

# Dietary of *Spirulina Platensis* through Feed Against Weight and Length Final, Hepatomatic Indek, and Visceral Organ Profile of Tilapia (*Oreochromis Niloticus*) and *Pangasius* Sp.

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ARTICLE INFO	ABSTRACT					
Article history:	Consumer needs for tilapia and pangasius can be met with high stocking					
DOI: 10.30595/pssh.v12i.834	densities in cultivation intensification, large amounts of feed on limited land. Poor management of intensive aquaculture causes leftover feed and faeces to settle to the bottom of the pond, producing high levels of					
Submited: May 31, 2023	ammonia which inhibits growth. This study aims to increase growth development of gonads and visceral organs with Spirulina platensis diet The observation method is used in research with a complete random design, applying 3 treatments and 1 control with 4x replications Observations included final length and weight, index of hepatosomatic (HSI), and index of visceral somatic (VSI). The results showed that the					
Accepted: August 24, 2023						
Published: October 05, 2023	Spirulina platensis diet increased the final weight and length, HSI of tilapi and catfish significantly, but not significantly to VSI. This shows that the Spirulina platensis diet can be used to increase the growth an development of gonads.					
Keywords:	<i>This work is licensed under a <u>Creative Commons Attribution 4.0</u></i>					
Tilapia, Pangasius, <i>Spirulina</i> <i>Platensis</i> , Growth, Gonads, Visceral Organ	International License.					
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## 1. INTRODUCTION

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The potential of aquaculture nationally is estimated at 15.59 million ha consisting of freshwater aquaculture, which is 2.23 million ha; brackish water 1.22 million ha; and seawater cultivation reaches 12.14 million ha. The utilization of aquaculture potential currently only reaches 10.1% for freshwater aquaculture; 40% for brackish water cultivation; and 0.01% for marine aquaculture. Therefore, the Government of Indonesia encourages increasing fish farming including important freshwater fish commodities such as tilapia [1].

Tilapia (Oreochromis niloticus) is one of the fishery commodities that is favoured by the community in meeting the needs of animal protein because it has thick meat and good taste. Tilapia is also a potential fish to be cultivated because it can adapt to environmental conditions with a wide salinity range [2]. In addition to tilapia, Pangasius sp. has good development prospects, important economic value, a fairly high selling price, and can be marketed in fresh and processed form. Pangasius farming continues to increase because it can increase income and profits obtained [3]. The development of tilapia and pangasius aims to meet the adequacy of nutrition in the community. But the area of farmers ponds is limited so that the intensification of cultivation needs to be done. In intensive cultivation, a high number of spreads is applied and a large amount of feed are used so that if the management is not good, it can damage water quality. Declining water quality can inhibit the growth and reproduction of fish. To overcome this, it is done by giving supplements. Supplements of terrestrial

origin have been studied quite a lot while those of aquatic origin are relatively few such as the use of Spirulina platensis.

Spirulina platensis is a blue-green microalgae, containing very beneficial nutrients including antioxidant pigments such as carotenoids and phycocyanin, various kinds of vitamins and minerals, essential fatty acids such as  $\gamma$ -linolenic acids, and various essential amino acids [4]. Spirulina can be used as a useful supplement to increase growth, and feed efficiency, improve carcass quality, and physiological response to stress and disease in fish [5], modulation of the host's immune system or immunomodulator [6]. In addition to increasing growth, feed attractant, pigmentation, and immunomodulatory, spirulina can enhance reproductive development [7], [8]. However, reports related to the effect of spirulina administration on the reproductive profile of some fish are still few [8], [6]. In addition to the utilization of supplements to promote growth, declining water quality in intensive cultivation can be overcome by the application of a biofloc system. Aquaculture research using biofloc on tilapia can significantly reduce total ammonia-N and Nitrite-N. In addition to reducing ammonia levels, the use of biofloc can improve growth as indicated by an increased average final weight, and reduce the feed conversion ratio (FCR). The application of biofloc can also significantly increase the spleen index (SSI) in intensive cultivation with high stocking densities. This proves that the application of biofloc in fish farming can improve water quality stability and fish growth performance [9].

