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Evaluation of dietary coffee *Coffee canephora* husk supplementation on the growth, blood chemicals, and antioxidative activity of red Nile tilapia *Oreochromis* sp.

Evaluasi pemanfaatan tepung kulit kopi *Coffee canephora* dalam pakan terhadap kinerja pertumbuhan, kimia darah dan antioksidan ikan nila merah *Oreochromis* sp.

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ABSTRACT

This study aimed to evaluate the utilization of different dietary coffee husk supplementation dosages on the growth, blood chemicals, and antioxidative activity of red Nile tilapia (*Oreochromis* sp.). Red Nile tilapia (initial weight 10.59 ± 0.29 g) were raised in 15 aquaria at a density of 15 fish per aquarium and treated in five different diets: P0 (control), P1 (1% coffee husk/kg), P2 (2% coffee husk/kg), P3 (3% coffee husk/kg), and P4 (4% coffee husk/kg) for eight weeks. The results determined that coffee husk powder inclusion improved final weight, daily growth rate, feed efficiency, protein retention, and lipid retention compared to the control. Dietary supplementation of coffee husk significantly decreased total cholesterol and triglycerides in serum (P<0.05) followed by a decrease in LDL and significantly increased HDL compared to the control. Dietary supplementation of coffee husk significantly increased superoxide dismutase (SOD) activity compared to the control (P<0.05). Furthermore, coffee husk supplementation was also significantly able to reduce the malondialdehyde (MDA) compared to the control (P<0.05). All coffee husk supplementation treatments produced a lower hepatosomatic index than the control. Furthermore, coffee husk supplementation significantly increased liver glycogen compared to the control (P<0.05). All dietary coffee husk supplementation treatments showed better results than the control. The application of a dose of 4% coffee husk in the diet provides better results than other treatments. Then, this study concluded that the dietary of 4% coffee husk supplementation could be applied in feed.

Keywords: antioxidant, coffee husk, diet, growth, tilapia

ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi pemanfaatan tepung kulit dengan dosis berbeda terhadap kinerja pertumbuhan, kimia darah dan antioksidan ikan nila merah (*Oreochromis* sp.). Ikan nila merah (bobot awal 10,59 ± 0,29 g) disebar di dalam 15 akuarium dengan padat tebar ikan sebanyak 15 ekor per akuarium dan diberi pakan perlakuan yaitu, P0 (kontrol), P1 (1% kulit kopi/kg), P2 (2% kulit kopi/kg), P3 (3% kulit kopi/kg) dan P4 (4% kulit kopi/kg) yang dipelihara selama delapan minggu. Hasil penelitian menunjukkan bahwa, suplementasi tepung kulit kopi meningkatkan bobot akhir, laju pertumbuhan harian, efisiensi pakan, retensi protein dan retensi lemak dibandingkan kontrol. Suplementasi tepung kulit kopi secara signifikan menurunkan total kolesterol dan trigliserida darah (P<0,05) diikuti juga terjadinya penurunan nilai LDL dan secara signifikan mampu meningkatkan HDL dibandingkan kontrol. Suplementasi tepung kulit kopi secara signifikan meningkatkan aktivitas superoxide dismutase (SOD) dibandingkan dengan kontrol (P<0,05). Selanjutnya, suplementasi tepung kulit kopi juga secara signifikan mampu menurunkan nilai malondialdehyde (MDA) dibandingkan kontrol (P<0,05). Semua perlakuan suplementasi tepung kulit kopi menghasilkan nilai hepatosomatic indeks yang lebih rendah dibandingkan kontrol. Selanjutnya, suplementasi tepung kulit kopi secara signifikan meningkatkan glikogen hati dibandingkan kontrol (P<0,05). Semua perlakuan suplementasi tepung kulit kopi menunjukkan hasil yang lebih baik dibandingkan kontrol. Penerapan dosis 4% kulit kopi dalam pakan memberikan hasil yang lebih baik dibandingkan perlakuan lainnya. Sehingga penelitian ini menyimpulkan bahwa, penggunaan tepung kulit kopi sebesar 4% dapat diaplikasikan di dalam pakan.

Kata kunci: antioksidan, kulit kopi, pakan, pertumbuhan, ikan nila

INTRODUCTION

Nile tilapia is one of the most economic freshwater cultured fish commodities. The Indonesian Ministry of Marine and Fisheries Affairs (2020) mentioned that the production of tilapia in Indonesia has been increasing annually, as the tilapia's world production has been predicted at 4,525,400 tons (FAO, 2020). Increased tilapia demand triggers massive intensification and expansion in culture activities. An intensive culture system applied by culturists is proposed for optimal production and fulfilling high market demand. Intensive culture application emerges risks against water quality decrease due to organic waste accumulation.

