



Journal of Aquaculture and Fish Health  
ISSN : 2301-7309  
E-ISSN : 2528-0864

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**JOURNAL OF  
AQUACULTURE AND FISH HEALTH**

VOLUME 10 NO. 2/JUNE 2021

**FACULTY OF FISHERIES AND MARINE  
UNIVERSITAS AIRLANGGA**

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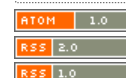
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## The Use of Rubber Seed Oil as an Alternative Plant Lipid Source for Stripped Catfish (*Pangasianodon hypophthalmus*) Diet

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Received : 2020-06-11

Accepted : 2021-03-09

Keywords :

Essential fatty acids, Stripped catfish, Rubber seed oil, Fat sources

### Abstract

Dietary lipid in the forms of fish oil and corn oil are known as the best lipid sources. An effort to find an alternative to lipid sources other than both forms of oil can be done through the use of rubber seed oil. The study was conducted to evaluate rubber seed oil as a lipid source in the diet for increasing the growth of striped catfish (*Pangasianodon hypophthalmus*) fingerlings. A tested diet having isoprotein ( $30.14 \pm 0.01\%$ ) and isoenergy ( $271.26 \pm 0.08$  DE kcal/100g) was used in this study. Fish oil, corn oil, and rubber seed oil at a total of 3% were used as the diet's lipid sources. Rubber seed oil was added to the diet at 0, 1, and 3%, respectively. After acclimatized to the experimental condition, striped catfish fingerlings ( $9.72 \pm 0.01$  g) were randomly stocked in 12 aquariums ( $69 \times 29 \times 35$  cm<sup>3</sup>; Volume 50 L) with a density of 15 fingerlings/container and fed on the tested diet at satiation for 40 days. The use of rubber seed oil as a source of lipid in the diet does not affect the survival rate and body fat ( $P > 0.05$ ). The composition of 2% rubber seed oil in the feed gives the best growth in striped catfish fingerlings, with feed intake of  $233.00 \pm 1.00$  g, a specific growth rate of  $2.01 \pm 0.05\%$  day<sup>-1</sup>, feed efficiency of  $75.45 \pm 1.18\%$ , protein efficiency ratio of  $2.45 \pm 0.11\%$  and body protein of  $44.03 \pm 2.42\%$ . There is a tendency that higher rubber seed oil content in the diet, produce better the fatty acid profile in the body of the striped catfish.

### INTRODUCTION

The cultivation of striped catfish (*Pangasianodon hypophthalmus*) is growing rapidly, among others, by utilizing peatlands (Sunarno and Marson, 2012). The support of quality seeds in sufficient quantities and commercial feed has led to an increase in the production of

striped catfish culture. However, the increasing price of commercial feed reduces the profit of cultivators (Syamsunarno and Sunarno, 2016). Therefore, striped catfish farmers try to overcome the problem of feed prices by making feed independently even though

the quality is lower than that of commercial feed (Sunarno *et al.*, 2013). Efforts to improve the quality of farmers' feed include using local raw materials as a source of essential fats and fatty acids, protein and essential amino acids, energy sources, vitamins, and minerals (Sunarno *et al.*, 2014; Sunarno *et al.*, 2012).

Lipid is a non-protein source of energy as an effort to reduce feed prices. In addition, the lipid can dissolve several vitamins, and it contains essential fatty acids which will increase feed efficiency growth (NRC, 2011). Fish oil and corn oil are sources of lipids that are often used in feed. Efforts to find ingredients other than the two oils are needed to overcome fluctuations in their products which have an impact on the increase of feed prices and limitation of their use in feed (Komariyah *et al.*, 2014).