The biofloc technology system (BFT) can be used as a strategy in an environmentally friendly fishing industry to create stable water quality, providing additional feed in the form of biomass that fish can consume [10], [11]. Biofloc works by increasing the ratio of carbon to nitrogen, through the addition of a stimulating carbon source natural growth of heterotrophic bacteria in culture water [12], [13]. These bacteria then convert harmful nitrogenous metabolites such as ammonia into microbial biomass known as biofloc aggregates [10], [14]. Biofloc aggregates consist of various organic materials such as bacteria, microalgae, food scraps, and zooplankton which can serve as additional nutrition for aquatic organisms including fish that are reared [15], [11]. This study aims to create the effect of the biofloc system on tilapia and catfish hatcheries by adding ginger supplements through the feed to increase growth.

### 2. RESEARCH METHODS

This study used tilapia and catfish fry for 60 days with the addition of Spirulina platensis supplements mixed into the feed. Experiments are used in this research method through a random design complete by applying 3 treatments, 1 control and 4x tests. Supplementation of Spirulina platensis supplements based on previous research doses was 2%, 3%, and 4% [16]. The full treatment is:

T0: control (without diet spirulina) T1: diet spirulina 2%

T2: diet spirulina 3%

T3: diet spirulina 4%

Measured parameters

The parameters observed in the study are growth, development of the gonads, and profile of the visceral organs. Growth indicators are the final weight and length, hepatosomatic index (HSI), for gonadal development is gonadosomatic index (GSI), while the visceral organ profile is visceral somatic index (VSI), see equation and formula (1), (2) and (3) as follows:

$$HSI = ----- x 100\%.$$
 (1)  
Body weight

Gonad weight  

$$GSI = ----- x \ 100\%.$$
 (2)  
Body weight

Visceral weight  

$$VSI = ---- x 100\%.$$
 (3)  
Body weight

### Statistical analysis

The microsoft SPSS program is used to analyse data from analysis of variance (ANOVA) research results at a 95% confidence level. If the results show a noticeable difference in the F test, then proceed with the Duncan Multiple Range Test (DMRT) test at a 95% confidence level so that the difference between treatments can be known.

## 3. **RESULT AND DISCUSSION**

Growth

The results of this study showed the diet of Spirulina platensis can increase the growth of length and weight of tilapia and catfish during the 90 days of study (Table 1).

Table 1. Spirulina diet against final length and weight of tilapia and pangasius for 90 days in biofloc system							
cultivation							

			cultivation			
Treatm	nent	Final w	eight (g)		Final length (cm)	
	g kg <sup>-1</sup> feed			Pangasius	Tilapia	
		_	Pangasius	-	-	
T0	38.65	$5\pm8.90^{\mathrm{a}}$	13,37±2,95 <sup>a</sup>	$12.18\pm1.41^{\rm a}$	13,12±1,23 <sup>a</sup>	
T1	49.60	$\pm 8.00^{ab}$	$15,97{\pm}2,85^{ab}$	$13.61\pm0.13^{ab}$	13,57±0,97 <sup>a</sup>	
T2	59.97	± 12.87 <sup>b</sup>	$20,88 \pm 4,68^{b}$	$14.50\pm1.36^{b}$	$15,80\pm1,18^{b}$	
T3	82.80	$\pm 1.00^{\circ}$	28,05±5,77°	$16,16 \pm 0,11^{\circ}$	14,31±1,42 <sup>ab</sup>	

Studies prove *Spirulina platensis* can significantly improve weight growth performance. Spirulina contains phycocyanin and polysaccharides that can act as immunomodulators. Spirulina is an antioxidant that can reduce the effects of excessive ROS-free radical damage. The effect of spirulina application on fish is not only species-specific but also depends on the dose of administration of the amount in the diet [17]. This suggests a spirulina diet can improve non-specific immune responses. Improved immunity is the basis that is thought to cause growth for the better.

As reported by [18], the spirulina flour diet may increase growth indicators in *Astronotus ocellatus* fish. Indicators of increased growth include the daily growth rate, feed conversion value, the relative percentage of weight gain, and the final weight. Next, the addition of diet *Spirulina* sp. can improve the growth of comet fish at a dose of 2.1 (g%) by producing 4.33 g of weight gain and the survival of 8.33% [19]. Furthermore, [20] reported that goldfish dieted with *Spirulina platensis* flour supplements can improve length and weight growth, and feed efficiency. The found dose of spirulina amounted to 6% and was suggested for cultivation of the koki carp.