High culture waste concentration can cause oxidative stress in tilapia, marked by increased reactive oxygen species (ROS) or endogenous free radical exposure (Ardiansyah & Indrayani, 2007). The free radical and antioxidant imbalance in the body will cause oxidative stress, which will damage the cell component and disrupt the cell metabolic activities. Oxidative stress that causes cell damage due to free radical reactions can be prevented by applying antioxidant supplementation. Intracellular antioxidants or endogenous antioxidants in the body are composed of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). SOD is one of the endogenous antioxidants to catalyze superoxide anion (O_2^-) dismutation to hydrogen peroxide and oxygen molecules (Miller, 2012). An alternative material that contains antioxidative compounds is a coffee husk.

Coffee husk is a coffee bean by-product from 30-40% of coffee production that remains unutilized optimally. For these reasons, several studies have been conducted on the potential of coffee husks. Coffee husk has been used to produce biofuels and ethanol, as an absorbent of contaminants in the water and as a source of dietary fiber (Hoseini *et al*., 2021; Gouvea *et al*., 2009; Paredes-Laverde *et al*., 2018). The coffee husk contains important nutrient sources, such as protein, carbohydrates, lipids, vitamins, and minerals (Bondesson, 2015; Iriondo-Dehond *et al*., 2020; Esquivel & Jiménez, 2012). The coffee husk also contains secondary metabolites as bioactive compounds, namely phenolic compound (chlorogenic acid) and alkaloid group (caffeine), which affect the antioxidant activity and free radical antidote (Rahimnejad *et al*., 2015).

This condition followed the results of Xu *et al*. (2022), who reported that chlorogenic acid could improve the antioxidative activities, namely SOD, CAT, GPx, and GSH, reducing MDA (malondialdehyde) in koi carp fish. Increasing GPx enzyme activity in olive flounder *Paralichthys olivaceus* (Rahimnejad *et al*., 2015). Coffee husk affects reducing total cholesterol, triglycerides, and low-density lipoprotein (LDL), previous studies reported that coffee peel was able to reduce total cholesterol, LDL, and triglycerides in tilapia fillets by feeding the fermented coffee peel supplemented diet (Fitria *et al*., 2020). No other information is related to the mechanism of cholesterol reduction, it is predicted the role of secondary metabolite compounds in the coffee peel are chlorogenic acid, polyphenols, ferulic acid, and caffeic acid (Fitria *et al*., 2020). Chlorogenic acid has an effect in inhibiting the work of β-hydroxy-β-methyl glutaric acyl-coenzyme A reductase (HMG CoA reductase enzyme) that cholesterol synthesis is inhibited (Meng *et al*., 2013).

In addition, a similar study reported by Fatimatuzzahro & Prasetya (2018) that the role of chlorogenic acid in the spent coffee ground could prevent the oxidation of free fatty acid oxidation and decrease triglyceride value. From the explanation above, dietary coffee husk supplementation affects antioxidant activity and cholesterol absorption. On the other hand, there has been no information available that explains the effect of coffee husk on antioxidants and cholesterol absorption in red Nile tilapia. Therefore, this study was conducted to evaluate the dietary coffee husk supplementation with different doses on the growth, blood chemicals, and antioxidative activity in red Nile tilapia.

MATERIALS AND METHODS

Preparation of coffee husk

Coffee husks were used as a type of robusta obtained from Muaradua City, Ogan Komering Ulu Selatan Regency, South Sumatra Province. Coffee husks were dried in the sun for six hours. After drying starch using a disk mill. The content of coffee husk phytochemical compounds consists of caffeine 29.36%, 9.12-*Octadecadienoic* acid 8.14%, *Chlorogenic* acid 9.19%, *Hexadecanoic acid* 12.51%, and Vitamin E 1.70%. Phytochemical analysis of coffee husk was obtained using the GC-MS (Gas Chromatography-Mass Spectrometry) instrument

in Health Laboratory DKI Jakarta Province.

Experimental diets

Five experimental diets were applied in this study, namely: P0 (0% coffee husk flour/kg diet), P1 (1% coffee husk flour/kg diet), P2 (2% coffee husk flour/kg diet), P3 (3% coffee husk flour/kg diet), P4 (4% coffee husk flour/kg diet) were formulated to contain 28% crude protein (Table 1). Proximate analysis of experimental diet following the Association of Official Analytical Chemists (AOAC, 2012).

Experimental design

Red Nile tilapia were obtained from Integrated-fish farming, academic business unit, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University. Nile Tilapia were reared in a fiberglass tank for two weeks for environmental adaptation. Furthermore, 200 fish (10.59 ± 0.29 g) were distributed in 15 aquaria (60×50×40 cm^3) at a stocking density of 15 fish per aquarium, composed of five treatments and three replications, based on the completely randomized design experimental method. During the experiment, fish were until apparent satiation three times a day at 08.00, 13.00, and 17.00 WIB (GMT+7). Water quality parameters remained at a temperature of 30.7-31.4 °C, pH of 6.8-7.6, Dissolved Oxygen of 5.1-5.9 mg/L, and Total Ammonia Nitrogen (TAN) 0.25-0.40 mg/L.

Growth performance

After maintenance for eight weeks, body weight was calculated before the final sampling. The parameters measured are weight gain (WG),

Table 1. Experimental diet formulation with different dosages.

