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Combination of fish oil with rubber seed oil to the growth performance of catfish (*Clarias* sp.)

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Abstract. The rubber seed oil contains essential fatty acids, which may be required by cultured freshwater fish. Therefore, the research aimed to determine a combination of fish oil and rubber seed oil for catfish's best growth performance (*Clarias* sp.). Isonitrogenous (33.23 ± 0.19%) and isoenergy (266.50 ± 0.09 DE kcal/100g) tested diets were used in the experiment. The fish oil was replaced by rubber seed oil at 0%, 15%, 25%, 35%, and 50%, respectively. Cyanide acid of rubber seed oil was reduced its content by stirring at 110 °C for 90 minutes to have as high as 0.47 ppm. Catfish fingerlings of 6.91 ± 0.04 g in individual weight were randomly distributed into 15 aquariums (60x40x40cm) each at a rate of 20 fingerlings and fed on the tested diet ad satiation for 40 days during the experimental period. The results showed that the increase in rubber seed oil to replace fish oil in the feed was not significantly different from the survival rate and nutrient retention in body catfish (P> 0.05). Furthermore, catfish were not poisoned by cyanide acid, indicated by catfish blood profile in the normal range at the end of rearing (P>0.05). The combination of fish oil and rubber seed oil by 50% gives optimal growth in catfish.

1. Introduction

Catfish require a nutritionally complete and balanced diet for growth. The quality of fish feed is determined by the nutrients available to support basic metabolic, health status, and optimal growth [1]. Lipid is one of the macronutrients that must be available in the feed, serves as a source of essential fatty acids, and if insufficient amounts in the feed, it can reduce the use of protein as an energy source [2]. Fish oil is a major component of fat in food because of its complete fatty acid profile and can be a source of fish's metabolic energy [3]. However, its use is currently limited to meet the growing demand for the aquafeed industry [4]. That leads to increased feed prices affecting profit freshwater fish farmers, especially in catfish [5].

Vegetable oil is a potential source of lipid to replace fish oil in the diet because of its abundant availability, acceptable price, relatively low organic contamination, and high fatty acid [6]. Furthermore, types of agro-industrial by-products have the potential as alternative feed ingredients [7]. Rubber seed is a by-product in rubber cultivation that has not been utilized, with an oil content of around 40 - 50%



[8]. Rubber seed oil is a by-product of processing rubber seed meal, and it is estimated that rubber seed oil production in Indonesia is in the range of 192.2 - 199.08 million tons per year [10].

Rubber seed oil is a semi-drying oil containing essential fatty acids, namely, oleic acid (22.95%), linoleic acid (46.28%), and linolenic acid (19.22%) [10]. Similarly, the result was found by [11], who reports that oleic acid levels, linoleic acid, and linolenic acid were 25.33%, 37.50%, and 14.21%, respectively. The rubber seed oil has a higher fatty acid than soybean oil [12] and almost close to corn's fatty acid composition [13]. The rubber seed oil's essential fatty acid composition is 52% of the fatty acid composition [14].

Unprocessed rubber seed oil in the diet will limit feed intake, lower growth and survival rates, affecting blood profiles of fish [9] [15]—that is thought to be due to an anti-nutritional substance in cyanide acid-containing rubber seed oil. The most prominent anti-nutritional content in rubber seeds is cyanide acid [16]. There are also phytic, oxalic, tannin, saponins, and trypsin inhibitors [17] [18]. Several studies have reported that cyanide acid content can reduce through physical treatment, including soaking, heating, boiling, and roasting [19] [20]. Furthermore, [21] reported that stirring at 100°C could reduce cyanide acid in rubber seed oil. Therefore, the research aimed to determine a combination of fish oil and rubber seed oil for catfish's best growth performance (*Clarias sp.*).

