since 2009 – 2013, increased from 46 254 tonnes in 2009 to 94 605 tonnes in 2013. The Banyumas district is the center of gurami with production reaching 3,057 tons in 2012 (Ministry of Fisheries and Marine Resources, 2014). Thus, the cultivation of gurami are good prospects for development. Production of gurami each year has increased, but not enough to meet market demand. However, lately less interested fish farmers to cultivate gurami. This is due to the slow growth of gurami, to achieve 500 g size takes 18 months (Sitanggang & Sarwono, 2001). Gurami are also susceptible to infections and maintenance gurami larva that have not been intensively, so that the production is still low (Effendi et al., 2006). The human need for protein is derived from fish, if it is only rely on capture fisheries, will not be sufficient, so that fish production needs to be increased.

Some studies of the growth of compensation in various species of fish have been carried out. Compensatory growth by limiting the feed (Dmitriew & Rowe, 2007; Turano et al., 2008; Gao & Lee, 2012; Eslamloo et al., 2012), the restriction of feed with different kinds of feed (Cho, 2011) and compared between the two species of striped bass, Morone chrysops x Morone saxatilis (Turano et al., 2007). However, research on the stimulation cycle of feed deprivation with feed supplemented *S. platensis* 4 g/kg at feeding gurami has not been done.

Some researchers also provide additional on fish feed to promote growth and the survival. Muchlisin et al., (2016) examined the effectiveness of various levels of papain in fish Keureling (*Tor tambra*) found that the application of papain in the diet significantly influence growth performance and survival rate. Leaf extract of *Terminalia catappa* L. enhance survival and blood profile of ornamental fish (*Betta sp*) (Nugroho et al., 2016). Incorporation of *S. platensis* and *Lactobacillus rhamnosus* in Nile tilapia diets increase the growth rate and biochemical performance. Giving *S. platensis* more effective in stimulating the growth of fish (Zeinab et al., 2015).

Therefore, this research needs to be done in order to get stimulation cycle of feed deprivation with feed supplemented *S. platensis*, the best to increase growth (weight and body length), hematological (the number of erythrocyte, leukocyte count, hematocrit value and hemoglobin concentration) and body composition of gurami, *Osphronemus gouramy*.

An important finding of this study is *S. platensis* can improve the physiological response of gurami fish, because of the high nutritional content. This research beneficial to the development of science and for fish farmers for the management of feeding.

METHODS

The research was conducted at the Experimental station Study Program Diploma III in Management of Fisheries Resources, Biology Faculty, Jenderal Soedirman University Purwokerto. Research implementation took place from March to June 2016.

The research materials were gurami, *S. platensis* powder, aquarium fiber, stationery, paper labels, air circulation equipment, microscope, digital scales, 1 cc syringe, EDTA (anticoagulant), Hayem solution, Turk solution, HCl 0.1 N, microcentrifuge haematocrite, hemocytometer, hemometer, oven, spryer, commercial feed, petri

disk, blender, millimeter block paper.

Gurami fish used in this study was 3 months old and came from a pair of parent spawning gurami. As many as 24 gurami is placed in the aquarium fiber 12 each with a density of 2 fish with an average weight of 2.62 ± 0.64 g (Figure 1). Gurami were placed in the aquarium at random and in acclimation for one week, at the end of acclimation, fish were fasted for one day. Gurami fish, ready for treatment.

Figure 1. Gurami fish (*Osphronemus gouramy* Lac.)

The study was conducted experimentally by treatment differences stimulation cycle of feed deprivation with feed supplemented *S. platensis* and each repeated three times. The treatments were tested, namely: C (control) = fish fed every day; P1 = fish fasted every Monday; P2 = fish fasted every Monday and Thursday; P3 = fish fasted every Monday, Wednesday and Friday. The feed has been supplemented with 4 g *S. platensis* in 1 kg of commercial feed (Simanjuntak et al., 2003).

Spirulina platensis supplementation on feed made with the protocol as follows: 150 ml of distilled water were placed in a spray (sprayer), then added 4 g of dried *S. platensis* then shaken until homogeneous. Commercial feed as much as 1 kg placed in the tray, *S. platensis* solution is sprayed on feed while inverted slowly so evenly. Food that has been supplemented with 4 g/kg *S. platensis* dried under the sun to dry, after a dry cooled at room temperature. The feed placed on a clean container, sealed and ready to be tested on the gurami (Figure 2 and Figure 3).