*Spirulina platensis* algae supplementation in tilapia affects the growth performance at different dose levels (0, 5, 10, and 15 g kg<sup>-1</sup> feed). Spirulina algae diet through feed at different feed concentrations can produce better growth performance and be able to improve the immunity and health of *O. niloticus* seeds. The spirulina diet can significantly reduce the ratio of feed conversion, but increases the ratio of protein efficiency significantly (p <0.05) compared to control [21].

### Hepatosomatik Indek (HSI) dan Viscerasomatik Indek (VSI)

Research using the spirulina diet can have a significant effect on HSI in tilapia and catfish, but it has no significant effect on GSI tilapia while in pangasius it has not been seen the development of gonads (GSI). In VSI, the spirulina diet increased in tilapia and catfish but not significantly. In general, there is a positive correlation relationship between HSI, GSI, and VSI in tilapia and catfish (Table 2).

cultivation										
Treatment	HSI (g%)		GSI (g%)	VSI (	g%)					
g kg <sup>-1</sup> feed	Tilapia	Pangasius	Tilapia	Tilapia	Pangasius					
Т0	$1,61 \pm 0,92^{b}$	$0,22\pm0,06^{a}$	0,43±0,33ª	$9,60 \pm 0,69^{a}$	1,37±0,29ª					
T1	$1,91 \pm 0,78^{b}$	0,28±0,02 <sup>a</sup>	$2,66\pm 2,62^{a}$	11,99± 1,94ª	1,68±0,54ª					
T2	$0,93 \pm 0,33^{a}$	0,41±0,89 <sup>b</sup>	$2,66 \pm 2,62^{a}$	$10,51\pm 2,22^{a}$	1,91±0,47 <sup>a</sup>					
T3	$0,43 \pm 0,25^{a}$	0,59±0,09°	$1,18 \pm 0,51^{a}$	$9,86 \pm 1,43^{a}$	2,25±1,03ª					

Table 2. Spirulina diet against the HSI, GSI, and VSI of tilapia and catfish for 90 days in biofloc system

Diet spirulina can significantly increase weight growth, SGR, feed efficiency, and FCR, but not significantly against HSI. Giving spirulina at the right dose does not have a negative impact, and can be used as a substitute for good fish meals, and as a growth promoter in fish and shrimp farming [22]. Furthermore, the same study also proved [23], the diet of *Spirulina platensis* in feed can be used as a substitute for fish meal and can significantly improve the growth, reproduction, and quality of meat and colour of fish, but does not affect HSI and GSI. However, this study showed, HSI experienced a significant improvement with the spirulina diet in tilapia and catfish.

The article review written by [22] did not mention the age of the fish, while the study [23] used goldfish measuring  $45\pm2$  g, while this study used tilapia and catfish 7-9 cm weighing  $19\pm1$  g. This shows that the fish used in this study (tilapia and pangasius) are smaller so their growth is faster. Along with the growth of fry and the age of fish, metabolism tends to be more active for the activity, growth, and development of gonads. Increased metabolism is generally correlated with the heaviness of the liver. Such conditions can be seen in HSI tilapia and catfish which increase as the diet dose of spirulina increases.

HSI may increase during the growth and development of the ovaries but then decrease as the period of ovarian development ends [24]. The results of other studies state, HSI is related to fish growth. In the period of growth, it takes a lot of energy for activity, and growth in weight, length, and volume. The increasing weight, length, and volume of fish causes the HSI value to be greater. Spirulina is known to increase growth corresponding to the increase in HSI [25]. Spirulina in fish can be a growth promoter, as [19], comet fish given a spirulina dose of 2100 mg kg<sup>-1</sup> feed can promote growth. The same experimental results prove the supplementation of spirulina can increase weight growth and specific growth rate in clownfish (*Amphipriono cellaris*) at a dose of 3-5 g kg<sup>-1</sup> feed [26].

