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Dietary *Terminalia catappa* leaves reduced growth performance but increased hematological profiles and survival rate of *Pangasianodon hypophthalmus*

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Abstract. The aim of this study was to evaluate the effects of different levels dietary supplementation of *Terminalia catappa* leaves (TCL) viz: 250; 500; and 1000 g kg⁻¹ on the growth, blood profile, and survival of *Pangasianodon hypophthalmus*. Four groups including control of four replications of forty fish (Initial weight 0.230±0.004 g; initial length 31.43±0.18 mm) per tank were fed the test diets for 12 weeks. Results showed that dietary 1000 g kg⁻¹ of TCL negatively affected all growth parameters, WBC, Hb and Plt. Feed conversion ratio (FCR) was found significantly higher than other groups, while RBC and Htc were not affected by any concentration of TCL supplementation. Further, fish fed 250 g kg⁻¹ TCL in the diet showed significantly higher survival. This finding concluded that TLC which contains tannin, flavonoid, and some important phytochemicals reduced the growth of fish but significantly increased survival and FCR.

1. Introduction

The *Terminalia catappa*, belongs to Combretaceae family is widely distributed in tropical, including Asian [1]. In Indonesia, *T. catappa* L. is an attractive, long-lived tree well suited to ornamental and amenity plantings. The water extract of *T. catappa* leaves (TCL) has been also known as a traditional medicine for antipyretic, hemostatic, hepatitis and liver-related diseases purposes in Indonesia [2, 3].

Besides as the traditional medicine, TCL has been also used in fish culture. Nugroho et al. [4] proved that TCL can be applied in ornamental fish such as *Betta* sp. culture in order to enhance survival and hematological profile. Moreover, the extract of TCL at 500 ppm effective as an antibacterial against *Aeromonas hydrophila* [5]. In contrast, the use of plant based diet for fish that contains phytochemical might reduce the growth performance and other negative effects on fish [6, 7]. Inclusion rubber seed meal in the diet of *Cyprinus carpio* also caused decline in fish haematological



parameters [8]. Further, the role of TCL on the growth, morphometric analysis, survival, and hematological profile of juvenile striped catfish (*Pangasianodon hypophthalmus*) is unknown.

The growth performance and health of fish can be determined by using various physiological tools such as: average weekly gain (AWG), specific growth rate (SGR) [9, 10], Feed efficiency (FE) [11]; survival rate [12] have been applied in various of fish. Besides growth performance, the immune status of fish can be monitored by hematology indices which express as the health status of fish [13]. Hematological indices such as total red blood cells (RBC), white blood cells (WBC), hemoglobin count (Hb) and differential leukocyte count such as lymphocyte, monocyte, granular, neutrophil of fish is an effective tool that can be used to evaluate physiological in fish [14]. Moreover, WBC has been regularly used as indicators of health status in fish because WBC is key components of innate immune defense and involved in regulation of immunological function in fish [15].

Although the study regarding the effects of many plants leaves has been confirmed in several fish, the information regarding the effects of TCL on the growth performance, survival, and blood profile of striped catfish is limited. Thus, the current study was designed to evaluate, final weight (FW), average weekly gain (AWG), Specific growth rate (SGR), survival rate, RBC, WBC, Hemoglobin (Hb), hematocrit (Htc) of striped catfish fed different concentration of TCL in the diet. In addition, the phytochemical compounds in the extract which probably responsible on the growth indices and blood profiles properties were also identified.

2. Materials and Methods

2.1. Plant material

The *T. catappa* dried brown leaves were collected from, Samarinda, East Kalimantan, Indonesia. To eliminate extraneous matter, collected *T. catappa*'s leaves was washed with deionized water and immediately dried in an oven at 40°C for 12 h. To obtain *T. catappa* leaves powder (TCL), The leaves were cut, grinded and powdered using a mill.

2.2. Preliminary phytochemical tests

The preliminary phytochemical tests such as alkaloid, saponin, steroid, triterpenoid, quinone, phenolic, tannin, and flavonoid were performed to detect the presence of possible phytochemicals in the TCL. The qualitative phytochemical test was done following methods that previously used by Nugroho et al [4].