2. Material and Methods

2.1. Rubber seed oil preparation

The number of 18 kg of rubber fruit was taken from smallholder plantations in Semerangkai Village, Sanggau Regency, West Kalimantan, and broken down to get a seed. The rubber seed was then boiled for 20 minutes, dried in an oven at 60°C for 60 minutes, and ground into meal form. The meal was oiled out using a pressing machine at 80°C for 30 minutes [22]. Cyanide acid of rubber seed oil was reduced its content by stirring at 110°C for 90 minutes to have as high as 0.47 ppm. [23] recommends that cyanide acid content in food products is 1 ppm. The process of removal of cyanide acid refers to [21].

2.2. Preparation experimental diet

Isonitrogenous ($33.23 \pm 0.19\%$) and isenergetic (266.50 ± 0.09 DE kcal/100g) tested diets were used in the experiment. The primary sources of protein in the experimental diet were fish meal and soybean meal. The total lipid in the test diet was 5% [24], from a combination of fish oil and rubber seed oil, namely 0%, 15%, 25%, 35%, and 50%. Raw materials are weighed according to the formula and mixed evenly and molded-in pellets with a 3 mm diameter, then oven at 60°C [9]. After drying, the experimental diet was put into a plastic bag labeled, and the samples were taken as the subject of proximate and fatty acid analysis. The formulation and analysis results of the experimental diets are present in Table 1 [25].

2.3. Experimental design

Catfish of 6.91 ± 0.04 g fish in initial weight were reared at 20 individuals/aquarium. The fishes were raised in 15 aquariums of 60 x 40 x 40 cm in dimension size, each for 40 days. After acclimatized for seven days, the fish was fasted for 24 hours to eliminate the body's remaining feed [26]. Feeding was carried out three times a day at satiation. Water changes were carried out as much as 50% of the aquarium's total volume, aiming to maintain water quality during the study. At harvest, several fish samples were taken as subjects for proximate analysis and blood profiles.

2.4. Growth parameters

At the end of the study, the growth parameters of catfish were analyzed. The specific growth rate (SGR), feed efficiency (FE), survival rate (SR), and protein/lipid retention (PR/LR) were calculated using the formula according to [25].

$$\text{SGR (\%)} = 100 \times (\ln W_t - W_o) / \text{time (days)} \quad (1)$$

$$\text{FE (\%)} = 100 \times [\text{wet weight gain (g)} / \text{dry feed intake (g)}] \quad (2)$$

$$\text{SR (\%)} = \text{NT}/\text{N0} \times 100 \quad (3)$$

$$\text{PR/LR (\%)} = ((F - I) / P) \times 100 \quad (4)$$

Where W_t and W_o indicate the final and initial weight (g), respectively, NT is the number of fish at the end (individuals), N0 is the number at the beginning (individuals). F is total protein/lipid in the final body (g), I is the total protein/lipid in the initial body (g), and P is total protein/lipid consumption (g).

Table 1. Composition of tested diets, and their proximate analysis and fatty acids

Composition	Experimental diet (%)				
	0	15	25	35	50
Fish meal	30.00	30.00	30.00	30.00	30.00
Soybean meal	27.50	27.50	27.50	27.50	27.50
Tapioca	13.00	13.00	13.00	13.00	13.00
Lard	9.45	9.45	9.45	9.45	9.45
Wheat flour	7.50	7.50	7.50	7.50	7.50
Fish oil	5.00	4.25	3.75	3.25	2.50
Flax seed oil	0.00	0.75	1.25	1.75	2.50
Vitamin and mineral premix	5.00	5.00	5.00	5.00	5.00
Casein	2.00	2.00	2.00	2.00	2.00
Vitamin C	0.05	0.05	0.05	0.05	0.05
PMC	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00
Proximate compositions (dry base)					
Crude protein (%)	33.07	33.16	33.48	33.39	33.06
Crude lipid (%)	6.43	6.56	6.46	6.44	6.45
Ash (%)	13.62	13.92	13.80	13.74	13.63
Crude fiber (%)	7.44	7.45	7.41	7.42	7.48
Free extract nitrogen (%)	39.44	38.91	38.85	39.01	39.38
DE (kcal/100g)*	266.43	266.47	266.63	266.55	266.41
Fatty acids (%)					
Σ SFA**	18.72	18.59	18.51	18.42	18.29
Σ MUFA***	19.84	19.57	19.38	19.20	18.92
Σ n-6	20.46	20.71	20.88	21.05	21.31
Σ n-3	10.83	10.65	10.53	10.40	10.22
Σ n-3/Σ n-6	0.53	0.51	0.50	0.49	0.48