Feed was given twice daily in the morning and afternoon (08.00 and 16.00) for 56 days. Feed given 5% of body weight, every two weeks, made adjustments to the weight of the feed that is, after the measurement of body weight of gurami.

Growth measurements of gurami (weight and body length) were done every two weeks. Body length measurements were performed using millimeter blocks paper while the body weight measurement was done using a digital scale with a precision of 0.01 g.

Figure 2. *Spirulina platensis* powder.

Figure 3. Feed supplementation *S. platensis*

The hematological data of gurami (number of erythrocyte, number of leukocyte, hemoglobin concentration and hematocrit value) were calculated at the end of the study. Blood was drawn from the heart using a 1 ml syringe that had been moistened with anti-coagulant (EDTA). Calculation of the total number of erythrocytes and leukocytes using haemocytometer "Improved Double Naubauer's", measurement of hemoglobin levels using haemometer "Assistant" and measurement of haematocrit value (Hct) by microhematocrit "Hawkskey haematocrit reader" (Chairlan & Lestari, 2011)

Proximate analysis of the feed was tested to determine the composition of feed given. Feed supplementation of 4 g / kg *S. platensis* was smoothed by a blender then analyzed in Faculty of Animal Husbandry, Jenderal Soedirman University, Purwokerto (Result Table 1)

Body composition analysis was done as follows: gurami samples were placed on a petri

dish and dried in an oven with a temperature of 70 ° C. Gurami that have been dried, cooled at room temperature and smoothed using a blender, then body composition analyzed in Faculty of Animal Husbandry, Jenderal Soedirman University, Purwokerto (Result Table 3).

The data was analyzed by analysis of variance (ANOVA) to identify the significantly different groups at ($P < 0.05$) by one way ANOVA with post hoc LSD multiple comparison test using a statistic program of SPSS software (SPSS for windows version 21).

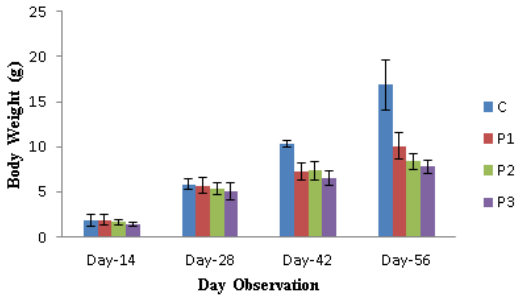
RESULTS AND DISCUSSION

The composition of the feed supplemented *S. platensis* 4g/kg of commercial feed can be seen in Table 1.

Table 1. Feed composition

Parameters (%)	Feed Composition
Water	10.71
Protein	39.22
Lipid	6.04
Moisture	7.14
Ash	8.94

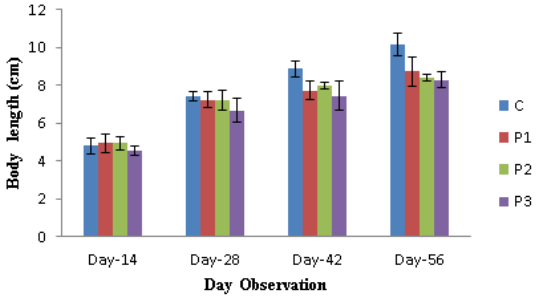
Data Growth measurements of gurami (weight and body length) were measured every 14 days, for 56 days is shown in Figure 4 and Figure 5.



Description: C (control), is not fasting; P2 = fasting on Mondays and Thursdays; P1 = fasting on Mondays; P3 = fasting on Mondays, Wednesday and Friday

Figure 4. Average the length of gurami during 56 days of observation.

The results of this study showed that the highest growth was achieved by treatment gurami control (feeding every day, not fasting) which means that the stimulation cycle deprivation with feed supplemented *S. platensis* 4g/kg commercial diets did not generate compensatory growth. This is evident from the data results weight and body length of gurami; the highest generated in control, followed by treatment P1, P2 and P3 (Figure 1 and Figure 2). However, the time difference provide data retrieval weight and body length concomitant with increasing time of data collection.