2.3. Preparation of basal and test diet

Basal diet that contains 22.45% protein, 7.15% lipid, 45.9% carbohydrate, 15.5% ash and 8.95% moisture was obtained from local market. To make test diet, basal diet was mixed with various concentration of TCL (250; 500; and 1000 mg kg⁻¹). Both dried basal and test diet were then allowed to cool at room temperature, packed and stored in a dark room before being used as a test diet and control-basal diet.

2.4. Fish and experimental setup

Six hundred forty fish (Initial weight 0.230±0.004 g; initial length 31.43±0.18 mm), 50 days-old, were purchased from Local Breeding Farm, Samarinda East Kalimantan and acclimated at Animal Physiology, Development, and Molecular Laboratory, Mulawarman University, East Kalimantan for a week. Fish were then randomly distributed into four groups with four replications (tanks) of forty fish each tank. Each group of fish was then placed in plastic tank. All tanks were supplied with recirculating water at the rate of 0.34 L min⁻¹. The experiment was carried out in 16 tanks. For 12 weeks, fish in each group was fed with various concentrations of TCL *viz*; 250; 500; and 1000 g kg⁻¹ feed supplementation. Temperature, pH, and Dissolve Oxygen (DO) were measured once a week using routine thermometer, pH meter, and TOA-dkk pH HM-7, TOA instrument, Japan. Nitrate, nitrite, and ammonia were detected using Sera test kits (Sera test kitTM, SERA GMBH, Germany) once

a week. The fish in each tank was fed with basal or test diet at a rate of 5% of body weight four times per day. Uneaten food and faeces were siphoned out.

2.5. Growth analysis

For measuring fish weight, an electronic balance (GX-4000, A&D Company, Ltd., Japan) was used. Both initial and final weights of fish were used to calculate (BWG), (DWG) averages weekly gains (AWG), Specific growth rate (SGR) [9, 16]. The length of the fish was determined using digital calliper. Meanwhile, Feed conversion rate (FCR) was calculated following equation that previously used by [17].

2.6. Blood parameters and survival rate

At the end of the research, blood samples were collected from each experimental group. Blood samples were taken by caudal venipuncture after anaesthetizing the fish. Total RBC ($10^6 \mu\text{L}^{-1}$), WBC ($10^3 \mu\text{L}^{-1}$), Hb (g dL⁻¹), Htc (%), and Plt ($10^3 \mu\text{L}^{-1}$) were determined using Auto Hematology Analyzer Mindray BC2800, Mindray® Shenzhen, China. Meanwhile, the survival rate of fish in each group was recorded every 4 weeks.

2.7. Data analysis

Results are expressed as means \pm standard error (SE) and data were analyzed using SPSS version 22 (SPSS, Inc., USA). The data of Survival was transformed to arcsine before statistically analyzed. Meanwhile, Growth analysis and hematological profile data at the end of the week 12 were subjected to analysis of variance (ANOVA), followed by Duncan post hoc test to evaluate significant differences among the groups of treatments. All significant tests were at $P < 0.05$ levels.

3. Results and Discussion

3.1 Results

There are many of phytochemical that have various biological activities to be supplemented in the diet of fish have been reported [18-22]. However, limited number of study that related to the phytochemical of *T. catappa* and its effect on the fish, especially juvenile striped catfish has been reported. Present research revealed that TCL contained saponin, triterpenoid, quinone, phenolic, tannins, and flavonoid. After 12 weeks of feeding trial, the inclusion of TCL in the diet higher than 500 g kg⁻¹ reduced significantly ($P < 0.05$) all growth parameters of juvenile striped catfish. However, FCR of juvenile striped catfish fed TCL supplementation above 500 g kg⁻¹ in the diet was increased (Table 1). Hematological profile of fish fed different concentration of TCL in the diet did not indicate significantly different on the RBC and Htc. However, the WBC, Hb and Plt of fish fed high concentration of TCL in the diet showed significantly reduced compare to other groups (Table 2). Meanwhile, supplementation with TCL in the diet, fish showed significantly higher survival. The group of fish fed 250 g kg⁻¹ of TCL in the diet had better survival rate than other groups (Figure 1).