Syamsunarno and Sunarno (2014) recommend rubber seeds as a potential local raw material for fish feed. About 80% of rubber fruits are not used as seeds (Rivai *et al.*, 2015). The rubber seeds contain 40 - 50% oil (Siahaan *et al.*, 2009). After removing the oil, the protein content of the rubber seeds increases, which is assumed to be able to act as a substitute for soybean meal (Suprayudi *et al.*, 2014a; Suprayudi *et al.*, 2014b; Syamsunarno, 2011). The rubber seed oil also contains essential fatty acids in the form of oleic acid (22.95%), linoleic acid (37.28%) and linolenic acid (19.22%) (Salimon *et al.*, 2012). The oil content of rubber seeds is higher than soybean oil (Ramadhas *et al.*, 2005) and is almost similar to corn oil (Hwang, 2009). The composition of essential fatty acids in rubber seed oil is 52% of the fatty acid composition (Onoji *et al.*, 2016).

Like other plant materials, rubber seeds contain cyanide acid. These compounds dissolve in rubber seed oil, which has an impact on reducing fish growth performance (Komariyah *et al.*, 2014; Shalihah *et al.*, 2019). The physical treatment process through soaking and boiling rubber seed oil can reduce the content of cyanide acid (Setyawardani *et*

*al.*, 2013; Karima, 2015; Rahmawati *et al.*, 2017). Furthermore, boiling rubber seeds at 100 °C for 30 minutes can reduce the cyanide acid content (Udo *et al.*, 2018). Rubber seed oil heated at 100 °C for 60 minutes can reduce 95% of cyanide acid (Kushayadi *et al.*, 2020). Based on this, an experiment was carried out to evaluate rubber seeds as a source of plant lipids in feed to improve the growth performance of catfish seeds.

## METHODOLOGY

### Place and Time

This research was conducted for 6 months in the Laboratory of Aquaculture, Faculty of Agriculture, Sultan Ageng Tirtayasa University and Baros Fish Seed Center, Serang, Banten Province.

### Research Materials

The experimental materials used included an aquarium with dimensions of 69x29x35 cm, a set of aeration, a water pump, a thermometer, a pH meter, a DO meter, a digital scale, a thermostat, a pellet printer, a rubber seed pressing machine, striped catfish seeds, rubber seed oil, corn oil, fish oil, commercial vitamin/mineral premixes, commercial vitamin C, rice bran, tapioca, fish meal, and soybean meal.

### Research Design

The treatments used in this experiment were differences in the composition of rubber seed oil in the feed, namely 0, 1, 2, and 3%. Each treatment had three replications. The experimental design used was a completely randomized design (CRD).

### Work Procedures

Rubber fruit originating from rubber plantations in Pandeglang and Lebak Districts were broken to obtain the rubber seeds. Afterward, the rubber seeds were washed, boiled at a temperature of 100°C for 30 minutes, chopped and then stored for one day (Udo *et al.*, 2018). In the next process, the rubber seeds were dried for 30

minutes in an oven at a temperature of 100°C and floured. The oil from the rubber seed flour was extracted using an automatic pressing machine. Afterward, the rubber seed oil was put into a container with a lid and was stored in the refrigerator.

Before the making of test feed was conducted, the flouring was carried out for solid feed ingredients, and then the sample was taken as the subject of proximate analysis. The test feed was composed of fish meal and soybean meal as a source of protein, rice bran as a source of carbohydrates, tapioca as an adhesive, various commercial vitamins and minerals, commercial vitamin C, fish oil, corn oil, and rubber seed oil as a source of

fat (Table 1). Moreover, the protein and energy contents were made relatively the same, namely  $30.14 \pm 0.01\%$  and  $271.26 \pm 0.08$  DE kcal/100g (Syamsunarno *et al.*, 2011). The lipid content in the test feed was set at 3%. Variation in fish oil and corn oil content was carried out to suit the content of rubber seed oil treatment. Furthermore, the raw materials were weighed according to the formula, mixed evenly, and made into pellets with a diameter of 3mm. The test feed was then dried for 24 hours in an oven at a temperature of 60°C. After drying, the test feed was put into a labeled plastic bag, and the samples were taken as the subject of proximate analysis and its fatty acids (Table 2).

Table 1. Test feed formulation for striped catfish seeds (*P. hypophthalmus*).

