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Effects of Propolis (*Trigona* Sp.) Extract Supplementation on The Growth and Blood Profile of *Pangasius djambal.*

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Abstract. The study was conducted to examine the effects of propolis extract (PE) on the growth (G), growth rate (GR), specific growth rate (SGR) and blood profile (erythrocyte, leukocyte, and hemoglobin) of catfish (*Pangasius djambal*). five groups of fish with three replicates, containing 10 fish each group were fed various concentration of PE, *viz* 2 (P1), 4 (P2), 6 (P3), 8 (P4) and 10 (P5) g kg^{-1} of PE in a basal diet and compared to control (C) fish without PE at a rate 3% of body weight for 4 weeks. At the end of the trial, G, GR, SGR, and blood profile (erythrocyte, leukocyte, and hemoglobin) of all groups of fish were examined. The results showed that PE in the fish diet significantly increased G, GR, SGR, erythrocyte, hemoglobin while leukocyte was not affected by dietary any concentration of PE. The dietary 10 g kg-1PE in the diet of fish showed the highest growth while the highest number of erythrocyte and hemoglobin activity was found in the fish fed 6 g kg^{-1} PE in the diet. These findings suggested that the inclusion of PE higher than 8 g kg^{-1} in the diet is beneficial to improve growth, growth rate, specific growth rate, erythrocyte and hemoglobin of *Pangasius djambal*.

INTRODUCTION

Propolis is a resinous hive product collected by honeybees from various plant sources and the composition of raw propolis varies with the geographical origin and the bearing plants. In general, it is composed of 50% resin and vegetable balsam, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% various organic and mineral compounds [1].

Propolis has long been used folk medicine for curing infections [2] and in European ethnopharmacology as an antiseptic and anti-inflammatory agent for healing wounds and burns [3]. Besides its traditional uses, propolis has recently gained popularity as a supplement in numerous countries, claimed to improve health and prevent diseases [4,5]. Propolis has plenty of biological and pharmacological properties such as antibacterial [2], antifungal [6], antiviral [7], antiprotozoae [8], anti-inflammatory [9], antioxidant [10], and immunostimulant [11].

Propolis is a non-toxic natural product with multiple pharmacological effects and complex chemical compositions [4]. Recently, propolis has been extensively used as a growth promoter for poultry and fish [12,13] an adjuvant for mammals and poultry [14,15], or an immunostimulant for fish [1,16,17.18]. In view of these properti propolis was selected for use in this experiment. The objective of the present study was to determine the effect of propolis (*Trigona* sp) extract supplementation on the growth and blood profile of Pangasius djambal.

RESEARCH METHODS

Preparation of ethanolic extracts of propolis

Propolis samples were supplied by Faculty of Mathematics and Natural Science, Mulawarman University, Indonesia. 200 gram of propolis sample was cut into small pieces and extracted at room temperature with 2 L of 95% ethanol for 3 days. The propolis extract (PE) was evaporated under vacuum at 50°C and stored at 4°C until used.

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Experimental procedure

Feed used is commercial feed brand Prima Feed PF-1000. The feed mixed with propolis extract in accordance with the concentration being used i.e. 2, 4, 6, 8 and 10 g kg^{-1} of pellets. 180 (initial weight 0.93 g) fish distributed randomly to 6 tanks with 5 replications, each tank containing 10 fish.

Sample collection and hematological

Every 2-week a total number of mean body weight of fish in each tank were measured to calculate the growth performance and At the end of the feeding trial blood was collected from the caudal vein with a syringe containing EDTA and transferred into a tube and analyzed using Maindray.

Statistical analysis

Data were analyzed by using one-way analysis of variance (ANOVA) and Manny Whitney to determine whether significant differences occurred in fish fed the different diets and Hematological Profiles. If a significant difference was identified, differences among means were compared by Tukey's multiple range tests ($P<0.05$). Statistical analysis was performed using the SPSS 22.0 for Windows.