The results of this study also show that HSI profile is correlated with GSI. In low HSI and GSI controls, then in treatments 1 and 2 (T1 & T2), it increased and then decreased in treatment 4 (T4). This can be explained according to [25], there is a correlation between the growth of metabolic organs in the liver and the development of the reproductive organs and gonadal maturity. This is due a relationship between liver growth and gonadal development. This is because the liver as a place of metabolism of the body is further used for the activity, growth, and development of the gonads. HSI shows liver weight, while GSI describes gonadal weight along with reproductive development and fish growth [27] (Kjesbu, Hunter, & Witthames, 2003).

In female fish, a high GSI value is an indication of the mature stage of the ovary, the shape of the egg is round and loaded with yolk [28]. In the mature condition of the ovaries, HSI generally rises because there is a process of vitellogenesis in the liver to synthesize vitellogenin. Vitellogenin is a precursor to yolks that are synthesized in the liver and subsequently transferred into the egg into an egg yolk [29]. This is to explain, in the mature condition of eggs, the HSI and GSI values are generally high and then fall along with the final development of the ovaries. Research conducted by [25] showed that the increase in weight and length of fish was correlated with liver and gonadal weight which could be known from the index of gonadosomatic and index of visceral somatic.

The results of this experiment as stated in Table 2 prove that the spirulina diet in tilapia and catfish can increase VSI even if it is not significant. The report from [8] proves spirulina diet can improve the profile of the visceral organs indicated by increasing VSI values. The index of visceral somatic (VSI) tends to decrease with time or growth period, which indicates the presence of an overhaul and transfer of fat from the digestive organs to the liver organs. There are still few reports related to the effect of spirulina administration on the VSI profile. However, there is one study that uses spirulina flour substitution results that can improve the growth and profile of VSI. According to the results of the study of [30], the substitution of fish oil flour with spirulina and corn oil or soybean oil can be a useful solution to reduce costs in the maintenance of sturgeon including a better VSI value than control even if it is not significant.

### 4. CONCLUSION

The addition of *Spirulina platensis* supplements through feed in fish farming for 60 days significantly enhances the final length and weight, hepatosomatic index in tilapia and catfish, but not significantly to the visceral somatic index. The results of this study prove that the addition of *Spirulina platensis* supplements through feed can be used to improve the growth and development of gonads.

### ACKNOWLEDGMENTS

Thank you to Muhammadiyah Purwokerto University for funding this research through Letter of Agreement No: A.11-III/478-S.Pj. /LPPM/XII/2021. Thank you to Mr. Rijal and Mr. Ihsan for the technical assistance and discussion, also to Mr. Aan who helped in the biofloc wet laboratory.

### REFERENCES

- Angriani, R., Halid, I., & Baso, H. S.: Analisis Pertumbuhan Dan Kelangsungan Hidup Benih Ikan Nila Salin (*Oreochromis niloticus* Linn) Dengan Dosis Pakan Yang Berbeda. *Fisheries of Wallacea Journal*, 1(2), 84–92 (2020)
- [2] Agustono, A., Hadi, M., & Cahyoko, Y.: Pemberian Tepung Limbah Udang yang Difermentasi dalam Ransum Pakan Buatan Terhadap Laju Pertumbuhan, Rasio Konversi Pakan Dan Kelangsungan Hidup Benih Ikan Nila (*Oreochromis niloticus*) <Br><I>[The Given Fermentation The Prawn Waste Flour In Artificial Fee. Jurnal Ilmiah Perikanan Dan Kelautan, 1(2), 157–162. https://doi.org/10.20473/jipk.v1i2.11682 (2009)