Table 1. Mean \pm SE growth and feed efficiency of Juvenile *Pangasianodon hypophthalmus* fed dietary *Terminalia catappa* leaves supplementation in the diet for 12 weeks

Parameters	Groups (g kg ⁻¹)			
	Control	250	500	1000
Initial length (mm)	31.15 \pm 0.29 ^a	31.70 \pm 0.42 ^a	31.06 \pm 0.39 ^a	31.83 \pm 0.34 ^a
Final length (mm)	59.26 \pm 2.74 ^a	55.03 \pm 3.2 ^{ab}	52.61 \pm 0.93 ^{ab}	45.80 \pm 3.07 ^b
Length gain (mm)	28.11 \pm 2.64 ^a	23.33 \pm 3.38 ^a	21.55 \pm 0.87 ^{ab}	13.97 \pm 2.85 ^b
DLG (mm)	0.31 \pm 0.02 ^a	0.25 \pm 0.03 ^a	0.23 \pm 0.009 ^{ab}	0.15 \pm 0.03 ^b
Initial weight (g)	0.226 \pm 0.007 ^a	0.233 \pm 0.008 ^a	0.220 \pm 0.010 ^a	0.236 \pm 0.008 ^a
Final weight (g)	1.81 \pm 0.17 ^a	1.41 \pm 0.26 ^{ab}	1.14 \pm 0.09 ^{bc}	0.80 \pm 0.16 ^c
Total biomass (g)	14.02 \pm 0.26 ^a	14.33 \pm 0.11 ^{ab}	15.71 \pm 0.23 ^b	11.43 \pm 0.57 ^c
BWG (g)	1.58 \pm 0.17 ^a	1.13 \pm 0.22 ^{ab}	0.92 \pm 0.09 ^{bc}	0.57 \pm 0.15 ^c

Parameters	Groups (g kg ⁻¹)			
	Control	250	500	1000
DWG (g)	0.017±0.001 ^a	0.012±0.002 ^{ab}	0.010±0.001 ^{bc}	0.006 ±0.0017 ^c
AWG	0.132±0.014 ^a	0.094±0.018 ^{ab}	0.077±0.007 ^{bc}	0.047 ±0.012 ^c
SGR	2.24±0.09 ^a	1.91±0.22 ^a	1.82±0.09 ^{ab}	1.26±0.17 ^b
FCR	0.65±0.019 ^a	0.96±0.19 ^{ab}	0.99±0.07 ^{ab}	1.42±0.33 ^b

Different alphabets (a, b, c) indicate significantly different means for different treatments at $P < 0.05$. Control group = without *Terminalia catappa* leaves supplementation. DLG = Daily length gain, BWG = Body weight gain, DWG = Daily weight gain, AWG = Average weekly gain, SGR = Specific growth rate, FCR = Feed conversion ratio.

Table 2. Blood profile of *Pangasianodon hypophthalmus* fed various concentrations (g kg⁻¹) of *Terminalia catappa* leaves supplementation for 12 weeks

Parameters	Groups			
	Control	250	500	1000
WBC (10 ³ μL ⁻¹)	1.46 ± 0.26 ^a	1.56± 0.56 ^b	1.58± 0.23 ^b	1.21± 0.17 ^b
RBC (10 ⁶ μL ⁻¹)	0.06 ± 0.01 ^a	0.06 ± 0.01 ^a	0.04 ± 0.01 ^a	0.05 ± 0.005 ^a
Hb (g dL ⁻¹)	0.33 ± 0.08 ^a	0.30 ± 0.05 ^a	0.36 ± 0.03 ^a	0.26 ± 0.06 ^b
Htc (%)	0.19±0.01 ^a	0.23 ± 0.02 ^a	0.20 ± 0.01 ^a	0.20 ± 0.06 ^a
Plt (10 ³ μL ⁻¹)	213.0 ± 6.20 ^a	189.0 ± 2.60 ^{ab}	190 ± 1.70 ^{ab}	145.0 ± 1.00 ^c

Data are given as mean values (Mean ± SE). Different superscript (a, b, c) in the same row indicates significantly different mean values at $P < 0.05$. WBC = White Blood Cells, RBC = Red Blood Cells, Hb = Hemoglobin, Htc = Hematocrit, Plt = Platelet