Ingredients (%)	Treatments				
	P0	P1	P2	P3	P4
Fish meal	5	5	5	5	5
Meat bone meal	5	5	5	5	5
Soybean meal	20	20	20	20	20
Poultry by-product meal	6	6	6	6	6
Rice bran	23	23	22.5	22	21.5
Bran pollard	24.7	23.7	23.2	22.7	22.2
Wheat flour	10	10	10	10	10
Fish oil	1	1	1	1	1
Crude palm oil	1	1	1	1	1
Vitamin mix	1	1	1	1	1
Mineral mix	1	1	1	1	1
Choline chloride	0.5	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.5	0.5	0.5	0.5	0.5
L- Lysine	0.5	0.5	0.5	0.5	0.5
L- Methionine	0.5	0.5	0.5	0.5	0.5
Polymethylolcarbamide	0.3	0.3	0.3	0.3	0.3
Coffee husk powder	0	1	2	3	4
Diet proximate contents (%)					
Protein	28.68	28.48	28.33	28.20	28.14
Lipid	6.70	6.42	6.22	6.51	6.40
Moisture	10.63	10.80	11.01	10.89	10.59
Ash	8.32	8.39	8.85	8.13	8.41
Crude fiber	5.86	6.03	6.27	6.42	6.58
NFE[1]	39.81	39.88	39.32	39.85	39.88
GE[2] (kcal/kg diet)	4709.50	4669.33	4665.37	4663.94	4648.18

Note: [1] NFE = nitrogen free extract; [2] GE = Gross energy = (%protein × 5.6 kcal) + (%NFE × 4.1 kcal) + (%lipid × 9.4 kcal) (Watanabe, 1988).

specific growth rate (SGR), feed efficiency (FE), protein retention (PR), lipid retention (LR), and survival rate (SR).

$$SGR = \left(\sqrt[t]{\frac{Wt}{Wo}} - 1\right) \times 100$$

Note:
SGR = specific growth rate (%/day)
Wt = average fish weight at the final maintenance period (g)
Wo = average fish weight at the initial maintenance period (g)

Feed efficiency is calculated based on NRC (1993), namely:

$$FE = \frac{(Wt + Wd) - Wo}{F} \times 100$$

Note:
FE = feed efficiency (%)
Wt = total fish biomass on the final maintenance period (g)
Wd = total dead fish weight (g)
Wo = total fish biomass on the initial maintenance period (g)

Blood chemical analysis

Blood chemicals were evaluated based on triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and total blood cholesterol (TBC). Blood chemicals were performed at the final rearing. Fish were anesthetized with clover oil at 0.1 ml/4 L water (Rifai *et al*., 2022). Blood was taken with a syringe, after rinsing with an anticoagulant. Microtube was filled with a blood sample, before being centrifuged for 15 minutes at 3000 rpm. Furthermore, blood plasma was taken with a micropipette and moved new microtube. Measurement was performed through enzymatic colorimetric test for cholesterol with lipid clearing factor cholesterol kit liquid color, Human Merck.

Liver performance and antioxidative activity

Liver performance was evaluated based on liver glycogen. The liver glycogen levels were measured following Watanabe (1988) method. The hepatosomatic index was measured based on divided between liver weight and body weight. Antioxidative status was analyzed following the MDA and SOD levels from two fish samples in each aquarium. The MDA level analysis was performed according to Ulhusna *et al*. (2019) method. The 0.5 g liver was minced in a cold condition.

The minced liver was homogenized with 5 ml phosphate-buffered saline (PBS). The homogenate was included in a reaction tube and centrifuged at 4 °C and 3.500 rpm for 10 minutes. For MDA measurement, 1 ml supernatant was added with cold HCl, containing 15% TCA (trichloroacetic), 0.38% TBA (thiobarbituric acid), and 0.5% BHT (butylated hydroxytoluene). The mixture was heated at 800 °C for an hour and centrifuged at 40 °C and 3.500 rpm for 10 minutes. Absorbance was measured with a spectrophotometer at λ 532 nm. A standard solution used was TEP (1,1,3,3-tetraethoxypropane). The SOD analysis was measured with a SOD Test kit Sigma Aldrich and spectrophotometer at 553 μm wavelength.

Data analysis

Data analysis was performed using One-way analysis of variance (ANOVA) with Microsoft Excel 2013 and SPSS v.22 at a 95% confidence level. Treatments with a significant difference were further analyzed with DMRT (Duncan's Multiple Range Test).

RESULTS AND DISCUSSIONS

Results

Growth performance

The effect of coffee husk powder supplementation on growth performance is presented in Table 2. Based on one-way ANOVA analysis, the highest final weight and specific growth rate values were found in the P4 treatment, which was significantly different from the control treatment ($P<0.05$). Nevertheless, no significant difference ($P>0.05$) between P1, P2, P3, and the control. No significant total feed intake in all treatments ($P>0.05$). Feed efficiency in Nile tilapia fed with coffee husk-supplemented diets was not significant in P1, P2, and P3 compared to control except P4 has a higher value and is significantly different compared to control. Moreover, protein and lipid retention were not significant in P1, P2, and P3 compared to the control except P4 has a higher value and is significantly different compared to the control diet. The survival rate of all diet treatments had no significant difference ($P>0.05$).

