

Evaluation of processed rubber seed oil *Hevea brasiliensis* utilization as lipid source in *Clarias gariepinus* feed

Evaluasi pemanfaatan minyak biji karet *Hevea brasiliensis* yang telah diproses sebagai sumber lemak pada pakan ikan lele *Clarias gariepinus*

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ABSTRACT

Clarias gariepinus rearing is mostly done with intensive systems that use a lot of commercial feed. The price of raw material for the feed lipid source which is currently the main source of fatty acids in freshwater fish feed continues to increase and compete with human needs. Rubber seed oil (RSO) is considered feasible to be an alternative. This study aimed to evaluate the use of heated RSO as alternative lipid source in *C. gariepinus* feed. The results of this study indicated that heating could reduce HCN levels in RSO by 95%. Even after heated RSO was inserted into the feed formulation there is still HCN in feed, however it did not affect the survival rate of *C. gariepinus* statistically. Heated RSO based feed shows a specific growth rate that was higher than non-heating RSO-based feed. Feeding with heated RSO also did not affect nutrient retention in *C. gariepinus*. This also followed by blood profile, blood chemistry and body fatty acids profile, MDA levels and SOD levels which were almost same as treatment feed using fish oil and corn oil. From these results, it can be concluded that heating can reduce 95% HCN content in RSO and can be used as a lipid source in *C. gariepinus* feed.

Keywords: Aquaculture, essential fatty acids, growth performance, HCN, rubber seed oil.

ABSTRAK

Budidaya ikan lele *Clarias gariepinus* dengan sistem intensif menggunakan banyak pakan buatan. Harga bahan baku untuk sumber lemak pakan terus meningkat dan bersaing dengan kebutuhan manusia. Minyak biji karet (MBK) layak menjadi alternatif. Penelitian ini bertujuan mengevaluasi penggunaan MBK yang dipanaskan sebagai sumber lemak alternatif dalam pakan ikan lele. Hasil penelitian menunjukkan proses pemanasan dapat mengurangi kadar HCN pada MBK hingga 95%. Walaupun masih terdapat sejumlah kecil HCN pada pakan berbasis MBK yang dipanaskan, tetapi tidak memengaruhi tingkat kelangsungan hidup ikan lele ($P > 0,05$). Pakan berbasis MBK proses pemanasan menunjukkan laju pertumbuhan spesifik yang lebih tinggi dari pakan berbasis MBK yang tidak dipanaskan. Pemberian pakan berbasis MBK proses pemanasan juga tidak memengaruhi retensi nutrisi ikan lele. Demikian pula parameter profil darah, kimia darah, profil asam lemak tubuh, kadar MDA dan kadar SOD hampir sama dengan pakan perlakuan berbasis minyak ikan dan minyak jagung. Sehingga dapat disimpulkan MBK proses pemanasan dapat mengurangi 95% kandungan HCN dan dapat digunakan sebagai sumber lemak dalam pakan ikan lele.

Kata kunci: Akuakultur, asam lemak esensial, HCN, kinerja pertumbuhan, minyak biji karet.

INTRODUCTION

According to KKP (2017), national production of *Clarias gariepinus* in 2017 reached 1.095.969 tons and up to early 2018, it reached 531.521 tons. *C. gariepinus* rearing is mostly done with an intensive system culture that requires artificial feed. One important component as energy and essential fatty acids source in fish feed is lipid (Orsavova *et al.*, 2015). Vegetable oils, such as corn oil, canola oil, and palm oil are the main sources of vegetable fatty acids in freshwater fish feed (Ortiz *et al.* 2017; Shahrooz *et al.* 2018). Unfortunately, along with the increase in the price of vegetable oil and competition with human needs, a more economical alternative of lipid sources that contains adequate essential fatty acid content is needed.

Data from the Directorate General of Plantations (2010); Widyarani *et al.* (2014) said, Indonesia has around 3.3 million hectares of rubber plantations which can produce around 4 million tons of rubber seeds per year but have not yet been fully utilized. Processed rubber seed meal can be used as a protein source in fish feed (Syamsunarno & Sunarno, 2014), such as in pangasius (Suprayudi *et al.*, 2014), carp (Suprayudi *et al.*, 2015), and tilapia (Yusuf *et al.*, 2016). Rubber seeds contain 50–60% rubber seeds oil (RSO) (Kumar & Sharma, 2011). According to Salimon *et al.* (2012) RSO has 37.28% of linoleic acid (LA 18:2n-6) and 19.22% of linolenic acid (LNA 18:3n-3). RSO has greater LNA content than similar vegetable oils, such as corn oil (0.023–0.39%) and palm oil (0.08–0.23%). It also has LA content which is fairly similar to corn oil (45.92–54.21%) (Hwang, 2009; Dowd *et al.*, 2010; Bami *et al.*, 2017).