- [3] Hendrik, H.: Analisis Usaha Budidaya Ikan Patin dan Prospek Pengembangannya di Desa Koto Masjid Kabupaten Kampar Provinsi Riau. *Jurnal Perikanan Dan Kelautan*, 27(2), 174–179 (2022)
- [4] Jaime-Ceballos, B. J., Hernández-Llamas, A., Garcia-Galano, T., & Villarreal, H.: Substitution of *Chaetoceros muelleri* by *Spirulina platensis* meal in diets for *Litopenaeus schmitti* larvae. *Aquaculture*, 260(1–4), 215–220. https://doi.org/10.1016/j.aquaculture.2006.06.002 (2006)
- [5] Nakagawa, H.: Effect of dietary algae on improvement of lipid metabolism in fish. *Biomedicine and Pharmacotherapy*, *51*(8), 345–348. https://doi.org/10.1016/S0753-3322(97)88053-5 (1997)
- [6] Takeuchi, T., Lu, J., Yoshizaki, G., & Satoh, S.: Effect on the growth and body composition of juvenile tilapia *Oreochromis niloticus* fed raw Spirulina. *Fisheries Science*, 68, 34–40 (2002)
- [7] Regunathan, C., & Wesley, S. G.: Pigment deficiency correction in shrimp broodstock using Spirulina as a carotenoid source. *Aquaculture Nutrition*, 12(6), 425–432. https://doi.org/10.1111/j.1365-2095.2006.00444.x (2006)
- [8] Güroy, D., Güroy, B., Merrifield, D. L., Ergün, S., Tekinay, A. A., & Yiğit, M.: Effect of dietary Ulva and Spirulina on weight loss and body composition of rainbow trout, *Oncorhynchus mykiss* (Walbaum), during a starvation period. *Journal of Animal Physiology and Animal Nutrition*, 95(3), 320–327. https://doi.org/10.1111/j.1439-0396.2010.01057.x (2011)
- [9] Hwihy, H., Zeina, A., Husien, M. A., & El-Damhougy, K.: Impact of biofloc technology on growth performance and biochemical parameters of *Oreochromis niloticus*. In *Egyptian Journal of Aquatic Biology and Fisheries* (Vol. 25). https://doi.org/10.21608/EJABF.2021.149930 (2021)
- [10] Avnimelech, Y.: World AquAculture Control of microbial activity in aquaculture systems: active suspension ponds. *Reprinted from World Aquaculture*, *34*(4), 19–21 (2003)
- [11] Bossier, P., & Ekasari, J.: Biofloc technology application in aquaculture to support sustainable development goals. *Microbial Biotechnology*, 10(5), 1012–1016. https://doi.org/10.1111/1751-7915.12836 (2017)
- [12] Hargreaves, J. A.: Photosynthetic suspended-growth systems in aquaculture. *Aquacultural Engineering*, 34(3), 344–363. https://doi.org/10.1016/j.aquaeng.2005.08.009 (2006)
- [13] De Schryver, P., Crab, R., Defoirdt, T., Boon, N., & Verstraete, W.: The basics of bio-flocs technology: The added value for aquaculture. Aquaculture, 277(3–4), 125–137. https://doi.org/10.1016/j.aquaculture.2008.02.019 (2008)
- [14] Ebeling, J. M., Timmons, M. B., & Bisogni, J. J.: Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. *Aquaculture*, 257(1–4), 346–358. https://doi.org/10.1016/j.aquaculture.2006.03.019 (2006)
- [15] Emerenciano, M., Gaxiola, G., & Cuzo, G.: Biofloc Technology (BFT): A Review for Aquaculture Application and Animal Food Industry. In *Biomass Now - Cultivation and Utilization*. https://doi.org/10.5772/53902 (2013)
- [16] Purbomartono, C., Panuntun, L. W., & Mulia, D. S.: Dietary impact of *Spirulina platensis* powder supplementation on the growth and immunity of *Clarias gariepinus*. AACL Bioflux, 15(5), 2717–2724. (2022)
- [17] Kumar, R. S. P., & Sibi, G.: Spirulina as Fishmeal to Improve Growth, Immune Response, Antioxidant Defense and Pigmentation. 12(2), 47–52. https://doi.org/10.5829/idosi.wjfms.2020.47.52 (2020)
- [18] Mohammadiazarm, H., Maniat, M., Ghorbanijezeh, K., & Ghotbeddin, N.: Effects of spirulina powder (*Spirulina platensis*) as a dietary additive on Oscar fish, *Astronotus ocellatus*: Assessing growth performance, body composition, digestive enzyme activity, immune-biochemical parameters, blood indices and total pigmentation. *Aquaculture Nutrition*, 27(1), 252–260. https://doi.org/10.1111/anu.13182 (2021)
- [19] Rosid, M. M., Yusanti, I. A., & Mutiara, D.: Tingkat pertumbuhan dan kecerahan warna ikan komet (*Carassius auratus*) dengan penambahan konsentrasi tepung Spirulina sp. pada pakan. Jurnal Ilmu-Ilmu Perikanan Dan Budidaya Perairan, 14(1), 37–44. https://doi.org/10.31851/jipbp.v14i1.3368 (2019).
- [20] Nazhiroha, N., Mulyana, & Mumpuni, F. S.: Pengaruh penambahan tepung Spirulina platensis dalam pakan terhadap pertumbuhan dan efisiensi pakan ikan mas koki (*Carassius auratus*). Jurnal Mina Sains, 5(1), 50–57. https://doi.org/10.30997/jms.v5i1.1773 (2019)
- [21] Sherif, A. H., EL-Sheekh, M. M., & Abdel-Halim, S.: Effect of spirulina algae on the health status and growth performance of Nile tilapia *Oreochromis niloticus* cultured at Kafr El-Sheikh Governorate. *Egypt. J. Basic Appl. Physiol*, 11(1), 57–68. (2012)
- [22] Li, L., Liu, H., Xie, S. Q., & Zhang, P.: Effects of taurine supplementation on growth performance and feed utilization in aquatic animals: A meta-analysis. *Aquaculture*, 551, 1–15. https://doi.org/10.1016/j.aquaculture.2022.737896 (2022).
- [23] Abdulrahman, N. M., Hama, H. J., Hama, S. R., Hassan, B. R., & Nader, P. J.: Effect of microalgae spirulina spp. as food additive on some biological and blood parameters of common carp *Cyprinus*