Blood chemicals

Table 3 presents that the dietary supplementation of coffee husk obtains a lower

cholesterol level and is significantly different from the control treatment (P<0.05). A lower triglyceride value is also presented in coffee-husk dietary supplementation and is significantly different from the control treatment. The HDL in all coffee husk powder treatments obtains a higher value than the control treatment. Meanwhile, the LDL is also lower in all coffee husk powder treatments than in the control treatment. Based on all blood biochemical parameters, the P4 obtained the best value among other treatments.

Liver performance and antioxidative activity

Table 4 indicates that coffee husk dietary supplementation can significantly lower the HSI level, compared to the control (P<0.05). The liver glycogen also has a significantly decreased value, compared to the control. The highest glycogen value was found in the P4 treatment. The one-way ANOVA analysis indicates that the coffee husk dietary supplementation can significantly increase the SOD value, compared to the control (P<0.05). The highest SOD value is found in the P4 treatment, followed by the P3, P2, and P1 treatments. Meanwhile, the MDA value of coffee husk dietary supplementation presents a significantly different value, compared to the control (P<0.05). The lowest MDA value is found

Table 2. Growth performance of red Nile tilapia after feeding treatments.

Parameters	Treatments				
	P0	P1	P2	P3	P4
Wo (g)	10.87 ± 0.14^{a}	10.56 ± 0.27^{a}	10.57 ± 0.28^{a}	10.37 ± 0.16^{a}	10.61 ± 0.35^{a}
Wt (g)	33.55 ± 0.92^{a}	33.67 ± 4.46^{a}	34.91 ± 1.48^{a}	35.53 ± 0.18^{a}	46.95 ± 1.22^{b}
FI (g)	595.53 ± 20.81^{a}	608.54 ± 57.29^{a}	648.25 ± 68.33^{a}	656.13 ± 40.52^{a}	704.55 ± 40.04^{a}
SGR (%/day)	1.88 ± 0.02^{a}	1.92 ± 0.25^{a}	1.99 ± 0.03^{a}	2.05 ± 0.03^{a}	2.48 ± 0.03^{b}
FE (%)	46.25 ± 5.05^{a}	50.02 ± 11.39^{ab}	55.49 ± 5.59^{ab}	59.18 ± 4.21^{ab}	65.37 ± 2.49^{b}
LR (%)	44.72 ± 2.93^{a}	51.14 ± 9.15^{ab}	50.35 ± 3.91^{ab}	53.07 ± 9.07^{ab}	63.72 ± 3.03^{b}
PR (%)	32.21 ± 3.72^{a}	35.07 ± 10.08^{a}	40.52 ± 7.48^{ab}	42.11 ± 2.75^{ab}	51.80 ± 7.74^{b}
SR (%)	82.22 ± 3.85^{a}	86.67 ± 11.55^{a}	91.11 ± 7.70^{a}	88.89 ± 3.85^{a}	86.67 ± 11.55^{a}

Note: the mean ± standard deviation (n=3) followed by different superscript letters on the same line shows a significant difference (P<0.05). Wo = Initial weight; Wt = Final weight; FI = Feed intake; SGR = Specific growth rate; FE = Feed efficiency; LR = Lipid retention; PR = Protein retention; SR = Survival rate.

Table 3. Cholesterol, triglycerides, HDL, and LDL of red Nile tilapia after feeding treatments.

Blood chemicals (mg/dL)	Treatments				
	P0	P1	P2	P3	P4
Cholesterol	121.08 ± 0.89^{a}	94.87 ± 1.24^{b}	89.82 ± 1.48^{c}	80.26 ± 0.51^{d}	80.09 ± 0.59^{d}
Triglycerides	106.83 ± 0.93^{a}	99.70 ± 0.62^{b}	98.56 ± 0.47^{b}	89.22 ± 1.24^{c}	66.22 ± 1.29^{d}
HDL	24.61 ± 0.40^{a}	28.38 ± 0.48^{b}	41.62 ± 0.74^{c}	41.74 ± 0.91^{c}	42.97 ± 0.77^{c}
LDL	49.38 ± 0.68^{a}	47.55 ± 0.74^{a}	43.33 ± 1.97^{b}	43.03 ± 2.35^{b}	38.21 ± 1.24^{c}

Note: the mean ± standard deviation (n=3) followed by different superscript letters on the same line shows a significant difference (P<0.05). HDL = High-density lipoprotein; LDL = Low-density lipoprotein.

Table 4. Liver performance and Antioxidative activity of red Nile tilapia after feeding treatments.

