



Effects of Spirulina Meal Supplementation on Growth and Survival Rate of Royal Whiptail Catfish Fry (*Sturisoma panamense*)

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Abstract

Royal Whiptail Catfish (*Sturisoma panamense*) have high-stress levels in the early stages of their life. This is a significant concern in the field of fish farming. One of the crucial factors in the cultivation of royal whiptail catfish is the addition of vegetable or algae protein to the feed according to the habits of herbivorous fish. This study aimed to determine the best dose of spirulina that can enhance the growth and survival rates of royal whiptail catfish fry. There were four treatment groups, i.e. feed without spirulina (K), a dose of 3% (SP-3%), a dose of 6% (SP-6%), and a dose of 9% (SP-9%). The study was conducted at the Tetra Aquaria Company in Sukabumi City in April - May 2024. The spirulina culture was dried in an oven at 60 °C for 24 hours and ground using a mortar. The powdered feed was mixed with spirulina according to the treatment dose. The fish larvae used were newly hatched larvae, and the maintenance period during the treatment was 21 days. The maintenance container uses a tray measuring 40 cm x 30 cm x 15 cm. The study results showed that the best group was SP-6% with an average survival rate of 87% ± 4.62, significantly different compared to the K, SP-3%, and SP-9% treatments. The daily growth rate value of SP-9% showed the best significant difference ($P < 0.05$) from the control treatment and 3% dose, however not significantly different ($P > 0.05$) with SP-6%.

INTRODUCTION

The royal whiptail catfish (*Sturisoma panamense*) is known as panamense. This fish is native to Central and South America (Ren *et al.*, 2019). Panamense is a calm fish and has a slender and elongated body shape. It is brown or yellow with a small black pattern along the sides so that it becomes its

attraction. Most of them are herbivores that feed on algae, vegetables, and biofilms (Londoño-Burbano and Britto, 2022). This commodity has high prospects for cultivation because it is unique and has a high selling price (Akbar, 2021). However, panamense cultivation is still difficult because many fail

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in larval stadia. Panamense larvae have a high level of stress if their needs are not met, one of which needs to be considered is feed.

Aquadiction World (2023) states that to accelerate the growth of *panamense* larvae, a high-protein diet is needed, and this fish needs vegetables or algae when it is still in the stadia larvae. This study will examine the composition of algae for panamense fish larval feed. The algae used is a type of *Spirulina platensis*. Spirulina has a high protein content of between 59-63%, fatty acids of around 7-10%, carbohydrates 13.6%, multivitamins, especially vitamin B12, and minerals (Grosshagauer *et al.*, 2020; Shekharam *et al.*, 1987; Lupatini *et al.*, 2016; Annapurna *et al.*, 1991). Spirulina has been shown to increase protein in fish feed and has reportedly shown promising results in several species, such as tilapia *Oreochromis niloticus* (Lu and Takeuchi, 2004; Mahmoud *et al.*, 2018), guppies *Poecilia reticulata* (Dernekbasi *et al.*, 2010; Perera *et al.*, 2024), goldfish *Carassius auratus* (Vasudhevan and James, 2011), barred knifejaw *Oplegnathus fasciatus* (Kim *et al.*, 2013), catfish *Clarias batrachus* (Dar *et al.*, 2014), rainbow trout *Oncorhynchus mykiss* (Yeganeh *et al.*, 2015), olive flounder *Paralichthys olivaceus* (Kim *et al.*, 2015), beluga *Huso huso* (Adel *et al.*, 2016), and oscar *Astronotus ocellatus* (Mohammadi azarm *et al.*, 2021).

The dose of spirulina that will be used for panamense has not been studied in depth, so it needs to be researched. The dosage of spirulina refers to the research of Nazhiroh (2019) and Agung *et al.* (2021), who provide spirulina for carp and zebrafish feed starting from doses of 3%, 6%, and 9%. This dose has a good effect on the growth performance and survival of chef fish and zebras. Thus, the addition of spirulina with the right dose to the commercial feed of panamense fish larvae needs to be researched to get optimal growth and increase survival.

METHODOLOGY

Ethical Approval

All procedures carried out in this study are under the standard procedures and the fish were properly kept. The animals used are harmless and do not cause environmental pollution.

Place and Time

The research was carried out in March - April 2024. Spirulina culture and simplicia manufacturing were carried out at the Laboratory of the Fish Hatchery Technology and Management Study Program, IPB Vocational School, Sukabumi Campus. Meanwhile, the treatment of fish is carried out at the Tetra Aquaria Company located at Situ Endah, Lembursitu District, Sukabumi City, West Java.