Description: C (Control), is not fasting; P2 = fasting on Mondays and Thursdays; P1 = fasting on Mondays; P3 = fasting on Mondays, Wednesday and Friday

Figure 5. Average the length of gurami during 56 days of observation.

Body weight and length of gurami the highest were generated at P3, followed by treatment of P2, P1 and control. All treatments could increase the growth of body length and body weight, but treatment control showed the highest growth. Thus, it can be said that the growth of gurami increases concomitant with increasing age of gurami.

Growth (weight and body length) was significantly different between stimulation cycle of feed deprivation and the control ($P<0.05$). Sampling time was significantly different between stimulation cycle of feed deprivation and re-feeding and the control ($P<0.05$).

The results of the study reported that restrictions or feed deprivation on different types of fish to induce the growth of compensation. Research Ruan et al., (2013) reported that there is a growth of compensation excessive due to schedule a cycle of feed belonging to the short time lack of feed, two and eight days starvation induced compensatory growth (Eslamloo et al., 2012), when applied at low or high temperatures water (Turano et al., 2008).

Other investigators have reported that feed restriction does not induce compensatory growth (Turano et al., 2007), compensatory growth in weight, but not length, in gilthead sea bream (*S. aurata*) (Bavcevic et al., 2010), fish in food deprived treatments presented the same growth (Cho, 2012), compensatory growth only in the gratification obtained in a short time (1 week) (Sevgili et al., 2013), complete growth compensation can

not be achieve at the lack of feed for longer than 1 week in hybrid tilapia (Wang et al., 2000), gratification in the short term does not significantly increase growth (Mirea et al., 2013).

Feed restrictions can also cause fish to experience hyperphagia (Mattila et al., 2009; Cho, 2011; Eslamloo, 2012). Hyperphagia occurs in several fish species during compensatory growth (Wang, 2000).

Stimulation cycle of feed deprivation do not lead to hyperphagia and does not generate compensatory growth. This is reflected in the growth results obtained that treatment control showed the highest growth, seen from the increase of weight and body length.

This is due to the feed given is a feed that has been supplemented with *S. platensis*. Blue green algae, *S. platensis* is receiving increasing attention for its bioactive components such as vitamins (especially vitamin A and B12), minerals and polyunsaturated fatty acids (PUFAs) (Wang, 2007), rich in Gamma Linolenic Acid (GLA) and enzyme (Demir & Tukel, 2010). In accordance with this research, Ibrahem et al., (2013) reported that supplementation of *S. platensis* significant increase growth performance parameters. Increasing level of *Spirulina* in guppy diet provided better growth comparing to the other commercial feeds (Dernekbasi et al., 2010). Giving *S. platensis* in diets more effective in stimulating the growth of fish Nile tilapia if compared with *Lactobacillus rhamnosus* in diets (Zeinab et al., 2015). Jana et al., (2014) reported that *Spirulina* need to add the feed to promote growth and the survival in *Pangasius sutchi*. Data from the calculation of erythrocyte, leukocyte, hemoglobin concentration and hematocrit values shown in Table 2.

The results of this study indicate that hematological of gurami fed supplementation *S. platensis* daily (control) compared with hematological gurami that stimulation cycle of feed deprivation and re-feeding to the same feed formula (P1 - P3) (Table 2). This indicates that the stimulation cycle of feed deprivation of gurami do not provide a significant difference to the hematology gurami.

Deprivation of feed treatment or lack of feed will cause the fish in stressful conditions, where conditions of stress will cause physiological changes in fish, especially in the blood profile. This can be seen by the increase in WBCs that played a role in the immune response. In contrast, the results showed no significant difference on all treatments and the highest hematological was in control.

This is presumably because the feed has been supplemented with *S. platensis* 4 g/kg of commercial feed. This research is in accordance with Promya & Chitmant (2011) who reported that received feed fingerlings roomates 5% *A. platensis* showed an increase red and white blood cell counts and immunity stimulating capacity. This increase occurred due *S. platensis* contain C-phycocyanin that can help built the immunity capacity (Vonshak, 1997). *Spirulina platensis* contain carotenes and other pigments that have antioxidants activity (Wang, 2007), the use of *Spirulina* can improve the immunity capacity of the animals which consume it (Bermejo et al., 2008).