Hydrogen cyanide (HCN) is a limiting factor in RSO utilization. Each kilogram of rubber seeds contained about 1.64 g HCN (Widyarani *et al.*, 2016). Salimon *et al.* (2012) stated that HCN is classified as a chemical compound that is unstable to heat, so that the physical treatment of heating can evaporate HCN. Therefore, this study used RSO which has been through physical treatment in the form of heating with a temperature of 100°C as an intention to reduce HCN levels in the RSO.

MATERIALS AND METHODS

Rubber seed oil (RSO) preparation

Rubber seeds were obtained from a rubber plantation in Setu Village, Jasinga, Bogor, West

Java. To get RSO, rubber seeds are pressed using hydrolic pressure at 80°C with a pressure of 8 bar for 15 minutes. RSO then given 500 mg/L TN Ox as an antioxidant. Some RSO then heated in an oven at 100°C for 60 minutes (Zhang *et al.*, 2013). RSO with and without heating were analyzed for HCN content, peroxyde number and free fatty acids (AOAC, 2012). The HCN and peroxyde value (POV) content of RSO with and without heating are presented in Table 1 and fatty acids profiles are presented in Table 2.

Table 1. Hydrogen cyanide (HCN) and peroxyde value (POV) content in rubber seed oil with and without heating.

Heating duration (minutes)	HCN (mg/L)	POV (meq/kg)
0	44.87	4.63
60	2.02	1.97

Table 2. Heated rubber seed oil and rubber seed oil without heating fatty acids profile.

Fatty acids	Heated rubber seed oil (%)	Rubber seed oil without heating (%)
ΣSFA*	17.79	22.81
ΣMUFA*	23.17	22.18
ΣPUFA*	57.71	53.76
18:2n-6 (Linoleic)	40.01	37.45
18:3n-3 (Linolenic)	17.6	16.3
20:4n-6 (AA)*	0	0
20:5n-3 (EPA)*	0.01	0.1
22:6n-3(DHA)*	0	0

% sample fatty acids: % area x % sample lipid.

*SFA: saturated fatty acid; MUFA: monosaturated fatty acid; PUFA: polyunsaturated fatty acid; AA: arachidonic acid; EPA: eichosapentainoic acid; DHA: docosahexanoic acid.

Experimental design

The experimental design in this study was a one-factor randomized complete design (CRD). This experiment consisted of five treatments and was repeated three times, so that 15 treatment units were obtained. Test feeds were isocalories and isoproteins. The combination of feed lipid sources given are as follows (Table 3):

Experimental diets

The tested feed used in this study originated from different lipid source. Feed raw materials

Table 3. Experimental design

Treatment	Dossage
CPO:FO*	Feed with 50% palm oil+50% fish oil
CPO:CO*	Feed with 50% palm oil +50% corn oil
CPO:RSOH*	Feed with 50% palm oil+50% RSOH
PRSO*	Feed with 100% RSOH
URSO*	Feed with 100% RSO

*CPO:FO : 50% palm oil + 50 % fish oil; CPO:CO: 50% palm oil + 50% corn oil; CPO:RSOH: 50% palm oil + 50% RSOH; PRSO: 100% RSOH; URSO:100% RSO.

*CPO: crude palm oil; FO: fish oil, CO: corn Oil; RSOH: heated rubber seed oil; RSO: unheated rubber seed oil; PRSO: 100% rubber seed oil with heating; URSO: 100% rubber seed oil without heating.

Table 4. Feed formulation, proximate, and HCN levels.

Ingredients (%)	Experimental diets				
	CPO:FO*	CPO:CO*	CPO:RSOH*	PRSO*	URSO*
Fish meal	6.5	6.5	6.5	6.5	6.5
Poultry by-product meal	16.5	16.5	16.5	16.5	16.5
Soybean oil	24.0	24.0	24.0	24.0	24.0
Pollard	30.0	30.0	30.0	30.0	30.0
Wheat flour	18.8	18.8	18.8	18.8	18.8
Fish oil <i>Chum salmon (Tassal®)</i>	1.0	-	-	-	-
Corn oil	-	1.0	-	-	-
Palm oil	1.0	1.0	1.0	-	-
Heated rubber seed oil	-	-	1.0	2.0	-
Rubber seed oil	-	-	-	-	2.0
Premix**	2.2	2.2	2.2	2.2	2.2
Total	100.0	100.0	100.0	100.0	100.0