Parameters	Treatments				
	P0	P1	P2	P3	P4
HSI (%)	1.95 ± 0.10^{a}	0.92 ± 0.26^{b}	0.93 ± 0.13^{b}	0.81 ± 0.02^{b}	0.79 ± 0.02^{b}
LG (mg/g)	0.62 ± 0.01^{a}	0.78 ± 0.05^{b}	0.80 ± 0.04^{bc}	0.88 ± 0.06^{bc}	0.89 ± 0.04^{c}
SOD (%)	45.13 ± 2.04^{a}	58.41 ± 1.54^{b}	62.63 ± 1.45^{c}	64.82 ± 1.70^{cd}	66.52 ± 0.79^{d}
MDA (mMol/g)	3.44 ± 0.04^{a}	1.72 ± 0.32^{b}	1.55 ± 0.27^{bc}	1.36 ± 0.16^{bc}	1.11 ± 0.19^{c}

Note: the mean ± standard deviation (n=3) followed by different superscript letters on the same line shows a significant difference (P<0.05). HSI = Hepatosomatic index; LG = Liver glycogen; SOD = Superoxide dismutase; MDA = Malondialdehyde.

in the P4 treatment, followed by P3, P2, and P1 treatments, then the highest value is obtained from P0 treatment (control).

Discussions

Red Nile tilapia rearing for eight weeks fed with coffee husk-supplemented diets increased the final body weight, specific growth rate, feed efficiency, protein retention, and lipid retention. The dietary coffee husk inclusion with 4% doses showed higher significant values ($P<0.05$) on all growth parameters compared to the control diet but had no significant difference ($P>0.05$) on treatment P1, P2, and P3. Although there is less information regarding the effect of coffee husk on growth, several bioactive compounds found in coffee husk are predicted to have antioxidative properties. A higher specific growth rate in coffee husk-supplemented diet treatments was thought due to low oxidative stress, which could proportionally expenditure lower energy. This condition was shown by SOD value in the P1, P2, P3, and P4 treatments followed by decreased MDA value ($P<0.05$). Chlorogenic acid and caffeine are antioxidative compounds in the coffee husk that are considered to have a strong effect on SOD increase and MDA decrease.

Increased antioxidative enzyme activities cause a lower energy expenditure to control oxidative stress (Hoseini *et al*., 2021). This condition was indicated by higher protein and lipid retention in coffee husk-supplemented diets than in the control diet, as antioxidative properties could actively promote growth by optimizing nutrient absorption in the body. Rahimnejad *et al*. (2015) reported that a 5% spent coffee ground by-product could improve growth performance and catalase level in olive flounder *Paralichthys olivaceus*. The use of coffee silverskin as a feed additive previously reported that the best growth performance in Nile tilapia by supplementing coffee silverskin at 20-40 g/kg diet, but showing a lower growth at high dose, i.e., 80 g/kg diet (Doan *et al*., 2021; Doan *et al*., 2022). In addition, dietary supplementation of 5% spent coffee ground could improve the growth performance of olive flounder *Paralichthys olivaceus* (Rahimnejad *et al*., 2015).

Coffee husk is one of the carbohydrate and fiber sources (Blinová *et al*., 2017) with a prebiotic potential that can be absorbed by the host but shows a good effect on microbiota growth in the intestine, such as *Lactobacilli* and *Bifidobacteria*, by effectively utilizing feed as the substrate and inducing digestive enzyme secretion, thus increasing gastrointestinal digestion process (Campos *et al*., 2012; Fiesel *et al*., 2014; Bhandarkar *et al*., 2021). This intestinal microflora will produce SCFAs (short-chain fatty acids), namely butyric and propionic acids. Propionic acid has roles in activating important SCFAs receptor (Gpr41 or Gpr43) to induce YY-peptides (PYY) and glucagon-like-peptide-I (GLP-1) that regulates digestive function, while butyric acid has roles in promoting proliferation, differentiation, and intestinal epithelium cell permeability (Chambers *et al*., 2015; Krumbeck *et al*., 2018; Perdijk *et al*., 2019). SCFAs produced during in vivo fermentation works by acidizing the intestinal lumen and triggering the intestinal wall widening to improve mineral absorption in the body. Intestinal microflora can selectively metabolize oligosaccharides to produce nutrients, such as protein, vitamins, and mineral ions that will be absorbed by the body (Whisner & Castillo, 2018).

SCFAs produced by intestinal microflora are related to AMPK (AMP-protein kinase) enzyme. AMPK is a regulatory enzyme for cellular energy homeostatic metabolism (Zhao *et al*., 2017). SCFAs are considerably capable of activating AMPK through GPCR (G-protein coupled receptors) signaling, i.e., Gpr41 and Gpr43, although their mechanism remains unclear. However, this activity is allegedly due to SCFAs role in regulating the body's immune system by binding the Gpr43 receptor, which dominantly resides in the body's immune system (Doan *et al*., 2021; Doan *et al*., 2022). Increased cellular immune system implicates the increased antioxidative activity in all coffee husk-supplemented diet treatments. Increased SOD enzyme indicates an exogenous antioxidant supply that can balance the endogenous antioxidant requirement in neutralizing free radicals, thus energy metabolism requirement through AMPK is lower to neutralize free radicals with a more nutrient retention process.