RESULTS AND DISCUSSION

The results of the analysis of proximate feed used for research are presented in **TABLE 1**

TABLE 1. The analysis of fish Feed Content proximate *Pangasius djambal* used in research with a wide variety of propolis supplementation (*Trigona* sp.).

Component	Control	P1	P2	P3	P4	P5
Moister (%)	7,24	8,74	8,35	8,21	8,12	7,82
Ash (%)	0,48	0,57	0,55	0,54	0,54	0,52
Fat (%)	1,05	1,05	1,03	1,08	1,07	1,06
Protein (%)	33,62	34,52	34,62	34,89	34,66	35,82
Fiber (%)	1,72	1,63	1,43	1,52	1,52	1,47
Carbohydrate (%)	57,61	55,12	55,45	55,31	55,61	54,78

Description: Propolis extract supplementation = 2 g kg^{-1} (P1), 4 g kg^{-1} (P2), 6 g kg^{-1} (P3), 8 g kg^{-1} (P4) and 10 g kg^{-1} (P5).

Analysis of the sample feed proximate resulted as follows: Sample feed on groups P1 has moisture content (8.74%) and ash (0.57%) had the highest among all the feed samples. However, the highest fat content found in the sample group P3 with the lowest value of 1.08 % and found on the sample feed group P2 (1.03%). Although the sample feed from the control group had the highest value of carbohydrates (57.61%), this group feed samples indicated moisture content (7.24%), ash (0.48%), and protein (33,62%), that was the lowest compare to the sample feed from other groups. Meanwhile, the lowest fiber obtained from the Group P2 (1.43%).

Growth Performance

Effects of propolis (*Trigona* sp.) extract supplementation on the growth of *Pangasius djambal* can be seen on **TABLE 2**. The feed intake was significantly higher in the 10 g kg^{-1} PE group as compare with control group ($p<0.05$). Dietary PE supplementation regardless of inclusion level significantly improved the growth rate and specific growth rate of fish.

TABLE 2. Mean ± SE (Standard error) of Growth performance of *Pangasius djambal* fed various concentrations (g kg^{-1}) of propolis extract in the diet for 4 weeks.

Parameters	Groups trial					
	Control	P1	P2	P3	P4	P5
Growth (gr)	0.09 ± 0.00^{a}	0.25 ± 0.02^{b}	0.33 ± 0.02^{bc}	0.32 ± 0.08^{bcd}	0.37 ± 0.038^{cde}	0.84 ± 0.11^{f}
Growth rate (%)	1.09 ± 0.017^{a}	1.26 ± 0.00^{b}	1.39 ± 0.06^{bc}	1.35 ± 0.07^{bcd}	1.43 ± 0.04^{cde}	2.01 ± 0.01^{f}
Specific growth rate ($x10^{-2}$) (%)	0.18 ± 0.08^{a}	0.20 ± 0.00^{a}	0.17 ± 0.04^{a}	0.02 ± 0.10^{a}	0.21 ± 0.03^{a}	0.57 ± 0.07^{b}

Description: Propolis extract supplementation = 2 g kg^{-1} (P1), 4 g kg^{-1} (P2), 6 g kg^{-1} (P3), 8 g kg^{-1} (P4) and 10 g kg^{-1} (P5).

Hematological parameters

The hematological values such as erythrocyte, leucocyte and hemoglobin are presented in **TABLE 3**. Dietary PE supplementation significantly (*P<0.05*) improved the erythrocyte and hemoglobin but leukocyte shown no significant difference.