Feed proximate

Protein %	30.34	30.09	30.17	30.52	30.44
Lipid (%)	5.27	5.33	5.43	5.24	5.37
Moisture (%)	8.09	8.47	7.96	8.18	8.89
Ash (%)	9.13	8.94	8.15	8.01	9.13
Crude fibre (%)	5.14	5.98	4.89	5.89	5.74
Free extract nitrogen (%)	42.03	40.38	43.4	42.16	40.43
GE*** (kkal/g)	3959.62	3927.38	4022.57	3972.46	3907.48
HCN (mg/L)	-	-	1.35	1.77	7.92

*CPO:FO : 50% palm oil + 50 % fish oil; CPO:CO: 50% palm oil + 50% corn oil; CPO:RSOH: 50% palm oil + 50% RSOH; PRSO: 100% RSOH; URSO:100% RSO.

*CPO: Crude palm oil; FO: Fish oil, CO: Corn Oil; RSOH: Heated rubber seed oil; RSO: Unheated rubber seed oil; PRSO: 100% Rubber seed oil with heating; URSO: 100% rubber seed oil without heating.

**Mineral and vitamin premix (Suprayudi *et al.*, 2014): retinol (A), 900 IU/kg; ascorbic acid (C), 200 mg/kg; cholecalciferol (D), 200 IU/kg; menadione (K3), 10.0 mg/kg; α -tocopherol (E), 100 mg/kg; choline, 1000 mg/kg; inositol, 100 mg/kg; thiamine (B1), 15 mg/kg; riboflavin (B2), 20 mg/kg; pyridoxine (B6), 15 mg/kg; d-pantothenic acid (B5), 50 mg/kg; nicotinic acid, 75 mg/kg; biotin, 0.5 mg/kg; cyanocobalamin (B12), 0.05 mg/kg; folic acid, 5 mg/kg; Co (as $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$), 0.5 mg/kg; Cu (as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), 5 mg/kg; Fe (as $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), 50 mg/kg; I (as KI), 4 mg/kg; Cr (as $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$), 0.1 mg/kg; Mg (as $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 150 mg/g; Mn (as $\text{MnSO}_4 \cdot \text{H}_2\text{O}$), 25 mg/kg; Se (as NaSeO_3), 0.1 mg/kg; Zn (as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), 100 mg/kg; Amino mix, 0.3 g/kg

***GE: Gross Energy; 1 g protein = 5.4 kcal GE; 1 g lipid = 9.4 kcal GE; 1g carbohydrate/free extract nitrogen = 4.2 kcal GE (NRC, 2011).

Table 5. Feed fatty acids composition.

Fatty acids (%)	Experimental diets				
	CPO:FO*	CPO:CO*	CPO:RSOH*	PRSO*	URSO*
∑SFA**	1.89	2.05	1.89	1.92	1.68
∑MUFA**	2.34	1.56	1.76	1.57	1.60
∑PUFA**	1.06	1.72	1.80	1.76	2.11
18:2n-6 (Linoleic acid)	0.34	1.53	1.28	1.29	1.56
18:3n-3 (Linolenic acid)	0.60	0.16	0.33	0.44	0.50
20:4n-6 (AA)**	0.02	0.00	0.00	0.00	0.00
20:5n-3 (EPA)**	0.02	0.00	0.00	0.00	0.00
22:6n-3(DHA)**	0.03	0.00	0.00	0.00	0.00

*CPO:FO : 50% palm oil + 50 % fish oil; CPO:CO: 50% palm oil + 50% corn oil; CPO:RSOH: 50% palm oil + 50% RSOH; PRSO: 100% RSOH; URSO:100% RSO.

*CPO: Crude palm oil; FO: Fish oil, CO: Corn Oil; RSOH: Heated rubber seed oil; RSO: Unheated rubber seed oil; PRSO: 100% Rubber seed oil with heating; URSO: 100% rubber seed oil without heating.

**SFA: Saturated fatty acid; MUFA: Monosaturated fatty acid; PUFA: Polyunsaturated fatty acid; AA: Arachidonic acid; EPA: Eicosapentainoic acid; DHA: Docosahexanoic acid.