Available carbohydrates and lipids optimize the utilization of non-protein energy sources, as producing energy from these sources will improve the protein-sparring effect capacity by maximizing protein roles for biological structure and activity improvements (Rifai *et al*., 2022), reducing protein catabolism as energy, elevating protein retention, and promoting fish growth (Doan *et al*., 2021). HDL is often called good cholesterol due to carrying cholesterols or removing bad cholesterol away from the arterial bloodstream to

the liver that will be metabolized as bile liquid, decreasing the blood cholesterol level. LDL is categorized as bad cholesterol due to the free fatty acid bulk existence in the blood. All coffee husk-supplemented diet treatments obtained an increased HDL value and were significantly different from the control diet treatment ($P<0.05$). Phenolic compounds in coffee husk trigger the activation of lecithin cholesterol acyltransferase (LCAT) enzyme activity involved in HDL cholesterol metabolism, mainly in cholesterol transport from tissue to liver.

LCAT also has an important role in excess unesterified cholesterol release from tissue lipoprotein to the liver (Meisyahputri & Ardiaria, 2017). This antioxidant compound can also influence the HDL increase by elevating the mRNA apolipoprotein A (*Apo A*) in the liver through the initiation process with the help of LCAT as a cofactor in the cholesterol esterification process in the bloodstream, which returns to the liver for bile acid excretion. Increased Apo A synthesis is followed by increased HDL level, thus providing no chance for LDL oxidation as the cause of oxidative stress in tissue (Meisyahputri & Ardiaria, 2017). Meanwhile decreased cholesterol, triglycerides, and LDL was thought due to chlorogenic acid and caffeine compounds that could suppress fatty acid synthesis and activate fatty acid oxidation. Chlorogenic acid and caffeine also improve the capacity of carnitine-acyltransferase (CAT) and acyl-CoA oxidase (ACO) which accelerates the lipolysis process (Zheng *et al*., 2014). Carnitine acyltransferase (CAT) and acyl-CoA oxidase (ACO) as a part of enzymes that has roles in elevating fatty acid β-oxidation and accelerating the lipolysis process (Zhao *et al*., 2017).

Farias-Pereira *et al.* (2019) described those bioactive compounds in coffee had roles in regulating fatty acid metabolism, reducing lipogenesis, inducing the lipolysis process, and increasing fatty acid β-oxidation. The effect of coffee husk dietary supplementation on cholesterol reduction has been reported by Fitria *et al*. (2020) in tilapia fillets. Moreover, available linoleic acid (omega-6) in coffee husk has roles in reducing cholesterol and triglyceride levels, thus preventing and removing blockage from a blood vessel, besides inhibiting monocyte adhesion, platelet aggression, and preventing blood vessel constriction (Froyen & Burns-Whitmore, 2020). The liver is the main organ for the body metabolism process. The results showed a decreased HSI value in all coffee husk-supplemented diets ($P<0.05$).

Decreased HSI value means that there is an inhibition activity of lipid accumulation in the liver. This result was positively correlated with the MDA value, which obtained a decreased value significantly. MDA is the final product of lipid peroxidation and free radical existence indicator in the body (Suarsana *et al*., 2013). MDA can be measured as a parameter to determine liver cell damage level due to oxidative stress. A high MDA level indicates the existence of an increased lipid peroxidation process in the liver, and vice versa. In this experiment, SOD activity showed the highest value in all coffee husk-supplemented diet treatments, which indicates the chlorogenic acid role in inhibiting lipid peroxidation by capturing free radicals, before invading lipid molecules.

This process will break free radical chained reactions and inhibit oxidative stress (Costa *et al*., 2018). Xu *et al*. (2022) reported that the supplementation of chlorogenic acid could improve the antioxidative activities of SOD, CAT, GPx, GSH, and reduce MDA levels in koi carp fish. The chlorogenic acid could activate the Nrf2 signaling pathway to reduce ROS levels and play Nrf2 antioxidant as an important transcription factor that regulated cellular oxidative response and sustained intracellular homeostasis. Nrf2 (nuclear factor-erythroid-2 related factor 2) is a protein that is found in cell biochemical pathways and acts as a master regulator of all antioxidative responses in the body. Nrf2 activated inductively could trigger antioxidative activation such as SOD, CAT, and GPx (Huang *et al*., 2015; Bischoff *et al*., 2019; Tu *et al*., 2019; Yang *et al*., 2019). Meanwhile, low SOD and high MDA values in the control diet treatment were thought to be associated with increased blood cholesterol and LDL level, which caused free fatty acid oxidation and triggered atherogenic activation (narrowing, thickening, and hardening of blood vessel walls), thereby expanding the intracellular oxidation, which implicated on liver cell damage due to fat accumulation.

CONCLUSION

The dietary supplementation of 4% coffee husk could be applied in the red Nile tilapia diet to improve growth and antioxidative activity.

REFERENCES

AOAC. 2012. Official methods of analysis of AOAC Intl. Association of Official Analytical

Chemists: Maryland (US).