Noted: * DE= Digestible energy = 1 g protein: 3.0 kkal DE; 1 g lipid: 8.1 g kkal DE; 1 g carbohydrate: 2.5 kkal

DE [27]

** SFA= Saturated fatty acids

*** MUFA = Monounsaturated fatty acids

2.5. Blood profiles

At harvest, three individuals from each treatment were taken out for collecting blood samples of fish. The blood samples were taken out using a syringe already containing anti-coagulant 0.1 mL of 3.8% Na citrate [28]. Furthermore, the blood profile consisting of total red blood cells (RBC), white blood cells (WBC), haemoglobin, and haematocrit were calculated with a microscope's aid. Determination of total

RBC and WBC according to the method [29], measurement of haemoglobin (Hb) levels by the Sahli method according to [30], and measure of haematocrit (Ht) levels according to the procedure [31].

3.4. Chemical analysis

The proximate composition of raw materials, experimental diet, and fish body samples were analyzed according to the standard method [32]. The proximate composition is crude protein, crude fat, ash content, crude fiber, and nitrogen-free extract.

2.7. Statistical analysis

Statistical analysis of growth parameters and blood profiles perform using the Statistical Package for the Social Sciences (SPSS) program for Windows (v.16.0). If a significant difference, then proceeds with the Tukey test at a level of 95% [33].

4.0 Results and Discussion

Based on the results of the research in Table 2, it was known that the increase in rubber seed oil in feed to replace fish oil could increase feed intake in catfish ($P < 0.05$). This increase is thought to be due to rubber seed oil, which can increase feed palatability. Similar results are reported in juvenile Malaysian mahseer (*Tor tambroides*) fed a vegetable oil-based feed to replace fish oil [34]. Unsaturated fatty acids influence feed palatability. According to [15], the increase of unsaturated fatty acids results in poor taste and the feed aroma, limiting feed intake by fish. In this study, the unsaturated fatty acid content in the test feed (Table 1) is thought to have increased feed consumption.

4.3

Table 2. The growth performance of catfish fed the experimental diet during the rearing period

Parameters**	Experimental diet (%)*				
	0	15	25	35	50
FI (g)	165.00±3.61 ^a	167.33±4.04 ^a	176.67±5.51 ^{ab}	181.67±5.86 ^b	183.67±2.08 ^b
FE (%)	40.31±1.78 ^a	41.35±2.87 ^{ab}	44.56±5.86 ^{ab}	49.08±2.80 ^{ab}	49.08±1.58 ^b
SGR (%)	1.00±0.06 ^a	1.03±0.06 ^a	1.14±0.12 ^{ab}	1.24±0.03 ^b	1.30±0.05 ^b
Protein retention (%)	67.09±2.44	67.61±2.72	71.59±2.84	72.40±3.14	73.95±3.41
Lipid retention (%)	64.03±5.34	62.42±3.21	63.12±5.62	64.05±5.33	65.61±5.02
SR (%)	96.67±2.89	96.67±2.89	98.33±2.89	100.00±0.00	96.67±2.89

Noted: *T values in the same row followed by similar superscript are not significantly different ($p > 0.05$).