Table 6. Growth and feed performance of *Clarias gariepinus* fed with different lipid sources

Parameters	Experimental diets				
	CPO:FO*	CPO:CO*	CPO:RSOH*	PRSO*	URSO*
Feed intake (g)	1796.94 ± 68.75 ^a	1761.13 ± 162.06 ^a	1866.07 ± 105.36 ^a	1787.49 ± 57.98 ^a	2183.49 ± 123.03 ^b
FCR	1.16 ± 0.03 ^a	1.12 ± 0.09 ^a	1.15 ± 0.06 ^a	1.10 ± 0.07 ^a	2.16 ± 0.13 ^b
SGR (%)	4.69 ± 0.06 ^b	4.71 ± 0.05 ^b	4.70 ± 0.13 ^b	4.56 ± 0.08 ^b	2.23 ± 0.7 ^a
Protein retention (%)	50.21 ± 0.79 ^a	51.14 ± 4.23 ^a	50.25 ± 2.88 ^a	53.07 ± 3.59 ^a	26.34 ± 0.96 ^b
Lipid retention (%)	119.90 ± 4.55 ^a	122.85 ± 9.41 ^a	116.73 ± 6.10 ^a	125.93 ± 8.82 ^a	65.10 ± 4.85 ^b
SR (%)	100 ± 0.00 ^b	97.78 ± 3.85 ^b	95.56 ± 3.85 ^b	88.89 ± 3.85 ^b	35.56 ± 13.88 ^a

*CPO:FO : 50% palm oil + 50 % fish oil; CPO:CO: 50% palm oil + 50% corn oil; CPO:RSOH: 50% palm oil + 50% RSOH; PRSO: 100% RSOH; URSO:100% RSO.

*CPO: Crude palm oil; FO: Fish oil, CO: Corn oil; RSOH: Heated rubber seed oil; RSO: Unheated rubber seed oil; PRSO: 100% Rubber seed oil with heating; URSO: 100% rubber seed oil without heating. Values are means ± SE ($n = 3$). Values that share different letters in the same column are significantly different (Duncan test; $P < 0.05$). FCR: Feed conversion ratio; SGR: Specific growth rates; SR: Survival rate.

were tested for proximate before formulation. The raw materials were combined using a mixer until it was homogeneous then printed using 2 mm diameter feed molding machine and dried with an oven at 40°C. Dry feeds were tested for proximate and HCN levels. Proximate and HCN analysis referred to AOAC (2012). Proximate values and feed HCN levels are presented in Table 4.

The use of different feed lipid sources will affect the fatty acids feed composition. Therefore, it was necessary to test the composition of feed fatty acids. The feed fatty acids composition are presented (Table 5).

Research procedures

This research was conducted from February to August 2019 which included the process of preparation for tested feed, fish maintenance, and final test results. The process of making test feed and maintaining fish was carried out in the Teaching Farm and the final test results were conducted at Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Bogor.

Rearing conditions

The fish being tested was *Clarias gariepinus*

Table 7. *Clarias gariepinus* body fatty acids that are fed with different lipid sources

Fatty acids	Unit	Experimental diets				
		CPO:FO*	CPO:CO*	CPO:RSOH*	PRSO*	URSO*
ΣSFA**	%	31.43 ± 0.48 ^a	37.22 ± 0.48 ^b	37.68 ± 0.72 ^b	37.00 ± 0.12 ^b	37.82 ± 0.75 ^b
ΣMUFA**	%	28.51 ± 0.61 ^a	33.65 ± 1.08 ^c	30.62 ± 1.11 ^{ab}	31.81 ± 1.60 ^{bc}	32.88 ± 1.56 ^a
ΣPUFA**	%	40.06 ± 1.08 ^c	29.14 ± 0.60 ^a	31.69 ± 1.82 ^b	31.19 ± 1.48 ^{ab}	29.30 ± 0.81 ^{ab}
Linoleic (18:2n-6)	%	27.31 ± 0.91 ^b	19.82 ± 0.98 ^a	20.83 ± 0.93 ^a	20.42 ± 0.85 ^a	19.58 ± 0.78 ^a
Linolenic (18:3n-3)	%	4.55 ± 0.17 ^b	1.84 ± 0.33 ^a	2.29 ± 0.29 ^a	1.73 ± 0.64 ^a	1.93 ± 0.38 ^a
AA** (20:4n-6)	%	1.28 ± 0.09 ^a	1.42 ± 0.03 ^b	1.56 ± 0.09 ^c	1.69 ± 0.05 ^c	1.41 ± 0.08 ^b
EPA** (20:5n-3)	%	0.22 ± 0.06 ^a	0.26 ± 0.02 ^a	0.22 ± 0.01 ^a	0.22 ± 0.01 ^a	0.20 ± 0.06 ^a
DHA** (22:6n-3)	%	1.46 ± 0.09 ^{ab}	1.44 ± 0.05 ^{ab}	1.52 ± 0.11 ^b	1.44 ± 0.15 ^{ab}	1.19 ± 0.22 ^a

*CPO:FO : 50% palm oil + 50 % fish oil; CPO:CO: 50% palm oil + 50% corn oil; CPO:RSOH: 50% palm oil + 50% RSOH; PRSO: 100% RSOH; URSO:100% RSO.