Research Materials

The tools used in this study were 2 L jars, 15 L mineral water gallons, rectangular trays measuring 40 cm x 30 cm x 15 cm, calipers (Tricle Brand, Indonesia), round mortars, aeration hoses and aeration stones, aluminum foil, and plankton. The materials used in this study were panamense larvae, spirulina seeds, Walne fertilizer, natrium thiosulfate, and chlorine.

Research Design

The research design used a Complete Randomized Design (RAL), which has 4 treatments and 3 replicates. The treatment group included test feed not given control spirulina supplementation (C), test feed with 3% spirulina supplementation (SP-3%), test feed with 6% spirulina supplementation (SP-6%), and test feed with 9% spirulina supplementation (SP-9%).

Work Procedure

Container Preparation

The container used for spirulina culture uses a 2 L jar and a gallon of mineral water measuring 15 L. Then the fish rearing container uses a rectangular tray with a size of 40 cm x 30 cm x 15 cm as many as 12 trays. Spirulina culture containers and fish

rearing were sterilized using chlorine at a dose of 0.01 g/L and then left for 24 hours. The aeration hose and aeration stone to be used are also put into a sterilization container containing a chlorine solution. After 24 hours, all equipment is rinsed with fresh water and then dried. The maintenance medium before use is sterilized with chlorine at a dose of 0.05 mL/L for 24 hours. Then, the maintenance medium is neutralized with Na-thiosulfate at a dose of 0.025 ml/L and waited 24 hours before it could be used.

Spirulina Culture

The spirulina used is a type of *Spirulina plantesis*. Spirulina culture uses the Buwono and Nurhasanah (2018) procedure. Spirulina is cultured on a laboratory scale through two stages. The first is a 1-liter scale using a 2-liter container, and the second stage is a 10-liter culture with a 15-liter container. The container to be used is washed first and then dried. The first stage of the 1-liter scale culture begins with a jar filled with 800 ml of water and 1 ml of Walne fertilizer (dose 1 ml/L) added. Spirulina seeds are added as much as 200 mL (20% of the total volume) and aerated. After 7 days, spirulina can be used for the second stage of culture. The second stage of the 10 L scale begins with a container filled with 8 L of water and 2 L of spirulina seeds added, then 10 ml of Walne fertilizer and aeration. After 14 days, spirulina is harvested by filtering using planktonet.

Spirulina Powder Preparation

Yields in 10 L cultures produce wet-weight spirulina weighing 225 g. The process of making spirulina powder is carried out by wet spirulina spread evenly on a heat-resistant container and covered with aluminum foil. Drying is done by heating it in the oven at 60°C for 24 hours. The dried spirulina is put into the mortar and then ground until smooth and powdered. The powder is stored in a closed, airtight container.

Test Feed

An amount of 100 g of commercial powder feed mixed with 3 g, 6 g, and 9 g of spirulina is stirred until well mixed, the characteristics of the feed have been mixed well, namely, the color of the powder pellets becomes darker than before mixing spirulina. As a binder, 5 g of gelatin is needed to be mixed with 100 ml of water, then the gelatin solution is cooked over low heat until it boils and a gel-like texture is obtained. The gelatin that is already in the form of a gel is mixed with spirulina feed and stirred until smooth until the texture of the feed becomes like paste. The test feed is analyzed proximate before it is carried out before fish rearing. The proximate analysis tested was fat, carbohydrate, protein, moisture content, and ash content.

Larval Maintenance

The fish used are newly hatched larvae that are 1 day old and kept until 21 days. One container is filled with 25 fish larvae. Fish are fed with ad satiation treatment with a frequency of feeding 2 times daily at 08.00 and 15.00. Spraying is carried out every morning, and the water changes by as much as 80% daily. During maintenance, water quality is maintained to meet SNI 6484.4 (2014) standards, namely temperatures ranging from 25-30°C; Ph 6,5-8; DO >3 mg/L; ammonia 0-0.5 mg/L; nitrite 0.1-0.5 mg/L. and nitrate <20 mg/L. Fish length data was collected every 7 days by measuring the total body length using a caliper.