Raw material (%)	% Rubber Seed Oil in Feed			
	0	1	2	3
Fish meal	20.00	20.00	20.00	20.00
Soybean meal	36.00	36.00	36.00	36.00
Rice bran	26.50	26.50	26.50	26.50
Tapioca	12.00	12.00	12.00	12.00
Fish oil	1.00	1.00	1.00	0.00
Rubber seed oil	0.00	1.00	2.00	3.00
Corn oil	2.00	1.00	0.00	0.00
Mix of vitamin and mineral mix	2.00	2.00	2.00	2.00
Vitamin C	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00

Table 2. Nutritional content and fatty acid profile of the test feed for striped catfish seeds (*P. hypophthalmus*).

Composition	% Rubber Seed Oil in Feed			
	0	1	2	3
Crude protein (%)	30.15	30.12	30.13	30.14
Crude Fat (%)	6.43	6.44	6.42	6.40
Ash (%)	11.91	11.92	11.90	11.91
BETN (%)*	47.26	47.20	47.26	47.22
Total Energy (kcal/100g)	271.31	271.32	271.27	271.14
DE-P ratio (kcal/gram protein) **	9.00	9.01	9.01	9.00
Saturated fatty acids (%)	28.46	29.53	30.55	27.94
Monounsaturated fatty acids (%)	33.42	33.16	31.06	28.01
ω-3 (%)	3.18	3.36	4.68	5.78
ω-6 (%)	23.54	24.01	23.59	27.71
ω-3/ω-6 (%)	0.13	0.14	0.18	0.21

Note: \*BETN- Nitrogen-Free Extract; \*\*1 g protein=3,0 kcal DE-digestible energy; 1 g fat=8,1 kcal DE and 1 g carbohydrate =2.5 kcal DE (NRC, 2011).

The striped catfish seeds from the hatcheries were maintained in a 500 L volume fiber tank and given the test feed

without rubber seed oil for five days as the fish acclimation period to the experimental conditions. After not being

fed for one day, the fish were randomly entered into 12 aquariums that had been labeled with treatment, and each aquarium was equipped with a thermostat and aerator. The density of each aquarium was 15 individuals. The sample of the fish was taken from the container as the subject of proximate analysis. Feeding was carried out at satiation 3 times a day in the morning, afternoon and evening. Before feeding in the morning, the cleaning process was done at each aquarium, and the water that was wasted was replaced with water that had been deposited. Furthermore, every five days, a 50% change of the water in the aquarium was carried out. At the end of the study, the number of fish was calculated, and the biomass and fish weight was weighed to calculate the growth performance of the fish. Also, fish sampling from each treatment was conducted as the subject of proximate analysis and fatty acids. The protein, lipid, and fatty acid content of striped catfish were then analyzed using the AOAC method (2012).

The experimental test parameters observed were the amount of feed consumption (JKP) and the survival rate (S) calculated based on Handayani and Widodo (2010), and the specific growth rate (LPS), feed efficiency (EP) and protein efficiency ratio (REP) calculated based on Nates (2016). The formula for each parameter is as follows:

$$JKP (g) = W_0 - W_1$$

Where:

$W_0$  = initial feed weight (g)

$W_1$  = final feed weight (g)

$$LPS (\%day) = \frac{\ln(W_t) - \ln(W_0)}{t}$$

Where:

$W_t$  = fish final average weight (g)

$W_0$  = fish initial average weight (g)

$t$  = cultivation period (day)

$$EP = \frac{(B_t + B_d) - B_0}{F} \times 100\%$$

Where:

$B_t$  = fish final biomass (g)

$B_d$  = dead fish absolute biomass (g)

$B_0$  = fish initial biomass (g)

$F$  = feed consumed by fish (g)

$$REP = \frac{W_t - W_0}{P_i} \times 100\%$$

Where:

$W_t$  = fish final average weight (g)

$W_0$  = fish initial average weight (g)

$P_i$  = weight of protein consumed (g)

$$S = \frac{\Sigma \text{fish at the end of cultivation}}{\Sigma \text{fish at the beginning of cultivation}} \times 100\%$$

## Data Analysis

Data for each test parameter included the amount of feed consumption, protein efficiency ratio, specific growth rate, feed efficiency, survival rate, and protein and body fat contents which were statistically analyzed using the variance-based fingerprint method (ANOVA) with a 95% confidence interval. If the test between treatments was significantly different, it was continued with the Duncan test. The fatty acid content of fish was then analyzed descriptively.