*CPO: Crude palm oil; FO: Fish oil, CO: Corn oil; RSOH: Heated Rubber Seed Oil; RSO: Unheated rubber seed oil; PRSO: 100% Rubber seed oil with heating; URSO: 100% rubber seed oil without heating.

**SFA: Saturated fatty acid; MUFA: Monosaturated fatty acid; PUFA: Polyunsaturated fatty acid; AA: Arachidonic acid; EPA: Eicosapentanoic acid; DHA: Docosahexanoic acid.

Values are means ± SE (n = 3). Values that share different letters in the same column are significantly different (Duncan test; P<0.05).

Table 8. The chemical values of *Clarias gariepinus* blood fed with different lipid sources

Parameters	Experimental diets				
	CPO:FO*	CPO:CO*	CPO:RSOH*	PRSO*	URSO*
Cholesterol (mg/dL)	133–144	85–106	105–108	108–117	84–90
HDL** (mg/dL)	85–104	78–94	86–87	79–86	67–72

*CPO:FO : 50% palm oil + 50 % fish oil; CPO:CO: 50% palm oil + 50% corn oil; CPO:RSOH: 50% palm oil + 50% RSOH; PRSO: 100% RSOH; URSO:100% RSO.

*CPO: Crude palm oil; FO: Fish oil, CO: Corn oil; RSOH: Heated rubber seed oil; RSO: Unheated rubber seed oil; PRSO: 100% Rubber seed oil with heating; URSO: 100% rubber seed oil without heating.

**high density lipoprotein.

weighing 7.5 ± 0.27 g per head, length 9.5 ± 0.23 cm, and reared in an aquarium with 105 L of water volume. Tested fish was obtained from Purwakarta, West Java. The fish were first acclimatize (feed and environment) for seven days. Stocking density used was 15 fish/aquarium. They were reared for 56 days and fed three times a day on 08.00, 12.00, and 16.00 in satiation feeding method. Sampling was carried out once every 14 days with weight, body length, and total biomass measurements. Every dead fish was weighed. The water quality was maintained and monitored in the maintenance container, a top filter, aerator set, thermostat, and thermometer installed on the inner front wall of the aquarium.

Experimental parameters

The parameters in this study consisted of chemical analysis, evaluation of feed utilization, stress indicators, and growth performance. Chemical analysis included proximate analysis

of raw materials, tested feed and fish, HCN content analysis, and analysis of fatty acid profiles. Evaluation of feed utilization included feed conversion ratio (FCR), protein and fat retention, muscle and liver glycogen, and blood chemistry. Stress indicator parameters consisted of the metabolic stress response in the form of blood images and oxidative stress response in the form of malanodealdehyde (MDA) and superoxide dismutase (SOD). Meanwhile, growth performance parameters consisted of the amount of feed consumption, daily growth rate, and survival rate.

Data analysis

Data were analyzed using analysis of variance (ANOVA) with 95% of confidence level (α : 5%) followed by normality test and homogeneity test using SPSS 20.0. If any significant differences were found, further tests would be conducted using the Duncan method.

RESULTS AND DISCUSSION

Results

Growth and feed performance

Based on this experiment, it was known that *Clarias gariepinus* in PRSO and CPO:RSOH treatments showed a decent growth and feed performance and were not statistically significant ($P>0.05$) with fish in CPO:FO and CPO:CO treatments (Table 6). Different things were shown by fish in the URSO treatment that showed lower growth performance and were statistically significantly different ($P<0.05$) from other treatments.

Body fatty acids

The results of body fatty acid analysis showed that *Clarias gariepinus* fed with different lipid sources had different body fatty acid compositions (Table 7). The SFA and PUFA content of fish fed with RSO based was not statistically different ($P>0.05$) from fish fed with CPO:CO. On the contrary, it is statistically different ($P<0.05$) from fish fed with CPO:FO.

Blood chemistry

The results of the blood chemistry test showed there were differences in the blood chemical value of *Clarias gariepinus* which was fed with different lipid sources (Table 8). Fish in CPO:RSOH and PRSO treatments had a higher range of cholesterol and HDL values compared to fish in URSO treatment.