TABLE 3. Mean ± SE (Standard error) Blood profile (erythrocyte, leukocyte, and hemoglobin) of *Pangasius djambal* fed various concentrations (g kg^{-1}) of propolis extract in the diet for 4 weeks

Parameters	Groups trial					
	Control	P1	P2	P3	P4	P5
Erythrocyte (10^{6} µL)	0.11 ± 0.01^{a}	$0,06\pm0^{b}$	0.05 ± 0.02^{bc}	0.20 ± 0.06^{acd}	0.1 ± 0.01^{acde}	0.14 ± 0.03^{acdef}
Leukocyte (10^{3} µL)	$0.9\pm0,3^{a}$	1.1 ± 0^{a}	0.6 ± 0.06^{a}	2.17 ± 0.83^{a}	1.8 ± 0^{a}	1.17 ± 0.48^{a}
Hemoglobin (g dL^{-1})	$0,93\pm0.03^{a}$	$0,7\pm0.08^{b}$	0.73 ± 0.03^{bc}	0.83 ± 0.03^{acd}	0.9 ± 0^{adef}	0.77 ± 0.07^{abcdg}

Description: Propolis extract supplementation = 2 g kg^{-1} (P1), 4 g kg^{-1} (P2), 6 g kg^{-1} (P3), 8 g kg^{-1} (P4) and 10 g kg^{-1} (P5).

Fish that were observed during the study experienced showed significantly growth performance after fed various supplementation of PE in the diet. Growth, growth rate and specific growth rate showed significantly increased and the highest growth showed on the group of fish fed PE 10 g kg^{-1} (P5) in the diet.

According to Mudjiman in Hariati [20], catfish are more likely to choose a protein as an energy source, so that the availability of protein can support optimally on the growth of fish. This finding is in accordance with the research of Deng *et al.,* [21], stating that propolis extract significantly improves the performance of growth and feed efficiency. Protein and fat are more widely used by the fish as a source of energy compared to carbohydrates. Group P5 supplementation with 10 g kg^{-1} PE increased the value of 35.82% and protein into fat 1.06%. Funjaya [19] also supported that the white blood cells helps to keep the body functioning of organisms, so that the nutrients obtained from a feed is not only used for growth but used to nourish the body and replace the damaged cells.

The Blood Profile

Circulatory system has a function as the transport of oxygen, carbon dioxide, nutrient and metabolic residue. The blood brings substance to all parts of the body and keep the body of fish properly function. Red blood cells carry oxygen, white blood cells to keep the body of the organism attacks pathogen. On each species, the number of blood cells also varies depending on the activity of the fish [19]. Propolis is can serve as an immunostimulant, which is used to increase the durability of the body of the fish [22]. Supplementing PE on the feed as a growth promoter in this research is also used to find out the health of catfish through their blood profile. The results of the analysis of the number of leukocytes catfish after fed any concentration of PE supplementation (**TABLE 3**) showed no significantly different. However, the number of erythrocytes and hemoglobin found significantly difference (*P<0.05*).

It is found that hemoglobin of fish fed various concentration of PE in the diet ranged from 0.70-0.93 g dL^{-1} while erythrocytes 0.06-0.20 x 10^6 µ L (**TABLE 3**). Lukistyowati *et al*., [23] stated that the number of catfish's erythrocytes ranged from 1.175-2.910 10^6 cell mm^{-1}. According to Dallman and Brown in Emu [24] factors that affects the number of erythrocytes is feed, nutrition, stem size, physical activity, and age. Lukistyowati [23] added that fish that fish size is one of the factors that affect the number of erythrocytes and hemoglobin in fish hematology test.

CONCLUSION

Propolis extract supplementation had positive influence on the growth of fish. Fish fed 10 gr kg^{-1} propolis extract in the diet resulted the highest growth (0.8367 ± 0.1146 g). After 4 weeks of treatment, the supplementation of propolis extract increased the number of erythrocytes and hemoglobin while the number of leukosit of fish was not affected by any various concentration of PE supplementation in the diet. The highest erythrocytes and hemoglobin value were found as follows 0.20 ± 0.0584 x10^6 µL (P3) and 0.93 ± 0.0333 g dL^{-1} (K).

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