## RESULTS AND DISCUSSION

Based on Table 3, fish feed intake decreases along with the increase in rubber seed oil ( $P < 0.05$ ). The lower unsaturated fatty acids along with the increase in rubber seed oil in the feed are thought to reduce the palatability of the test feed (Table 2). Sargent *et al.* (2002) state that low unsaturated fatty acids will be easily oxidized and cause a decrease in feed palatability. The same thing happens to goldfish (Shalihah *et al.*, 2019) and striped catfish (Suprayudi *et al.*, 2014b). A decrease in appetite in tilapia is observed in the use of rubber seed flour of more than 60% in feed (Lee and Wendy, 2017). In addition, the presence of cyanide acid in rubber seed oil can reduce feed palatability (Kushayadi *et al.*, 2020). The content of cyanide acid in the feed is 8.25 - 43.12 ppm (Deng *et al.*, 2017). However, it is suspected that the striped catfish seeds could tolerate the cyanide acid content in the rubber seed oil during maintenance. This is indicated by the survival rate that does not differ between treatments ( $P > 0.05$ ). Moreover, the increase in the concentration of cyanide in feed causes high mortality in fish (Komariyah *et al.*,

2014; Shalihah *et al.*, 2019). The cyanide content of 1.27 - 5.05 mg/kg does not affect on the survival rate in goldfish (Fawole *et al.*, 2016), and in general the mortality in freshwater fish occurs at

cyanide content at above 13.87 mg/kg (Sharma *et al.*, 2014). The minimum lethal dose of cyanide acid given via feed to land animals is 50 - 90 mg/kg (Newhouse and Chiu, 2010).

Table 3. The growth performance of striped catfish seeds during the cultivation period.

Parameter**	% Rubber Seed Oil in Feed *			
	0	1	2	3
JKP (g)	237.00 ± 1.00 <sup>a</sup>	237.00 ± 1.53 <sup>a</sup>	233.00 ± 1.00 <sup>b</sup>	227.00 ± 1.00 <sup>c</sup>
REP (%)	1.92 ± 0.18 <sup>c</sup>	2.06 ± 0.23 <sup>b</sup>	2.45 ± 0.11 <sup>a</sup>	1.80 ± 0.15 <sup>c</sup>
LPS (%day <sup>-1</sup> )	1.77 ± 0.03 <sup>c</sup>	1.86 ± 0.04 <sup>b</sup>	2.01 ± 0.05 <sup>a</sup>	1.75 ± 0.05 <sup>c</sup>
EP (%)	60.63 ± 2.93 <sup>bc</sup>	65.68 ± 4.13 <sup>b</sup>	75.45 ± 1.18 <sup>a</sup>	58.20 ± 1.49 <sup>c</sup>
S (%)	95.56 ± 3.85	95.56 ± 3.85	97.78 ± 3.85	95.56 ± 3.85

Where: \*Different superscript letters indicate significantly different values (P<0,05); \*\* JKP: the amount of feed consumption, REP: protein efficiency ratio, LPS: specific growth rate, EP: feed efficiency and S: survival rate.

Table 4. Crude protein and crude fat contents (%) in the body of striped catfish at the end of the cultivation

Parameter	% Rubber Seed Oil in Feed			
	0	1	2	3
Crude protein	38.56 ± 2.44 <sup>ab</sup>	37.56 ± 3.20 <sup>b</sup>	44.03 ± 2.42 <sup>a</sup>	41.56 ± 3.43 <sup>ab</sup>
Crude fat	35.13 ± 2.42	35.19 ± 2.74	36.22 ± 2.36	38.25 ± 2.44

Note: \* Different superscript letters indicate significantly different values (P<0,05).