Ardiansyah, Indrayani. 2007. Natural antioxidants dietary and lipid oxidation analysis in zebrafish (*Brachydanio rerio*) tissue. Hayati Journal of Biosciences 14: 87–92.

Bhandarkar NS, Mouatt P, Majzoub ME, Thomas T, Brown L, Panchal SK. 2021. Coffee pulp, a by-product of coffee production, modulates gut microbiota and improves metabolic syndrome in high-carbohydrate, high-fat diet-fed rats. Pathogens 10.

Bischoff LJM, Kuijper IA, Schimming JP, Wolters L, Braak B ter, Langenberg JP, Noort D, Beltman JB, van de Water B. 2019. A systematic analysis of Nrf2 pathway activation dynamics during repeated xenobiotic exposure. Archives Toxicology 93: 435–451.

Blinová L, Sirotiak M, Bartošová A, Soldán M. 2017. Review: Utilization of waste from coffee production. Research Papers, Faculty Materials Science Technology, Slovak University Technology 25: 91–101.

Bondesson E. 2015. A nutritional analysis on tea by- product coffee husk and its potential utilization in food production. Faculty of Natural Resources and Agricultural Sciences Department of Food Science 415: 1–25.

Campos D, Pallardel IB, Chirinos R, Galvez AA, Norrato G, Padreschi R. 2012. Prebiotic effect of yacon (*Smallanthus sonchifolius* Poepp. & Endl), a source of fructooligosaccharides and phenolic compounds with antioxidant activity. Food Chemistry 135: 1592–1599.

Chambers ES, Viardot A, Psichas A, Morrison DJ, Murphy KG, Zac-Varghese SEK, MacDougall K, Preston T, Tedford C, Finlayson GS, Blundell JE, Bell JD, Thomas EL, Mt-Isa S, Ashby D, Gibson GR, Kolida S, Dhillo WS, Bloom SR, Morley W, Clegg S, Frost G. 2015. Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. Gut Microbiota 64: 1744–1754.

Costa ASG, Alves RC, Vinha AF, Costa E, Costa CSG, Nunes MA, Almeida AA, Santos-Silva A, Oliveira MBPP. 2018. Nutritional, chemical and antioxidant/pro-oxidant profiles of silverhusk, a coffee roasting by-product. Food Chemistry 267: 28–35.

Doan HV, Lumsangkul C, Hoseinifar SH, Harikrishnan R, Balasundaram C, Jaturasitha S. 2021. Effects of coffee silverskin on growth performance, immune response, and disease resistance of Nile tilapia culture under biofloc system. Aquaculture 543.

Doan HV, Lumsangkul C, Hoseinifar SH, Jaturasitha S, Tran HQ, Chanbang Y, Ringø E, Stejskal V. 2022. Influences of spent coffee grounds on skin mucosal and serum immunities, disease resistance, and growth rate of Nile tilapia (*Oreochromis niloticus*) reared under biofloc system. Fish and Shellfish Immunology 120: 67–74.

Esquivel P, Jiménez VM. 2012. Functional properties of coffee and coffee by-products. Food Research International 46: 488–495.

Farias-Pereira R, Park CS, Park Y. 2019. Mechanisms of action of coffee bioactive components on lipid metabolism. Food Science Biotechnology 28: 1287–1296.

Fatimatuzzahro N, Prasetya RC. 2018. Effects of robusta coffee brewing on blood lipid profile and body weight of high-fat diet-induced rats. Brawijaya Journal Medical 30: 7.

Fiesel A, Gessner DK, Most E, Eder K. 2014. Effects of dietary polyphenol-rich plant products from grape or hop on pro-inflammatory gene expression in the intestine, nutrient digestibility and faecal microbiota of weaned pigs. BMC Veterinary Research 10: 1–11.

Fitria PD, Amin M, Lokapirnasari WP, Lamid M. 2020. Supplementation of fermented coffee-husk flour to increase high-density lipoprotein (HDL) cholesterol, docosahexaenoic acids (DHA) and eicosapentaenoic acids (EPA) deposition in tilapia fillet. Biocatalysis Agricultural Biotechnology 24.

[FAO] Food and Agriculture Organization. 2020. State of world fisheries and aquaculture. Sustainability in action. Rome.

Froyen E, Burns-Whitmore B. 2020. The effects of linoleic acid consumption on lipid risk markers for cardiovascular disease in healthy individuals: A review of human intervention trials. Nutrients 12: 1–19.

Gouvea BM, Torres C, Franca AS, Oliveira LS, Oliveira ES. 2009. Feasibility of ethanol production from coffee husks. Biotechnology Letters 31: 1315–1319.

Hoseini M, Cocco S, Casucci C, Cardelli V, Corti G. 2021. Coffee by-products derived resources. Biomass and Bioenergy 148.

Hoseini SM, Hoseinifar SH, Van Doan H. 2021. Growth performance and hematological and antioxidant characteristics of rainbow trout, *Oncorhynchus mykiss*, fed diets supplemented

with Roselle, *Hibiscus sabdariffa*. Aquaculture 530: 735827.