** feed intake (FI), feed efficiency (FE), specific growth rate (SGR), survival rate (SR)

3.6

The increased composition of rubber seed oil in the feed affects feed efficiency and can further increase growth. The results of statistical tests showed that the feed without rubber seed oil (0%) was significantly different on feed efficiency and specific growth rate at 50% treatment ($P < 0.05$). The combination of fish oil with rubber seed as much as 50% produces an adequate supply of essential fatty acids to increase catfish growth. The use of vegetable oil to replace fish oil in aquafeed can be done more than 50% if the fatty acids found in the feed are sufficient to meet the needs of essential fatty acids in fish [4]. Based on Table 1, the n-6 fatty acids increased with the additional rubber seed oil in the diet. In general, freshwater fish need linoleic acid (18: 2n-6), or linolenic acid (18: 3n-3), or both to support optimum growth [1]. According to [35], catfish need more n-6 than n-3 fatty acids to accelerate their growth. The increasing growth of catfish is thought to be related to the decreasing ratio of n-3/n-6 in the diet. According to [36], the n-3 and n-6 fatty acid requirements in the lower n-3/n-6 rate promote better growth at the striped catfish (*Pangasius hypophthalmus*).

Protein and lipid retention at the end of the study had no significant effect with different ratios of rubber seed oil and fish oil ($P > 0.05$). The presence of saturated fatty acids in the appropriate test diet

required by catfish causes the energy derived from these fatty acids to be used as an energy source to maintain the fish's body. According to [29], higher saturated fatty acids can be easily oxidized. The energy derived from these fatty acids can be used to maintain body maintenance and protein for growth. This study's results are following the results of a research according to [37] [36] [38].

Table 3. The value of blood profiles in catfish fed the experimental diet at the end of the study

Blood profile**	Experimental diet (%)*				
	0	15	25	35	50
RBC ($\times 10^6$ cell/mm ³)	1.49 \pm 0.14	1.56 \pm 0.12	1.56 \pm 0.11	1.55 \pm 0.13	1.57 \pm 0.13
RWC ($\times 10^4$ cell/mm ³)	3.20 \pm 0.06	3.21 \pm 0.08	3.28 \pm 0.15	3.26 \pm 0.03	3.28 \pm 0.07
Hb (g%)	6.47 \pm 0.15	6.50 \pm 0.20	6.43 \pm 0.15	6.50 \pm 0.30	6.43 \pm 0.21
Ht (%)	25.60 \pm 0.44	25.40 \pm 0.17	25.83 \pm 0.31	25.73 \pm 0.65	25.47 \pm 0.31

Noted: *T values in the same row followed by similar superscript are not significantly different ($p > 0.05$).

** red blood cell (RBC), white blood cell (WBC), Haemoglobin (Hb), Ht (Haematocrit)

Table 3 shows that the blood profiles did not significantly differ between treatments ($P > 0.05$). The level of cyanide acid will affect fish health and fish blood profile. According to [15], fish exposed to cyanide acid in their body causes the fish to be under metabolic stress, making it susceptible to disease. The process of cyanide acid poisoning in fish involves cellular cytochrome enzymes so that haemoglobin in the blood cannot bind to oxygen and causes low haemoglobin [39]. High cyanide acid will affect blood chemistry and blood profile, such as low haematocrit, low total red blood cells and white blood cells [40] [15]. The study results [9] reported a low fish survival rate due to high cyanide acid, but in this study, the survival rate was not different, namely between 96.67-100% ($P > 0.05$). The content of cyanide acid of rubber seed oil used in this study can make tolerant of catfish. Cyanide acid below 5.05 ppm in the feed does not affect fish survival rate [41]. Reducing cyanide acid to fish tolerance level can improve rubber seed oil quality combined with fish oil up to 50% (1:1). These results suggest that rubber seed oil can be used as an alternative source of vegetable oil mixed with fish oil in feed, such as corn oil and palm oil. The combination of fish oil and corn oil is the best as a lipid source in the diet [27] [1]. According to [4], the vegetable oils most commonly used for fish feed production include soybeans, corn, rapeseed, sunflower, palm oil and olive oil.

4. Conclusion

The best combination of fish oil and rubber seed oil at 1:1 (50%) in the diet composition resulted in catfish's optimal growth performance.

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