*carpio* L. *Iraqi Journal of Veterinary Sciences*, 33(1), 27–31. https://doi.org/10.33899/ijvs.2019.125527.1049 (2018)

- [24] Lubis, S., Windarti, W., & Riauwaty, M.: The effects of photoperiod manipulation on morpho-anatomy and growth of Clarias gariepinus. *Berkala Perikanan Terubuk*, 47(2), 60–68. https://doi.org/10.31258/terubuk.46.3.60-68(2019)
- [25] Putra, W. K. A., Yulianto, T., Miranti, S., Zulpikar, & Ariska, R.: Tingkat Kematangan Gonad, Gonadosomatik Indeks Dan Hepatosomatik Indeks Ikan Sembilang (*Plotus* sp.) Level Gonad Maturity, Gonadosomatic Index And Hepatosomatic Of Gray Eeltailed Catfish (*Plotosus* sp.). Jurnal RUAYA, 8(1), 1–9. (2020).
- [26] Hadijah, Junaidi, M., & Lestari, D. P.: Pemberian tepung Spirulina platensis pada pakan terhadap kecerahan warna ikan badut (Amphiprionocellaris). Jurnal Perikanan Unram, 10(1), 41–49. https://doi.org/10.29303/jp.v10i1.187 (2020).
- [27] Kjesbu, O. ., Hunter, J. ., & Witthames, P.: Modern approaches to assess maturity and fecundity of warmand cold-water fish and squids. (2003).
- [28] Prasad, S., & Nath, P.: Study of Gonadosomatic Index and Maturation of An Indian Major Carp Labio rohita (Ham) in Bihar. International Archive of Applied Sciences and Technology, 11(1), 37–41. https://doi.org/10.15515/iaast.0976-4828.11.1.3741 (2020)
- [29] Cerdá, J., Calman, B. G., LaFleur, G. J., & Limesand, S. Pattern of vitellogenesis and follicle maturational competence during the ovarian follicular cycle of *Fundulus heteroclitus*. *General and Comparative Endocrinology*, 103(1), 24–35. https://doi.org/10.1006/gcen.1996.0090 (1996).
- [30] Palmegiano, G. B., Gai, F., Daprà, F., Gasco, L., Pazzaglia, M., & Peiretti, P. G.: Effects of Spirulina and plant oil on the growth and lipid traits of white sturgeon (*Acipenser transmontanus*) fingerlings. *Aquaculture Research*, 39(6), 587–595. https://doi.org/10.1111/j.1365-2109.2008.01914.x (2008)