Simanjuntak et al., (2006) reported that supplementation of *S. platensis* in fish feed significant increase hematological fish nilem (*Osteochilus hasselti* C.V.), as well as hematological mice given the extract methanol of *S. platensis* (Simanjuntak et al., 2011). Supplementation of *S. platensis* has been able to increase RBCs, WBCs, Hb and PCV. Supplementation of *S. platensis* 2 g / kg of feed for 14 days can boost immunity jambal catfish (*Pangasius djambal* Bleeker). Wounds caused by pathogenic bacteria *Aeromonas hydrophila* infections occur recovery (Simanjuntak, et al., 2002; 2003). Supplementation of *S. platensis* 6 g / kg of feed increases RBCs, level 2 and 4 g / kg of feed increases WBCs, while the increase in hemoglobin present in all treatments compared with control (Zeinab et al., 2015).

Hematologic examination is needed to determine the health condition of an animal. Hematocrit value measurement to check the condition of anemia, animals infected with the disease and loss of appetite have a low hematocrit values. Hemoglobin is the oxygen binding function of blood

Table 2. Hematological values of gurami

Parameters	Treatments			
	Control	P1	P2	P3
RBCs (cells/mm^3)	3.38 x 10^6	3.26 x 10^6	3.20 x 10^6	3.14 x 10^6
WBCs (cells/mm^3)	2.97 x 10^5	2.65 x 10^5	2.47 x 10^5	2.28 x 10^5
Hb (g/dL)	8	8	7.6	7
Hct (%)	47	44.5	45	42

pigment. When hemoglobin levels are low, then the binding of oxygen will also be low. Results of the proximate analysis of the body composition of gurami can be seen in Table 3.

Table 3. Body composition of gurami.

Parameters (%)	Treatments			
	Control	P1	P2	P3
Water	4.08	10.38	9.36	7.31
Protein	47.45	55.81	51.88	53.24
Lipid	27.87	15.20	23.27	20.25
Moisture	1.14	2.98	2.91	3.68
Ash	6.37	14.29	12.53	16.02

When compared between feed composition (Table 2.) and body composition gurami (Table 3.), then an improvement total protein and lipid, however, a decline in water levels and moisture. Differences were recorded for all parameters in all treatments. Water content, which was higher in P1 treatment, as well as the protein content, however, the highest lipid content in the control treatment. In contrast, some inconsistent results with our study were observed in Barb all parameters (Eslamloo et al., 2012), unaffected by deprivation/re-feeding treatment (Tian et al., 2010), cyclic deprivation did not affect the proximate body composition in young yellow catfish (Ruan et al., 2013).

The content of the nutrients found in *S. platensis* can improve body composition of gurami. *Spirulina platensis* is rich in protein, vitamins, minerals, amino acids and essential fatty acids, all of which play a role in increasing the body's composition. Research Blavan et al., (2010) reported that feed *Artemia nauplii* enriched with *Spirulina* on *Macrobrachium rosenbergii* PL significantly improve the body's biochemical composition. Abdulrahman & Ameen (2014), feeding *Spirulina* to 20% significantly increase carcass body composition in *Cyprinus carpio*. It affects the protein and lipid content of the entire body of the fish. Changes in proteins and lipids in the body of the fish are affected by changes in the synthesis and deposition levels in muscle (Karakatsouli, 2012). Ungsethaphand et al., (2010) research shows that up to 20% of *S. platensis* can be used to feed the hybrid red tilapia without causing side effects to the proximate composition carcass.

CONCLUSION

The results of this study showed that stimulation cycles of feed deprivation with feed supplementation of *S. platensis* 4 g/kg feed commercial could not improve growth (weight and body length) and hematological, but could improve body composition. This could perhaps be a suitable feeding strategy for the rearing of this species. Although, stimulation cycles of feed deprivation of gurami were still undergoing growth and hematological, however, it is advisable for the cultivation of gurami, farmers should give feed on the gurami every day with feed supplementation of *S. platensis* 4 g/kg commercial feed. Thus, it is necessary to add *S. platensis* in gurami feed to promote growth, survival and body composition. Further studies on stimulation of deprivation cycle of gurami with varying levels extracts of *S. platensis*.

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