Huang Y, Li W, Su Z yuan, Kong ANT. 2015. The complexity of the Nrf2 pathway: Beyond the antioxidant response. Journal Nutritional Biochemistry 26:1401-1413

Indonesian Ministry of Marine and Fisheries Affairs. 2020. Rencana Strategis 2020-2024 DJPB. Jakarta

Iriondo-Dehond A, Iriondo-Dehond M, Del Castillo MD. 2020. Applications of compounds from coffee processing by-products. Biomolecules 10: 1–20.

Krumbeck JA, Rasmussen HE, Hutkins RW, Clarke J, Shawron K, Keshavarzian A, Walter J. 2018. Probiotic *Bifidobacterium* strains and galactooligosaccharides improve intestinal barrier function in obese adults but show no synergism when used together as synbiotics. Microbiome 6: 1–16.

Meisyahputri B, Ardiaria M. 2017. Effect of combination of hemp oil with sesame oil on high density lipoprotein (HDL) cholesterol levels in sprague dawley dyslipidemia mice. Journal Nutrition College 6: 35.

Meng S, Cao J, Feng Q, Peng J, Hu Y. 2013. Roles of chlorogenic acid on regulating glucose and lipids metabolism: A review. Evidence-based Complementary Alternative Medicine 2013: 1-11.

Miller AF. 2012. Superoxide dismutases: Ancient enzymes and new insights. FEBS Letters 586: 585–595.

NRC. 1993. Nutrient requirement of warm water fishes and shelfish. Nutritional Academy of Sciences, Washington D.C. 102 p.

Paredes-Laverde M, Silva-Agredo J, Torres-Palma RA. 2018. Removal of norfloxacin in deionized, municipal water and urine using rice (*Oryza sativa*) and coffee (*Coffea arabica*) husk wastes as natural adsorbents. Journal Environmental Management 213: 98–108.

Perdijk O, Baarlen P Van, Fernandez-Gutierrez MM, Brink E Vanden, Schuren FHJ, Brugman S, Savelkoul HFJ, Kleerebezem M, Neerven RJJ Van. 2019. Sialyllactose and galactooligosaccharides promote epithelial barrier functioning and distinctly modulate microbiota composition and short chain fatty acid production *in vitro*. Frontiers Immunology 10: 1–14.

Rahimnejad S, Choi J, Lee SM. 2015. Evaluation of coffee ground as a feedstuff in practical diets for olive flounder (*Paralichthys olivaceus*). Fisheris Aquatic Sciences 18: 257–264.

Rifai R, Jusadi D, Suprayudi MA, Alimuddin, Nuryati S. 2022. Evaluation of dietary α-lipoic acid supplementation on the growth performance and physiological status of striped catfish *Pangasianodon hypophthalmus*. Indonesian Journal Aquaculture 21: 198–206.

Suarsana IN, Wresdiyati T, Suprayogi A. 2013. Oxidative stress response and isoflavone administration on superoxide dismutase enzyme activity and lipid peroxidation in rat liver. *Jitv*. 18: 146–152.

Tu W, Wang H, Li S, Liu Q, Sha H. 2019. The anti-inflammatory and anti-oxidant mechanisms of the keap1/Nrf2/ARE signaling pathway in chronic diseases. Aging Disease 10: 637–651.

Ulhusna FA, Winarto A, Wresdiyati T. 2019. The profile of superoxida dismutase and malondialdeyde level in the liver tissue of hypercholesterolemic rats treated with holothuria nobilis polysaccharide. Journal Veterinary Sciences 13: 37–44.

Watanabe T. 1988. Fish nutrition and mariculture. The General Aquaculture Course, Department of Agriculture Bioscience, Tokyo University of Fisheries, Japan, 233 p.

Whisner CM, Castillo LF. 2018. Prebiotics, bone and mineral metabolism. Calcified Tissue International 102: 443–479.

Xu G, Xing W, Yu H, Jiang N, Ma Z, Luo L, Li T. 2022. Evaluation of chlorogenic acid supplementation in koi (*cyprinus carpio*) diet: growth performance, body color, antioxidant activity, serum biochemical parameters, and immune response. Aquaculture Nutrition 2022:1-10

Yang SY, Pyo MC, Nam MH, Lee KW. 2019. ERK/Nrf2 pathway activation by caffeic acid in HepG2 cells alleviates its hepatocellular damage caused by t-butylhydroperoxide-induced oxidative stress. BMC Complementary Alternative Medicine 19: 1–13.

Zhao Y, Yang L, Huang Z, Lin L, Zheng G. 2017. Synergistic effects of caffeine and catechins on lipid metabolism in chronically fed mice via the AMP-activated protein kinase signaling pathway. European Journal Nutrition 56: 2309–2318.

Zheng G, Qiu Y, Zhang QF, Li D. 2014. Chlorogenic acid and caffeine in combination inhibit fat accumulation by regulating hepatic lipid metabolism-related enzymes in mice. British Journal Nutrition 112: 10341040.