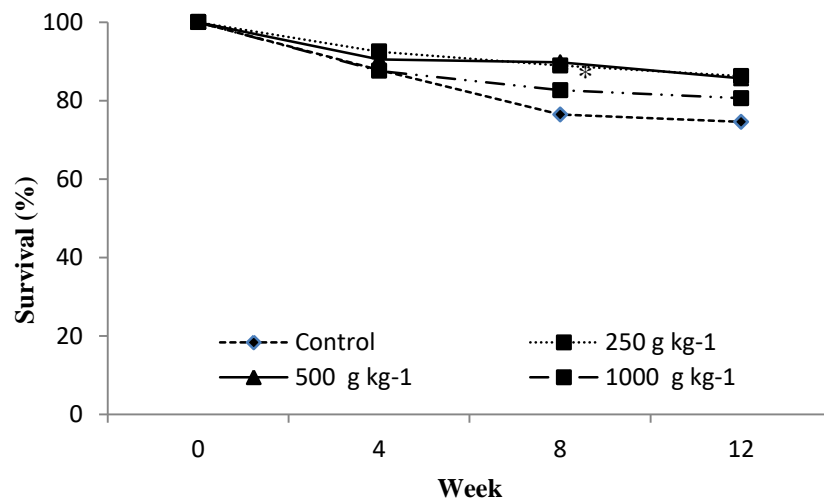


Figure 1. Survival rate (%) of juvenile *Pangasianodon hypophthalmus* fed dietary *Terminalia catappa* leaves (TCL) supplementation (g kg⁻¹) for 12 weeks. C = Control without supplementation. (*) The highest survival rate (250 g kg⁻¹ of TCL).

3.2 Discussion

Body weight is the major indicator of growth and to determine whether the feeding level is adequate or not. The growth of fish is influenced by internal and external factors [23]. External factor, such as feed supplementation has been recognized as an important factor that influences on the growth of fish. Current results found that the inclusion of TCL in the diet generally decreased growth value of fish. This finding might be affected by high level carbohydrate in the diet and level of supplementation of TCL which contain phytochemicals. de Francesco et al [24] stated that high content of carbohydrate in the diet of fish is generally not well digested. In addition, plant ingredients also contain various levels of non-starch polysaccharides [25] whose possible negatively effects are unclearly mechanism in fish [26]. It is also possible that other ANFs might be responsible for the low growth rate [24].

The application of phytochemical on the blood profile is a current trend in fish culture. Blood parameters are gaining in popularity and an important variable in aquatic field, expressing the health status of fish [27-30]. Blood parameters such as RBC, WBC, Hb, Htc, and Plt are major indicators in assessing the health statuses of the fish. Current results showed that groups of fish fed with TCL any concentration had higher WBC than control and inclusion above 500 g kg⁻¹ resulted higher Plt than any others group whereas RBC and Htc of fish fed TCL were not affected by TCL inclusion.

The phytochemical such as flavonoid can be a catalyst in leukocytes production and leukocytes stimulator as nonspecific cellular immunity. In addition, flavonoid has a beneficial effect to decrease RBC hemolysis, protecting biological membranes of RBC against free radical and oxidative damage [31, 32]. The flavonoid in the TCL is also capable as an antioxidant which may contribute to maintain the heme iron in its ferrous state [33-35]. Present finding also found that juvenile stripped catfish fed 250 g kg⁻¹ TCL which contains phytochemicals in the diet had better survival than other groups. This finding was in line with previous research that there was no significant evidence on the mortality of *Betta sp* after exposure of phytochemicals [36] and had higher survival after immersion with 500 ppm TCL extract than control group [37, 38]. Chakraborty and Hancz [39] revealed that phytochemicals such as alkaloids, flavonoids, pigments, phenolics, and terpenoids are secondary metabolites in plants that might increase the innate immune system of fish culture. In addition, the phytochemical could enhance the survival rate of the fish. In contrast, tannin which presents dominantly in plant had also negative effects to *Channa striatus* and *Cyprinus carpio* [40]. Although saponin has a negative effect on the growth of fish, the application of 1 and 2 mg L⁻¹ saponin on white shrimp *Litopenaeus vannamei* improve the survival rate [41]. Nevertheless, pure high levels of saponin (200 mg L⁻¹) can be harmful and cause stress and mortality [42].

4. Conclusion

Inclusion high concentration of *Terminalia catappa* leaves above 250 g kg⁻¹ which contains active phytochemicals in the diet of juvenile stripped catfish reduces growth performance and blood profile such as white blood cells, hemoglobin, and platelet, but shows high survival and increases FCR. Further research needs to be conducted to determine the antioxidant enzymes activity, such as glutathione peroxide, superoxide dismutase and catalase as well as levels of lipid peroxidase that are related to the health and immunity of juvenile stripped catfish.

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