Blood profile

The blood profile results show that *Clarias gariepinus* fed with different lipid sources have different blood profile (Table 9). Fish in the PRSO and CPO:RSOH treatments have a higher range of Hb, Ht, and erythrocytes and lower range of leukocytes compared to fish in the URSO treatment.

MDA and SOD values

MDA and SOD test results showed that the *Clarias gariepinus* in the PRSO treatment had lower MDA values and were statistically significantly different ($P<0.05$) from the fish in the URSO treatment (Table 10). Meanwhile, fish in PRSO treatment had higher SOD value and statistically significantly different ($P<0.05$) with fish in URSO treatment.

Table 9. The blood profile of *Clarias gariepinus* fed with different lipid sources

Parameters	Experimental diets				
	CPO:FO*	CPO:CO*	CPO:RSOH*	PRSO*	URSO*
Hemoglobin (Hb) (%)	10.40–11.20	11.80–12.00	14.00–15.00	12.20–12.60	10.10–10.90
Hematocrit (Ht) (%)	29.70–31.60	29.20–33.80	36.70–42.30	33.40–34.00	24.80–27.90
Erythrocytes	1.85–2.24	2.06–2.09	2.65–3.00	2.20–2.23	1.79–1.98
Leukocytes	38.00–55.30	43.60–50.70	34.60–71.90	46.50–52.40	45.20–56.90

*CPO:FO : 50% palm oil + 50 % fish oil; CPO:CO: 50% palm oil + 50% corn oil; CPO:RSOH: 50% palm oil + 50% RSOH; PRSO: 100% RSOH; URSO:100% RSO.

*CPO: Crude palm oil; FO: Fish oil, CO: Corn oil; RSOH: Heated rubber seed oil; RSO: Unheated rubber seed Oil; PRSO: 100% Rubber seed oil with heating; URSO: 100% rubber seed oil without heating.

Table 10. MDA and SOD tests for *Clarias gariepinus* fed with different lipid sources.

Parameters	Experimental diets				
	CPO:FO*	CPO:CO*	CPO:RSOH*	PRSO*	URSO*
MDA (uMol/L)	1.26 ± 0.01 ^a	1.38 ± 0.06 ^{ab}	1.44 ± 0.04 ^{ab}	1.70 ± 0.02 ^b	3.45 ± 0.27 ^c
SOD (U/g prot)	9.41 ± 0.89 ^b	9.94 ± 1.51 ^b	9.10 ± 0.21 ^b	8.86 ± 1.11 ^b	5.56 ± 1.40 ^a

*CPO:FO : 50% palm oil + 50 % fish oil; CPO:CO: 50% palm oil + 50% corn oil; CPO:RSOH: 50% palm oil + 50% RSOH; PRSO: 100% RSOH; URSO:100% RSO.

*CPO: Crude palm oil; FO: Fish oil, CO: Corn oil; RSOH: Heated rubber seed oil; RSO: Unheated rubber seed oil; PRSO: 100% Rubber seed oil with heating; URSO: 100% rubber seed oil without heating. Values are means ± SE ($n = 3$). Values that share different letters in the same column are significantly different (Duncan test; $P<0.05$).

Liver and body glycogen

Liver and body glycogen test results showed that feeding with different feed lipid source affects fish body and liver values (Table 11). Fish in PRSO treatment have a higher body and liver glycogen values compared to fish in URSO treatment.

Discussion

Five types of tested feed were made to observe the effect of different types of raw material sources of feed lipids by using heated rubber seed oil (RSO). Based on the results of this study, it is known that the heating process of RSO using an oven at a temperature of 100°C with a duration of 60 minutes could reduce levels of hydrogen cyanide (HCN) in RSO up to 95% (Table 1). Rubber seeds have by-products of plant metabolites in the form of linamarin. Linamarin can form a stable complex in the form of HCN when it meets with the enzyme linamarase (Salimon *et al.*, 2012). In the study of Kushayadi *et al.* (2019), heating at 100°C for 60 minutes can reduce HCN levels in rubber seed oil by 95%. Although HCN can be evaporated at 26°C, a higher temperature used to shorten the evaporation time (Nebiyu & Getachew, 2011). The heating process did not show any effect in the form of a significant decrease in the value of fatty acids (Table 2).