Data Collection

At the end of the rearing, all the fish were measured for the total length and the total live fish. The growth parameters to be analyzed include length growth parameters (Zonneveld *et al.*, 1991), average daily length growth (Sari *et al.*, 2017), and survival rate (Effendie, 2002) using the following formula:

Length growth (cm) = final body length – initial body length

$$\text{Average daily length growth (cm/day)} = \frac{\text{final body length} - \text{initial body length}}{\text{duration of experiment}}$$

$$\text{Survival rate(\%)} = \frac{\text{Final number of fish}}{\text{initial number of fish}} \times 100$$

Feed proximate was analyzed with the standard methods (AOAC, 1995). Moisture content analysis was carried out by drying at 105 °C. Crude protein, lipid, and ash content were determined using the Kjeldahl method (Kjeltec 2100), the ether extraction method (SER 148/3 Solvent Extractor, Velp, Italia), and muffle furnace at 550 °C for 8 hours.

Water quality parameters consist of water physics measured daily such as temperature, pH, and dissolved oxygen (DO) using a thermometer, pH meter, and DO meter. Chemical water was measured weekly using ammonia using a water test kit.

Data Analysis

The field data obtained were processed and analyzed, then tested for normality and homogeneity before conducting a variety analysis (ANOVA). The Smallest Real Difference follow-up test with a confidence interval of < 95% using the Duncan test.

RESULTS AND DISCUSSIONS

Water quality parameters

Water quality parameters during 21 days of maintenance are presented in Table 1. The water quality parameters including temperature, pH, DO, and ammonia, were not remarkably ($p > 0.05$) impacted by different levels of Spirulina dose. The level of ammonia in the dose 9% was higher compared to the 3% and 6% doses.

Table 1. Growth and survival in royal whiptail catfish *Sturisoma panamense* fish fed without the addition of spirulina (C), fed with spirulina dose 3% (SP-3%), dose 6% (SP-6%), and dose 9% (SP-9%).

Parameters	Dose of Spirulina			
	C	SP-3%	SP-6%	SP-9%
Temperature (°C)	27,29 ± 0,48	27,26 ± 0,48	27,22 ± 0,44	27,21 ± 0,45
pH	7,42 ± 0,21	7,45 ± 0,22	7,47 ± 0,26	7,45 ± 0,30
DO (ppm)	6,84 ± 0,65	6,24 ± 0,20	6,31 ± 0,24	6,44 ± 0,58
NH ₃ (ppm)	0,25 ± 0,02	0,35 ± 0,03	0,42 ± 0,04	0,55 ± 0,02

DO: Dissolved oxygen.

Growth Performance

Panamense fish fed spirulina supplementation feed had better growth and survival performance than feed without spirulina (Table 2). The most optimal daily length growth rate and absolute length growth were obtained in the SP-9% treatment, which was significantly different

from the control treatment and SP-3% but did not differ significantly from the SP-6% treatment. The highest survival rate was obtained from feed supplemented with SP-6% spirulina, significantly different from the control treatment, SP-3%, and SP-9%. The lowest survival rate was in feed treatment, which was not given spirulina.

Table 2. Growth and survival in *Sturisoma panamense* fish fed without the addition of spirulina (C), fed with spirulina dose 3% (SP-3%), dose 6% (SP-6%), and dose 9% (SP-9%).

Parameters	Dose of Spirulina			
	C	SP-3%	SP-6%	SP-9%
Length growth (cm)	1,48 ± 0,03 ^a	1,69 ± 0,05 ^b	1,79 ± 0,03 ^c	1,86 ± 0,06 ^c
Average daily length growth (cm/day)	0,070 ± 0,0013 ^a	0,080 ± 0,0025 ^b	0,085 ± 0,0015 ^c	0,089 ± 0,0029 ^c
Survival rate (%)	59 ± 4,62 ^a	75 ± 2,31 ^b	87 ± 4,62 ^c	68 ± 4,00 ^b

The results of the feed-proximate analysis can be seen in Table 3. Feed

proximate with the addition of spirulina was known to have the highest protein content

obtained in SP-9% treatment. The highest fat value was obtained in the SP-3% group; the highest crude fiber was obtained in the SP-6% treatment.

Nutrient Composition

The results of the proximate analysis are presented in Table 3. The addition of spirulina to feed has provided a significant

increase in carbohydrates, proteins, and fats. The highest carbohydrate content was found at a dose of 6% amount of 17.46%. The highest protein content was found in the 9% dose treatment amount of 26.07%. The highest lipid content was found in the 9% dose treatment amount of 6.23%. In addition, the moisture content in feed given spirulina was lower than in feed without spirulina.

Table 3. Feed proximate analysis of experimental diets (% dry weight).