The growth of striped catfish seeds increases until a concentration of 2% rubber seed oil and decreases at a concentration of 3% (P <0.05). This is related to the adequacy of the essential fatty acids of feed in rubber seed oil 2% to the increase of the growth rate. The adequacy of essential fatty acids increases fluidity as a medium for transferring nutrients and enzymes to the cell membrane (Steffens and Wirth, 2007). Striped catfish need ω-6 fatty acids more than ω-3 to accelerate their growth (Asdari *et al.*, 2011; Juliana *et al.*, 2016). ω-6 fatty acids are mostly found in vegetable oils, and they can increase the growth rate in African catfish (*Clarias gariepinus*) (Szabó *et al.*, 2009). The results of the growth of striped catfish seeds in 0 and 3% rubber seed oil, which are not significantly different, indicate that fish oil and corn oil could be replaced by rubber seed oil which contains ω-6, but the feed contains cyanide acid which can suppress the release of oxygen in the fish blood, which causes a decrease in metabolism (Komariyah *et al.*, 2014).

Cyanide acid poisoning is characterized by a low feed efficiency value (Satimehin and Tiamiyu, 2019). The fish are thought to have cyanide poisoning in 3% rubber seed oil, so they are unable to use feed efficiently due to low feed intake, feed efficiency and protein efficiency when compared to 2% rubber seed oil (P <0.05). The low efficient ratio of protein results in low fish growth (Munisa *et al.*, 2015).

Cyanide acid of rubber seed affects the storage of feed protein in the body (Oluodo *et al.*, 2018). The protein in the fish body in 2% rubber seed oil gives a significant result with other treatments (P <0.05) (Table 4). The increase in rubber seed oil is thought to increase anti-nutritional substances that inhibit metabolic processes and nutrient storage in fish bodies (Kushayadi *et al.*, 2020). However, the use of rubber seed oil does not affect the body fat content of striped catfish seeds at the end of the cultivation (P> 0.05). Body fat in fish is not influenced by differences in the source of fat in the feed (Turchini *et al.*, 2013). Feed

oil is thought to be used to fulfill metabolic energy so that protein can be used for growth (Syamsunarno *et al.*, 2011). Moreover, feed protein should be used for

replacement of damaged tissue and growth and not be used as an energy source (Putra, 2017).

Table 5. Composition of the fatty acid in the body of the striped catfish at the end of the cultivation (%).

Fatty acid	% Rubber Seed Oil in Feed			
	0	1	2	3
12 : 0	0.54	0.62	0.69	0.79
14 : 0	5.72	5.98	5.82	6.14
15 : 0	0.24	0.26	0.25	0.24
16 : 0	29.50	30.98	29.50	29.36
17 : 0	0.31	0.33	0.31	0.30
18 : 0	6.17	6.60	6.49	6.96
20 : 0	0.18	0.19	0.17	0.16
21 : 0	0.00	0.00	0.00	0.00
22 : 0	0.08	0.08	0.07	0.08
23 : 0	0.02	0.03	0.02	0.03
24 : 0	0.06	0.06	0.06	0.05
14 : 1	0.04	0.09	0.09	0.04
16 : 1	1.40	1.44	1.35	1.32
17 : 1	0.03	0.03	0.15	0.15
18 : 1n9	29.08	30.52	27.59	28.69
20 : 1	0.67	0.71	0.62	0.59
24 : 1	0.04	0.04	0.04	0.04
18 : 2n6	11.40	11.64	11.10	11.93
20 : 3n6	0.57	0.62	0.59	0.60
20 : 4n6	0.45	0.54	0.50	0.48
20 : 3n6	0.57	0.62	0.59	0.60
18 : 3n3	0.69	0.78	0.99	1.34
20 : 5n3	0.21	0.20	0.17	0.17
22 : 6n3	0.57	0.52	0.44	0.46
Σ saturated fatty acids	42.82	45.13	43.38	44.11
Σ monounsaturated fatty acids	31.26	32.83	29.84	30.83
Σ ω-6	12.63	13.01	12.40	13.22
Σ ω-3	1.47	1.50	1.60	1.97
ω-6/ω-3	8.59	8.67	7.75	6.71
ω-3/ω-6	0.12	0.12	0.13	0.15