Added heated RSO in feed (CPO:RSOH and PRSO) did not affect fish SR, SGR, protein retention value, and lipid retention. Tested fish in CPO:RSOH and PRSO treatments did not show SR, SGR, protein retention value, and lipid retention that were significantly different from tested fish in CPO:FO and CPO:CO treatments (Table 6). SGR is influenced by the amount of nutrients that can be retained by the body. Suprayudi *et al.* (2016) and Fawole *et al.* (2016) also showed that the decrease of HCN in feed raw materials was

able to maintain the value of protein retention and lipid retention in tilapia and rohu. The SR, SGR, protein retention value, and lipid retention in URSO treatments were lower and significantly different from other treatments. The high level of HCN in the URSO treatment is thought to be the cause of the nutrients absorption inhibition. The decreases of SR and SGR fish because the nutrients derived from feed were insufficient for body maintenance. If this continues, the fish will utilize stored nutrients as energy to maintain the body so that the amount of stored nutrients become lower. The inhibition of nutrient absorption cause fish require more amount of feed to complete their nutrients need so that the ratio of FCR in the URSO treatment becomes greater. The inhibition of nutrient absorption is caused by inactive zymogens. HCN is able to block the active sites of enzymes, especially peptidase and lipase so that the enzyme activity decreases. Enzymes play a role in cutting complex nutrient bonds into simple bonds that are easily digested by the intestine (NRC, 2011). The same thing happened in the Komariyah *et al.* (2014), showed the value of SR, SGR, protein retention value, and lipid retention of fish decreased with increasing HCN concentrations in feed.

In addition to affecting SR and SGR, the amount of nutrients that are retained also affects the fatty acid profile of the fish's body. This can be seen from the content of saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) in the tested fish body fatty acid profile (Table 7). The SFA and PUFA content of tested fish fed with heated RSO-based feed is relatively not significantly different from tested fish fed with palm oil-based feed. Ng *et al.* (2013) study also showed that tilapia fed with linseed oil and palm oil had PUFA values that were relatively similar to tilapia which were fed with palm oil based and

Table 11. Liver glycogen and body glycogen value for *Clarias gariepinus* fed with different lipid sources.

Parameters	Experimental diets				
	CPO:FO*	CPO:CO*	CPO:RSOH*	PRSO*	URSO*
Liver glycogen (mg/g)	7.98 ± 0.03 ^a	5.05 ± 0.62 ^{ab}	3.49 ± 0.33 ^{ab}	3.13 ± 0.55 ^b	2.59 ± 0.31 ^c
Body glycogen (mg/g)	3.83 ± 0.26 ^a	1.90 ± 0.01 ^b	1.71 ± 0.01 ^b	1.74 ± 0.01 ^b	1.66 ± 0.00 ^b

*CPO:FO : 50% palm oil + 50 % fish oil; CPO:CO: 50% palm oil + 50% corn oil; CPO:RSOH: 50% palm oil + 50% RSOH; PRSO: 100% RSOH; URSO:100% RSO.

*CPO: Crude palm oil; FO: Fish oil, CO: Corn oil; RSOH: Heated rubber seed oil; RSO: Unheated rubber seed oil; PRSO: 100% Rubber seed oil with heating; URSO: 100% rubber seed oil without heating. Values are means ± SE ($n = 3$). Values that share different letters in the same column are significantly different (Duncan test; $P < 0.05$).

showed the same weight gain between treatments. This indicates that RSO-based feeds can provide the same profile of body fatty acids and growth as other vegetable oil-based feeds.

Nutrients absorbed by the body will be circulated in the blood. This can be seen in the difference of blood chemical values (cholesterol and HDL) in each treatment (Table 8). Tested fish in the PRSO and CPO:RSOH treatments had higher ranges of cholesterol and HDL values compared to tested fish in the URSO treatment. The low amount of nutrients absorbed is thought to be the cause of the low range cholesterol and HDL values of tested fish in the URSO treatment. The main function of HDL is to transport blood lipid from body tissue to the liver (Luo *et al.* 2014). According to Stepanowska *et al.* (2006) fish with deficient nutrient conditions tend to experience decreased blood lipid concentrations. The low cholesterol and HDL values are also related to the low protein retention value of the URSO treatment resulting in a low apoprotein value. Apoprotein is an albumin group that is used to circulate lipid in the bloodstream. As an insoluble compound in polar solvents, lipids need apoprotein to emulsify lipids in the form of lipoproteins and circulate in the bloodstream (NRC, 2011). Besides being influenced by the amount of nutrients absorbed, blood chemical levels are also influenced by the feed raw material used. Some previous studies stated that blood cholesterol levels are closely related to cholesterol in the feed consumed. Suprayudi *et al.* (2016) study's also mentioned that fish fed with rubber seed based on low HCN showed an increase of cholesterol levels in blood plasma. The presence of HCN in blood can cause cholesterol and HDL to be low (Aviram *et al.*, 2000; Brites *et al.*, 2017).