Composition	Dose of Spirulina			
	C	SP-3%	SP-6%	SP-9%
Moisture (%)	50,93	50,52	48,77	48,2
Ash (%)	6,07	6,09	6,14	6,23
Crude protein (%)	23,51	24,19	25,47	26,07
Crude lipid (%)	2,12	2,97	2,17	2,73
Crude fiber (%)	3,02	16,235	17,46	16,78

This research was conducted on ornamental fish larvae, so the parameters emphasized absolute length growth and survival. The results show that the larger the dose of spirulina added to the feed, the higher the daily length growth rate and the absolute length growth. This is suspected because the protein content of feed increases with increasing doses. Protein is the most important source of nutrients for fish, and it functions for protein synthesis, energy, and growth. It is also a substrate for the central metabolic pathway (Andersen *et al.*, 2016; Kristinsson and Rasco, 2000). Early stadia fish need protein for growth and maintenance; after maturity, protein is used for reproduction (Tilami and Samples, 2017). Panamense fish is a type of herbivore fish that have a sucking mouth structure so that they can scrape away the algae layer from glass, stone, or driftwood. The protein needed for herbivorous fish is undoubtedly lower than for carnivores (Andersen *et al.*, 2016).

The results showed that there was an increase in protein in the feed with spirulina, and carbohydrates and fats also increased. The use of carbohydrates in feed depends on fish-eating habits, environmental conditions, and types of carbohydrates (Honorato *et al.*, 2010; Moro *et al.*, 2016).

The ability to digest carbohydrates varies significantly between species, but herbivorous fish can digest and metabolize carbohydrates better than carnivorous fish (Wilson, 2002; NRC, 2011). Increased carbohydrates can be one of the low-cost sources of energy compared to protein. In addition, fat is also one of the primary sources of energy, especially for fish reproduction. It provides fish with essential fatty acids, a key nutrient for developing fish stadia (Chou *et al.*, 2001). Fats contain energy and are easily digested by fish, especially carnivores (NRC, 2011). Fat needs depend highly on protein and carbohydrate levels (Watanabe, 1982).

Larvae fed spirulina had higher survival compared to controls. The highest survival was possessed by a 6% dose treatment with a survival value of 87%. Spirulina can not only improve human health but also has the benefit of improving the health of fish so that it can increase the chances of survival is high. Spirulina has several active substances such as phenols, beta carotene, and chlorophyll that function to increase the immune system in fish (Macias-Sancho *et al.*, 2014), anti-inflammatory and prevent stress (Rana *et al.*, 2024), and anti-microbial (Metekia *et al.*, 2021). High survival will provide more

significant income and profit levels for cultivators. Based on the Akbar report (2021), the selling price of a 4.5 cm panamense is IDR 11,000.00/ind, while the selling price of a 1.5 cm panamense is IDR 1,500.00/head.

During the experiment, feed with a dose of 9% experienced a decrease in survival. It is suspected that there is an effect of a very high value of unionized ammonia (NH₃) at a dose of 9%, which is around 0.55 ppm. The lethal ammonia threshold value is reported in the range of 0.2 – 0.4 (EIFAC, 1973). Zeinab *et al.* (2016) conducted a similar study, which found that the LC50 value of ammonia in tilapia larvae was in the range of 0.8 ppm. Ammonia is an aquatic pollutant from nitrogen metabolism (Evans *et al.*, 2006). According to Zaenab *et al.* (2016), fish larvae begin to show toxicity symptoms after exposure to ammonia ranging from 0.5 – 0.6 ppm. Clinical symptoms are characterized by larvae starting to lose their balance and appearing on the water's surface to take in oxygen. The skin appears pale in some larvae, and mucus increases in the skin and gills. Furthermore, the larvae do not want to eat, so the mortality increases.

CONCLUSION

The best dose of spirulina added to the larval feed of Panamense ornamental fish was obtained at 6%. Survival at a dose of 6% had the highest value and significant difference compared to other treatments. However, the value of absolute length growth and the rate of daily length growth were not significantly different from the 9% treatment. Feed with the addition of spirulina provides improved feed quality proximate protein, fat, and crude fiber.

CONFLICT OF INTEREST

There is no conflict of interest among all authors upon writing and publishing the manuscript.

AUTHOR CONTRIBUTION

The author's contribution are described as follows: Amalia Putri Firdausi directed the research design, conducted research, and drafting manuscript. Annas Yudha Mauladina conducted research and collected data in the field. Ega Aditya Prama and Risma Arafah Tunisa drafting the manuscript.

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