Table 5 presents fatty acid profiles in the body of striped catfish seeds in various rubber seed oils. The differences in the composition of the dietary lipid result in the differences in the fatty acid profile of the fish body. The fatty acid in the body of striped catfish seeds at the end of the cultivation is dominated by 16: 0 fatty acids. The high level of fatty acids in the body is the characteristic of Teleostei fish and other freshwater fish (Asdari *et al.*, 2011; Dernekbaşı *et al.*, 2017). The high ω-9 content in the fish body is thought to be related to the higher ω-3 composition

of the feed with the addition of rubber seed oil. The difference in ω-3 content affects the ω-9 content of the body (Mukti *et al.*, 2014). The high-3 content causes a low ω-9 composition in the body (Tocher, 2010). Moreover, monounsaturated fatty acids and saturated fatty acids are the dominant body fatty acids in catfish (Juliana *et al.*, 2016). Rubber seed oil also contains a lot of oleic, linoleic and linolenic and 16: 0 and 18: 0 (Eka *et al.*, 2010; Karima, 2015). In 2% and 3% rubber seed oil, fish body contains 20: 5n – 3 and 22: 6n – 3 fatty acids lower than

in the other treatments. Striped catfish are thought to be able to convert 18: 3n-3 fatty acids to 20: 5n-3 and 22: 6n-3. Striped catfish do not need 18: 3n-3 unsaturated fatty acids for growth because they have elongase and desaturase enzymes so that striped catfish need 18: 2n-6. This is shown in the high  $\omega$ -6 fatty acid composition of striped catfish at the end of the cultivation. The use of high vegetable oil in feed results in a decrease in monounsaturated fatty acids and  $\omega$ -3 and an increase in fish bodies (Dernekbaşı *et al.*, 2017; Ayisi *et al.*, 2018). The ratio of  $\omega$ -3/ $\omega$ -6 in the body of striped catfish seeds is influenced by the ratio of  $\omega$ -3/ $\omega$ -6 in the feed. The higher the content of the rubber seed oil results in a relatively similar value of  $\omega$ -3/ $\omega$ -6 fatty acid ratio in the feed and in the body of the striped catfish. Moreover, the use of vegetable oil in feed results in the low  $\omega$ -3/ $\omega$ -6 content in the fish body when compared to the use of fish oil (Piedecausa *et al.*, 2007). Striped catfish require a low  $\omega$ -3/ $\omega$ -6 feed ratio to produce a high level of fish growth (Asdari *et al.*, 2011). Meanwhile, the ratio of  $\omega$ -6/ $\omega$ -3 in fish bodies at the end of the cultivation for all treatments is 6.71-8.59. According to Kartono (2012), a ratio of  $\omega$ -6/ $\omega$ -3 of 4.0 - 8.1 can prevent chronic degenerative diseases in humans.

Apart from the content of cyanide acid in the feed which is thought to be tolerated by striped catfish seeds, the optimal media water quality results in the survival rate of striped catfish seeds between treatments not significantly different. The results of water quality measurements for 40 days of cultivation were DO of 5.16 - 6.00 mg/L, ammonia of 0.013 - 0.015 mg/L, a water temperature of 29 - 30°C and water pH of 7.3 - 7.5. The results of these measurements are in accordance with BSN (2000) that the optimal water quality for striped catfish cultivation is at a temperature of 27-30°C, pH 6.50-8.50, and DO > 5 mg/L.

## CONCLUSION

Rubber seed oil can be used as an alternative source of plant lipids in the

feed if the cyanide content can be removed. The use of 2% rubber seed oil in the formula feed gives the best growth in striped catfish seeds, and there is a tendency that the fatty acid profile of striped catfish is getting better in line with the increase in the content of rubber seed oil in the feed.

## ACKNOWLEDGMENT

The authors would like to offer special thanks to IsDB (Islamic Development Bank), Universitas Sultan Ageng Tirtayasa for the research grant on the Basic Research scheme of Excellence of Science and Technology Year 2018. The authors would also thank the Baros Fish Seed Center, Serang Banten Province for permitting the accomplishment of this research.

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