HCN that enters the body can cause metabolic stress in fish. The effect of metabolic stress in fish can be seen with secondary responses in fish blood profile (Wahyu *et al.*, 2015). There is no significant differences in erythrocytes, leukocytes, Hb and Ht value found between the CPO:RSOH and PRSO treatments with the CPO:FO and CPO:CO treatments (Table 9). The same thing was found in several studies (Deng *et al.*, 2015; Fawole *et al.*, 2016; Suprayudi *et al.*, 2016) with the results of fish fed with low rubber seed based HCN not showing any difference in erythrocytes and leukocytes values. However, the URSO treatment showed different result, which were

higher leukocytes value and lower erythrocytes value when compared to other treatments (Table 9). Suprayudi *et al.* (2016) suggested that the provision of high seed-based rubber HCN can increase leukocytes and reduce human resources in fish. Stressful conditions in fish can suppress the health conditions of fish and fish are increasingly vulnerable to disease. The leukocytes in fish is the first level of protective response to infection or tissue damage due to stress oxydative (Ezike *et al.*, 2019).

The low proportion of erythrocytes can be caused by the lipid peroxidation process. Lipid peroxidation that occurs in the blood cell membrane will disrupt the permeability of blood cells so the blood cells become lysis. Simultaneously with the lipid peroxidation process a malanodealdehyde (MDA) compound is formed as the final product of the lipid peroxidation process on the cell membrane (Fawole *et al.*, 2016). *Clarias gariepinus* which were given URSO treatment had higher MDA levels and were significantly different from other treatments. In contrast, the tested fish treated with CPO: RSOH and PRSO had MDA levels that did not differ significantly from CPO: FO and CPO: CO (Table 10). MDA is known as one of the markers for oxidative stress (Arinola *et al.*, 2010; Nwani *et al.*, 2010) related to stress caused by an increase in reactive oxygen species (ROS) including HCN (Nwani *et al.*, 2010; Lushchak, 2011; Deng *et al.*, 2015; Fawole *et al.*, 2016). According to David and Kartheek (2015) only with HCN levels of 0.2 mg/L in water can increase MDA in blood vessels.

When oxidative stress occurs, the body will produce antioxidants as a form of protection against free radical damage. Antioxidants are substances that have the task of delaying or preventing cells from being oxidized (Franco & Martinez-Pinilla, 2017). Some of the antioxidant enzymes produced by the body are superoxide dismutase (SOD), glutathione peroxidase, and catalase (Sharma *et al.*, 2014). Tested fish treated with CPO:RSOH and PRSO showed SOD values that were not significantly different from CPO:FO and CPO:CO treatments, while tested fish treated with URSO had lower SOD values and were significantly different from other treatments (Table 10). The low SOD value and high MDA value in fish treated with URSO indicates the fish experienced oxidative stress. According to Yang *et al.* (2015), low levels of SOD and high MDA

are a dangerous conditions for aquatic organisms and can reduce growth rates. SOD is an enzyme that acts as an intracellular antioxidant. SOD will convert free radicals into hydroperoxide (H₂O₂). Low levels of SOD can be caused by increased lipid peroxidation due to the amount of free radicals absorbed by the body. The higher SOD enzyme activity indicates the lower lipid oxidation products that occur (Johansson *et al.*, 2018).

The energy used to overcome oxidative stress and release SOD is the stored energy called glycogen. Tested fish treated with URSO had lower levels of liver glycogen and were significantly different from other treatments (Table 11). This showed that there were a decrease in glycogen value along with an increase in HCN consumption. Also a decrease in liver glycogen values that decreased significantly in rats with high levels of oxidative stress (Abdullah & Salimon, 2010). Decreased glycogen levels are caused by an increase in fish glucose requirements in the HCN detoxification process, so that more glycogenolysis processes occur in the liver and muscle of the fish (Suprayudi *et al.*, 2015).

CONCLUSIONS

Based on this study it can be concluded that heating can reduce HCN levels in rubber seed oil by 95%. Giving 100% heated rubber seed oil (PRSO) has no side effects on growth performance and feed intake in *Clarias gariepinus*. Thus, 100% of heated rubber seed oil can be used as a raw material source of feed lipid substitute for corn oil and palm oil.

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